Acta Biol. Univ. Daugavp. 17 (2) 2017
ISSN 1407 - 8953

AN EFFECTIVENESS OF *ARTEMIA NAUPLII* ENRICHMENT WITH POLYUNSATURATED FATTY ACIDS USING A SUPPLEMENT EASY DHA SELCO

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Khudyi O., Khuda L., Kushniryk O., Prusinska M., Kolman R., Marchenko M. 2017. An effectiveness of *Artemia nauplii* enrichment with polyunsaturated fatty acids using a supplement easy DHA selco. *Acta Biol. Univ. Daugavp., 17 (2): 169 – 183.*

The technologies of targeted products bioencapsulation into live feeds have been widely used in modern larviculture. However, the question of assessing the side effects from the use of such technologies remains opened. This study is devoted to complex evaluation of the effectiveness of *Artemia* saturation with PUFA using a supplement Easy DHA Selco, and includes not only an investigation of fatty acids profile in enriched nauplii, but also the assessing of their nutritional value and the level of hydrolytic activity during saturation procedure. It was shown that the procedure of nauplii bioencapsulation with a supplement for 24 h provided the increase in the share of docosahexaenoic acid in fatty acids profile of *Artemia* several dozen times. Nevertheless, the content of total proteins and lipids had been changing, and the carotenoids content was significantly reduced in the fodder organisms during bioencapsulation. An introduction of large amounts of polyunsaturated fatty acids to the incubation medium under conditions of enhanced oxygenation provokes the development of free-radical oxidation and the accumulation of lipid peroxidation products. Additional maintenance of nauplii for bioencapsulation within 24 hours after hatching leads to 10-25% of their loss. The procedure of saturation ambiguously effects on the level of hydrolytic activity in the nauplius body that should be considered when feeding of fish larvae with live feeds. According to the obtained results, an improved scheme of the procedure of brine shrimp saturation using a supplement Easy DHA Selco has been suggested.

Key words: bioencapsulation, PUFA, *Artemia*, nutritional value, Easy DHA Selco, hydrolytic activity, TBARS.

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INTRODUCTION

The use of live feeds is a required element in the technology for successful cultivation of fish juveniles due to their high nutritional value and easy digestibility. The widespread application of live feeds in aquaculture is caused not only by advantages of their nutrient composition (Abowei & Ekubo 2011), but also by a complex of hydrolytic enzymes contained inside (Das et al. 2012). In the initial stages of fish larvae development their digestive system is characterized by low enzymatic activity. In this regard, the digestion in fish organism during transition to exogenous feeding is largely ensured by hydrolytic enzymes of the consumed live feed, that provide autolysis (Ostroumova 2012). The consumption of zooplankton has a positive impact on the formation of general enzymatic activity in the intestine of fish juveniles (Meyers 1994).

However, the live feed is not only the source of nutrients and hydrolytic enzymes, but also can serve as a means for delivering into the body of fish larvae of different targeted products, namely therapeutic agents, probiotics, essential compounds, providing the increase in their survival rate and acceleration of growth (Hafezieh et al. 2009, Akbary et al. 2011). The advantage of such technology like supplement introduction (bioencapsulation) is that feed organisms simultaneously possess both nutritional value and provide «delivery» of the targeted substances.

Used in larviculture *Artemia* sp. nauplii are the traditional live starter feed with high nutritional value (Aragao et al. 2004). Their use can significantly reduce the mortality of fish larvae and accelerate their growth rates and development (Leger et al. 1986, Kadhar et al. 2014). An important advantage of *Artemia* using is the ability to synchronized obtaining of nauplii in the desired quantity. Despite numerous technological advantages of their application, the problem is a small amount of polyunsaturated fatty acids (PUFAs) in their composition (Navarro & Sargent 1992).

Fish organism can not synthesize key PUFA's – linoleic and linolenic fatty acids, they are essential and must be received with feeds. Insufficient intake of PUFA with feeds causes a variety of physiological disorders in young fish. In particular, lower levels of linoleic, linolenic acid, docosahexaenoic (DHA) and eicosapentaenoic acid (EPA) in the diet of fish larvae causes the metabolic disorders, slowing their growth and skeletal abnormalities (Lall & Lewis-McCrea 2007). One of the possible solutions to this problem is enriching of *Artemia* nauplii with PUFA-containing supplement.

However, the question whether the procedure of live feeds saturation with PUFA causes the reduction in certain groups of nutrients, including protein, as well as other undesirable metabolic changes, still remains opened. This study is devoted to complex evaluation of the effectiveness of *Artemia* saturation with PUFA using a supplement Easy DHA Selco, and includes not only an investigation of fatty acids profile in enriched nauplii, but also the assessing of their nutritional value and the level of hydrolytic activity during saturation procedure.

