The composition of monounsaturated fatty acids of artemia enriched with biologically active substances

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Abstract. The work is devoted to the study of monounsaturated fatty acids (MUFA) content in artemia enriched with biologically active substances at different stages of ontogenesis. Artemia cysts are used in the preparation of dietary supplements, highly effective feed additives for farm animals, fertilizers for crop production, chitosan for pharmaceutical industry and cosmetology, as raw materials in many other fields. When growing fish, crustaceans, shellfish in aquaculture, artemia nauplii are used as live starter feeds, which are obtained in situ when cultivating artemia cysts. Recently, the direction of enrichment of artemia nauplia used for feeding fish larvae and fry with biologically active substances has begun to develop: vitamins, essential amino acids, essential fatty acids, probiotics. In our work, we used a feed additive for the enrichment of artemia nauplia, including: vitamin-amino acid complex – «Chiktonic», probiotic – «Vetom-1», adaptogen – «Trekrezan», hemp oil. Enrichment was carried out through the skin at the stage of early nauplia. The aim of the work was to study the composition of MUFA at different stages of ontogenesis in intact artemia and artemia enriched with biologically active substances. The main function of MUFA is to activate metabolic processes and maintain homeostasis. When identifying monounsaturated fatty acids of artemia, the race we studied, the following were identified: oleic, palmitoleic, eicosenic, elaidic, erucic, nervonic, myristoleic acids. Oleic, palmitoleic and eicosenic fatty acids played a dominant role in the structure of MUFA. Two fatty acids, erucic and elaidic, can be distinguished in the composition of MUFA, the content of which was minimal.

1 Introduction

Use The strategy of scientific and technological development of the Russian Federation focuses on the transition to a highly productive and environmentally friendly agro- and aquatic economy. The solution to this problem is inextricably connected with the development of aquaculture [1].

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In recent decades, Russian aquaculture has been increasingly focused on high-tech fish farming systems, which have become increasingly introduced into practice, using innovations with a clear scientific basis [1-3]. Science is an important factor that can ensure the targeted and accelerated development of the industry [3].

The introduction of sanctions regime by the European Union has significantly complicated the situation with the development of aquaculture in Russia, depriving it of high-quality feed for the development of fish farming. It is well known that Rus-sian aquaculture was 60% dependent on European feed. Even before the introduction of the sanctions regime, the production of domestic fish feed barely provided 40% of the needs of the national market [1].

Russian factories that produced feed, which included imported components, faced with the problem of forced search for domestic analogues - substitutes for imports [1, 4].

Against this background, the problem of starter feeds, which for the most part were imported from abroad, sharply aggravated, which required an operational solution [1, 4].

In the practice of world aquaculture, nauplia, as well as decapsulated artemia eggs, are the internationally recognized and most popular starter food [5, 6].

Artemia is a gill-footed crustacean, widespread in salty aquatic ecosystems. The high demand for artemia as starter feeds for aquaculture is due to its high nutritional value, soft and thin outer covers, small size.

In the global fish market, the demand for artemis eggs is growing from year to year, but the volume of their global production in natural reservoirs does not provide even half of the existing demand [5, 6].

Russia is among the leading suppliers of artemia cysts to the world market, 95% of artemia cysts extracted in Russian hyperhaline lakes are exported abroad. This is due to the fact that for enterprises producing artemia cysts, large exports are much more profitable than retail trade on the Russian market. Today there is a paradoxical situa-tion when the price of artemia cysts in the domestic market is many times higher than in the international market [5, 6].

Artemia, sold by retail on the domestic Russian market, is practically inaccessible to domestic consumers, since prices for its cysts range from 2 thousand rubles / kg (ECOFUND LLC) - up to 2500 rubles/kg (Rybovod Altai LLC), and in pet stores prices exceed 3 thousand rubles, which at today's rate is about 40Artemia, sold by: $50. At the same time, the wholesale price of artemia cysts on the international mar-ket for the last decade has not exceeded 10-15 dollars/kg [5, 6].

