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USE OF *ACUTODESMUS DIMORPHUS* (TURPIN) TSARENKO AS A FODDER ORGANISM FOR *DAPHNIA* GROWING

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This investigation was devoted to the study of the possibility of using microalgae *Acutodesmus dimorphus* (Turpin) Tsarenko as a fodder organism for *Daphnia* growing. It was proposed to use as a nutrient medium for algae waste water from RAS (Recirculating Aquaculture System). Algae was cultivated for 21 days with a 16-hour photoperiod, illuminated with fluorescent lamps of 2500-4000 lux and at the temperature of 24 ± 2°C. A sufficiently high content of protein, lipids and carotenoids in *A. dimorphus* biomass has been established, which makes it possible to use it as a feed substrate for growing *Daphnia*. The amino acid profile of the algae biomass was analyzed. The dominant content of alanine, glutamic acid and leucine is established. There were approved two schemes of *Daphnia* cultivation, namely co-cultivation with *A. dimorphus* and regular replenishment of *Daphnia* culture by *A. dimorphus* biomass. The rate of increase in the number of *Daphnia* individuals and their consumption of microalgae culture was analyzed. In the biomass of *Daphnia* obtained by joint cultivation with *A. dimorphus* algae, an increased content of lipids and carotenoids was established. The content of total proteins was not significantly different with both approved growth regimens. By the rate of increase in the number of *Daphnia* individuals and their nutritional profile, the scheme of co-cultivation was recognised as optimal.

Key words: *Daphnia magna*, *Acutodesmus dimorphus* (Turpin) Tsarenko, waste water from RAS, co-cultivation.

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INTRODUCTION

In the structure of aquatic bioresources, unicellular algae are mainly studied as a forage base of plankton-eating fish, mollusks and zooplankton (Minyuk, 2008). Microalgae have a high nutritional value, their biomass is rich in proteins, lipids and carbohydrates. A large group of representatives of algal flora is capable for controlled biosynthesis of pigments, in particular carotenoids (Guedes, 2011). The nutritional value of fodder algae is also caused by substantial content of macro- and microelements in their cells (Brown, 2002). As a result of feeding hydrobionts with fodder algae, protein and pigment assimilation is improved both in crustaceans and in herbivorous fishes.

Obviously, algal proteins are readily available, well digested, and characterized by a balanced amino acid composition (Mente, 2006). In addition, the use of algae as feed substrates in aquaculture makes it possible to reduce the cost of the products obtained.

Among a large number of branchiate crustacean's species for cultivation under conditions of aquaculture, species that are distinguished by high productivity, adaptation to the specific conditions of cultivation, and high nutritional value are recommended. The representatives of the family Daphniidae have a particular importance for mass cultivation and occupy one of the first places in terms of use as live feed for fish. *Daphnia* are characterized by high fertility, rapid growth rates, well suited to cultivation.

The main problem in their cultivation is the need for constant feeding. Algae are the optimal feed for the growth of branchiate crustaceans, despite the fact that alternative feeds such as yeast, bacteria, algal paste or concentrates have been found (Tuchapska, 2014). At the same time, phytoplankton not only serves as a full-fledged source of nutrients, but also contributes to the enrichment of the aquatic environment with oxygen, participates in the circulation of substances.

As an algal feed for zooplankton, *Chlorococcales* are often used, namely *Chlorella* and *Scenedesmus*, the introduction of which into the diet of hydrobionts significantly affects on the growth of their biomass (Brown, 2002). However, among other representatives of green algae there is a large number of promising genera that can be attracted to cultivation in aquaculture. Thus, the biomass of protococcal microalgae from the genera *Desmodesmus*, *Acutodesmus* is a valuable source of proteins, amino acids, pigments, in particular carotenoids, vitamins and polyunsaturated fatty acids (Cheban, 2015).

In addition, small cell sizes make it possible to use their individual species as a balanced fodder supplement or starting live feed for young fish, both directly and indirectly (through enrichment of zooplankton) (Minyuk, 2008).

In this regard, the purpose of the work was to assess the possibility of using the species *A. dimorphus*, cultivated on waste water from RAS as a fodder organism for *Daphnia* growing.