MATERIAL AND METHODS

Obtaining of *Artemia* nauplii and their enrichment

Studies of *Artemia* nauplii incubation and enrichment procedure were performed in Inland Fisheries Institute (Olsztyn, Poland). Incubation of brine shrimp cysts (Sep-Art *Artemia* Cysts from «Ocean Nutrition», Belgium) was carried out in the Weiss's flasks with a volume of 8 liters within 24 hours under the constant illumination, aeration and water temperature of 28°C. The bioencapsulation of nauplii with polyunsaturated fatty acids (PUFA) was conducted using Easy DHA Selco supplement («INVE Aquaculture», Belgium). The percentage of PUFA in the supplement is about 34% of total fatty acids, and the ratio of docosahexaenoic/eicosapentaenoic acid (DHA/EPA) is approximately 2.5. Before the enrichment procedure, a stable emulsion of

Easy DHA Selco was preliminarily prepared with a small amount of water, followed by inputting of obtained emulsion into the Weiss's flasks with *Artemia*.

Four schemes of bioencapsulation procedure have been tested: Easy DHA Selco was applied once at the beginning of enrichment at doses of 0.6 g / l (scheme 1, according to manufacturer's recommendation), 0.9 g/l (scheme 2) and 1.2 g/l (scheme 3); in scheme 4, an emulsion at a dose of 0.6 g/l was added twice in equal parts at the beginning and after 12 hours of cultivation. The control group (without enrichment) was consisted of nauplii, that did not receive Easy DHA Selco, but were cultured under the same conditions as in the experimental groups.

The mortality rate of *Artemia* nauplii was determined at 6, 12, 18 and 24 hours of the experiment. For this purpose, the number of dead and living individuals in 1 ml of the incubation medium was calculated using a binocular microscope. Mortality was expressed in % as a ratio of dead individuals to the total number of all selected nauplii.

Biochemical analysis

Biochemical analyses were performed in Yuriy Fedkovych Chernivtsi National University (Chernivtsi, Ukraine). The samples selected for the study were preliminary dried on filter paper, weighed on a Precisa 120A analytical balance (PAG Oerlikon, Zurich, Switzerland) to the nearest 0.1 mg, and frozen in liquid nitrogen. Analyses of all biochemical parameters were conducted in six replicates. The biochemical parameters were determined at 6, 12, 18 and 24 hours of the experiment.

All calculations were performed on dry weights. The samples were dried at 60 °C for 24 h until they reached a constant weight (Postel et al. 2000). Samples weighing 1-1.5 g were homogenized at + 4 °C in a Potter-Elvehjem homogenizer using a phosphate buffer with a pH of 7.4. The homogenate was centrifuged at 1500 g for 15 min. Lipid extraction was performed with the Folch method (Folch et al. 1957). Total lipids were determined with acid hydrolysis followed by the reaction between the decomposition products and phospho-vanillin reagent (Knight et al. 1972). The total carotenoid content of the samples was determined by spectrophotometry at λ 450 nm after deposition with Carrez solution I and Carrez solution II, acetone extraction, followed by purification with petroleum ether (GOST R 54058-2010 2011).

Lipase activity was determined with the unified method (Sklyarov et al. 2008). Proteolytic activity was investigated with the modified Anson's method (GOST 20264.2-88 1988). The activity of α-amylase was determined with the Caraway amylolytic method (Caraway 1959). The total protein content was determined with Lowry's method (Lowry et al. 1951).

Fatty acids were determined with gas chromatography on an HRGC 5300 chromatograph (Italy) in a 3.5 m glass column filled with Chromosorb W / HP with the application of a 10% liquid phase of Silar 5CP at a programmed temperature of 140-250°C (Kates 1973, Baidalinova et al. 1977). The identification of individual fatty acids was conducted using the respective standards by Sigma; their contents were expressed as the percentage of the total amount of fatty acids.

Determination of the content of thiobarbituric acid reactive substances (TBARS) was carried out spectrophotometrically with an absorption maximum at λ = 532 nm by the content of trimethyl colored complex of lipid peroxidation products with thiobarbituric acid (Rakhmanova et al. 2009).

All the data are presented as mean ± SEM. Effects of water temperature and dietary treatment were analyzed by a one-way analysis of variance (ANOVA), followed by Tukey's or Student's post hoc test in order to determine significant differences. Previous to statistical analysis, data were transformed with natural logarithm if identified as non-homogenous (Levene's test) to meet the assumptions for statistical methods.

Mean values were considered significantly different at $P \leq 0.05$. Statistical analysis was computed using MS Excel software and STATISTICA 6.0 application package.