Russian artemia is valued on the international market. Its main consumers are China, Thailand, Vietnam. Kurgan Agricultural Resource enterprise supplies decapsulated artemia eggs to the world's leading European producers of fish feed – Inve (Belgium), Coppens (Netherlands), who consider artemia the best starter food for many species of aquatic organisms. In the starter feeds for fish and shrimp of these producers, artemia in its pure form reaches 95%.

Artemia is also in demand not only in aquaculture, but also in other sectors of na-tional economy, enterprises engaged in processing artemia and obtaining valuable dietary supplements from it in Russia are still isolated. The most famous scientific and production enterprise «Bio Building» in Novosibirsk, which brought to the market such dietary supplements as «Artemia Gold» and «Astaxanthin+Omega-3+Omega-6+Iodine» based on Artemia raw materials.

The research aim: to investigate the composition of monounsaturated fatty acids at different stages of ontogenesis in intact artemia and artemia enriched with biologically active substances.

2 Research materials and methods

The research object was cysts, decapsulated eggs and artemia nauplia, which, in order to increase their biological value, were treated with a complex of biologically active substances, which included: probiotic «Vetom 1.1» based on Bacillus subtilis, adaptogen «Trekresan» vitamin-amino acid preparation «Chiktonic», hemp oil.

The following composition was used to enrich cysts or decapsulated artemia eggs per 1 kg of biomaterial: 1 g of probiotic + 5 mg of adaptogen trekresan + 1 ml of vitamin-amino acid preparation Chictonic + 1 ml of hemp oil, which was diluted in 100 ml of water was strongly shaken and the resulting fine suspension from a spray was sprayed with cysts or decapsulated artemia eggs scattered in a monolayer, which were then dried at room temperature.

When enriching artemia nauplia, the same component composition of enriching complex was used, but the enrichment was carried out twice during cultivation. The first enrichment was carried out after 12 hours of incubation of cysts. The formulation of enriching complex introduced into the culture medium included: 0.1 g/l of Vetom-1 probiotic, 5 mg/l of adaptogen, 0.1 ml/l of vitamin-amino acid preparation «Chiktonic». The second enrichment was carried out after 24 hours of incubation, the formulation of the second stage of enrichment included: 1 g / l of probiotic, 5 mg / l of adaptogen, 1 ml / l of vitamin-amino acid preparation «Chiktonic», 0.5 ml / l of hemp oil.

To analyze the composition of monounsaturated fatty acids, enriched and intact cysts, enriched and intact decapsulated artemia eggs, enriched with artemia nauplia were used.

The analysis of fatty acid composition of the studied biomaterial was carried out using a hardware and software complex for medical research based on the chromato-graph «Chromatek-Crystal 5000.1», a plasma ionization detector in accordance with GOST 31663-2012 and GOST 31665-2012.5

The research was carried out on the basis of a certified educational, scientific and testing laboratory for determining the quality of food and agricultural products of FSFEI HE «Saratov SAU named after N.I. Vavilov».

3 The research results

The formation of the fatty acid composition of artemia is influenced by food sources and a complex complex of abiotic factors. Therefore, different ecomorphs or races of artemia, differing in biological and nutritional value, live in different natural ecosystems.

Lipids, which serve as a plastic and energy material, have a great influence on the biological and nutritional value. Artemias from different natural sources have a unique set of lipids in terms of qualitative and quantitative composition [5, 6], which has distinctive features at different stages of ontogenesis [5, 6].

The main function of monounsaturated fats is activation of metabolic processes and maintenance of homeostasis [7]. The lack of monounsaturated fats for the body is fraught with deterioration of brain activity, disruption of the cardiovascular system, deterioration of well-being [8].

During the study of fatty acid composition of artemia, monounsaturated fatty acids (MUFA) were isolated into a separate group) [7, 8].

The list of MUFA which we have identified includes fatty acids: oleic, palmitoleic, elaidic, eicosene, erucic, nervonic, myristoleic.

The data of the research results indicate that oleic acid takes a dominant place among monounsaturated fatty acids. Its content in the biomaterial of artemia varied in the range of 23.6-27.6% (Figure 1).

