UDK 577.112.34:582.736.3-15

NUTRITIONAL VALUE OF *DAPHNIA MAGNA* (STRAUS, 1820) UNDER CONDITIONS OF CO-CULTIVATION WITH FODDER MICROALGAE

L. M. CHEBAN, O. E. GRYNKO, M.M. MARCHENKO

Chernivtsi National University named after Y. Fedkovych,
Ukraine, 58012, Chernivtsi, Kotsiubynsky 2 Str.
e-mail: larisa.cheban@chnu.edu.ua

The possibility of using Desmodesmus armatus (Chod.) Hegew and Acutodesmus dimorphus (Turpin) Tsarenko algae as fodder substrates for growing Daphnia magna (Straus, 1820) has been investigated. The biochemical composition of algae cultivated in wastewater from a recirculating aquaculture system (RAS) fish-farm has been analyzed. It is shown that both algae cultures are characterized by a similar amount of protein - at a level of 50%. The amount of lipids predominates in A. dimorphus biomass and is 21%. In the biomass of both algae, a high carotenodine content of 12 mg/g of dry weight was noted. Also, the nutrient value of Daphnia magna was analyzed in the conditions of co-cultivation with these fodder algae. It is shown that co-cultivation of Daphnia magna with D. armatus allows to obtain daphnia biomass with an increased content of total protein (82.6%) and carotenoids (15,24 mg/g of dry weight). Use of A. dimorphus as a feed substrate leads to an increased accumulation in the biomass of daphnia lipids - 10.3%. The biomass of D. armatus algae is assumed to be used as an optimal fodder substrate for co-cultivation with Daphnia magna.

Keywords: Desmodesmus armatus (Chod.) Hegew, Acutodesmus dimorphus (Turpin), co-cultivation, protein, lipids, carotenoids.

Introduction. The current trend of work in aquaculture today is the development of technologies for obtaining live food. Live food is an important and necessary component of fish nutrition when they go to exogenous nutrition (Ostroumova, 2012). Natural feeds are the source of the introduction of essential amino acids in fish, unsaturated fatty acids, vitamins, minerals and other components that are necessary for the life and growth of fish (Khudyi, 2014).

The widespread use of live food in aquaculture is due not only to the benefits of their nutritional composition (Abowei and Ekubo, 2011), but also to the content of the hydrolytic enzyme complex (Das et al., 2012). At the initial stages of development of fish larvae, their digestive system is characterized by low enzymatic activity. In this regard, digestion in fish during the transition to external nutrition is largely provided by hydrolytic enzymes of consumed live food that provide autolysis (Lavens and Sorgeloos, 1996). In addition, the exogenous intake of hydrolases into the intestines of the larvae can cause additional activation of a number of propriete proteases by limited proteolysis (Kolkovski, 2001).

The mobility of zooplankton is also an important factor, as most fish respond to catch movements. Living organisms actively move and can be adapted to the size and shape of the mouth of fish immediately after capture, since they contain 85-95% water (Ostroumova, 2012).

From the large number of species of branchy crustaceans species that are distinguished by high productivity, adaptation to specific conditions that are created during cultivation and high nutritional content are recommended for cultivation in aquaculture conditions. One of the first places in terms of use as live food for fish is occupied by representatives of the family *Daphniidae*. Daphnia are characterized by high fertility, rapid growth rates, and are well suited to cultivation (Tuchapska and Krazhan, 2014). An important factor in the further use of zooplankton as food for fish is the nutritional value of *D. magna*. They are characterized by a sufficient content of nutrients that can provide the fish nutritional needs (Bogut et al., 2010). Crustaceans are able to accumulate a significant amount of proteins and lipids in biomass, the content of which will depend on the feed regime (Suantika et al., 2016). It is known that depending on the composition of the feed and the physiological characteristics of the organism, the content of proteins in *daphnia* can fluctuate within 45-70%, and lipids - 11-27% (Macedo and Pinto-Coelho, 2001). As a fodder substrate for daphnia, yeast, algae or mixtures thereof can be used. However, the use of algae as a feed substrate has several advantages. The biomass of algae is easily digested, accessible to animals and provides zooplankton with all necessary nutrients (Duong et al., 2015; Becker, 2007).

