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EFFECT OF ALGAL MONOCULTURES AND COMBINED ALGAL DRUG ON THE SURVIVAL OF ARTEMIA NAUPLII

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The maintenance of live feed in automatic feeders is accompanied by fasting of fodder organisms, resultedin a lose of their nutritional value and even death. The way out of this situation can be the joint maintenance of Artemia nauplii and their fodder objects – microalgae. Accordingly, an influence of three microalgae monocultures (Desmodesmus armatus, Chlorella vulgaris, Dunaliella salina) and the AlgaMac Protein Plus drug on survival of Artemia nauplii was analyzed. It was shown that the lowest mortality rates of brine shrimp were observed when it was co-cultivated with monoculture of Chlorella. The use of the AlgaMac Protein Plus algal drug provided a significant reduction in the mortality rate of Artemia nauplii at 12 hours of cultivation. Cell size of studied algal species is a key point for their using as a fodder object for brine shrimp. Maximum diameter of Chlorella cells reaches 10 μm. Cells of D. armatus are single nucleated autospores with coccoid shape. Their size is 10-20 microns in length; 3.5-8 microns in width. However, exept of single cells, Desmodesmus forms conglomerates in 2-4 cells, which complicates its capture by Artemia. Algae of the genus Dunaliella are the component of the natural feed base for Artemia in salt and hypersaline lakes. Despite the fact that these algae are actively consumed by adult Artemia, for their early stages Dunaliella remains practically inaccessible, as it has large enough cells of a pear shaped form up to 25 microns in length and 4 to 10 microns in width. The initial length of Artemia nauplii after hatching was about 551 μm. In the control sample after 24 hours, this parameter was increased to 625 microns. In the experimental sample, co-cultivated with Dunaliella the size of brine shrimp was 617 μm, while enriching with Chlorella this parameter was 611 μm. The smallest size was observed in nauplii co-cultivated with D. armatus.

Keywords: live feed, Artemia, microalgae, mortality, AlgaMac Protein Plus.

Introduction. Live feeds are often used in aquaculture during fish larvae transition to external feeding. This is due to an easy assimilation of nutrients by larvae which were accumulated in the tissues of fodder organisms. Accordingly, survival of fish larvae is much higher when using live feed than artificial one (Akbary et al., 2010; Kadhar et al., 2014).

Artemia nauplii are often used as the starting live feed, which is associated with the simplicity of their cultivation. However, nauplii do not always contain all necessary set of essential compounds, in relation to the early youth of certain species of fish. Bioencapsulation technology provides a correction of the native composition of live feeds. The point of such a methodological approach is that the fodder organism acts not only as a source of nutrients, but also as a living capsule, where you can "place" a certain target product (polyunsaturated fatty acids, essential amino acids, vitamins, probiotic microorganisms, etc.), and deliver in the organism of fish larva (Akbary et al., 2011; Immanue, 2016). Bioencapsulation of target products into the live feed increases the digestibility of these substances by fish larvae.

The use of dearly expensive and highly purified drugs of essential compounds in the bioencapsulation technologies of live feed is often economically inappropriate. Instead, the use of biomass producers of such compounds allows significantly reduce the cost of enriched feed. It is known that producers of a significant amount of essential nutrients are microalgae, but they are quite difficult to digest by the organism of fish larvae. The increase of the bioavailability of algae for young fish can be achieved through the use of zooplankton, which is used as a starting feed for fish larvae.

Microalgae are used in aquaculture as a feed for all growth stages of bivalve molluscs, for larval stages of commercial crustaceans species and certain species of fish, as well as for feeding of live feeds, in particular zooplankton (Ovie, Egborge, 2002; Brown, Blackburn, 2013; Napiórkowska-Krzebietke, 2017).

Algae should have a number of key features for being used in aquaculture. Their sizes should be in the range of 1 to 15 microns in order to be available for filtering feeders and about 10-100 microns – for organisms with an oral apparatus of sucking type (Brown, 2002). Fodder algae should have a rapid rate of biomass increasing, to be subjected to mass

cultivation and be resistant to any fluctuations in temperature, light and nutrient content. Finally, they must have an optimal composition of nutrients, have no toxins, and also be easily absorbed by the body of fish or other aquaculture objects (Tuchapska and Krazhan, 2014).

