Evaluation of the content of polyunsaturated fatty acids in artemia at different stages of ontogenesis

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Abstract. The work is devoted to the study of the composition of polyunsaturated fatty acids (PUFA) at artemia of different stages of ontogenesis. The content of PUFA in intact cysts and decapsulated artemia eggs was studied, and the content of PUFA after enrichment of cysts, decapsulated eggs, and artemia nauplia with a multicomponent complex of biologically active substances was also studied. The composition of the enriching complex included probiotics, adaptogens, vitamins, amino acids and hemp oil. The results of the research showed that the use of the enriching complex significantly increased the content of polyunsaturated fatty acids of the omega–3 and omega-6 families. The content of eicosapentaenoic, docosahexaenoic, linolenic fatty acids from Omega-3 family, and the content of linoleic and arachidonic acids from Omega-6 family increased to the greatest extent, primarily in nauplia, decapsulated eggs and artemia cysts, thereby significantly increasing their biological and nutritional value while using as starter feeds for fish. Keywords: Artemia, live starter feeds for fish, enrichment, biologically active substances, polyunsaturated omega-3 and omega - 6 fatty acids.

1 Introduction

Artemia is a gill-footed crustacean belonging to the Artemiidae family that lives in an aquatic environment of increased salinity [1-3].

All currently known artemia species are considered in the world aquaculture to be the best starter food for fish larva and other aquatic organisms [4-5] therefore, their cysts in natural ecosystems are the object of fishing.

Artemia has a high nutritional value and a unique fatty acid composition, which has a stimulating effect on the growth of fish and ensures their survival at different stages of postembryonic ontogenesis [6-8].

The biological and nutritional value of artemia used as feed for fish, crabs and shrimps in aquaculture conditions depends on many factors, including the chemical composition of water, salinity level, food sources, temperature, etc. [9].

In this respect, artemia races (ecomorphs) from various natural sources have different chemical composition and, consequently, different biological and nutritional value. Lipids,

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which serve as plastic and energy material in organisms cultivated in aquaculture, have a great influence on the biological and nutritional value [10].

Almost all living organisms have the ability to synthesize saturated fatty acids, but for synthesis of unsaturated fatty acids, species–specific enzymes are necessary - desaturases and elongases [11,12].

In most animal species, including humans, these enzymes that form a double bond at the position of ω3 and ω6 are absent.Therefore, for these species, such fatty acids as linoleic acid (18:2ω6) and α-linolenic acid (18:3ω3) are essential.These fatty acids are filled up only by substances coming from food sources [13].

Polyunsaturated fatty acids: 20:4n-6 and 20:5n-3 play an important role in fish metabolism. DHA is necessary for the formation of cell membranes, including nerve tissues [14, 15]

From arachidonic acid (20:4), eicosanoids are formed - a large group of mediators with a wide range of biological activity; in fish, they participate in the regulation of early stages of postembryonic ontogenesis and metamorphosis; they play a leading role in the realization of mechanism of inflammatory process [16-19].

Freshwater fish are able to synthesize long-chain polyunsaturated fatty acids from C18 polyunsaturated fatty acids [20]. It is known that in freshwater fish the essential fatty acids are 18:2n-6 and 18:3n-3 [20]. They must necessarily be present in fish feed within one percent.

Nowadays, the study is based not only on the high importance of polyunsaturated fatty acids for the vital activity and growth of the fish organism, but it is also relevant to humans as fish consumers.

As nauplia of artemia and artemia of other stages of ontogenesis are the most important feed object of fish, we made attempts to increase their biological and nutritional values by enriching biologically active substance complex which we developed, including probiotics, adptogens, vitamins, amino acids, adding to it hemp oil - Cannabis ruderalis of the first cold-pressed.

The introduction of probiotic based on Bacillus subtilis into the enriching complex was necessary for the formation of full-fledged microbiocenosis in fish larvae and fry, which will be fed with decapsulated eggs and artemia nauplii and to protect them from infectious diseases, since Bacillus subtilis produces polyene antibiotics.

The introduction of adaptogen into the enriching complex is due to the fact that trekrezan protects the body from all types of stress, increases the endurance of the body, activates the immune system.

The introduction of amino acids and vitamins into the enriching complex does not require explanation.