The aim of the work is to estimate the nutritional value of *Daphnia magna* (Straus, 1820) under conditions of co-cultivation with fodder algae.

Materials and Methods. As fodder, crops of green algae *D. armatus* (Chod.) Hegew and *A. dimorphus* (Turpin) Tsarenko were used. Algae were pre-cultivated under storage culture conditions to the exponential phase of growth in wastewater from recirculating aquaculture system (RAS) (Cheban et al., 2015).

Wastewater before use was standardized by pH values (U-160 MU ion meter) and total mineralization (Water Quality Tester COM-100 conductivity meter).

All manipulations related to the sowing of algae cultures were carried out under sterile conditions. The ratio Inoculum: nutrient medium was 1:10.

The research material was also the culture of *Daphnia magna*, which is kept in the collection of the Institute of Biology, Chemistry and Bioresources of the ChNU.

Simultaneous cultivation of daphnia with *D. armatatus* or *A.dimorphus* was performed for 15 days. In each of the experiment variants, the initial daphnia culture at the beginning of the co-cultivation was 50 specimen of microalgae 100 ml in 1 liter of cultivation medium. Control schemes were those in which daphnia were fed once every three days with the same algae or yeast *S. cerevisiae* (Kushniryk, 2015).

Cultivation took place in a climatic room under a 16-hour photoperiod, illuminated with fluorescent lamps of 2500-4000 lux and a temperature of 24±2 °C.

The density of microalgae culture was determined spectrophotometrically by the indirect optical index at 750 nm on SF-46. To calculate the absolutely dry biomass (ADB), the empirical coefficient k was used: ADB = k × D750, which was determined for each of the microalgae cultures in three independent repeats. k = g/l/unit of optical density (Hevorhyz, 2008).

Isolation of algal cells from the culture medium was carried out by centrifugation at 8,000 rpm within 15 minutes on "Herauses" Biofuga stratos. After that, the microalgae biomass was disintegrated by ultrasound on USDN-2T, in the presence of a suitable buffer or solvent.

The pigments were extracted from the watered microalgae cells with a mixture of chloroform: ethanol (2:1), centrifuged at 3000 rpm to discoloration of the extract. The pigment spectra were measured in the combined supernatant. The pigment concentration was calculated according to the formulas (Sanchez et al., 2008) by the values of optical density at wavelengths corresponding to absorption maxima of chlorophyll a and b and total carotenoids.

The content of total proteins in the *D.magna* culture was estimated by the Lowry method (Lowry et al., 1951) and was calculated per 1 g of dry matter. The determination of total lipids (Knight et al., 1972) and carotenoids (Sanchez et al., 2008; Tanaka, 1978) was carried out according to conventional methods and was calculated for 1 g of dry matter. The results obtained were recalculated to an absolutely dry mass.

Statistical processing of the results was carried out using Microsoft Excel software. Differences in the results discussed in this paper are possible at a significance level of $p \leq 0.05$ by the Student's criterion. Quantitative determinations were carried out in three independent repeats. In the table and in the figures, the data are represented as the mean ± SD.

Results. Algae, which are recommended for use as fodder in aquaculture, should be characterized by a high content of proteins, amino acids, fatty acids and carotenoids. Also, they should be small in size and not have specific outgrowths on the surface of the cells (Tuchapska and Krazhan, 2014). These criteria correspond to the protococcal algae *D. armatus* and *A.dimorpus*.

Earlier, we showed the possibility of cultivation of algae on wastewater from RAS (Cheban et al., 2015). As a result of our work, we obtained a biomass of *D. armatus* and *A.dimorpus* algae, which was characterized by such initial parameters (table 1.).

Table 1.

