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CHANGES IN THE BIOCHEMICAL COMPOSITION OF *ARTEMIA SALINA* (L.) IN RELATION TO DIFFERENT FEEDING CONDITIONS

PROMJENE U BIOKEMIJSKOM SASTAVU *ARTEMIA SALINA* (L.) S OBZIROM NA RAZLIČIT HRANIDBENI TRETMAN

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The biochemical composition of early stages of the starved *Artemia salina* was analysed and compared with the biochemical composition of *Artemia* fed with the phytoplankton monoculture *Chlorella* sp. and baker yeast. Changes in the protein, carbohydrate, lipid, ash and water contents were observed during 96-h period.

During the first 48 h proteins show an apparent increase under all three feeding treatments with a slight decrease thereupon. *Artemia* fed yeast shows significantly higher protein levels after 96 h than *Artemia* fed phytoplankton. This is probably due to the higher protein percentages in the baker yeast. The increase of the percentage of protein in the beginning, both in fed and unfed *Artemia* nauplii, may be due to their synthesis in a growing organism.

Relative total lipid and carbohydrate contents decrease during the 96-h period of *Artemia* nauplii development under all treatments, whereas the percentage of water shows increase. The ash mainly follows the patern of protein changes.

INTRODUCTION

Since *Artemia salina* inhabit highly saline waters they are not a natural diet of either fish (Kristensen and Hulscher-Emeis, 1972) or crustaceans (Roberts, 1974). However, *Artemia* nauplii are widely used as a food for early stages of a large number of different commercially important fish and crustacean species.

Even though widely acclaimed as an inevitable food source in aquaculture *Artemia* food value seems to be limited. It was shown that a long-term feeding of early fish stages with *Artemia* nauplii caused the defficiency of some fatty acids (Das, 1981). This accounted for the high mortality of the japanese red sea-bream, *Chrysophrys major* (Fujita et al., 1980). It was also established that the postlarvae of sea bass *Dicentrarchus labrax* fed

exclusively *Artemia* napulii over a longer period were liable to higher mortality accompanied with the hyperthrophy of swimbladder (Katavić, 1984).

Food value of early *Artemia salina* stages varies significantly (Benijits et al., 1975). With the body weight losses early developmental stages lose a considerable part of their caloric value (Paffenhöfer, 1967). At relatively low density of cultured fish postlarvae, not taken *Artemia* accumulate from one day to another. Therefore their food value is constantly reduced due to starvation. This results in a significant growth retardation of postlarvae and finaly in the mortality increase (Katavić, 1984).

It was also found that with respect to energy value losses of starved *Artemia* the postlarvae of fish should consume 27% more metanauplii than nauplii (Benijits et al., 1975). Food value of *Artemia* may as well significantly depend on its geographical origin. Thus Shelbourne (1968) noted unespectedly high mortality of sole *(Solea solea)* postlarvae fed with *Artemia* nauplii originating from the Great Salt Lake (Utah, USA) as distinct from those fed with *Artemia* nauplii from salt pans near San Francisco Bay (California, USA). Many authors tried to explain this by the difference in the biochemical composition of *Artemia* (Little, 1969; Reeve, 1969) or attributed it to the unfavourable food value of Utah nauplii not excluding the possibility of their toxicity due to the increased pesticide concentrations (Bookhout and Costlow, 1970). Wickins (1972), with an aim of increasing the food value of Utah *Artemia,* established the positive effect of phytoplankton monoculture *Isochrysis galbana.*

This paper is an attempt to observe the changes in basic biochemical composition of *Artemia* napulii in starvation. With an aim of improving their food value the changes of biochemical macroconstituents of *Artemia* living on organic feeds (baker yeast).

MATERIALS AND METHODS

Decapsulation of *Artemia* cysts (Great Salt Lake, Utah, USA) was carried out according to the method of Sorgeloos et al. (1977).

The cysts of *Artemia* were incubated in the sea water of 35 ppt (35×10^{-3}) salinity and constant 22°C temperature in plastic bags of 30 l volume with aeration. Live nauplii were harvested by filitrating the culture medium through a 150 μm net 0, 12, 24, 72 and 96 h after hatching.