RESULTS

Due to research results, availability of 32 fatty acids (FA) in the freshly hatched brine shrimps has been established. Saturated, monounsaturated and polyunsaturated fatty acids were in approximately equal shares. In the fraction of saturated fatty acids (SFA) of all experimental groups of *Artemia* the palmitic (C16:0), stearic (C18:0) and arachidic (C20:0) fatty acids were the dominating ones. Among the monounsaturated (MUFA) the largest share belonged to oleic (C18:1) and palmitoleic (C16:1) fatty acids, and of polyunsaturated (PUFA) - linolenic (C18:3 ω-3) and linoleic (C18:2 ω-6) fatty acids. The share of docosahexaenoic acid was insignificant (Table 1).

Table 1. Fatty acid profile of *Artemia* nauplii after 24 h bioencapsulation with a supplement Easy DHA Selco

Fatty acids		C, %						
		Easy DHA Selco	after hatching	without enrich-ment	Experimental groups			
					1	2	3	4
Enanthic	C7:0			0.022± 0.0014		0.026± 0.0011	0.004± 0.0002[2]	0.014± 0.0010[2]
Caprylic	C8:0	0.007± 0.0003	0.046± 0.0018	0.046± 0.0029	0.052± 0.0020[1,2]	0.066± 0.0041[1,2]	0.042± 0.0020	0.027± 0.0016[1,2]
Pelargonic	C9:0	0.031± 0.0014		0.005± 0.0003	0.004± 0.0002	0.005± 0.0002	0.011± 0.0007[2]	0.003± 0.0001[2]
Caprinic	C10:0	0.057± 0.0034	0.013± 0.0010	0.013± 0.0005	0.008± 0.0004[1,2]	0.008± 0.0002[1,2]	0.006± 0.0004[1,2]	0.009± 0.0004[1,2]
Isolauric	Ci12:0		0.043± 0.0025	0.026± 0.0013[1]	0.023± 0.0012[1,2]	0.011± 0.0007[1,2]	0.011± 0.0006[1,2]	0.019± 0.006[1,2]
Lauric	C12:0	0.049± 0.0019	0.074± 0.0024	0.057± 0.0018[1]	0.047± 0.0027[1,2]	0.045± 0.0027[1,2]	0.052± 0.0023[1,2]	0.050± 0.0031[1,2]
Tridecylic	C13:0	0.040± 0.0023	0.017± 0.0007	0.014± 0.0007[1]	0.015± 0.0005[1]	0.015± 0.0009[1]	0.023± 0.0015[1,2]	0.014± 0.0005[1]
Isomyristic	Ci14:0	0.019± 0.0006	0.143± 0.0070	0.109± 0.0055[1]	0.101± 0.0026[1]	0.080± 0.0033[1,2]	0.074± 0.0024[1,2]	0.101± 0.0060[1]
Myristic	C14:0	4.696± 0.1527	1.031± 0.0525	0.892± 0.0498[1]	1.072± 0.0252[2]	1.133± 0.0333[1,2]	2.816± 0.1996[1,2]	1.036± 0.0389[1]
Pentadecanoic	C15:0	0.250± 0.0100	1.324± 0.0677	1.032± 0.0555[1]	1.007± 0.0568[1]	0.686± 0.0528[1,2]	0.696± 0.0389[1,2]	0.967± 0.0598[1]
Isopalmitic	Ci16:0	0.110± 0.0071	0.838± 0.0292	0.705± 0.0265[1]	0.681± 0.0422[1]	0.512± 0.0287[1,2]	0.463± 0.0265[1,2]	0.648± 0.0259[1,2]
Palmitic	C16:0	16.635± 1.0833	13.797± 0.9251	14.013± 1.0574	14.457± 1.1424	11.860± 0.9448	14.791± 0.9154	13.902± 0.9451
Margaric	C17:0	1.530± 0.0580	1.016± 0.0584	0.886± 0.0287	0.921± 0.0534	0.819± 0.0170[1,2]	1.172± 0.0708[1,2]	0.875± 0.0410
Isostearic	Ci18:0		1.073± 0.0621	0.663± 0.0235[1]	0.654± 0.0523[1]	0.357± 0.0163[1,2]	0.290± 0.0182[1,2]	0.563± 0.0325[1,2]
Stearic	C18:0	4.052± 0.1673	5.820± 0.3201	5.521± 0.2492	5.348± 0.2054	4.330± 0.1240[1,2]	5.155± 0.2760	5.250± 0.2854