Biochemical parameters of algae (M±m, n=3, p≤0,05)

Parameters	*D. armatus*	*A. dimorphus*
Biomass, g/l	7,92	6,5
Total proteins, %	46,8 ± 2,15	45,4 ± 3,12
Total lipids, %	23,3 ± 0,74	16,6 ± 0,99
Total carbohydrates	15,4 ± 0, 94	21,0 ± 1,38
chlorophyll *a*, mg/g	11,17 ± 0,42	11,23 ± 0,14
chlorophyll *b*, mg/g	7,07 ± 0,29	7,01 ± 0,21
Total carotenoids, mg/g	12,65 ± 0,31	11,98 ± 0,21

Thus, the total protein content at the level of 50% is noted in the biomass of both microalgae. It is known that the total protein content in the biomass of algae depends on the systematic features, while the transition to a steady state of the culture, the protein content can be from 30% to 55% of dry weight (González-López et al., 2010; Kim and Wijesekara, 2010). It is a sufficient amount of protein that will determine the effectiveness of further use of biomass *D. armatus* and *A. dimorphus* in food chains in aquaculture conditions.

Algae can also produce various kinds of lipids, such as glycolipids, phospholipids (polar lipids), glycerolipids. They have large reserves of neutral lipids and free fatty acids. The lipid content in microalgae ranges from 10% to 50%. The biomass of *A. dimorphus* was characterized by a lipid content of 21%. In biomass of *D. armatus* this index reached the amount of 16%.

The value of algae as a food is also increased due to the fact that they contain pigments: carotenoids, xanthophylls, chlorophylls. The content of carotenoids in *D. armatus* and *A. dimorphus* biomass is about 12 mg/g of dry weight.

So, according to biochemical indicators, the biomass of these algae can serve as a feed substrate for growing *Daphnia magna*.

It is known that the biochemical parameters of *Daphnia magna* will depend substantially on the quality of the feed substrate used. Therefore, it is important to monitor the parameters of total protein, lipids and carotenoids in *Daphnia magna*, fed with various feeds. We compared the number of basic daphnia nutrients grown under co-cultivation conditions and those traditionally regularly (once every 3 days) fed with *S. cerevisiae* or algae cultures.

An important indicator when using zooplankton as a food object in aquaculture is a sufficient amount of protein in the biomass of the branchy crustaceans (Das et al., 2012). Based on the results of the studies, the greatest amount of protein was obtained in the biomass *D. magna* under conditions of co-cultivation with *D.armatus* (Fig. 1).

Thus, with simultaneous co-cultivation of daphnia and algae *D. armatus*, the amount of protein in the biomass of daphnia was 82.5%. This is 1.2 times more than when feeding daphnia with yeast (67.6%). In the conditions of complementary feeding of daphnia by the *D. armatus* culture, the protein content was 1.5 times less and was 55%.

When using *A. dimorphus* as a food substrate, the amount of protein in the biomass of daphnia was significantly less. Regardless of the scheme of application of such algae, the protein content was in the range of 50-60%. When using the traditional feed substrate (yeast *S. cerevisiae*), the protein content was also high enough, but did not reach the amount of daphnia characteristic for co-cultivation with *D. armatus*.

The largest amount of lipids (11%) was noted in the biomass of *Daphnia magna*, fed with yeast (Fig. 2).

The use of *A. dimorphus* as feed also made it possible to obtain a sufficiently high content of lipids in the biomass of Daphnia magna. However, important in this case is not the actual amount of lipids, but the quantity and ratio of fatty acids of the feed substrate (Brett et al., 2006).

A positive result of our scheme of growing daphnia together with the fodder substrate is a rather high content of carotenoids (Fig. 3). In the organism of crustaceans, they are not synthesized, so they must come with food organisms that are capable of carotenogenesis (algae, yeast, etc.) (Ostroumova, 2012; Bogut et al., 2010). The greatest amount of carotenoids (15.24 mg/g) is characteristic for the biomass of daphnia grown together with *D. armatus*.