The following food was used during the experiment:

experimental food	source and description of treatment
1. Phytoplankton monoculture *Chlorella* sp.	The concentration of phytoplanktoners was tried to be kept constant at 500 000/ml by adding daily new quantities. Initial nauplii concentration was 30 ind./ml
2. Baker yeast	Fresh yeast from local bakery was diluted and added daily in the quantity of 10 g/bag. Initial nauplii concentration was 30 ind./ml
3. Starvation	No food was added during the experiment. Initial nauplii concentration was 30 ind./ml

Three grams of *Artemia* obtained by washing the sample with distilled water and filtered by vacuum pump to remove all the remaining water were used for biochemical analysis. Ten ml of water were added to the weighed sample which was then homogenized at Ultra-Turrax homogenizer at 20 000 rpm for one minute. Aliquots were used for determination of dry weight, proteins, total lipids, carbohydrates and ash content.

The protein was determined by Lowry et al. (1951) method with the bowine serum albumin as standard. The lipids were extracted from 1 ml residue by the method of Folch et al. (1957). After drying of chloroform extract at a temperature of 60°C the total lipids were determined by gravimetry. Carbohydrate was determined from a residue with 5% TCA after the hydrolisis of the supernatant by the Dubois et al. method (1956) with glucose as standard. Dry weight was determined by drying the 2 ml residue at 105° for 24 hours. Ash was obtained by igniting the dry residue at 550°C.

The analysis of each constituent was repeated three times. The results for proteins, total lipids and carbohydrates are given as the percentages of the dry weight minus ash, whereas the ash dry weight was used as a basis for ash percentages.

Results were statistically analysed by analysis of variance. Homogeneous categories of means of different feeding treatments were established by the Student-Newman-Keuls (SNK) procedure (Sokal and Rohlf, 1969).

RESULTS

The differences in length were not significant ($P > 0.05$) in either fed (phytoplankton, yeast) or starved *Artemia* cultures 12 h after eclosion. However, 24 h after eclosion fed nauplii were greater than the starved nauplii (Table 1). The length of starved *Artemia* was significantly smaller than that of fed *Artemia* not earlier than after 48 and 96 h respectively. The 180 h old *Artemia* grew significantly slower on phytoplankton culture *Chlorella* sp. diet than on baker yeast.

Length increment and mean percentages of biochemical macroconstituents of *Artemia salina* in relation to different feeding conditons (*Chlorella* sp., yeast, starvation) during the 96-h experiment are presented in Figs. 1, 2 and 3.

Tested differences in the percentage protein levels between different feeding treatments 12, 24, 48 and 72 h after eclosion were not statistically significant. After 96 h protein levels were significantly higher in *Artemia* fed baker yeast than in *Artemia* fed phytoplankton and in starved animals (Table 2).

The difference in carbohydrate contents between *Artemia* reared on three different feeding treatments was statistically significant during the first 12 h when the relative content of carhobydrates was highest in *Artemia* fed with *Chlorella* sp. (Table 3). For the longer period tested differences were not statistically significant for the 95% confidence limit.

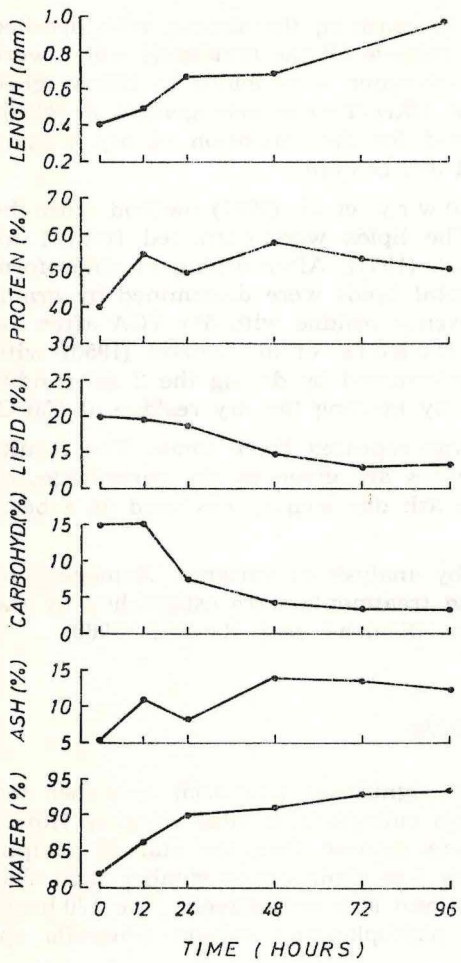

Fig. 1. Growth in length and the percentage protein, lipid, carbohydrate (on the ash-free dry weight basis), ash (dry-weight) in *Artemia* fed phytoplankton monoculture *Chlorella* sp.