Different types of algae can vary significantly in their nutritional value, which depends primarily on the conditions of cultivation (Cheban et al., 2015, González López et al., 2010, Hu et al., 2008). Nevertheless, carefully selected monoculture or a mixture of microalgae can be offered as an excellent fodder substrate for fish larvae. Algae can be introduced into the diet of fish by direct feeding or indirectly by their bioencapsulation into fodder zooplankton (Duong et al., 2015; Becker, 2007).

In large volumes of fish-breeding material automatic feeders are usually used, thus providing noctidial dosage of fodder organisms to the fish larvae which are grown. Accordingly, during this time a part of the nauplii dies as a result of starvation, and in those who survived the nutritional value decreases. The way out of this situation may be the joint maintenance of *Artemia* nauplii and their fodder objects – yeast, infusoria, microalgae and / or drugs based on them.

An influence of three microalgae monocultures, namely *Desmodesmus armatus* (Chod.) Hegew., *Chlorella vulgaris* Veijerinck, *Dunaliella salina* Teod. and AlgaMac Protein Plus, on the survival of *Artemia* nauplii was analyzed.

Materials and methods. Microalgae cumulative cultures were obtained by growing them up to the exponential phase of growth on the waste water from recirculating aquaculture system (RAS) (Cheban et al., 2015).

All manipulations of culture passage were carried out under conditions of laminar boxing. The cultivation was conducted in a climatic room at 16-hour illumination with fluorescent lamps of 2500-4000 lux and a temperature of 24 ± 2 °C.

After the cultivation of biomass, algae were concentrated and washed off from the residues of the nutrient medium with sterile distilled water. Allocation of algal cells from the culture medium was carried out by centrifugation at 6.000 rpm for 15 minutes in Biofuga stratos "Herauses". The incubation of *Artemia* cysts (Sepia Art *Artemia* cysts from Ocean Nutrition, Belgium) was conducted in Weiss apparatus with a volume of 8 liters for 24 hours at constant aeration, illumination and a constant temperature of 28 °C. The required level of salinity was provided by adding sodium chloride to water at a rate of 15 g / l. After 24 hours there was a massive release of nauplii.

After clearing from the shells for 200.000 hatched *Artemia* nauplii, they were transferred to 1.2 liter containers with salty water. In a container with *Artemia*, monocultures of *D. armatus*, *C. vulgaris*, *D. salina* algae were introduced at a rate of 3×10^6 cells / l. The Alga Mac Protein Plus drug was administered at three concentrations of 0.1 g / l; 0.2 g / l; 0.4 g / l. In the control group, brine shrimp was kept without feeding. The experiment lasted for 24 hours, providing a constant aeration and a water temperature of 28 °C.

Every 6 hours from the beginning of the experiment, the total number and the number of dead crustaceans in the experimental and control groups were counted. For this purpose, the dead organisms were firstly counted in 0.2 ml of medium in the Bogorov Counting Chamber. After that, the Lugol's solution was added and several minutes had been waiting until all individauls died. After that, the total number of *Artemia* brine shrimps was counted in a sample. The mortality rate was estimated by the Cumulative Mortality Index (CMI), which was calculated as a ratio of summarized dead individuals at this and previous hours to the sum of the total number of individuals in the sample at this and previous hours:

$$\mathrm{CMI}_i = \frac{\sum_0^{24} n_i}{\sum_0^{24} N_i} \times 100 \qquad (1)$$

n – number of dead individuals at i hour
N – total number of individuals at i hour
i – cultivation period (0, 6, 12, 24 hours)

All the data are presented as mean ± SE. Mean values were considered significantly different at $P < 0.05$. The significance of differences in the results was evaluated with one-way ANOVA. Statistical analysis was computed using MS Excel software and STATISTICA 6.0.

Results and discussion. During the first six hours of cultivation, the mortality rate of *Artemia* in all experimental groups was on the same level and did not differ significantly from the values of this indicator in the control group (Fig. 1 and 2). The explanation of this fact is in the peculiarity of the life cycle of *Artemia* – during the first 8-12 hours after hatching from the cyst nauplii endogenously feed nutrients from the the yolk sac (Simon, 2016; Lavens, Sorgeloos, 1996). Considering that aquaculture uses cysts, hatching synchronization of which can last up to 7 hours (Sorgeloos et al., 2001), the fodder substrate was introduced immediately after the separation of the nauplii from cysts to prevent starvation of *Artemia*.