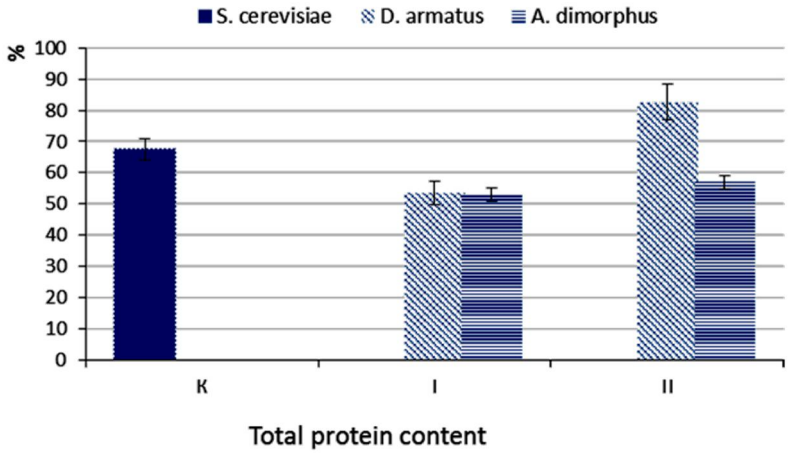

Fig. 1. Total protein content of Daphnia magna using different feeding schemes

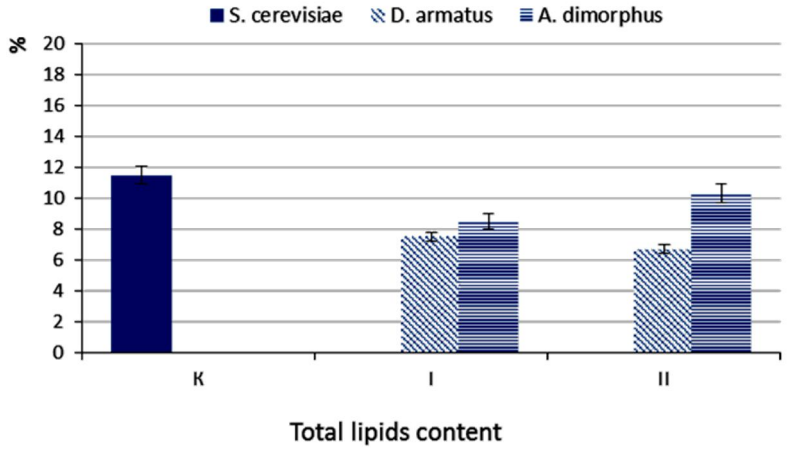

Fig. 2. Total lipids content of Daphnia magna using different feeding schemes

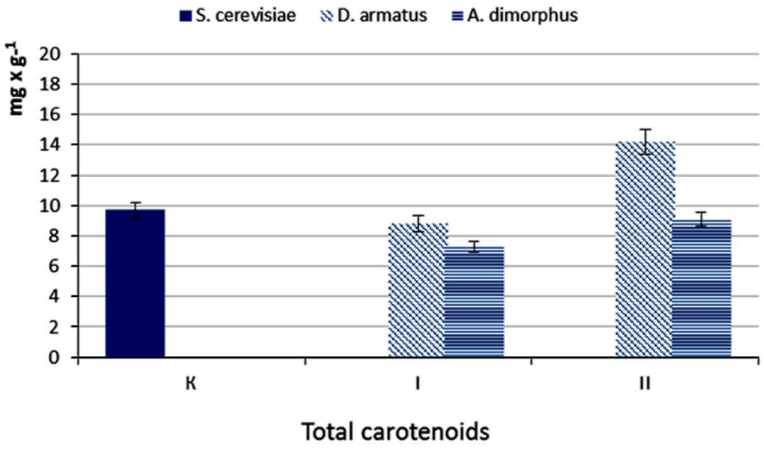

Fig. 3. Total carotenoids content of Daphnia magna using different feeding schemes

Almost the same level of accumulation of carotenoids was noted in daphnia biomass in the conditions of complementary feeding both with yeast and with the culture of microalgae *A. dimorphus,* 7-9 mg/g of dry weight.

Conclusions. Thus, the use of *D. armatus* and *A. dimorphus* results in the accumulation of a large number of proteins and lipids in daphnia biomass in comparison to their complementary feeding with yeast. This makes it possible to assume that the combination of live feeds of the protococcal algae *D. armatus* or *A. dimorphus* with *D. magna* can be used as an alternative to protein and lipid sources for fish larvae, and will increase the growth and survival of larvae.

