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INCREASING THE NUTRITIONAL VALUE OF ARTEMIA SALINA NAUPLII BY ENRICHING HAEMATOCOCCUS PLUVIALIS

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Abstract

This article presents materials on the results of an experiment on the enrichment of Artemia salina with the unicellular alga Haematococcus pluvialis to increase its nutritional value. For fry of marine fish, such as mullet and kalkana, and freshwater fish, such as clary catfish, carp and trout, the supply of fatty acids and the energy value of Artemia is still insufficient. Therefore, it is necessary to enrich brine shrimp with additional nutrients The use of Haematococcus pluvialis as a component of salmon food allows an increase in the intensity of the red color of the muscle tissue. Research has shown that using Haematococcus pluvialis as a starter feed supplement can provide an additional source of protein and fat for fish fry. Experiments have shown that A. Salina, enriched with hematococus, increased the increase in size of zebrafish by 17.27% for 10 days of feeding in comparison with feeding with pure brine shrimp, which shows the effectiveness of using H. Pluvialis as a feed additive in the starter feed of fish fry and increased overall survival in the experimental group by 20%.

Keywords: Artemia salina, enriched brine shrimp, Haematococcus pluvialis, nauplii, starter feed

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1. Introduction

Live food is an essential component of the starter diet of many fish species. Most often, Artemia salina is used for these purposes, since it has a high energy value and is easily cultivated during the day (Spiller & Dewell, 2003).

The nutritional value of brine shrimp can be increased by adding fatty acids, which are found in fish oil, as well as krill meal, vegetable oils and green algae (Olsen, 2000; Ponomarev, 2003).

The exit of crustaceans from the chorion is observed in the first hours after entering the water. Over time, the caloric content of the crustacean decreases, since the internal reserves are spent on its growth, and a day after hatching, the nutritional value of Artemia Salina decreases by more than 2 times (Chepurkina et al., 2014).

For fry of marine fish, such as mullet and kalkana, and freshwater fish, such as clary catfish, carp and trout, the supply of fatty acids and the energy value of Artemia is still insufficient. Therefore, it is necessary to enrich brine shrimp with additional nutrients (Guiry & Guiry, 2020; Merchie, 1996).

Haematococcus pluvialis (Figure 1) is a species of freshwater green algae from the Haematococcaceae family, of the order Chlamydomonadaceae (Li et al., 2011). It is known for its high content of the strong antioxidant astaxanthin, which is used as a food additive in aquaculture, and is also used as a dietary supplement and a component of cosmetics (Shah et al., 2016).