Hemp oil is included in the enriching complex not by chance, but due to the high content of polyunsaturated fatty acids. The content of linoleic fatty acid in it ranges from 50% to 70%, linolenic and gamma-linolenic from 15% to 25%. In smaller quantities, it also contains other equally significant polyunsaturated fatty acids.

Research aim: Evaluation of the content of polyunsaturated fatty acids of artemia at different stages of ontogenesis against the background of enrichment with a complex of biologically active substances.

2 Materials and methods of research

The objects of the study were 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 + 40 mg of adaptogen + 1 ml of vitamin-amino acid preparation + 1 ml of hemp oil, which were diluted in 100 ml of water and the resulting fine suspension from a spray sprayed cysts or decapsulated eggs of artemia, scattered 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. The formulation of enriching complex introduced into the culture medium included: 0.1 g /l probiotic, 0.005 g / l adaptogen, 0.1 ml / l vitamin-amino acid preparation. 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, 0.5 ml / l of hemp oil.

For the analysis of amino acid composition, enriched and intact cysts, enriched and intact decapsulated artemia eggs, enriched with artemia nauplia were selected.

The analysis of fatty acid composition of the biomaterial under study was carried out using a Hardware and software complex for medical research based on chromatograph "Chromatek-Crystal 5000.1", a plasma ionizer detector in accordance with GOST 31663-2012 and GOST 31665-2012 p.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 FSBEI HE «Saratov State Agrarian University named after N.I. Vavilov».

3 Research results

When studying the fatty acid composition of artemia (cysts, decapsulated eggs and nauplia) first of all, we focused our attention on the content of Omega-3 and Omega – 6 fatty acids, which were found in artemia at different stages of ontogenesis.

The composition of Omega –3 includes such acids as linolenic, docosahexaenoic and eicosapentaenoic, the composition of Omega–6: linoleic, gamma-linoleic, tricosan, arachidonic.

Linoleic acid. Cysts enriched with biologically active substances contained 16.1% linoleic acid. Intact cysts contained 4.1% linoleic acid, which was 3.9 times less.

In enriched decapsulated eggs, the linoleic acid content was 36.1%, which was 7.8 times more than in intact decapsulated eggs. The content of linoleic acid in the enriched nauplii was 25,5%.

The enriched decapsulated eggs contained 2.2 times more of linoleic acid than the enriched cysts. The content of linoleic acid in enriched nauplias was 1.5 times higher than in enriched cysts, but it was 41.6% lower compared to enriched decapsulated eggs (Fig. 1).

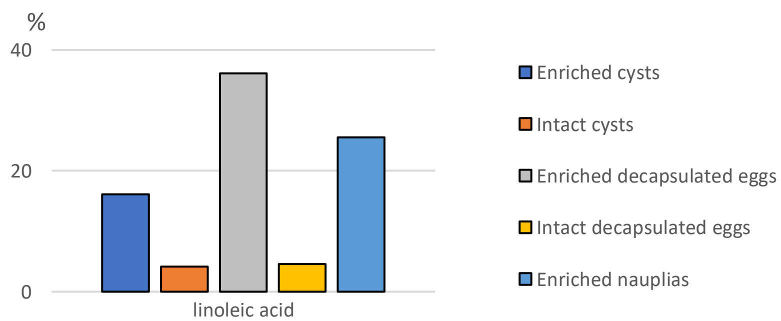

Fig. 1. The content of linoleic acid, %.

Gamma-linoleic acid. The highest content of gamma-linoleic acid was observed in enriched nauplias, where it was 1.3% (Table 1). The content of gamma-linoleic acid in enriched artemia cysts was 7 times higher compared to intact cysts. There was an increase in gamma-linoleic acid in enriched decapsulated eggs relative to intact eggs. The difference was 40%. The content of gamma-linoleic acid in enriched nauplias was 1.9 times higher than in enriched cysts and decapsulated eggs.

Table 1. The content of gamma-linoleic acid.

Indicators	C18:3n6 gamma-linoleic acid, %
Enriched cysts	0.7±0.17
Intact cysts	< 0.1
Enriched decapsulated eggs	0.7±0.34
Intact decapsulated eggs	0.5±0.13
Enriched nauplii	1.3±0.55

The total content of acids included in the composition of Omega – 6 is shown in figure 2.

