

# The content of nutrients and biogenic elements in enriched artemia salina

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**Abstract.** The work is devoted to the development of technology for enriching artemia with biologically active substances. The development of technology for enriching artemia with biologically active substances is important as an opportunity to obtain starter feeds for aquaculture with the desired properties. High content of high-grade protein, vitamins, amino acids, fatty acids, biogenic elements is of great importance. During artificial cultivation of artemia in a closed cycle in the conditions of aquaculture, there is a problem of sufficient accumulation of substances in its body that provide high nutritional and biological value. To solve this problem, we have developed a technology for enriching artemia with biologically active substances at the nauplius stage. The enrichment of artemia with vitamins, amino acids, probiotics, adaptogens, essential lipids allows us to create a new generation of bio-feeds containing a living symbiotic microbiota and ingredients capable of forming balanced complex of functional nutrition for fish. The aim of the study was to evaluate the biological and energy value, the content of proteins, fats, carbohydrates, moisture, ash, micro- and macroelements, heavy metals in artemia at different stages of ontogenesis against the background of its enrichment with a complex of biologically active substances. Intact and enriched cysts, intact and enriched decapsulated artemia eggs, enriched artemia nauplii were analyzed. It was found that against the background of enrichment with biologically active substances, the indicators of metabolic energy increased, the mass fraction of crude protein, the mass fraction of fat and the content of minerals and biogenic elements increased.

## 1 Introduction

Use High-quality starter feeds are necessary for the development of Global aquaculture. For this purpose, microscopic invertebrates are most often used, primarily *Artemia salina*, which is grown in artificial conditions in situ [1-4].

The choice of the gill-legged crustacean *Artemia Salina* as starter feeds for many species of aquatic organisms grown in aquaculture is due to the valuable qualities of artemia. These include: unpretentiousness, rapid growth and reproduction of crustaceans, the possibility of long-term storage and transportation of dry artemia cysts to different continents, available methods of cultivating artemia cysts in situ, their rich chemical

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composition with a high content of high-grade protein, vitamins, essential fatty acids necessary for the growth, development and increased survival of aquaculture facilities on the larval and juvenile stages of development [4].

Cultivation of artemia in artificially created conditions allows to enrich artemia nauplia with biologically active substances, such as probiotics, adaptogens, vitamins, amino acids, fatty acids. The artemia enrichment process is used to increase the nutritional and biological value of this recognized starter feed [5, 6]

Biologically active substances for feed enrichment should give fish useful product properties and have a positive effect on the body of fish [5, 6].

Thus, feed additives containing lyophilized culture of *Bacillus subtilis* and *Bacillus licheniformis* have the properties of antibiotics that protect from infections, participate in the removal of tissue breakdown products; synthesize a number of amino acids, vitamins and biologically active substances, immunomodulators, digestive regulators [7-10].

To increase the growth rate, stimulate the immune system and metabolism of fish in the manufacture of feed, feed additives are used, which are vitamin-amino acid complexes that show their effectiveness at certain stages of the technological process [11, 12].

In the conditions of aquaculture, the lack of vitamins A, D and E in farmed fish leads to various developmental anomalies and a decrease in reproductive function [9-12].

Biologically active additives in the feed increase the efficiency of aquaculture and have a positive effect on fish metabolism. Therefore, the development of new recipes for effective feed additives for use in aquaculture is always relevant. Especially relevant is the development of starter feeds, which play an important role at critical stages of postembryonic development of fish.

We have developed a feed additive based on probiotics, adaptogens, vitamins, essential amino acids and enterosorbents to create a new generation of bio-feeds containing a living symbiotic microbiota and ingredients capable of forming a balanced complex of functional nutrition for fish.

The development of innovative starter feeds for aquaculture based on the use of microscopic hydrobionts, in particular, artemia crustacean, which can be obtained using cultivation biotechnologies and enriched with biologically active substances in the process of obtaining directly at fish farms is important for the development of modern aquaculture.

In order to increase the nutritional value of artemia, *Artemia Salina* was enriched at various stages of ontogenesis with a complex of biologically active substances which we developed containing vitamins, amino acids, lipids, probiotics and adaptogens.