MATERIAL AND METHODS

The studies were carried out using the pure culture of protococcal microalgae *A. dimorphus*, obtained from the collection (IBASH-A) of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine. As a nutrient medium for the cultivation of microalgae, waste water from RAS was standardized by indicators of pH (U-160 MU ion meter) and total mineralization (Water Quality Tester COM-100 conductivity meter) (Khudyi et al., 2016). All manipulations related to the culture inoculation were performed under sterile conditions. The ratio inoculum : nutrient medium was 1:10.

Also a material of the research was the monoculture of cladocerans *Daphnia magna* (Straus, 1820) that is in the collection of the Institute of Biology, Chemistry and Bioresourses of Yuriy Fedkovych Chernivtsi National University. Cumulative cultivation of *Daphnia magna* initial culture was conducted under the previously developed conditions (Khudyi et al., 2016). Cultivation was carried out in a climatic room under a 16-hour photoperiod, illuminated with fluorescent lamps of 2500-4000 lux and at the temperature of 24 ± 2°C. Cultivation lasted 21 days followed by using of the cumulative culture as a feed for *Daphnia*. Two feeding schemes were laid, namely simultaneous co-cultivation of *Daphnia* and *A. dimorphus* and gradual feeding of daphniids with biomass of microalgae once every three days in the calculation of $2.4x10^4$ cells/ml of cultivation medium. During the cultivation and at its terminal stages, the number of zooplankton individuals and phytoplankton cells was counted. The phytoplankton cell counting was performed using a Fuchs-Rosenthal camera. The crustaceans were counted using the aliquot method with a Bogorov camera under a MicroMed XS-3300 binocular microscope.

The sampling for biochemical analysis was carried out in the phase of maximum productivity. The concentrated samples of algae and zooplankton were treated with a USDN-2T ultrasonic desintegrator. Homogenization

of the test material was performed at +4°C using phosphate buffer pH 7. The homogenate was centrifuged at 1500 g per 15 min with Biofuge Stratos (Heraeus Instruments). The content of main nutrients was defined in the studied samples. Total lipids extracted with Folch method (Folch, 1957) were determined after acid hydrolysis of the samples with the subsequent reaction between decomposition products and the phospho-vanillin reagent (Knight, 1972). Total protein content was defined by Lowry method (Lowry, 1951). The content of carbohydrates was determined by a color reaction with the anthrone reagent (Roe, 1955). Extraction of carotenoids was carried out with acetone from hydrated algal cells or zooplankton biomass after treatment with USDN-2T ultrasonic disintegrator and analyzed spectrophotometrically at the optimum wavelength (Sanchez, 2008; Tanaka, 1978).

Hydrolysis of amino acids was carried out with concentrated hydrochloric acid at a temperature of 106°C for 24 hours. The total amino acid composition was determined by the method of ion-exchange liquid column chromatography on an T-339 automatic analyzer of amino acids (Prague, Czech Republic) on the basis of the A.V. Palladin Biochemistry Institute, NAS of Ukraine. To detect amino acids in the eluates, the method of detection with ninhydrin was used. The content of individual amino acids was expressed as a percentage of their total mass and in milligrams per gram of dry matter of the organisms under study. Determination of tryptophan was not carried out. The expression of the content of asparagine and glutamine was conducted in combination with aspartic acid and glutamic acid, respectively. All calculations were performed on dry weights. To determine humidity and dry weights, previously weighed samples were dried at 60°C for 24 hours to constant mass (Harris, 2000).

Mean values were considered significantly different at $P<0.01$ according to Student's criterion. The results were analyzed statistically with Microsoft Excel software in accordance with generally accepted methods.

RESULTS

Attempts were made to develop a scheme for co-cultivation, where in one cultivation system, both *Daphnia* and fodder algae coexisted in balance. In this case, it is necessary to take into account a large number of factors, including the composition of the optimal culture medium. The selected medium should meet the needs of both microalgae and branchiate crustaceans. We have shown the possibility of cultivation of both species under study on permanent waste water from RAS. It has been established that the use of waste water during the cultivation of crustaceans allows not only to delay the deterioration of culture growth, but also to substantially increase in zooplankton abundance and biomass. For