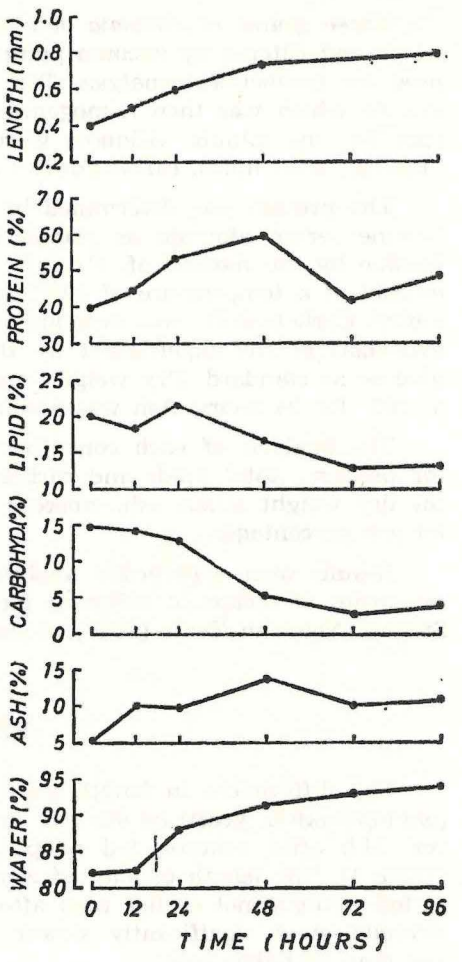

Fig. 2. Growth in length and the percentage protein, lipid, carbohydrate (on the ash-free dry weight basis), ash (dry weight) and water (fresh weight) in water (fresh weight) in *Artemia* fed yeast.

Statistically significant differences were established in 12, 24, and 4 h old *Artemia* reared under different feeding conditions (Table 4a, 4b and 4c).

Significantly higher lipid content was established by SNK method in 12 h old *Artemia* fed with phytoplankton. Differences in lipid contents between *Artemia* fed on phytoplankton and *Artemia* fed on yeast were not statistically significant; neither were the differences between *Artemia* fed on yeast and starved *Artemia*. The lipid content was also highest in *Artemia* fed with phytoplankton 24 and 96 h after eclosion (Table 4).

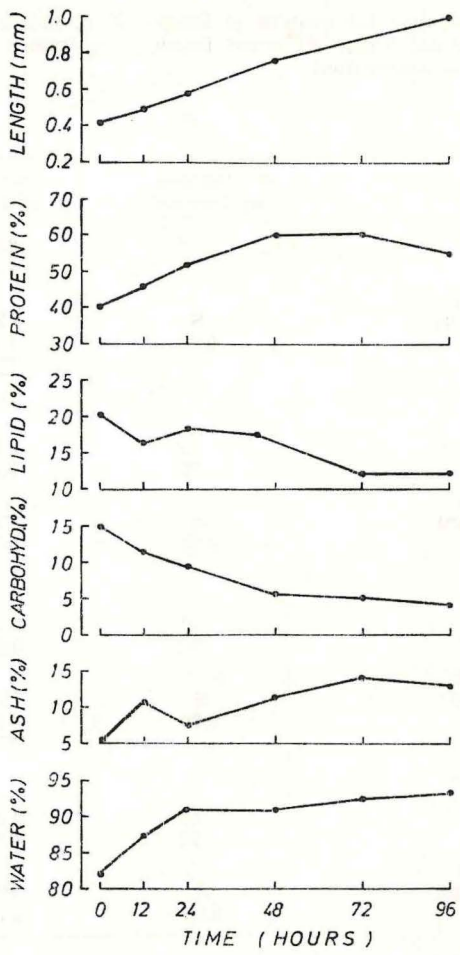

Fig. 3. Growth in length and the percentage protein, lipid, carbohydrate (on the ash-free dry weight basis), ash (dry weight) and water (fresh weight) in the starved *Artemia*.