The aim of the work was to study the biological and energy value, the content of proteins, fats, carbohydrates, moisture, ash, micro- and macroelements, heavy metals in artemia of different stages of ontogenesis during enrichment with biologically active substances.

## **2 Research materials and methods**

The study object was cysts obtained from OOO Dinat-Vneshtorg, decapsulated artemia eggs and cultured artemia nauplii, which were treated with a biologically active complex with the conditional name «Pravad» in order to increase the biological value. To enrich cysts or decapsulated artemia eggs, 1 g of probiotic + 40 mg of adaptogen + 1 ml of vitamin-amino acid preparation, 1 ml of hemp oil were used, which were diluted in 100 ml of water, strongly shaken and irrigated with 1 kg of cysts or decapsulated artemia eggs. Then the enriched biomaterial was dried at room temperature.

At the beginning of the experiment, Artemia cysts were weighed on ViBRA HT-224RCE analytical scales with an accuracy of 0.0001 grams. The number of artemia eggs in one hitch was determined by direct counting under a Micromed 2 binocular var. 3-20.

To incubate artemia cysts, a saline solution was prepared at the rate of 30 g of salt (not iodized) per 1 liter of fresh water. Before laying the eggs, the saline solution was aerated using a compressor, an air duct and a diffuser. The air duct with a diffuser was lowered into the lower layers of the salt solution. Before loading, dry artemia cysts were thoroughly mixed and loaded into an incubation tank without prior soaking and disinfection.

Incubation of cysts was carried out in Weiss apparatus for 24-48 hours at constant, intensive aeration, illumination of 2000 Lx and water temperature of 26-27°C. The draining of the working solution from the apparatus was carried out using a lower tap through a discharge pipe, at the outlet of which artemia naupliuses were concentrated in a gas tank. Then the crustaceans were placed in fresh water in order to separate the hydrobionts from the shells and non-hatched cysts.

During the enrichment of artemia nauplia, the same component composition of enriching complex was used, but the enrichment was carried out twice. The first enrichment was carried out after 8 hours of incubation. The formula of enriching complex introduced into the culture medium included: 1 g/l of probiotic, 0.005 mg/l of adaptogen, 1 ml/l of 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 the probiotic «Vetom-1», 40 mg/l of the adaptogen «Trecezan», 1 ml/l of the vitamin-amino acid preparation «Chiktonic», 1 ml of hemp edible oil.

For the analysis of nutritional value, enriched and intact cysts, enriched and intact decapsulated artemia eggs, enriched with artemia nauplia were selected.

The content of mass fraction of crude protein was determined by the Kjeldahl titrimetric method. The organic matter of the sample is mineralized by boiling sulfuric acid in the presence of a catalyst. Sodium hydroxide is added to the resulting ammonium sulfate to isolate ammonium. As a result of distillation and titration, ammonia is released and the mass fraction of nitrogen in the test sample is calculated in terms of the mass fraction of crude protein [13].

The analysis of lipid content (fat in terms of dry matter, %) was carried out in accordance with the international standard ISO 6492:1999 "Animal feeding stuffs – Determination of fat content", MOD. The method consists in the extraction of crude fat from the test sample with petroleum ether, followed by the elimination of solvent and weighing of resulting residue.

The fiber content in the studied samples was studied by determining dry residue from the sample after treatment with nitric and acetic acids, alcohol and ether. The mass fraction of moisture was determined according to the international standard ISO 6496:1999 «Animal feeding stuffs — Determination of moisture and other volatile matter content».

The chemical analysis of the studied cysts and nauplia artemii for the content of biogenic elements was carried out according to unified methods. The mass fraction of ash was determined by the standard method «Fodder, mixed fodder and mixed fodder raw material. Methods for determination of raw ash». The method was to determine the mass of the residue after burning and subsequent calcination of the sample. Determination of the content of iron, calcium, copper, zinc, manganese, phosphorus, nickel, lead, cadmium, arsenic and mercury was carried out by atomic absorption spectrometry in accordance with the interstate standard ISO 6869:2000 Feeds, compound feeds; «Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc by atomic absorption spectrometry method».