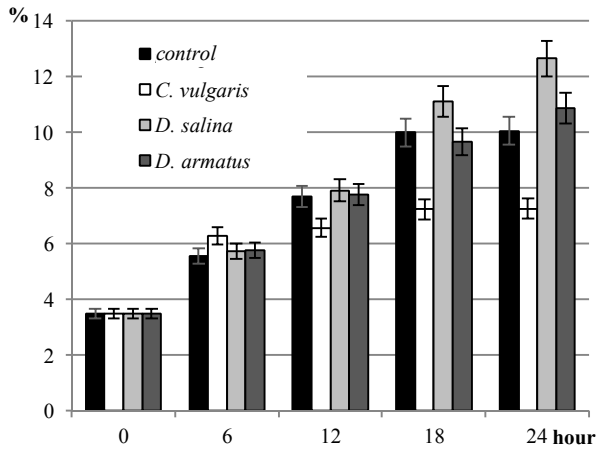


Fig. 1. Dynamics of the Cumulative Mortality Index of the brine shrimp nauplii co-cultivated along with microalgae

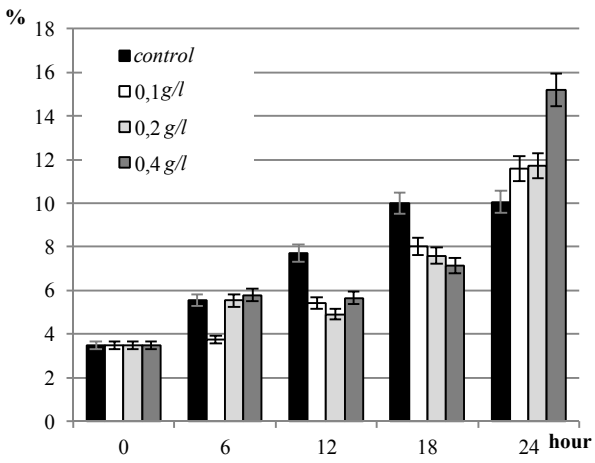


Fig. 2. Dynamics of the Cumulative Mortality Index of the brine shrimp nauplii under the influence of AlgaMac Protein Plus of various concentrations

The transition to exogenous feeding of *Artemia* occurs after the first molting, when nauplii turn into metanauplii. At this stage brine shrimp passes to exogenous feeding, actively filtering out small fodder objects such as bacteria, algae, protozoans, detritus. Sizes metanuplii usually do not exceed 50 microns, however, the optimal size of fodder objects for *Artemia* at all stages of development is within the range of 3-8 microns (Makridis, Vadstein, 1999).

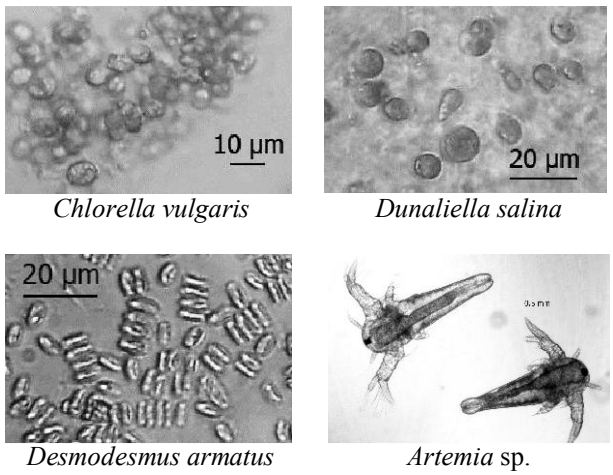
The lowest mortality rates of *Artemia* crustaceans were observed during their co-cultivation with monoculture of *Chlorella*. In spite of our expectations, the use of monocultures of *Dunaliella* and *Desmodesmus* did not provide a reduction in the mortality rate of *Artemia* (Fig. 1).

Obviously, this situation is related to the cell size of studied algal species. It is known that the maximum diameter of *Chlorella* cells reaches 10 μm (Heimann, Huerlimann, 2015). In addition, *C. vulgaris* cells have a shape close to the correct sphere that facilitates their absorption during the filtration process. Cells of *D. armatus* are single nucleated autospores with coccoid shape. Their shell contains an additional sporopollenin layer and no sharp ends. Their size is 10-20 microns in length; 3.5-8 microns in width. However, exept of single cells, *Desmodesmus* forms conglomerates in 2-4 cells, which complicates its capture by *Artemia*.