Fatty acids		C, %						
		Easy DHA Selco	after hatching	without enrich-ment	Experimental groups			
					1	2	3	4
Arachidic	C20:0	2.108± 0.0711	6.280± 0.2186	5.365± 0.1950[1]	5.195± 0.2920[1]	3.803± 0.1709[1,2]	3.478± 0.2442[1,2]	4.818± 0.2125[1,2]
Heneicosylic	C21:0	0.271± 0.0129	0.256± 0.0106	0.250± 0.0122	0.222± 0.0125	0.169± 0.0103[1,2]	0.248± 0.0139	0.229± 0.0112
Behenic	C22:0	0.412± 0.0133	0.864± 0.0556	0.880± 0.0380	0.863± 0.0606	0.777± 0.0459[2]	0.678± 0.0397[1,2]	0.731± 0.0273[1,2]
∑ SFA		30.266	30.266	32.635	30.500	30.670	24.701	30.011
Lauricoleic	C12:1		0.034± 0.0016	0.024± 0.0015[1]	0.022± 0.0009[1]	0.015± 0.0010[1,2]	0.014± 0.0007[1,2]	0.024± 0.0014[1]
Myristoleic	C14:1		0.364± 0.0244	0.272± 0.0147[1]	0.285± 0.0180[1]	0.216± 0.0101[1,2]		0.269± 0.0151[1]
Pentadecenoic	C15:1	0.662± 0.0328	0.210± 0.0116	0.135± 0.0066[1]	0.190± 0.0109[2]	0.170± 0.0109[1,2]	0.424± 0.0128[1,2]	0.194± 0.0088[2]
Palmitoleic	C16:1	6.682± 0.3293	5.836± 0.3955	4.829± 0.2545[1]	4.931± 0.1631[1]	4.497± 0.1938[1]	5.488± 0.2507[2]	4.626± 0.2513[1]
Heptadecenoic	C17:1	0.934± 0.0252	2.086± 0.0908	1.670± 0.0738[1]	1.606± 0.0986[1]	1.340± 0.0651[1,2]	1.234± 0.0604[1,2]	1.529± 0.0790[1]
Oleic	C18:1	17.493± 1.2034	20.758± 1.3550	23.481± 1.7478	22.738± 1.6865	26.739± 1.8066[1]	19.054± 1.1618[2]	23.969± 1.8163
Gadoleic	C20:1	4.393± 0.2379	0.770± 0.0482	0.768± 0.0517	0.932± 0.0530[1,2]	1.084± 0.0654[1,2]	2.514± 0.1411[1,2]	0.891± 0.0472[1,2]
Erucic	C 2 2 : 1 ω-9	5.160± 0.2975	3.237± 0.1847	3.055± 0.1025	3.745± 0.1964[1,2]	0.455± 0.0297[1,2]	2.281± 0.1455[1,2]	0.388± 0.0178[1,2]
∑ MUFA		35.323	35.323	33.294	34.233	34.449	34.517	31.009
Hexadecanoic	C 1 6 : 2 ω-6	0.255± 0.0107	1.762± 0.0853	1.523± 0.0923[1]	1.370± 0.0990[1,2]	1.106± 0.0700[1,2]	0.998± 0.0670[1,2]	1.321± 0.0470[1,2]
Linoleic	C 1 8 : 2 ω-6	4.829± 0.2881	9.790± 0.4107	8.396± 0.5450[1]	8.195± 0.5855[1]	6.783± 0.3977[1,2]	7.570± 0.5341[1]	7.901± 0.3238[1]
Linolenic	C 1 8 : 3 ω-3	1.381± 0.0497	18.978± 1.0894	21.801± 1.5058	20.622± 1.4192	23.749± 1.5173[1]	14.940± 1.2893[1,2]	21.659± 1.6736
Eicosatrienoic	C 2 0 : 3 ω-6	0.188± 0.0109			0.015± 0.0010		0.041± 0.0012	0.028± 0.0014
Arachidonic	C 2 0 : 4 ω-6	1.445± 0.0664	1.763± 0.1125	1.928± 0.1187	1.663± 0.1170[2]	1.802± 0.1440	1.769± 0.1160	1.758± 0.0787
Eicosapentaenoic	C 2 0 : 5 ω-3	7.293± 0.5779	3.237± 0.1681	3.055± 0.1661	3.745± 0.2766[1,2]	3.929± 0.2017[1,2]	5.188± 0.2158[1,2]	3.407± 0.1375[2]
Docosadienoic	C 2 2 : 2 ω-6	0.018± 0.0009	0.091± 0.0056	0.129± 0.0066[1]	0.100± 0.0022[2]	0.121± 0.0039[1]	0.054± 0.0030[1,2]	0.071± 0.0044[1,2]
Docosatrienoic	C 2 2 : 3 ω-3	0.462± 0.0211					0.140± 0.0093	0.014± 0.0005
Docosatetraenoic	C 2 2 : 4 ω-6	0.074± 0.0030	0.298± 0.0143	0.273± 0.0174	0.276± 0.0165	0.128± 0.0063[1,2]	0.178± 0.0082[1,2]	0.210± 0.0124[1,2]
Docosapentaenoic	C 2 2 : 5 ω-3					0.044± 0.0026		
Docosahexaenoic	C 2 2 : 6 ω-3	17.643± 1.0852	0.059± 0.0033	0.210± 0.0104[1]	1.280± 0.0579[1,2]	2.083± 0.1200[1,2]	6.465± 0.2921[1,2]	1.402± 0.0820[1,2]