The calculation of exchange energy (EE) was carried out according to the formula:

$$EE=0,12*SP+0,3*CF+0,07*CK+0,13*NFES, \text{ MJ/kg} \quad (1)$$

where:

SP is the mass fraction (MF) of crude protein in dry matter, %;

CF - MF of crude fat in terms of dry matter, %;

CK - MF of crude fiber in dry matter, %;

NFES - nitrogen-free extractive substances, %;

$$\text{NFES} = 100 - \text{SP} - \text{CF} - \text{CK} - \text{DA} \quad (2)$$

DA - MF of ash in dry matter, %.

Artemia cultivation in an artificial environment in a closed cycle was carried out on the basis of the Laboratory of experimental biology and aquaculture of the department of «Biology, ecology, parasitology, aquatic bioresources and aquaculture of FSBEI HE Ulyanovsk SAU».

The analysis of lipid content, fiber content in the studied samples, and chemical composition of Artemia Salina crustacean at different stages of development were determined in a certified laboratory for determining the quality of food and agricultural products of the Saratov State Agrarian University.

### 3 Research results

When evaluating feed and feed additives, their main characteristic is the chemical composition and the ratio of individual nutrients: fiber content, protein ratio, the presence of biologically active substances, anti-nutrients, etc. Unilateral excess or lack of nutrients negatively affects digestibility.

In the practice of world aquaculture, the opinion about high nutritional value of nauplius artemia as a starter feed for larvae of various species of fish and crustaceans has been firmly established. The value of artemia primarily depends on its chemical composition [14, 15]. Let's consider the components of chemical composition of artemia.

**Water.** Water is the liquid medium of any living organism, it is part of cells and tissues, it accounts for about 80% of cellular matter. It is difficult to overestimate the importance of water for hydrobionts: it is a habitat, gives stability and elasticity to connective tissues; participates in metabolic reactions (hydrolysis, oxidation); maintains normal osmotic pressure; promotes the dissolution and absorption of nutrients during metabolism; all toxic substances and metabolic products are removed from the body with water.

**Ash.** Ash is not a source of energy for the animal body and its value is determined by the amount of mineral elements contained in it, which are part of the tissues, maintain normal osmotic pressure of tissue fluids and water balance in hydrobionts body.

**Protein.** It is well known that the main structural and functional component of all living organisms is protein. The protein contained in live feeds is valuable for the content of essential amino acids necessary for hydrobionts for growth, accumulation of muscle mass, normal activity of digestive, hormonal, nervous and reproductive systems of the body. In addition, the need of fish for proteins is much higher than that of warm-blooded animals [16].

**Lipids.** Lipids, which are the most important source of energy, plastic material for building membranes, and a source of essential fatty acids that ensure the survival of fish in the early stages of ontogenesis, play an important role in the nutrition of fish [15, 16].

It is believed that full-fledged fish feeds should contain mainly liquid fats rich in unsaturated fatty acids, which are absorbed by 90-95%.

The results of studies of the nutrient content in intact (unenriched) and enriched biomaterial of artemia are presented in Table 1. The content of nutrients in cysts, decapsulated eggs and artemia nauplii was studied.

Analyzing the results obtained, it should be noted the tendency to increase crude protein in artemia cysts enriched with biologically active substances. The mass fraction of crude

protein in dry matter in decapsulated artemia eggs enriched with biologically active substances is higher than in intact decapsulated artemia.

There is a natural tendency to increase the mass fraction of fat in decapsulated enriched eggs.

We found no significant differences in the content of fiber and ash in the artemia cysts of the experimental groups.

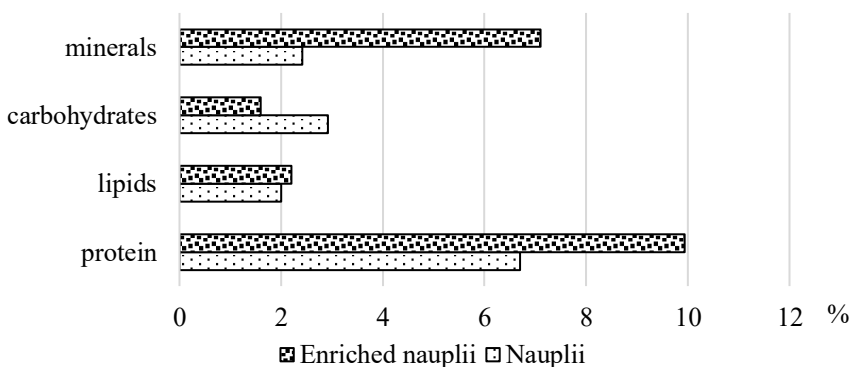
**Table 1.** The content of nutrients in cysts and decapsulated eggs of artemia.