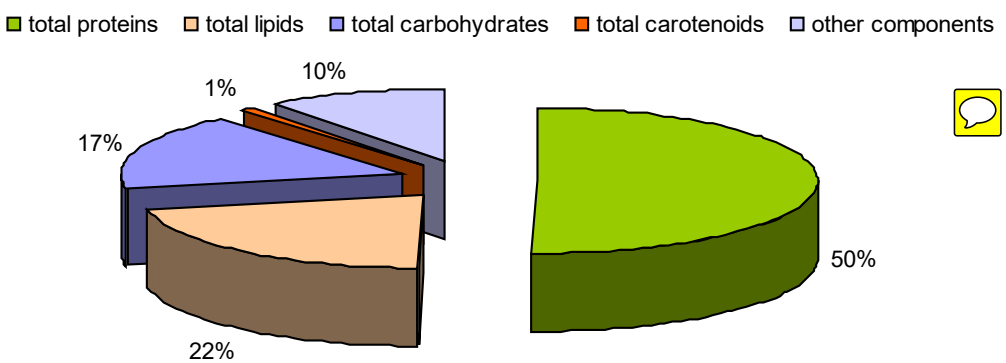

Fig. 1. The content of proteins, lipids, carbohydrates and carotenoids in *A. dimorphus* biomass at the terminal stage of cultivation on waste water from RAS.

algae, such a medium serves as a full-fledged source of all necessary elements and allows obtaining an actively growing productive culture (Khudyi et al. 2016). Under such cultivation conditions, intensive growth of *A. dimorphus* biomass was observed up to 21 days, which also was accounted for its maximum productivity in terms of biochemical parameters (Fig. 1). So, the protein content was noted at the level of 50%, the lipid content exceeded the carbohydrate content and was about 20%.

A rather high content of carotenoids in *A. dimorphus* biomass, namely about 1% is also positive. Such a quantity of carotenoids makes it possible to predict the use of these species as feed organisms for zooplankton, since representatives of the latter do not have carotene-synthesizing properties and require their intake with food.

Against the background of a high protein content, the distribution of amino acids in *A. dimorphus* biomass was quite interesting (Fig. 2). All proteinogenic amino acids, both interchangeable and essential, have been identified. For reasons of methodological nature, the essential amino acid cysteine was determined in the form of cystine, and an identification of tryptophan was not conducted. The quantitative characteristics of the amino acid profile directly depend on the specific features and conditions of cultivation. For *A. dimorphus* culture, the content of alanine and glutamic acid was 0.203 and 0.327 mgg^{-1} of dry weight, respectively. Among the essential amino acids, leucine has the maximum content, which was 0.16 mg/g in *A. dimorphus* biomass. It is known that the biomass of most algal species contains trace amounts of methionine and tryptophan (Zolotareva, 2008). Therefore, the fact of a sufficiently high methionine content in *A. dimorphus* biomass, 0.023 mgg^{-1}, was of interest.

So, the cell mass of *A. dimorphus* is characterized by a sufficient number of nutrients and can be used as a fodder object. Later, we tried to feed the zooplankton *Daphnia magna* (Straus, 1820) with the biomass of algae. According to the literature recommendations (Tuchapskaya, 2014), we tested two methods of feeding, namely, the simultaneous cultivation of *Daphnia* with *A. dimorphus* or replenishing the zooplankton culture with microalgal biomass once every three days (Fig. 3).

It is noted that *Daphnia* organisms adapt to the new feed substrate during the first three days at both applied schemes. Under conditions of co-cultivation, the number of *Daphnia* individuals increased in proportion to the cultivating duration and reached 650 ind. l^{-1} at the terminal stage. In conditions of bait feeding of daphniids with fodder substrate, the number of individuals was significantly smaller at each measuring point. At the final stage of detection, the number of *Daphnia* individuals was 525 ind. l^{-1}, which is 1.3 times less than in the previous scheme. The

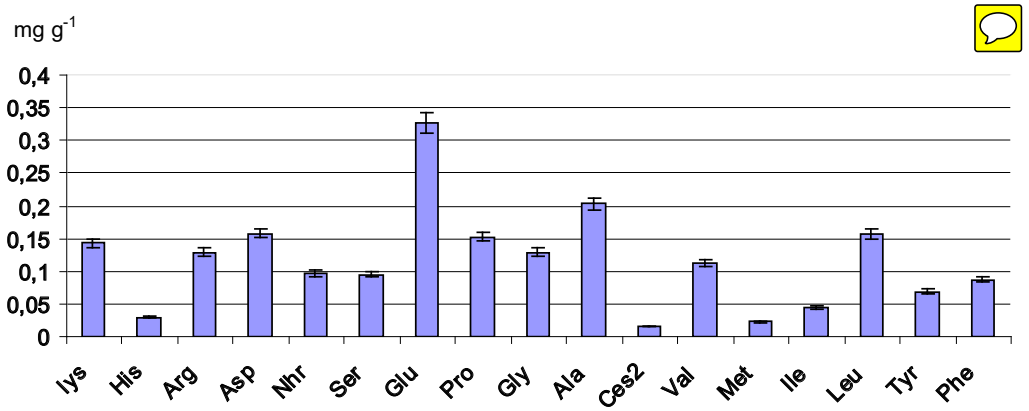

Fig. 2. Amino acid composition of A. dimorphus biomass cultivated on waste water from RAS.