The difference in ash contents between individual treatments were statistically significant only after 12 and 24 hours.

Twelve hours after eclosion ash content was highest in *Artemia* fed on phytoplankton and 24 h after eclosion in starved *Artemia*. (Table 5a and 5b).

Test of the differences in water content gave statistically significant differences for 12, 24, 48 and 96 h, as shown in Table 6.

After 12 and 24 h water content was lowest in starved *Artemia*. Water content was lower in *Artemia* fed on yeast for other time intervals (Table 6).

Tab. 1. Analysis of variance for growth in lenght of *A. salina* 24, 48, 96 and 180 — hrs after hatching under different feeding treatments (Ph — phytoplankton; Y — yeast; S — starvation).

a) 24 hrs

Source of variation	Degrees of freedom		Mean square		F - value
Among groups	2		0.04		8.17*
Within groups	72		0.01		
Least significant range					
Feeding treatment	S		Y		Ph
Lenght (mm)	0.60		0.65		0.68

b) 48 hrs

Source of variation					
Among groups	2		0.06		11.28**
Within groups	72		0.01		
Least significant range					
Feeding treatment	S		Y		Ph
Lenght (mm)	0.74	*	0.77	*	0.84

c) 96 hrs

Source of variation					
Among groups	2		0.32		31.47**
Within groups	70		0.01		
Least significant range					
Feeding treatment	S		Y		Ph
Lenght (mm)	0,79	*	0.98		1.00

d) 180 hrs

Source of variation					
Among groups	2		21.35		217.68**
Within groups	72		0.01		
Least significant range					
Feeding treatment	S		Ph		Y
Lenght (mm)	0.85	*	2.17	*	2.64

Underscored means are not singnificantly different ($P > 0.05$)

Table 2. Analysis of variance data on the percentage protein content after 96-hrs growth period under differnt feeding treatments (Ph — phytoplankton; Y — yeast; S — starvation)

Source of variation	Degrees of freedom		Mean square		F - value
Among groups	2		28.16		8.47*
Within groups	6		3.32		
Least significant range					
Feeding treatment	Ph		S		Y
Ranged means (%)	49.26		50.75	*	55.14

Underscored means are not singnificantly different ($P > 0.05$)

Table 3. Analysis of variance data on the percentage carbohydrates content after 12-hrs growth period under different feeding treatments (Ph — phytoplankton; Y — yeast; S — starvation)

Source of variation	Degrees of freedom	Mean square	F - value
Among groups	2	18.56	49.35**
Within groups	6	0.38	
Least significant range			
Feeding treatment	S	Y	Ph
Ranked means (%)	11.38	11.39 *	15.74

Underscored means are not singnificantly different ($P > 0.05$)

Table 4. Analysis of variance data on the percentage lipid content after a) 12, b) 24, c) 96-hrs growth period under different feeding treatments (Ph — phytoplankton; Y — yeast; S — starvation).

a) 12 hrs

Source of variation	Degrees of freedom	Mean square	F - value
Among groups	2	8.29	8.09*
Within groups	6	1.02	
Least significant range			
Feeding treatment	Y	S	Ph
Ranked means (%)	16.46	18.48	19.78

b) 24 hrs

Source of variation			
Among groups	2	9.63	11.42**
Within groups	6	0.84	
Least significant range			
Feeding treatment	S	Y	Ph
Ranked means (%)	18.57	18.71 *	21.73

c) 96 hrs

Source of variation			
Among groups	2	0.53	9.77*
Within groups	6	0.0047	
Least significant range			
Feeding treatment	Y	S	Ph
Ranked means (%)	12.34 *	13.44 *	14.68

Underscored means are not singnificantly different ($P > 0.05$)