The second place was taken by palmitoleic acid, the level of which was in the range of 1.8-16.2% (Figure 1). In the third place is eicosenic acid. Its share was 3.2-11.3%.

Erucic and elaidic acids (less than 0.1%) were characterized by the minimum con-tent of MUFA. This was typical for intact and enriched cysts, intact and enriched decapsulated eggs. The nauplii had a higher content – 0.4%.

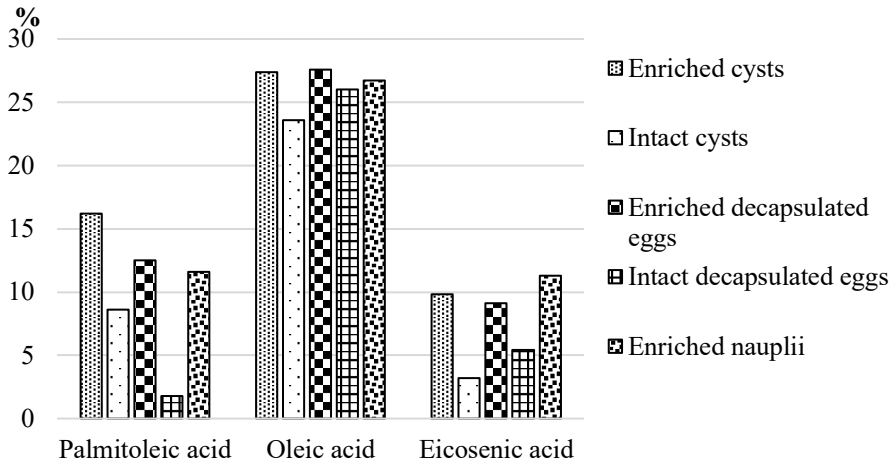

Fig. 1. The content of palmitoleic, oleic and eicosenic acids, %.

Palmitoleic acid refers to monounsaturated fats n-7, which are usually a product of palmitic acid metabolism in the body. It reduces insulin resistance, protects against inflammation and fat accumulation.

In the studies on humans and animals it has been shown that palmitoleic acid has an anti-inflammatory and hypolipidemic effect. Although the conversion of palmitic acid to palmitoleic acid may have some positive effect on metabolic markers, in some cases this acid can also potentially contribute to the development of cardiovascular diseases. With an increased content, it increases the level of low-density lipoproteins to a greater extent than other saturated fatty acids.

Palmitoleic acid is a part of human hypodermic fat, has an anti-inflammatory effect within normal limits and improves the sensitivity of liver and skeletal muscles to insulin.

According to the results of our studies, the content of palmitoleic acid in artemia varied in the range of 1.8-16.2%. Its highest level was noted in enriched cysts - 16.2%, and the content of palmitoleic acid in intact cysts was 1.9 times lower compared to enriched cysts.

Palmitoleic acid in intact decapsulated eggs was 6.8 times less than in enriched decapsulated eggs. There was a significant difference between intact cysts and intact decapsulated eggs. Thus, intact cysts contained 4.8 times more palmitoleic acid compared to intact decapsulated eggs. The content of palmitoleic acid in enriched cysts, decapsulated eggs and nauplias was 16.2%, 12.5% and 11.6%, respectively.

Oleic acid is a monounsaturated fatty acid that is synthesized in the cytoplasm of liver cells. It performs energy and plastic functions in the body. It makes up approximately 92% of all cis isomers of monounsaturated fats in the human diet. Oleic acid affects the composition of cell membranes and the activity of receptors on their surface.

According to the results of our studies, the content of oleic acid ranged from 23.6-27.6% (Figure 2).