nutrient content in *Daphnia* biomass was also analyzed at both used feeding regimens (Fig. 4). Regardless of the scheme used, the content of total proteins in *Daphnia* biomass did not differ significantly and was at 55%. The amount of total lipids and carotenoids of *Daphnia* under conditions of constant feed presence in the culture medium was slightly higher and amounted to about 10% lipids and 9 mg g^{-1} carotenoids on dry weight.

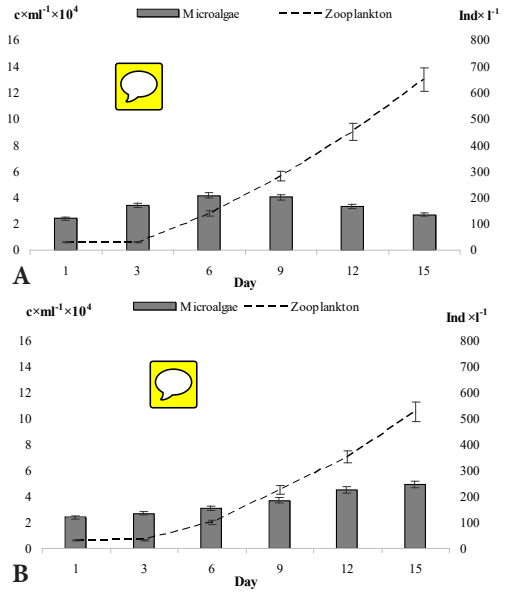

Fig. 3. Changes in the number of *A. dimorphus* cells and *D. magna* individuals under different growth patterns, where:
A – simultaneous introduction of phyto- and zooplankton;
B – feeding of zooplankton with algae once every three days.

DISCUSSION

Microalgae, involved in food chains in aquaculture, should be characterized by a high content of protein and essential amino acids. It is known that the biomass of protococcal microalgae can contain about 30-50% of the protein, and its amount and amino acid composition can differ significantly depending on the conditions of cultivation and the composition of the nutrient media used (Becker 2007, Cetin et al. 2015). It is a sufficient amount of protein and a qualitative composition of amino acids that will determine the effectiveness of the nutrient medium used and the possibility of further using of *A. dimorphus* biomass in food chains under aquaculture conditions (Chakri, 2014). The amino acid composition of this species is very similar to the composition, for example, of molluscan protein (Brown et al., 2002). It indicates that the protein and its amino acid composition is the factor that determines the nutritional value of fodder algal species.

Often the increased content of proteins in biomass at the terminal stages of cultivation is due to the redistribution of the nutrient profile due to the uneven use of mineral components of the nutrient medium. For example, an