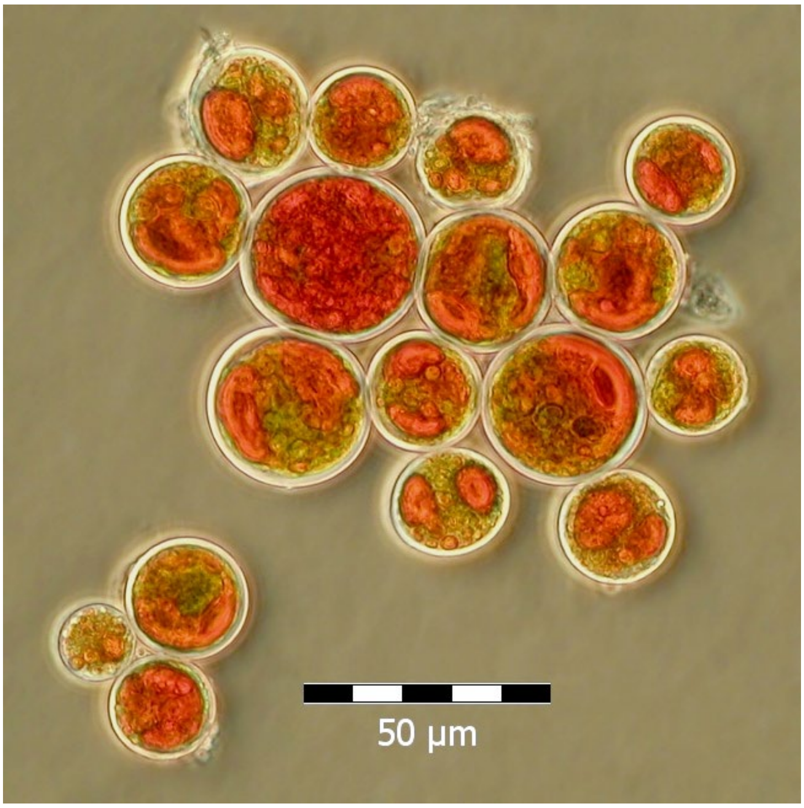

Figure 1. Haematococcus pluvalis

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The use of Haematococcus pluvialis as a component of food for salmonids makes it possible to achieve an increase in the intensity of the red color of muscle tissue, in particular, this alga is widely used in aquaculture in Southeast Asia, and its use is also allowed in the USA (with a restriction on the content of astaxanthin in the finished product). feed no more than 80 mg / kg (Gupta et al., 2007).

In good conditions, the alga is green in color, but if environmental conditions become unfavorable for normal cell growth (in particular, under the action of bright light, high salinity and low availability of nutrients), the cells enter the dormant phase and intensively produce and accumulate astaxanthin. becoming red (Gupta et al., 2007).

Haematococcus pluvialis is rich in proteins and consists of 29-45% of them. The amino acid composition is mainly represented by aspartic acid, glutamic acid, alanine and leucine with a total acid content of 10.02 / 100 mg. 46% of these amino acids are essential. Carbohydrates make up 15-17% of the composition. Lipids make up 20-25%, about 10% of which are short polyunsaturated fatty acids. The predominant ones are neutral lipids. A more detailed composition of the algae is presented in Table 2.

The above information suggests that using Haematococcus pluvialis as a starter feed supplement could provide an additional source of protein and fat for fish fry.

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2. Purpose of the Study

Study of the effect of Haematococcus pluvialis enriched with A. salina on survival and morphometric parameters of Danio rerio juveniles.

3. Research Methods

Cultivation of Haematococcus pluvialis (strain IBCE-17) was carried out on modified Rudik's medium at 14 hours of light - 10 hours of darkness and at a temperature of 28 ± 2 ° C, the density of the algae ready for use was 65 individuals / ml. The composition of Rudik's medium is shown in Table 1.

Table 1. Composition of Rudik's environment

Macronutrients, g / l		Trace elements, mg / l	
NaNO3	0,3	H3BO3	0,3
K2HPO	0,08	MnSO4 · H2O	1,5
KH2PO4	0,02	ZnSO4 · 7H2O	0,1
MgSO4 · 7H2O	0,01	(NH4)6Mo7O24 · 9H2O	0,3
CaCl2 · 2H2O	0,0585		
EDTA	0,0075	CuSO4 · 5H2O	0,08
NaCl	0,02	Co(NO3)2 · 6H2O	0,26
		FeCl3 · 6H2O	17

The incubation of A. salina was carried out in a 3% NaCl solution in a cultivator at a temperature of 25-26 ° C, pH 7-8, with intensive aeration and lighting (Chepurkina et al., 2014). After complete

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hatching of the nauplii, hematococcus was added to the reservoir with brine shrimp in a 1: 1 ratio and the brine shrimp was enriched within 2 hours. Feeding experiments were carried out on ~ 0.4 mm Danio rerio larvae. After that, they were fed according to their consumption - 6 times a day for 10 days. The control group was fed with unenriched brine shrimp bred under the same parameters.

The filling of the digestive tract of A. Salina nauplii with hematococcus took 1 to 2 hours, depending on the density of A. Salina in solution (Miyamoto et al., 1985).

The morphology of A. Salina nauplis was examined for abnormalities using microscopy.

For the experiment, 60 Danio rerio juveniles were selected from which 2 groups were selected: control and experimental, 30 individuals in each. The average size of a Danio Rerio specimen at the beginning of the experiment was 0.4 mm.

Measurements of the size of each individual were carried out at the beginning and at the end of the experiment, and on the basis of the data obtained, the average size of Danio Rerio individuals was obtained, as well as the increase in percentage.