Fatty acids	C, %						
	Easy DHA Selco	after hatching	without enrich-ment	Experimental groups			
				1	2	3	4
∑ PUFA	33.588	35.979	37.316	37.266	39.744	37.343	37.772
∑ ω-3	26.780	22.274	25.066	25.647	29.805	26.732	26.483
∑ ω-6	6.808	13.705	12.250	11.619	9.939	10.611	11.289
ω-3 / ω-6	3.93	1.63	2.05	2.21	3.00	2.52	2.35
DHA / EPA	2.42	0.02	0.07	0.34	0.53	1.25	0.41

Notes:
[1] – difference in comparison with group of *Artemia* nauplii after hatching is statistically significant at P≤0.05;
[2] – difference in comparison with appropriate group of *Artemia* nauplii without enrichment is statistically significant at P≤0.05.

Table 2. Mortality rate (%) of *Artemia* nauplii during bioencapsulation with Easy DHA Selco

Experimental groups	Dose of a supplement	after hatching	Enrichment procedure, h			
			6	12	18	24
1	0.6 g/l	5.8±0.3	10.3±0.9[1,2]	10.3±0.3[1,2]	11.1±1.1[1,2]	9.3±1.1[1,2]
2	0.9 g/l		13.3±0.6[1,2]	14.3±1.1[1,2]	17.1±0.5[1]	26.8±2.2[1,2]
3	1.2 g/l		12.9±0.1[1,2]	13.3±0.7[1,2]	13.3±3.6[1]	13.5±1.0[1,2]
4	0.3+0.3 g/l		10.7±0.1[1,2]	10.7±0.3[1,2]	16.5±0.9[1]	25.0±1.3[1]
Control	without enrich-ment		6.4±0.2	8.6±0.7[1]	15.9±0.5[1]	22.0±1.6[1]

Notes:
1 – difference in comparison with group of *Artemia* nauplii after hatching is statistically significant at P≤0.05;
2 – difference in comparison with appropriate group of *Artemia* nauplii without enrichment is statistically significant at P≤0.05.

An applied supplement Easy DHA Selco is characterized of the same ratio of the basic fatty acids groups as in *Artemia* nauplii. However, the docosahexaenoic acid prevailed in the PUFAs fraction in the supplement while the share of linolenic acid was dominated in nauplius organism. The share of EPA in the fatty acid profile of Easy DHA Selco was also significantly higher than in freshly hatched nauplii. The ratio DHA / EPA in the supplement was close to the ratio declared by the manufacturer. The conducted procedure of nauplii saturation using a supplement Easy DHA Selco within 24 h provided the increase in the share of DHA in the fatty acid profiles of a few dozen times. However, the share of EPA increased significantly only in the brine shrimps from the experimental group 3, where the highest dose was treated. The application of a supplement also contributed to the appearance of eicosatrienoic and docosatrienoic acids in fatty acid profiles of nauplii from some experimental groups, while in freshly hatched *Artemia* they were absent.

Keeping of intact *Artemia* nauplii from 12 to 24 h in terms of feed deprivation is accompanied by a gradual increase in the number of dead individuals. The procedure of brine shrimp saturation occurs with a significant increase in mortality at all experimantal groups compared to

Table 3. The content of total proteins, lipids and carotenoids in *Artemia* nauplii during bioencapsulation with Easy DHA Selco

Experimental groups		Total proteins, mg/g	Total lipids, mg/g	Total carotenoids, mg/g
After hatching		569.9±60.7	171.0±14.3	0.267±0.001
without enrichment	6 h	545.8±55.1	160.7±18.4	0.191±0.005[1]
	12 h	550.9±23.0	157.4±10.0	0.148±0.004[1]
	18 h	413.5±33.8[1]	140.7±12.4[1]	0.126±0.007[1]
	24 h	411.5±25.2[1]	132.0±9.6[1]	0.078±0.008[1]
group №1	6 h	522.6±28.6	169.0±17.1	0.195±0.007[1]
	12 h	445.7±41.4[1,2]	137.0±14.1[1]	0.069±0.002[1,2]
	18 h	388.2±33.6[1]	158.5±14.4	0.071±0.003[1,2]
	24 h	426.4±32.2[1]	163.3±31.2	0.046±0.004[1,2]
group №2	6 h	482.2±26.9	336.7±31.9[1,2]	0.070±0.003[1,2]
	12 h	464.8±39.7[1,2]	321.0±23.5[1,2]	0.056±0.014[1,2]
	18 h	420.7±36.3[1]	229.0±20.9[1,2]	0.016±0.001[1,2]
	24 h	423.6±27.5[1]	216.7±15.7[1,2]	0.006±0.001[1,2]
group №3	6 h	468.3±30.2[1]	158.0±14.3	0.080±0.002[1,2]
	12 h	370.5±32.2[1,2]	153.0±9.8	0.083±0.001[1,2]
	18 h	441.1±41.1[1]	297.9±26.4[1,2]	0.088±0.003[1,2]
	24 h	394.1±28.7[1]	170.7±17.2[2]	0.068±0.007[1]
group №4	6 h	590.4±51.6	221.6±18.2[1,2]	0.112±0.005[1,2]
	12 h	495.2±40.0	117.1±8.8[1,2]	0.129±0.010[1,2]
	18 h	553.2±50.3[2]	210.1±17.0[1,2]	0.116±0.007[1]
	24 h	572.0±48.2[2]	153.3±18.2	0.114±0.014[1,2]