Algae of the genus *Dunaliella* are the component of the natural feed base for *Artemia* in salt and hypersaline lakes. It is known that *D. salina* cells are deprived of hard cell walls, resulting in relatively easy digestion in the intestinal tract of zooplankton. Despite the fact that these algae are actively consumed by adult *Artemia*, for their early stages *Dunaliella* remains practically inaccessible, as it has large enough cells of a pear shaped form up to 25 microns in length and 4 to 10 microns in width.

As it is known, the presence of cellulose cell walls complicates the digestibility of microalgae by animal organisms, including zooplankton. AlgaMac Protein Plus is a dried and destroyed biomass of various algae and microorganisms species, with a particle size of less than 10 microns. We assumed that the use of non-native monocultures, and crushed biomass of algae would provide a better assimilation by *Artemia*. However, the results of the studies showed that after 24 hours of incubation, a significantly higher mortality rate of nauplii with the use of the studied drug, compared with alive algae (Fig. 2).

The most pronounced positive effect of using AlgaMac Protein Plus was observed from 12 hours at all concentrations studied. Further increases in mortality apparently are associated with a deterioration of the quality of water by organic matter, released from the drug residues that were not absorbed by *Artemia* during the first 12 hours of cultivation.



Chlorella vulgaris *Dunaliella salina*

Desmodesmus armatus *Artemia* sp.

Fig. 3. Brine shrimp nauplii and their forage objects

Using fodder substrates to cultivate *Artemia*, a negative consequence may be an excessive increase in the size of nauplii, and as a result it will make such feed unavailable for fish larvae. The initial length of the nauplii after hatching was about 551 μm. In the control sample after 24 hours, this parameter was increased to 625 microns. In the experimental sample, co-cultivated with *Dunaliella salina* the size of brine shrimp was 617 μm, with *Chlorella vulgaris* this parameter was 611 μm. The smallest size was observed in nauplii co-cultivated with *Desmodesmus armatus* – 608 μm.

Thus, the absence of significant differences in size between the nauplii of the control group and the nauplii, which were cultivated together with microalgae, against the background of a decrease in their mortality rates, makes it appropriate to add algae to the *Artemia* culture medium.

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ВПЛИВ МОНОКУЛЬТУР ТА КОМБІНОВАНОГО ПРЕПАРАТУ ВОДОРОСТЕЙ НА ВИЖИВАНІСТЬ НАУПЛІЙ АРТЕМІЇ

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Утримання живих кормів в автогодівницях супроводжується голодуванням кормових організмів, внаслідок чого вони втрачають свою поживну цінність і, навіть, гинуть. Виходом з даної ситуації може бути сумісне утримання науплій артемії та їх кормових об'єктів – мікроводоростей. Відповідно було проаналізовано вплив монокультур трьох видів мікроводоростей (Desmodesmus armatus, Chlorella vulgaris, Dunaliella salina) та альгопрепарату AlgaMac Protein Plus на виживаність науплій артемії. Показано, що найнижчі показники смертності артемії спостерігали при її сумісному культивуванні з монокультурою хлорели. Використання альгопрепарату AlgaMac Protein Plus забезпечило істотне зниження показників смертності науплій артемії станом на 12 годину культивування. Розмір клітин досліджуваних видів водоростей є ключовим моментом при їх використанні як кормового об'єкта для артемії. Максимальний діаметр клітин хлорели досягає 10 мкм. Клітини мікроводорості D. armatus – одноядерні, автоспорові, кокоїдні. Їх розміри становлять 10-20 мкм в довжину; 3,5-8 мкм завширшки. Проте, десмодесмус окрім поодиноких клітин формує конгломерати по 2-4 клітини, що ускладнює його захоплення артемією. Водорості роду Dunaliella є елементом природної кормової бази для артемії у солоних та гіперсолоних озерах. Незважаючи на те, що дана водорость інтенсивно виїдається дорослою артемією, для її ранніх стадій Dunaliella залишається практично недоступною, оскільки має достатньо крупні клітини грушевидної форми довжиною до 25 мкм та шириною від 4 до 10 мкм. Початкова довжина науплій після викльову була близько 551 мкм. У контрольній групі через 24 год цей показник збільшився до 625 мкм. У дослідних – при насиченні Dunaliella salina розмір становив 617 мкм, Chlorella vulgaris – 611 мкм. Найменшими розмірами володіли науплії, які культивували сумісно з Desmodesmus armatus – 608 мкм.

Ключові слова: живий корм, артемія, мікроводорості, смертність, AlgaMac Protein Plus.

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