4. Findings

Over the observation period, the average increase in the experimental group at the end of the experiment was 62.9%, while in the control group it was 45.63%. Artemia, enriched with Haematococcus pluvialis, increased the increase by 17.27% over the observed period in relation to the control group.

On the 3-4th day of the experiment, the experimental group greatly increased in size, and the difference was 8.4%. At the end of the first week of the experiment, the difference in size between the groups was 15.5% or 1.146 times. From day 7 until the end of observations, the average percentage difference between the experimental groups was 16.8%.

The survival rate was also recorded, at the end of the experiment the difference was 20% percent, in the experimental group there was zero mortality.

5. Conclusion

The results obtained show the effectiveness of the developed method to increase the nutritional value of Artemia due to the fact that in the process of feeding Artemia, H.pluvialis enters the red phase - the protective function of the microalgae cell, during which its nutritional composition greatly changes, Table 2 shows the composition of the hematococcus in comparison green (normal) and red (protective) phase of the cell.

Table 2. Composition of H. Pluvialis in the "green" and "red" stages "

Composition, %	Green stage	Red stage
Proteins (total %)	29-45	17-25
Lipids (total %)	20-25	32-27
• Neutral lipids	59	51,9-53,5
• Phospholipids	23,7	20,6-21,1
• Glycolipids	11,5	25,7-26,5
Carbohydrates (total%)	15-17	36-40

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Carotenoids (total%)	0,5	2-5
• Neoxanthin	8,3	n.d
• Violaxanthin	12.5	n.d
• β-carotene	16,7	1,0
• Lutein	56,3	0,5
• Zeaxanthin	6,3	n.d
• Astaxanthin (including esters)	n.d	81,2
• Adonixanthin	n.d	0,4
• Adonirubin	n.d	0,6
• Canthaxanthin	n.d	5,1
• Echinenon	n.d	0,2
• Chlorophyll	1,5-2	0

From the presented table, one can draw attention to a sharp increase in free lipids by 60% by 122% of glycolepids, and by almost 140% increase in carbohydrates. On the photomicrographs (Figure 2 B), one can pay attention to the presence of algae in the red stage. Figure 2 (A) demonstrates the complete filling of the intestine of A salina with a culture of microalgae.

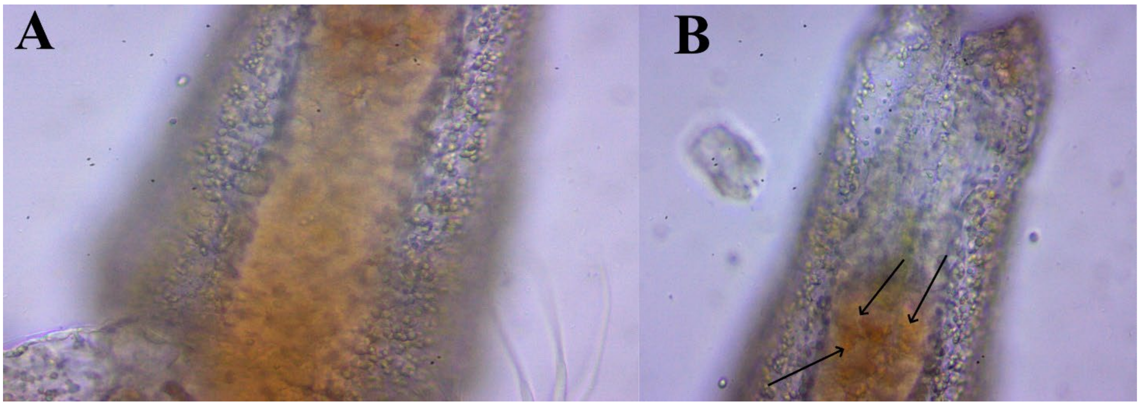

Figure 2. Artemia enriched with the culture of H. Pluvialis, in image B the arrows show the transition of the microalga to the red phase

A. Salina, enriched with hematococus, increased the increase in size of zebrafish by 17.27% for 10 days of feeding in comparison with feeding with pure brine shrimp, which shows the effectiveness of using H. Pluvialis as a feed additive in the starter feed of fish fry.

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