The obtained results showed that feeding of *Daphniidae* by algae using both proposed schemes allows obtaining a productive culture. The content of common proteins and lipids in biomass remains typical for the branching crustaceans. However, the predominance of lipids and carotenoids in the biomass of daphnia, co-cultivated with *A. dimorphus*, makes it possible to propose such a scheme as optimal for growing daphnia.

References:

1. Abowei J.F.N., Ekubo A.T. A review of conventional and unconventional feeds in fish nutrition // British Journal of Pharmacology and Toxicology. – 2011. – 2 (4). – P. 179–191.
2. Becker E. W. Micro-algae as a source of protein // Biotechnol. Adv. – 2007. – Vol. 25. – P. 207–210. doi: 10.1016/j.biotechadv.2006.11.002
3. Bogut I., Adameck Z., Puškadija Z., Galović D. Nutritional value of planktonic cladoceran Daphnia magna for common carp (Cyprinus carpio) fry feeding // Croatian Journal of Fisheries : Ribarstvo. – 2010. – 68 (1). – P. 1–10.
4. Brett M.T., Müller-Navarra D.C., Ballantyne A.P., Ravet J.L., Goldman C.R. Daphnia fatty acid composition reflects that of their diet // Limnology and Oceanography. – 2006. – 51(5). – P. 2428–2437. doi: 10.4319/lo.2006.51.5.2428
5. Cheban L., Malischuk I., Marchenko M. Peculiarities of cultivation *Desmodedesmus armatus* (Chocl.) Hegew. in

the wash water from RAS // Arch. Pol. Fish. – 2015. – V. 23 (3). – P. 155–162. doi: 10.1515/aopf-2015-0018
6. Das P., Mandal S.C., Bhagabati S.K. et al. Important Live Food Organisms and Their Role in Aquaculture // Frontiers in Aquaculture / Edited by M. Sukham. – New Delhi: Narendra Publishing House, 2012. – P. 69–86.
7. Duong V.T., Ahmed F., Thomas-Hall S.R. et al. High protein- and high lipid-producing microalgae from Northern Australia as potential feedstock for animal feed and biodiesel // Frontiers in Bioengineering and Biotechnology – 2015. – Vol. 3. – P. 1–7. doi: 10.3389/fbioe.2015.00053
8. González López C., García M., Fernández F. et al. Protein measurements of microalgal and cyanobacterial biomass // Bioresour. Technol. – 2010 – 101(19). – P. 7587–7591. doi: 10.1016/j.biortech.2010.04.077.
9. Hevorhyz R.H., Shchepachyov S.H. Metodyka yzmerenyia plotnosty suspenzyy nyzshykh fototrofov na dlyne volny sveta 750 nm [Method for measuring the density of a suspension of lower phototrophs at a wavelength of light of 750 nm]. – Sevastopol, 2008. – 10 p. (In Russian).
10. Khudyi O., Kolman R., Khuda L., Marchenko M., Terteryan L. Characterization of growth and biochemical composition of sterlet, *Acipenser ruthenus* L., juveniles from the Dniester population reared in RAS // Arch. Pol. Fish. – 2014 – 22 (4). – P. 249–256. doi: 10.2478/aopf-2014-0026
11. Kim S., Wijesekara I. Development and biological activities of marine-derived bioactive peptides: A review // J. Funct. Foods. – 2010 –2(1). – P. 1–9. doi: 10.1016/j.jff.2010.01.003
12. Knight J.A., Anderson S., Rawle J.M. Chemical basis of the sulfo-phospho-vanillin. Reaction for estimating total serum lipid // Clinical Chemistry. – 1972 – Vol. 18. – P. 199–202.
13. Kolkovski S. Digestive enzymes in fish larvae and juveniles – implications and applications to formulated diets // Aquaculture. – 2001. – 200(1-2). – P. 181–201. doi: 10.1016/S0044-8486(01)00700-1
14. Kushniryk O., Khudyi O., Khuda L., Kolman R., Marchenko M. Cultivating Moina macrocopa Straus in different media using carotenogenic yeast Rhodotorula // Arch. Pol. Fish. – 2015 – 23 (1). – P. 37–42. doi: 10.1515/aopf-2015-0004
15. Manual on the Production and Use of Live Food for Aquaculture. / Edited by P. Lavens, P. Sorgeloos, - FAO Fisheries Technical Paper № 361. – Rome: FAO, 1996 – 295p.
16. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. Protein measurement with the Folin phenol reagent // J. Biol. Chem. – 1951 – V. 193. – P. 265-275.
17. Macedo C.F., Pinto-Coelho R.M. Nutrition status of Daphnia laevis and Moina micrura from a tropical reservoir to different algal diets: Scenedesmus quadricauda and Ankistrodesmus glacilis // Braz. J. Biol. – 2001 – 61(4). – P. 555–562.
18. Ostroumova I.N. Biological principles of fish feeding. – SPb, State Research Institute of Lake and River Economy, 2012 – 564 p. (In Russian)
19. Sanchez D.M., Serrano C.M., Rodriguez M.R. at al. Extraction of carotenoids and chlorophyll from microalgae with supercritical carbon dioxide and ethanos as cosolvent // Journal of Separation Science. – 2008 – 31(8). – P. 1352– 1362. doi: 10.1002/jssc.200700503.
20. Suantika G., Rachminiwati N., Aditiawati P., et al. The use of Cyanobacteria Arthrospira platensis and Cladoceran Daphnia magna as complementary protein and lipid sources in transitional diet for Common Carp (Cyprinus carpio L.) Nursery // Natural Resources. – 2016. –7(7) – P. 423–433. doi: 10.4236/nr.2016.77037
21. Tanaka Y. Comparative biochemical studies on carotenoids in aquatic animals // Mem. Fac. Fish. – 1978 – 27(2). – P. 355–422.
22. Tuchapska A., Krazhan S. Cultivation of cladoceran for increasing provision of young-of-the-year carp with natural feeds (Review) // Ribogospod. nauka Ukr. – 2014 – 2(28). – P. 55–68. (in Ukrainian). doi: 10.15407/fsu2014.02.055