Arachidonic acid is an essential acid necessary for the functioning of the body. Its metabolites fulfill important regulatory functions, promote the growth and regeneration of skeletal muscles and muscle fibers. Arachidonic acid participates in metabolic processes, prevents the deposition of subcutaneous fats, improves metabolism, stimulates muscle growth [21].

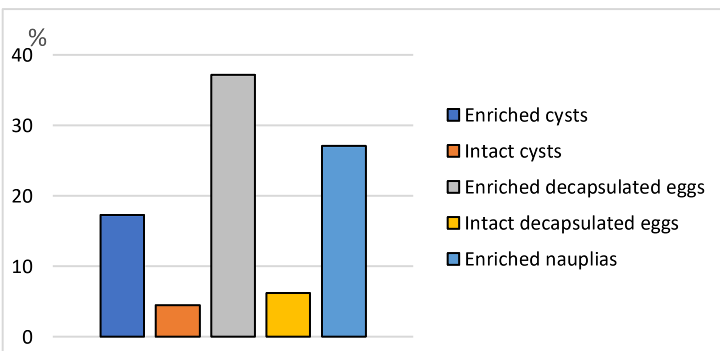

Fig. 2. Total values of fatty acids, in the composition of Omega 6, %.

The content of arachidonic acid in artemia on the background of enrichment ranged from 0.2 – 0.4% in enriched cysts, nauplias and decapsulated eggs. In intact cysts and intact decapsulated eggs, arachidonic acid was less than 0,1%.

Analyzing the data on the content of Omega - 6 fatty acids, it should be noted that their level in all types of artemia biomaterial increased after enrichment.

Fatty acids such as n-3 and n-6 are essential nutrition factors and must necessarily be in food. For cold-loving fish, this is mainly a family of linolenic (n-3) acid and to a lesser extent linoleic (n-6).

Polyunsaturated fatty acids (PUFA) of the n-6 series are precursors of eicosanoids.

Polyunsaturated fatty acids n-3 serve as physiological activators of the cardiovascular system. The imbalance in the ratio of essential fatty acids, of course, is one of the main reasons for the decrease in the growth rate of juveniles, physiological incidence, persistence, adaptive abilities. [22].

From polyunsaturated fatty acids, the Omega–3 family includes the following acids: linolenic acid, docosahexaenoic acid, eicosapentaenoic acid. The total value of Omega – 3 fatty acids is shown in Figure 3.

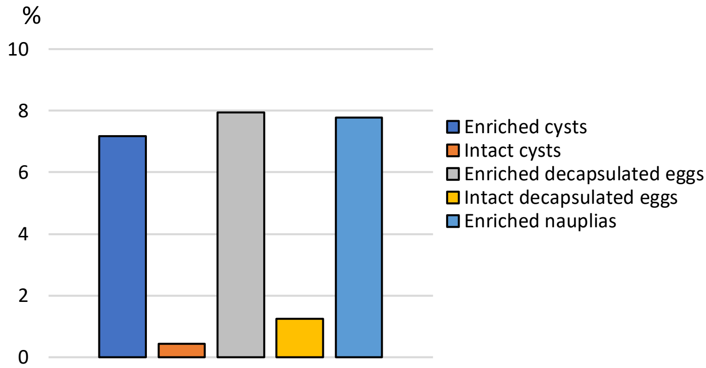

Fig. 3. Total content of Omega family acids – 3, %.

The content of linolenic acid ranged from 0.2-0.4%. The level of linolenic acid in enriched cysts, enriched decapsulated eggs, and intact decapsulated eggs was the same 0.2%. Less than 0.1% of linolenic acid was observed in intact cysts. In enriched nauplias, this indicator was 2 times higher compared to enriched cysts, enriched decapsulated eggs and intact decapsulated eggs and was 0,4%.

Eicosapentaenoic and docosahexaenoic acids. It has been proved that the incidence of coronary heart disease (CHD) is significantly less than the average among people who eat sea fish, seal meat and whales. This is due to the consumption of long-chain polyunsaturated fatty acids of omega-3 family - eicosapentaenoic and docosahexaenoic acids.