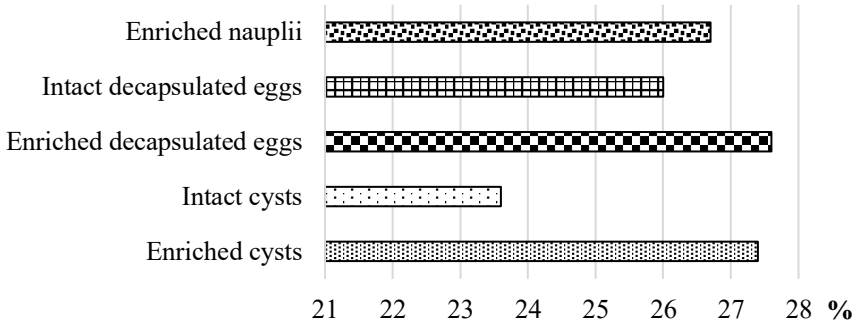

Fig. 2. The content of oleic acid, %.

The content of oleic acid in intact cysts was 23.6%, enriched cysts 27.4%. In intact and enriched decapsulated eggs, this indicator was 26.0% and 27.6%, respectively. The content of oleic acid in nauplia was 26.7%.

The content of oleic acid in intact cysts was 16% lower compared to enriched cysts. The content of oleic acid in intact decapsulated eggs was less than in enriched decapsulated eggs by 6.2%.

The content of oleic acid in enriched: cysts, decapsulated eggs and nauplias did not have a significant difference. The difference between intact cysts and intact decapsulated eggs was insignificant. It was 10.2%.

Eicosenic acids of C20 series are polyene fatty acids, from which biologically active substances called eicosanoids are synthesized. The main substrate for their synthesis in humans is arachidonic acid, since its content in the body is significantly higher than other polyenic acids – precursors of eicosanoids. In smaller quantities, eicosapentaenoic and eicosatetraenoic acids are used for the synthesis of eicosanoids [9]. An important role is assigned to the role of eicosenic acids in the pathogenesis of cardiovascular diseases [10].

In intact and enriched: cysts, decapsulated eggs, artemia nauplii, the content of eicosenic acid was in the range of 3.2 – 11.3%. The content of eicosenic acid in intact cysts was at the level of 3.2%, in enriched cysts - 9.8%. Thus, the content of eicosenic acid in intact cysts was 3 times lower than in enriched ones.

The content of eicosenic acid in intact decapsulated eggs was 5.4%, enriched with 9.1%. Consequently, it was 68% less in intact decapsulated eggs compared to enriched decapsulated eggs.

The content of eicosenic acid in enriched nauplia was 11.3%, which is 15.3% more compared to enriched cysts and 24.2% more compared to enriched decapsulated eggs. The difference in the content of eicosenic acid between intact cysts and decapsulated eggs was 68.7%.

Elaidic acid refers to Omega-9 unsaturated fatty acids. It helps to increase the activity of protein that transports cholesterol esters in plasma. This compound is important for lowering cholesterol.

There were no significant differences in the content of elaidic acid in all the studied objects. According to the results of our studies, elaidic acid was found only in enriched nauplias, where it was 0.4%. In all other samples of artemia, its content was less than 0.1% (Figure 3).

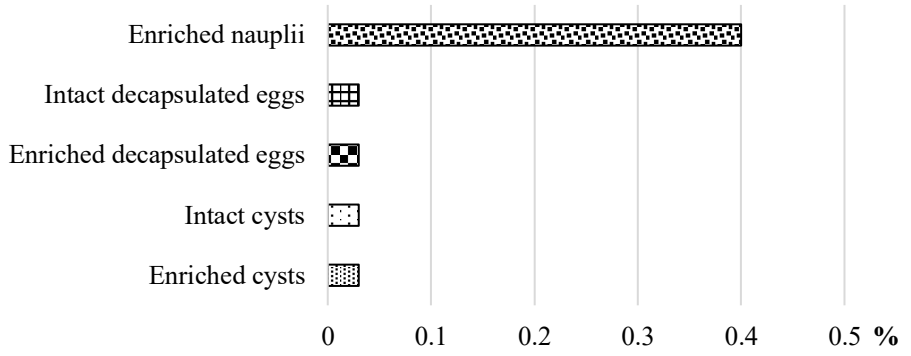

Fig. 3. The content of elaidic acid, %.

Erucic acid is produced in the body naturally. The question of its negative effect on the body is being debated, but there is no sufficient evidence to confirm this [11].