Table 5. Analysis of variance data on the percentage ash content after a) 12, b) 24 hrs growth period under different feeding treatments (Ph — phytoplankton; Y — yeast; S— starvation)

a) 12 hrs

Source of variation	Degrees of freedom		Mean square		F - value
Among groups	2		0.52		10.29*
Within groups	6		0.05		
Least significant range					
Feeding treatment	Y		Ph		S
Ranked means (%)	10.29	*	10.78	*	11.12
b) 24 hrs					
Source of variation					
Among groups	2		3.92		5.21*
Within groups	6		0.75		
Least significant range					
Feeding treatment	Y		Ph		S
Ranked means (%)	7.54		8.36		9.41

Underscored means are not singnificantly different ($P > 0.05$)

Table 6. Analysis of variance data on the percentage water content at different time intervals (Ph — phytoplankton; Y — yeast; S — starvation).

a) 12 hrs

Source of variation	Degrees of freedom		Mean square		F - value
Among groups	2		13.68		5.64*
Within groups	6		2.42		
Least significant range					
Feeding treatment	S		Ph		Y
Ranked means (%)	82.57	*	86.55		87.42
b) 24 hrs					
Source of variation					
Among groups	2		7.53		961.68**
Within groups	6		0.01		
Least significant range					
Feeding treatment	S		Ph		Y
Ranked means (%)	88.14	*	90.16	*	91.28
c) 48 hrs					
Source of variation					
Among groups	2		0.65		78.91**
Within groups	6		0.01		
Least significant range					
Feeding treatment	Y		S		Ph
Ranked means (%)	90.71	*	91.51		91.52

d) 72 hrs					
Source of variation					
Among groups	2		0.28		5.12
Within groups	6		0.05		
Least significant range					
Feeding treatment	Y		Ph		S
Ranked means (%)	92.51		92.91		93.11
e) 96 hrs					
Source of variation					
Among groups	2		0.96		165.53**
Within groups	6		0.01		
Least significant range					
Feeding treatment	Y		Ph		S
Ranked means (%)	92.83	*	93.57	*	93.93

Underscored means are not singnificantly different ($P > 0.05$)

DISCUSSION

Judging from the length increment and basic biochemical composition *Artemia salina* nauplii appear to be very resistant to starvation, particularly in the initial stages. No statistically significant differences in nauplii growth rate were recorded for the first 12 h after eclosion. Only 24-h old *Artemia* fed on phytoplankton exceeded significantly in size the starved ones. Later on the differences in length between fed and starved *Artemia* became greater. The comparison of length increment of 180-h old *Artemia* fed on phytoplankton monoculture *Chlorella sp.* and that of *Artemia* fed on yeast showed that this phytoplankton group is not quite fit for *Artemia* feeding. This is in agreement with the records of Sick (1976) who found that *Artemia* fed *Chlorella condutrix* had lower rates of assimilation of organic matter in relation to those fed other species such as *Chlamidomonas spahagnicolo, Dunaliella viridis, Plytimonas elliptica* and *Nitzschia closterium.* The same author, in addition, found positive correlation between the growth rate of *Artemia* larvae and the protein content of the algal food. Claus et al., (1979) explained significantly faster growth of the larvae fed on *Spirulina* diet than on *Scendesmus* by the higher protein content of *Spirulina.* In agreement with this, faster growth of *Artemia* fed yeast in our experiment in relation to *Artemia* fed phytoplankton monoculture *Chlorella* sp. may be explained in the same way.

The percentage of protein in the larvae increased for the first 48 h, both in the fed and starved nauplii. This is attributed to the rapid protein synthesis in growing organisms (Claus et al., 1979). After 48 h the percentage of protein is gradually reduced in the fed as well as in starved animals what lowers their food value to early life stages of fish and crustaceans. Somewhat less reduced percentage of protein in individuals fed yeast in relation to the group fed phytoplankton monoculture *Chlorella* sp. points to the necessity to utilise this food source to improve the food value of *Artemia.*

It was found that the percentage of carbohydrate is decreased with time. However, the differences in carbohydrate levels are significant only 12 hours

after eclosion when the carbohydrate level was highest in nauplii fed phytoplankton and lowest in the starved nauplii.

Lipids also show a general trend of decrease with aging under all treatments. Generally, the lipid content was lowest in the starved *Artemia*. This is in agreement with Claus et al. 1979, who found that during the 48-h starvation the percentage of lipid and carbohydrate decreased.