Notes: [1] – difference in comparison with group of *Artemia* nauplii after hatching is statistically significant at P≤0.05;

[2] – difference in comparison with appropriate group of *Artemia* nauplii without enrichment is statistically significant at P≤0.05.

the control group after the first 6 h of incubation. However, as of 18 h the mortality rates of both intact and enriched nauplii were about the same level (Table 2). During the next 6 hours a share of dead brine shrimps extremely increased in 2 and 4 experimental groups instead of 1 and 3 groups where the mortality rate remained virtually unchanged throughout the whole saturation period, and its end result was lower than in intact *Artemia* as of 24 h.

Biochemical analysis of intact *Artemia* nauplii showed consistently high levels of total proteins (about 55% of dry weight) during the first 12 h after hatching. Further starvation was accompanied by gradually decreasing in protein content to 40%. One-staged introduction of all studied doses led eventually to decrease in total protein content to the same level as in unenriched *Artemia* on the day after hatching. Instead, the two-staged introduction helped to keep in live feeds the original protein level (Table 3).

In intact *Artemia* the total lipids content gradually decreased, while brine shrimps from the experimental groups were characterized

of significant fluctuations in lipids content during the period of saturation. The keeping of brine shrimps for 24 hours was accompanied by a decrease in carotenoids content 3.4 times in the control group and 2,3-44 times in the experimental groups.

The analysis of the TBARS content in Artemia nauplii showed a significant increase in level of lipids oxidation in all studied groups where PUFA supplement was introduced into cultivation medium. The greatest values of this parameter were reached in *Artemia*, treated with a maximum dose studied (Fig. 1).

During investigations of total proteolytic activity, the values of its level in the brine shrimp nauplii from all studying groups were found the highest at pH 9.0. Saturation of *Artemia* with a supplement Easy DHA Selco once at a dose of 0.6 g / l increases the proteolytic activity in the alkaline range almost 4 times in 24 h from the beginning of saturation. At this period, the rest of the applied schemes was characterized of common trend – a reduction in total proteolytic activity compared with a group of unenriched *Artemia* (Fig. 2).

Since 6 h of saturation the dynamics of lipolytic activity in enriched *Artemia* nauplii has been changing in accordance with specific digestion of the different amounts of PUFA-containing supplement (Fig. 3, A).

Regarding to amylolytic activity it should be noted that the use of all the schemes with one-step enrichment leads to the growth of this indicator, while the enrichment regimen with two-step supplement introduction follows the trends in the control group (Fig. 3, B).

DISCUSSION

The fact that freshly hatched *Artemia* nauplii are characterized by low content of DHA is well known. The share of EPA can vary widely (Chakraborty et al. 2007, Adloo et al. 2012), depending on both the species of brine shrimp and the place of cysts origin. Feeding of fish larvae on *Artemia* with low content of these PUFAs may have negative consequences. Polyunsaturated fatty acids, especially DHA, have an important role in the adaptation of aquatic animals to various environmental factors – temperature, pressure, salinity, oxygen regime; they are participated in increasing of stress resistance, ensuring of successful metamorphosis, reducing of pigmentation disorders, formation of the visual analyzer (Hafezieh et al. 2009, Adloo et al. 2012). In addition, DHA takes an important