The indicator name, units of change.	Intact cysts	Enriched cysts	Intact decapsulated eggs	Enriched decapsulated eggs
Mass fraction (MF) of moisture, %	7.7	9.9	4.8	9.2
MF of crude protein, %*	47.94±1.69	48.63±1.69	52.25±1.81	52.5±1.81
MF of crude protein in dry matter, %	52.69±1.81	53.19±1.81	54.88±1.88	57.81±1.94
MF of fat, %*	1.2	2.3	14.0	15.9
MF of fat in terms of dry matter, %	1.3	2.6	15.4	16.7
MF of fiber, %*	3.0	2.9	3.1	2.9
MF of fiber in dry matter, %	3.1	3.1	3.4	3.0
MF of ash, %*	4.8±0.2	5.0±0.2	4.2±0.2	4.8±0.2
MF of ash in dry matter, %	5.4±0.3	5.4±0.3	4.7±0.2	5.0±0.2

\* - at actual humidity.

According to literature sources, the raw tissues of artemia contain: moisture — 85.20%, crude protein - 6.70%, total lipids - 2.00%, minerals — 2.42%, carbohydrates — 2.92% [17, 18], according to foreign sources, when enriching artemia with various biologically active substances, it is possible to increase the protein content - up to 6.9% [19-21].

The data on the content of proteins, fats, carbohydrates and minerals obtained during the study of enriched nauplia artemia are shown in the Figure 1.



**Fig. 1.** The proportion of proteins, fats, carbohydrates and minerals.

The results obtained show that the enriched nauplia of artemia have higher indicators of protein, lipids and minerals.

Minerals are necessary for fish and other hydrobionts to build an inorganic part of the body to perform a variety of physiological functions. In particular, magnesium participates

in energy metabolism, synthesis of proteins, nucleic acids, has a stabilizing effect on membranes, is necessary to maintain homeostasis of calcium, potassium and sodium.

Iron is a part of proteins of various functions, including enzymes. Participates in the transport of electrons, oxygen, ensures the course of redox reactions and activation of peroxidation.

Manganese is a part of coenzymes involved in the metabolism of amino acids, carbohydrates, and nucleotide catecholamines. Insufficient consumption of manganese is accompanied by a slowdown in growth, carbohydrate and lipid metabolism.

Copper is a part of enzymes that have redox activity and are involved in iron metabolism, stimulates the assimilation of proteins and carbohydrates, participates in the processes of providing human tissues with oxygen. The deficiency is manifested by disorders of the formation of the cardiovascular system and skeleton, the development of connective tissue dysplasia.

Zinc is a part of more than 300 enzymes, participates in the synthesis and decomposition of carbohydrates, proteins, fats, nucleic acids and in the regulation of the expression of a number of genes. Recent studies have revealed the ability of high doses of zinc to disrupt the absorption of copper and thereby contribute to the development of anemia in the animal body [15, 19].

The accumulation parameters of biogenic elements in the cysts and larvae of the *Artemia Salina* crustacean as a result of enrichment with biologically active substances are presented in the Table 2.

The data obtained showed that the highest nitrogen content is in enriched decapsulated artemia eggs and early nauplia. Slightly less nitrogen is contained in cysts.

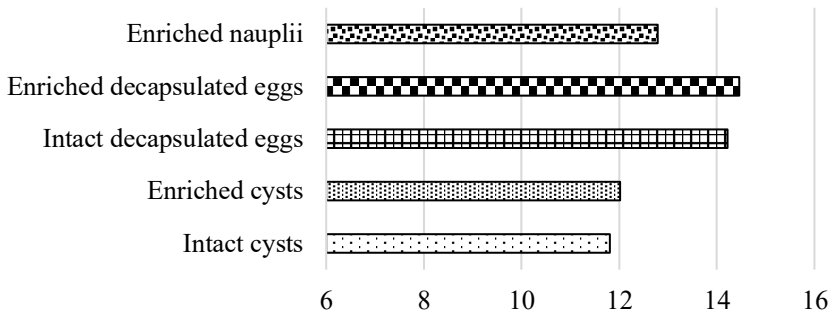
**Table 2.** The content of biogenic elements in cysts, decapsulated eggs and artemia nauplia.