According to the results of our studies, the content of erucic acid in both intact cysts and decapsulated eggs, as well as in enriched samples, including nauplia, was less 0.1%.

Nervonic acid or selacholic acid (cis-15-tetracosenic acid) is found in small amounts in the fats of marine animals and fish (salmon). Plays an important role in ensuring the normal functioning of the nervous system. Through amide bonds, it connects with sphingosins and forms sphingolipids, which are responsible for the transmission and recognition of the cellular signal. It accounts for 40% of the total amount of fatty acids in these substances.

The research results of the content of nervonic acid in artemia are shown in the Figure 4.

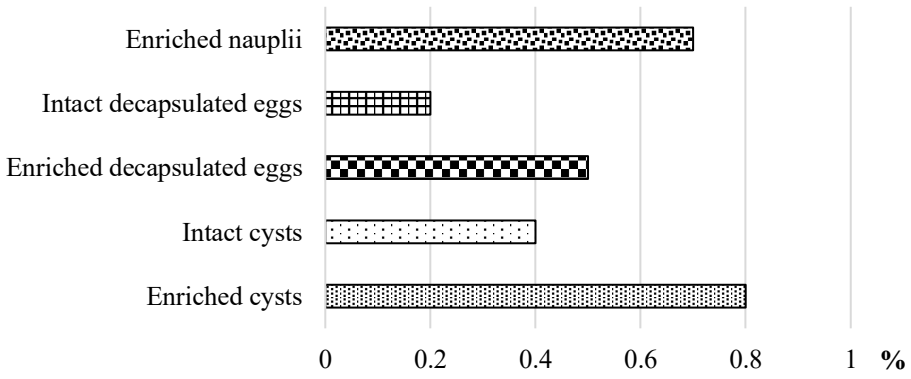

Fig. 4. The content of nervonic acid, %.

According to the results of our studies, the content of nervonic acid was in the range of 0.2-0.8%. Thus, the content of nervonic acid in enriched cysts was 0.8%, in intact cysts 0.4%. The content of this acid in intact decapsulated eggs was 0.2%, enriched - 0.5%. The content of nervonic acid in the nauplii was higher than in decapsulated eggs – 0.7%.

The content of nervonic acid in intact cysts was 2 times lower than in enriched cysts. In intact decapsulated eggs, the amount of nervonic acid was 2.5 times lower compared to enriched decapsulated eggs.

The content of nervonic acid in enriched nauplia was lower than in enriched cysts by 14.3%, and enriched decapsulated eggs - by 40%. There were also differences between

intact cysts and decapsulated eggs. The amount of nervonic acid in intact cysts was 2 times higher compared to intact decapsulated eggs.

Myristoleic acid is a monounsaturated fatty acid found in the fats of fish and marine mammals, in female milk in shark liver, eel and turtle fat, goat's milk.

According to the results of our studies, the content of myristoleic acid in artemia ranged from 0.4 - 2.1% (Figure 5).

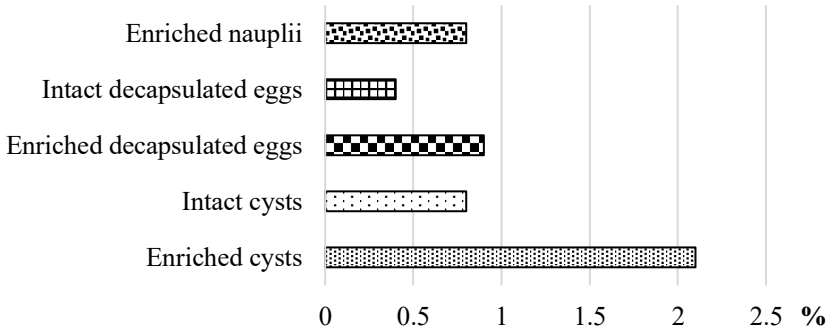

Fig. 5. The content of myristoleic acid, %.

The highest content was observed in enriched cysts and was 2.1%. The amount of myristoleic acid in enriched cysts was 2.3 times higher than in enriched decapsulated eggs, and 2.6 times higher than in enriched nauplii. The content of myristoleic acid in intact cysts was 2 times higher compared to intact decapsulated eggs.