Ash content increase in all the treatments may be most probably consequent to the loss of organic matter. Significantly higher ash content was found in the starved *Artemia* 24 h after eclosion. Similar increase of ash content in the starved *Artemia* was also recorded by Claus et al. 1979.

Water also icreases with time under all the treatments. After 96 h, starved nauplii show significantly higher water level than other groups in addition to the retardation of growth. This is in agreement with the generally well known fact that a relative decrease of an organic component, like rapid initial decrease of carbohydrate and lipid percentages in the starved animals in our experiment, is always accompanied by a relative increase of ash and water.

Results of biochemical analyses of early larval stages of starved *Artemia salina* are in agreement with those obtained by Benijits et al. (1975). They calculated that *Artemia* nauplii lost 27% of their food value during 24-h starvation with a contemporaneous decrease of lipid content for 26% and accompanying ash content increase. After 48-h starvation food value is still more reduced with the further decrease of the percentage protein and ash content increase from 40% to 100%. This decrease of energetic value of *Artemia* (Paffenhöfer, 1967). Thus it was found that individuals of Dover sole (*Solea solea,* L.) fed 24-h old *Artemia* grew significantly better than those fed 48-h old *Artemia* (Ablett and Richards, 1980).

During starvation *Artemia* significantly alters lipid composition (Benijitis et al., 1975). Relative decrease of some fatty acids (e. g. palmitic and linoleic acid) and increase of some others (stearic and oleic acids) Claus et al. (1979) explained by the fact that some acids are preferentially used in metabolism while the others may be synthesized. The increased mortality of red sea bream *Chrysophrys major,* after long-term *Artemia* feeding may be due to this. Fujita et al. (1980) brought this in connexion with the defficiency in some fatty acids proposing that the food value of *Artemia* is assessed on the basis of the contents of essential fatty acids. Similarly, sea bass *(Dicentrarchus labrax)* larvae were liable to increased mortality after beeing fed exclusively *Artemia* for a rather long time (Barnabe, 1973). Katavić (1984), however, established the connexion between *Artemia* food and hypertrophy of swimbladder of the late postlarval sea bass stage.

CONCLUSION

Relative protein content increases during the first 48 h after eclosion in *Artemia* under all feeding treatments and is gradually reduced thereupon to be statistically significantly higher in nauplii fed yeast after 96 h. Lipid decrease was recorded under all feeding treatments. Lipid content was statisti-

cally significantly higher in nauplii fed phytoplankton. Apart from the small differences in growth rate for the first 48 h after eclosion not a single feeding treatment may be distinguished as affecting more the food value of nauplii judging from the relative content of biochemical macroconstituents. Statistically significant differences in growth in length and protein content of nauplii after 96-h treatment point to the possibility to apply successfully the yeast as a food source for improvement of *Artemia* food value.

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PROMJENE U BIOKEMIJSKOM SASTAVU *ARTEMIA SALINA* (L.) S OBZIROM NA RAZLIČIT HRANIDBENI TRETMAN

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KRATKI SADRŽAJ

Analiziran je biokemijski sastav ranih stadija *Artemia salina* u starvaciji, u odnosu na one koje su hranjene fitoplanktonskom monokulturom *Chlorella sp.*, odnosno pekarskim kvascem. Promjene u sadržaju proteina, ugljikohidrata, lipida, pepela i vode praćene su u okviru 96 satnog perioda.

Proteini pokazuju prividni trend porasta u prvih 48 sati u sva tri tretmana, a nakon toga je uočljiv njihov umjereni pad. *Artemia* hranjena pekarskim kvascem nakon 96 sati pokazuje signifikantno veći sadržaj proteina u odnosu na one koje su hranjene fitoplanktonom. Ovo je najvjerojatnije rezultat većeg sadržaja proteina u kvascu. Činjenica da se postotak proteina u početku povećava, kako kod hranjenih nauplija tako i onih u starvaciji vjerojatno je posljedica njihove sinteze u organizmu koji raste.

Relativni sadržaj totalnih lipida i ugljikohidrata opada tokom 96 satnog perioda razvitka naplija *Artemia* u svim tretmanima, dok postotak vode pokazuje porast. Pepeo uglavnom prati trend promjena koje karakteriziraju proteine.