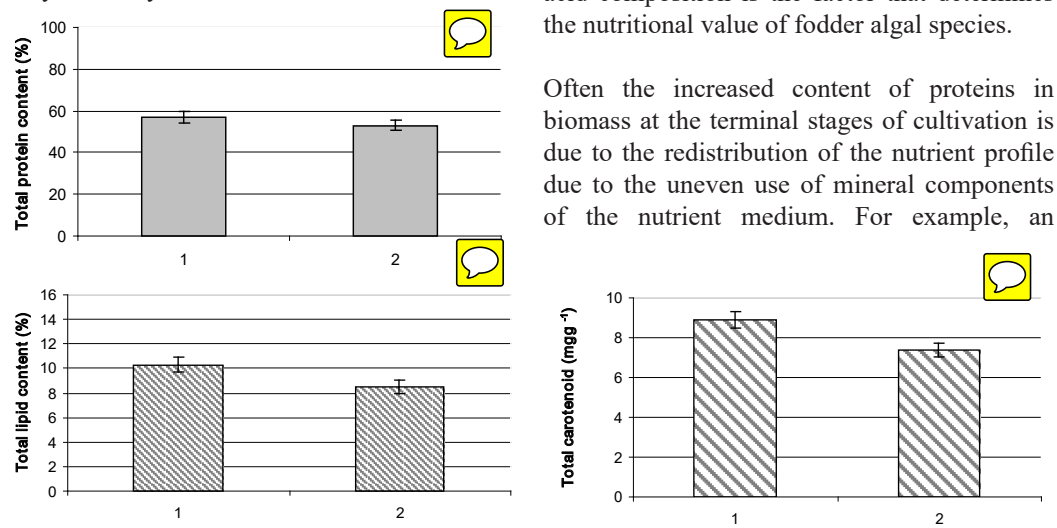

Fig. 4. Nutrients content in Daphnia magna biomass when using different feeding schemes, where: 1 – Daphnia, introduced simultaneously with algae; 2 – Daphnia using algae as a feed substrate

insufficient amount of nitrogen and phosphorus in the medium inevitably leads to an increase in the amount of lipids in the algal biomass (Minyuk, 2008). This fact is important when the accumulation of algal biomass is conducted with the goal of obtaining renewable energy sources. However, for fodder organisms, the maximum protein concentration in the cells is the determining one. Comparing the parameters of the nutrient profile, the prevalence of the proteins amount in the biomass of investigated culture was established under conditions of waste water from RAS using as a nutrient medium.

The ratio of the main nutrients in *A. dimorphus* biomass remained typical for the protococcal algae. A slightly higher level of lipids compared to carbohydrates in *A. dimorphus* biomass is probably caused by specific features of the algal culture. A sufficiently high content of carotenoids in the algal biomass is also an advantage. It is known that zooplankton, in particular *Daphnia*, is incapable of carotenoids synthesizing, but only of their accumulation. Their quantity in the biomass of the feed substrate defines the final level of accumulation of carotenoids in *Daphnia* biomass. A sufficiently high content of carotenoids, total proteins and a wide range of amino acids in *A. dimorphus* cell mass, cultivated in waste water from RAS, makes it possible to predict the use of these species of protococcal algae as feed for zooplankton under conditions of intensive aquaculture.

Among the number of methods for *Daphnia* cultivation proposed, two main directions are distinguished: the first is the co-cultivation of *Daphnia* and its feed (bacterio- and phytoplankton); the second is the separate cultivation of *Daphnia* and the organisms that are their feed, based on the creation of conditions typical for natural water bodies. Both schemes of *Daphnia* feeding with algae (regular feeding and simultaneous cultivation with fodder substrate) were tested. The quantity and availability of the feed substrate for zooplankton will determine its production indices. The largest number of *D. magna* individuals was obtained under conditions of simultaneous introduction of zooplankton and phytoplankton into the cultivation system. Obviously, this is a consequence of the constant availability of feed substrate for *Daphnia* under conditions of co-cultivation with microalgae. However, even in bait conditions, the number of algal cells was sufficient and even increased with time. It is obvious that under the conditions of feeding the fodder substrate, it is not possible to achieve a balance between the amount of algae biomass that has been introduced and the rate at which it is consumed by *Daphnia*. The cell mass of algae gradually accumulates, they begin to multiply in mass and gradually suppress of *Daphnia* growth. This suggestion is supported by a smaller number of crustaceans individuals in the culture system under this feeding pattern. Probably, the mass development of phytoplankton leads to qualitative changes in the cultivation medium where algal exometabolites are accumulated.

In addition to the rapid growth of *Daphnia* biomass, biochemical characteristics are also important indicators. This group of feed organisms is consumed by almost all fish species at the first stages of exogenous feeding, despite their further nutritional specialization. The obtained results show that feeding of daphniids with algae using both proposed schemes allows to obtain a productive culture. The content of total proteins and lipids in biomass remains typical for the branchiate crustaceans. However, the predominance of lipids and carotenoids in *Daphnia* biomass, co-cultivated with *A. dimorphus*, makes it possible to propose such a scheme as an optimal for *Daphnia* growing.

CONCLUSIONS

Therefore, the possibility of algae *A. dimorphus* use as a fodder substrate for *D. magna* growing have been demonstrated. The scheme of simultaneous introduction of phyto- and zooplankton proved to be effective. Under such conditions it was possible to obtain the largest number of *Daphnia* individuals and maintain the optimal ratio of the main nutrients. Crustaceans

obtained in this way are recommended to be used as a feed for youth of commercial fish species.

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