4 Discussion of the obtained results

Monounsaturated fatty acids (MUFA) are fatty acids, in the structure of molecules, which are allowed no more than one double carbon bond. MUFA have one important distinguishing feature - at room temperature they have a liquid structure, but when the temperature decreases, they thicken [9].

The most famous representative of MUFA is oleic acid (omega-9), which is found in large quantities in olive oil [9, 10].

In addition, palmitoleic acid, erucic acid, eicosenic acid, and aceterucic acid belong to MUFA. And eleven less common monounsaturated fatty acids. Monounsaturated fats are mostly considered very useful substances for the body. They actively participate in metabolic processes, which leads to well-coordinated work of the whole body, strengthens the immune system.

The main function of monounsaturated fats is to activate metabolic processes and maintain homeostasis. The lack of monounsaturated fats for the body is fraught with deterioration of brain activity, disorders of the cardiovascular system, feeling unwell [9, 10].

When identifying monounsaturated fatty acids of artemia at different stages of ontogenesis, 7 MUFA were identified: palmitoleic, oleic, elaidic, eicosene, erucic, nervonic, myristoleic.

Among the monounsaturated fatty acids of artemia, oleic acid occupies the main place. 23.6-27.6, palmitoleic acid 1.8-16.2% took the second place, eicosenic acid 3.2-11.3% took the third place. The wide range of fluctuations demonstrates how different the content of MUFA is in enriched and non-enriched artemia

Two fatty acids, erucic acid and elaidic acid, should be distinguished from MUFA with a minimum content. Their content was less than 0.1%. This was typical for intact and enriched cysts, intact and enriched decapsulated eggs. In nauplii, its content was higher and amounted to 0.4%.

In intact and enriched: cysts, decapsulated eggs, artemia nauplii, the content of eicosenic acid, which plays an important role in the pathogenesis of cardiovascular diseases, was in the range of 3.2 - 11.3% in intact and enriched artemia, respectively.

The content of nervonic acid necessary for the normal functioning of the nervous system was 0.2-0.8% in intact and enriched artemisia, respectively.

It is well known that the use of artemia as live starter feeds avoids the mass death of fish larvae during the transition to exogenous nutrition, a large role in this process is given to fatty acids [6, 9, 10]. This stimulates the growth of research on the enrichment of artemia and its expanded use as live starter feeds for a wide range of hydrobionts.

The main direction of scientific research on the use of artemia as starter feeds for fish is focused on finding ways to enrich it with various biologically active substances that play an important role in the vital activity of the fish organism [6, 12-14]. The world's largest manufacturers of starter feeds for fish also orient their business in this direction.

In Russia, few works on the enrichment of artemia are published; during a patent search on the problem under study, a single patent was found issued for a method of enriching artemia [RU 2 577 478 C1]. Artemia was enriched in it through the intestines.

We went our own way and used the method of enrichment through the skin at an early stage of the development of artemia larvae, before their transition to exogenous nutrition. Until now, the enrichment of early artemia nauplia through the skin has not been practiced. All known attempts to enrich artemia used enrichment through the intestine.

A complex of biologically active substances which we developed and patented was used as an enriching additive (patent RU 2777105 C1) [12].

The composition of enriching biologically active complex for the production of starter feeds - nauplii artemia includes the adaptogen trecresan, a probiotic based on Bacilus subtilis and the vitamin-amino acid complex «Chiktonic», balanced in essential amino acids and hemp oil [6, 12-15].

The research results showed that the enrichment process in the prevailing majority of cases increased the content of the most important MUFA in artemia of different stages of ontogenesis several times.

4 Conclusion

Enriched according to the developed technology, nauplia and decapsulated artemia eggs, when used as a starter feed, can not only ensure the survival and energy costs of the fish body, but also heal, stimulate immune protection, accelerate growth and development, increase the effectivity of feed utilization, reduce the level of cannibalism in fish larvae and fry.

The development of biotechnologies for the production of biologically active substances enriched live starter feeds is an urgent task of modern aquaculture.

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