Eicosapentaenoic acid. The use of artemia nauplia as starter feeds increases the survival of fish larvae due to eicosapentaenoic acid [88]. Eicosapentaenoic acid is an antioxidant and protects the body from harmful effects of free radical processes.

Besides, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are necessary for resolution of infections.

In fish, eicosapentaenoic and docosahexaenoic acids are not contained in pure form, but in the composition of phospholipids of cell membranes. This prevents the destruction of long-chain omega-3 polyunsaturated fatty acids at high temperatures. Therefore, during the heat treatment of hydrobionts, the content of eicosapentaenoic and docosahexaenoic acids does not decrease.

The EPA content in artemia of different stages of ontogenesis on the background of enrichment ranged quite widely from 0.43% to 7.14% (Figure 4). In enriched: cysts, decapsulated eggs and nauplias, an increased content of EPA was noted - 6,27%, - 7,14%, - 6,87% respectively, in comparison with intact forms.

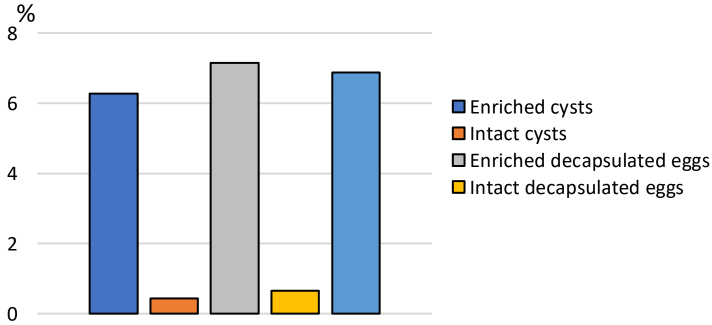

Fig. 4. Content of eicosapentaenoic acid, %.

The EPA content in enriched cysts was 14.6 times higher than in intact ones. This indicator in enriched decapsulated eggs was 11 times higher compared to intact decapsulated eggs.

The EPA content in the enriched cysts was 7.14% (Figure 4), in the enriched nauplias it was 9.6% higher compared to the enriched cysts. The difference in EPA content between intact cysts and intact decapsulated eggs was 33.8%.

Docosahexaenoic acid occupies a leading position in terms of importance for the body, it prevents the development of cardiovascular diseases, stimulates metabolism, and regulates the emotional background. The speed of linear-weight growth of fish in early stages of postembryonic ontogenesis depends on the content of docosahexaenoic acid [23].

According to the results of our studies, DHA in artemia was in the range of 0.4-0.7%. The number of docosahexaenoic acid in enriched cysts was 0.7%, in non-enriched cysts less than 0.1%. In enriched decapsulated eggs, this indicator was 33.3% higher compared to unenriched decapsulated eggs.

The content of DHA in the enriched nauplias was lower, compared with the enriched cysts, and with the enriched decapsulated eggs by 40% and 20%, respectively. DHA in intact cysts was less than 0.1%. The same indicator in intact decapsulated eggs was lower compared to enriched cysts and decapsulated eggs, as well as enriched nauplii by 75%, 50% and 25%, respectively.

Thus, based on the conducted studies, we can conclude that the enrichment process significantly increased the content of eicosapentaenoic and docosahexaenoic acids in cysts, decapsulated eggs and nauplias, compared with intact cysts and decapsulated eggs.

4 Discussions of results

Artemia cysts contain a significant amount of lipids, but their qualitative composition is not the same [24-25]. For example, in the eggs of crustaceans living in the reservoirs of Italy, triacylglycerols predominate, and in cysts from the Great Salt Lake (Utah, USA) - phospholipids and sterols [26]. In the process of ontogenesis, the composition of artemia lipids may change: the content of fatty acids increases with growth and development from 490-704 mg/g in cysts to 602-854 mg/g in nauplii [27,28].

Insufficient content of polyunsaturated fatty acids in the body slows down growth, causes capillarotoxicosis, leads to necrosis of investment, and a variety of other pathological disorders.

Among fatty acids for fish, the most physiological value is represented by long-chain polyunsaturated fatty acids of the n-3 family, namely, eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), and the n-6 family, namely, arachidonic acid (20:4n-6, ARK) [29]

Deficiency of ARC, EPA and DHA in fish feeds leads to inflammatory processes in the heart muscle, to fat deposition in the intestine, to fatty liver dystrophy, spinal column pathology, infertility, growth retardation [16-19].