The indicator name, units of change.	Intact cysts	Enriched cysts	Intact decapsulated eggs	Enriched decapsulated eggs	Enriched nauplii
Nitrogen, %	7.67±0.27	7.78±0.27	8.36±0.29	8.40±0.29	8.31±0.10
Iron, mg/kg	210.0	219.0	211.0	215.0	213.0
Cobalt, mg/kg	less 0.50	less 0.50	less 0.50	less 0.50	less 0.50
Nickel, mg/kg	less 0.50	less 0.50	less 0.50	less 0.50	less 0.50
Manganese, mg/kg	6.0±4.0	6.0±4.0	5.0±4.0	5.0±4.0	6.0±4.0
Copper, mg/kg	16.1±3.4	15.0±3.2	16.4±3.4	14.8±3.1	14.5±3.0
Zinc, mg/kg	53.1±11.2	51.8±10.9	50.1±10.5	49.3±10.4	49.1±10.3
Calcium, %	less 0.020	less 0.020	less 0.020	less 0.020	less 0.020
Phosphorus, %	0.77±0.13	0.69±0.12	0.22±0.04	0.35±0.07	0.24±0.04

In terms of iron content, enriched cysts are in the lead, they contain iron 219.0 mg/ kg and the iron content in enriched decapsulated eggs is slightly lower, enriched early nauplia are slightly behind their indicators. In enriched iron cysts by 9.0%, and in enriched decapsulated eggs by 4.0% more than in enriched nauplii.

There are no special differences in the content of copper, zinc and calcium between the studied material.

Enriched cysts, decapsulated eggs and nauplia, had increased metabolic energy indicators compared to intact ones (Figure 2).



**Fig. 2.** Exchange energy in intact and enriched artemia biomaterial, MJ/kg.

Heavy metal salts coming from the external environment or accumulating in water are dangerous for hydrobionts. The most common are: mercury, lead, arsenic, cadmium and others. In order to determine the degree of accumulation of toxic substances in artemia cysts, their study on the content of these elements was carried out. The results obtained are shown in the Table 3.

**Table 3.** The degree of accumulation of toxic elements in artemia cysts.

Studied artemia cysts	Mass fraction of toxic elements, mg/kg			
	Lead	Cadmium	Arsenic	Mercury
Intact artemia cysts	0.21±0.07	less 0.01	less 0.01	less 0.002
Enriched artemia cysts	0.17±0.06	less 0.01	less 0.01	less 0.002

Analyzing the data in table 3, it can be noted that it was found that cadmium (Cd), arsenic (As), mercury (Hg), lead (Pb) content was low and did not exceed the maximum permissible concentrations of toxic elements in the analyzed samples in acceptable limits.

## 4 Discussions

The research results showed that the mass fraction of crude protein in the dry matter of decapsulated eggs enriched with biologically active substances is 3.0% higher than in intact decapsulated eggs.

There is a tendency to increase the mass fraction of fat in artemia cysts enriched with biologically active substances. There were no large differences in the content of fiber and ash in intact and enriched cysts.

The analysis of the protein ratio indicator showed that the highest values of the ratio of protein to nitrogen-free substances are observed in enriched cysts and nauplii artemia.

Separately, it should be noted that during enrichment with biologically active substances, the energy value of artemia increases, compared with the intact form.

The study of the experimental material for the content of toxic elements showed that the use of biologically active which we have used is absolutely safe.

## 5 Conclusion

The use of artemia as live starter feeds is a modern trend in the development of global aquaculture. The search for ways to improve the quality of live starter feeds is associated

with the search for highly effective biologically active substances for the enrichment of artemia.

In their studies, as enriching biologically active substances, the following were used: vitamin–amino acid complex – «Chiktonic», probiotic «Vetom-1», adaptogen – «Trecrezan», edible hemp oil.

The conducted studies have shown that as a result of enrichment with biologically active substances, in artemia of different stages of ontogenesis, the nutritional, biological and energy value has increased, the content of protein, fats, biogenic elements has increased.

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