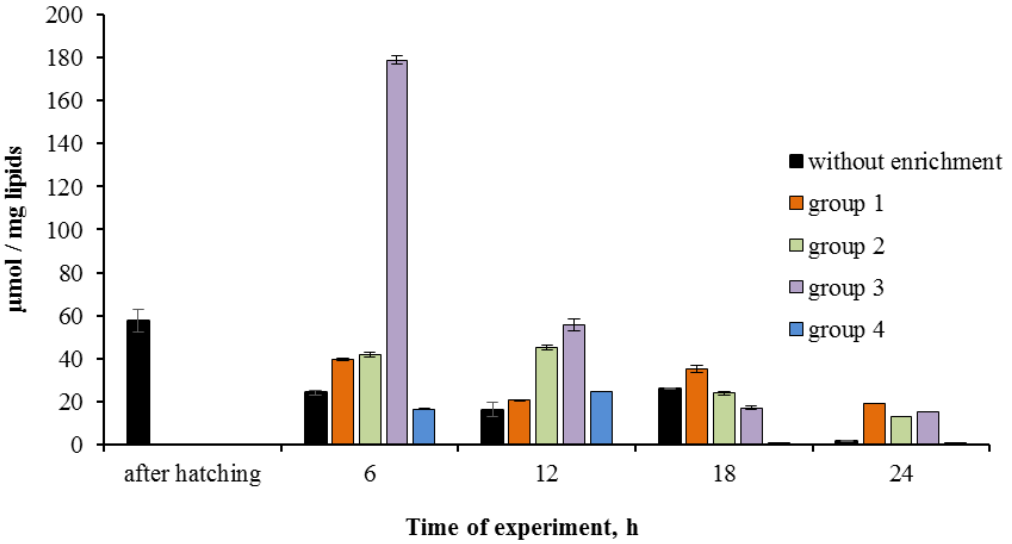

Fig. 1. The content of TBARS in *Artemia* nauplii during bioencapsulation with Easy DHA Selco.

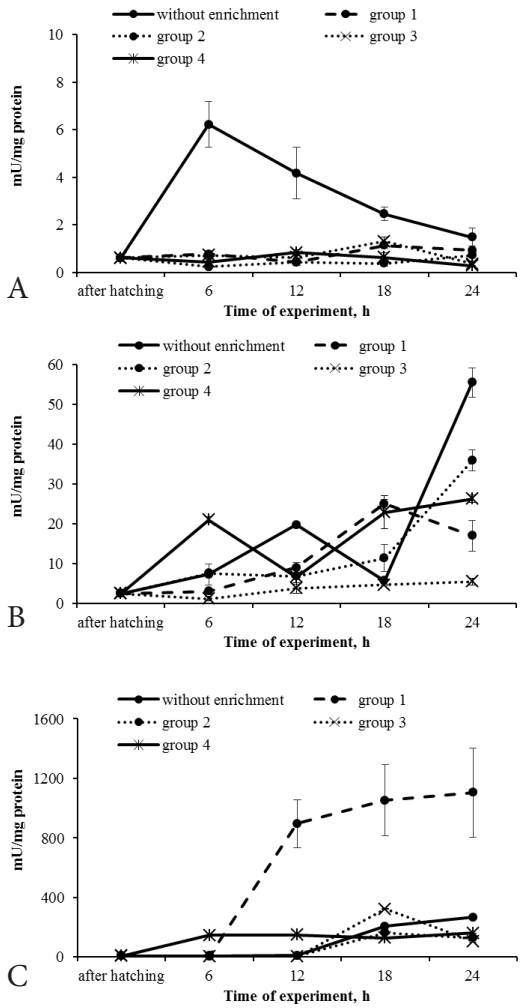

Fig. 2. Total proteolytic activity in *Artemia* nauplii at the different pH during bioencapsulation with Easy DHA Selco (A – pH 4.8; B – pH 7.4; C – pH 9.0).

role as a component of membrane phospholipids, providing membrane modulation and required level of viscosity, and participating both in the formation and functioning regulation of ion channels and in maintaining the proper operation of membrane receptors (Estevez & Kanazawa 1996). One of the largest depot of PUFAs, in particular DHA, in the fish body is a brain; the leading role of PUFA in the formation of the eye retina is also widely known (Estevez & Kanazawa 1996, Mourente 2003).

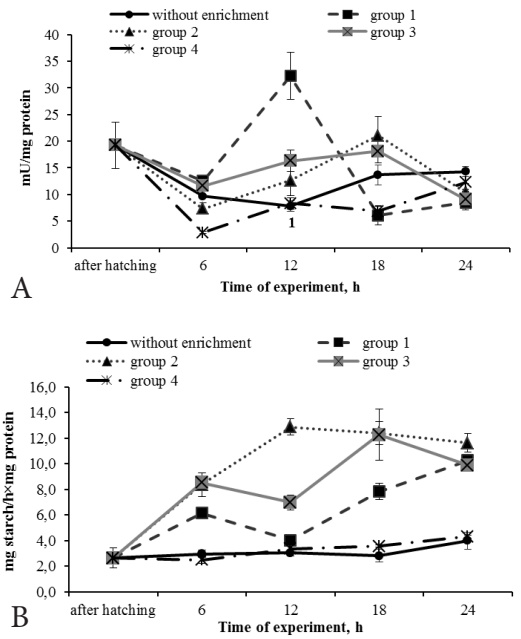

Fig. 3. Lipase (A) та α-amylase (B) activities in *Artemia* nauplii at the different schemes of bioencapsulation with Easy DHA Selco.