The needs of the fish body for certain fatty acids vary greatly at different stages of ontogenesis and depend on environmental factors [17, 19].

When analyzing the fatty acid composition, it was established that linoleic acid was exposed to the greatest fluctuations.

The content of linoleic acid at different stages of artemia ontogenesis and during its enrichment varied from 4.1% to 36.1%.

Linoleic acid belongs to polyunsaturated essential fatty acids, namely to the Omega-6 class. It performs a protective function and helps cells to adapt to adverse conditions and survive even in adverse situations.

Enriched cysts, decapsulated eggs and nauplia contained significantly more linoleic acid compared to intact cysts and decapsulated eggs.

Gamma-linoleic acid. Gamma-linolenic acid participates in the processes of intracellular respiration, supports the rheological properties of blood, regenerates cell membranes, normalizes lipid metabolism, improves the immune and nervous systems, is responsible for the synthesis of full-fledged sperm.

According to the results of our studies (Figure 1), the content of gamma-linoleic acid in artemia of different stages of ontogenesis on the background of enrichment ranged from 0.1% to 1.3%.

Gamma-linolenic acid participates in the processes of intracellular respiration, supports the rheological properties of blood, regenerates cell membranes, normalizes lipid metabolism, improves the immune and nervous systems, is responsible for the synthesis of full-fledged sperm.

Analyzing the data on the content of Omega - 6 fatty acids, it should be noted that their level in all types of artemia biomaterial increased after enrichment.

Fatty acids such as n-3 and n-6 are essential nutrition factors and must necessarily be present in food. For cold-loving fish, this is mainly a family of linolenic (n-3) acid and to a lesser extent linoleic (n-6).

Polyunsaturated fatty acids n-3 serve as physiological activators of cardiovascular system. The imbalance in the ratio of essential fatty acids, of course, is one of the main reasons for the decrease in the growth rate of larvae, fry, juveniles, physiological incident, resilience, adaptive abilities. [22].

Linolenic acid in the body is converted into long-chain omega-3 acids: eicosapentaenoic and docosahexaenoic, which play a leading role in the regulation of lipid levels, thrombosis, elimination of inflammation; participates in the processes of intracellular respiration, supports rheological parameters of blood, regenerates cell membranes, normalizes lipid metabolism, improves the immune and nervous systems, is responsible for the synthesis of full-fledged sperm.

The content of linolenic acid ranged from 0,2-0,4%.

The use of artemia nauplia as starter feeds increases the survival of fish larvae due to eicosapentaenoic acid [23]. Eicosapentaenoic acid is an antioxidant and protects the body from the harmful effects of free radical processes.

Besides, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are necessary for the relief of inflammatory processes.

The EPA content in artemia ranged from 0.43-7.14%, the highest content was noted in the enriched material.

Docosahexaenoic acid occupies a leading position in terms of importance for the body, it prevents the development of cardiovascular diseases, stimulates metabolism, regulates the emotional background. The speed of linear-weight growth of fish in the early stages of postembryonic ontogenesis depends on the content of docosahexaenoic acid [88]. According to the results of our studies, DHA in artemia was within 0,4-0,7%.

Thus, based on the conducted studies, we demonstrated that the enrichment of biologically active substances complex that we developed significantly increased the content of eicosapentaenoic and docosahexaenoic acids in cysts, decapsulated eggs and nauplias, compared with unenriched intact cysts and decapsulated eggs.

5 Conclusion

Among the list of polyunsaturated fatty acids which we identified, the dominant one in artemia at all stages of ontogenesis was: linoleic acid. Against the background of the enrichment of artemia, the total content of omega-3 and omega-6 fatty acids sharply increased.

In particular, it should be noted that the enrichment of artemia at different stages of life cycle with a complex of biologically active substances significantly increased the content of essential fatty acids from the Omega-3 family, such as eicosapentaenoic, docosahexaenoic, linolenic fatty acids and from the Omega-6 family, such as linoleic and arachidonic acids in nauplia, decapsulated eggs and artemia cysts, thereby significantly increasing their biological and nutritional value when used as starter feeds for fish.

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