НУТРІЄНТНА ЦІННІСТЬ *DAPHNIA MAGNA* (STRAUS, 1820) ЗА УМОВ СУМІСНОГО КУЛЬТИВУВАННЯ З КОРМОВИМИ МІКРОВОДОРОСТЯМИ

Л. М. Чебан, О. Е. Гринько, М. М. Марченко

У роботі досліджено можливість використання водоростей Desmodesmus armatus (Chod.) Hegew та Acutodesmus dimorphus (Turpin) Tsarenko як кормових субстратів для вигодовування Daphnia magna (Straus, 1820). Проаналізовано біохімічний склад водоростей, культивованих на скидній воді із рибоводної установки замкнутого водопостачання (RAS). Показано, що обидві культури водоростей характеризуються подібною кількістю білка - на рівні 50%. Кількість ліпідів переважає у біомасі A.dimorphus і становить 21 %. У біомасі обох водоростей відмічено високий вміст каротинодів – 12 мг/г сухої маси. Також в роботі проаналізовано нутрієнтну цінність Daphnia magna за умов кокультивування з цими кормовими водоростями. Показано, що кокультивування Daphnia magna з D. armatus дозволяє отримати біомасу дафній з підвищенним вмістом загального білка (82,6 %) та каротиноїдів (15,24 мг/г сухої маси). Застосування як кормового субстрату A. dimorphus призводить до збільшеного накопичення в біомасі дафній ліпідів – 10,3 %. Біомасу водорості D. armatus запропоновано використовувати як оптимальний кормовий субстрат при кокультивуванні з Daphnia magna.

Ключові слова: Desmodesmus armatus (Chod.) Hegew, Acutodesmus dimorphus (Turpin), сумісне культивування, білок, ліпіди, каротиноїди

Отримано редколегією 18.12.2017