A supplement Easy DHA Selco used in our study does not lead to a significant redistribution in the total shares of saturated, monounsaturated and polyunsaturated FA. It could be obviously due to the fact that the share of respective groups of fatty acids in the supplement and freshly hatched nauplii are similar. Despite of the stable FA ratio the *Artemia* enrichment with Easy DHA Selco contributed to the increase in content of ω-3 PUFA - DHA and EPA, the ratio of which was correlated with the amount of introduced emulsion Easy DHA Selco.

Sufficient amount of PUFAs in a fodder diet is especially important in the early stages of the fish ontogeny. They provide a positive impact on the growth and development of fish larvae, reducing of their mortality (Akbary et al. 2011) and the number of skeletal abnormalities (Cahu et al. 2003, Villeneuve et al. 2005, Lall & Lewis-McCrea 2007), contributing to the development of the digestive tract (Kamaszewski et al. 2014a) and increased activity of digestive enzymes (Kamaszewski et al.

2014b). Therefore, a prerequisite of complete live feeds is their enrichment with PUFA-containing supplements (Cure et al. 1996, Sargent et al. 1999, Copeman et al. 2002). An effective synthesis of linolenic acid in the natural conditions occurs by microalgae, where during desaturation and elongation of linoleic and linolenic acids other unsaturated fatty acid of ω6 and ω3 families are synthesized (Sargent et al. 1995, Henderson 1996, Tocher 2003), and they are characterized by a wide range of biological action in the body (Hrytsyniak et al. 2009). They provide the distribution of ω3 acids in aquatic animals - zooplankton, benthos, from where PUFA through the food chains apear in the fish organism. The inability of the fish to the transformation of saturated fatty acids into the unsaturated linoleic and linolenic acids with subsequent transformation into other polyunsaturated ω6 and ω3 fatty acids (arachidonic, docosahexaenoic and eicosapentaenoic fatty acid) is due to lack of appropriate enzymes that catalyze reactions of elongation and desaturation, namely elongases and Δ12, Δ15 desaturases. Despite the fact that a number of fish species, mostly freshwater, is able to convert linolenic acid to EPA and through a series of intermediate stages into DHA the growth and survival rates of larvae increased when these acids already present in the feeds, and the energy loses for conversion of linolenic acid to a long-chain EPA and DHA are not implemented (Tocher 2003). It should be noted that in *Artemia* organism the processes of DHA retroconversion to EPA could be observed (Navarro et al. 1999).

Reducing of linoleic, linolenic acids, EPA and DHA in the diet of fish larvae causes the delay of the growth and metabolic disorders (Hafezieh et al. 2009, Ostroumova 2012, Adloo et al. 2012). Arachidonic acid (ARA), EPA and DHA are required as precursors of biologically active components – eicosanoids (Kim et al. 2002, Hrytsyniak et al. 2009), providing tightness of the skin barrier and are involved in the transport of cholesterol and its metabolism (Hrytsyniak et al. 2009). In particular, ARA and EPA are the precursors of prostaglandins, which are synthesized in many tissues in response to various intracellular signals and participate in the functioning of the liver, nervous tissue, blood clotting, immune and inflammatory reactions (Ricciotti & Fitzgerald 2011). Use of Easy DHA Selco justified the attempts to improve the content of ω3 FA in comparison with freshly hatched *Artemia* nauplii.

The mortality rates of freshly hatched nauplii and *Artemia* from the control group were about the same at 6 h after hatching. It could be due to the fact that during the first 6-8 hours after hatching nauplii are in the first stage of development and consume nothing yet (Sorgeloos et al. 2001). The gradual increase in the share of dead individuals in the group of intact nauplii is observed since 12 hours after hatching. During this period nauplii are transformed into metanauplii (instar II), they have completed the formation of the digestive system and their exogenous feeding is already observed. Increased mortality of the intact individuals is caused, apparently, by the lack of feed and the death from starvation.

Obviously, that saturation process itself is stressful for *Artemia* nauplii, which manifests in significant increase in mortality compared to controls in all experimental groups after the first 6 h of incubation. It should be noted that within the next 12 hours magnitude of the brine shrimp mortality at all studied schemes of saturation was not increased as in a group of intact individuals, and remained approximately at the same level. It could be caused by the fact that nauplii in the experimental group began to assimilate the supplement emulsion introduced in the cultivation medium. In general, as of 24 h after hatching the brine shrimp mortality in all groups were within 9-27%, which is consistent with other studies (Harel et al. 2002, Prusinska et al. 2015).

The nutritional value of live feeds for fish is primarily determined by the content of proteins and lipids. According to different authors, the content of main nutrients in the brine shrimp nauilii varies widely (Bengtson et al. 1991, Moraiti-Ioannidou et al. 2009). At the initial stage of development the nauplii have used primarily lipids from the yolk sac, not proteins, as an energy

substrate. Supposedly it causes a relatively stable level of proteins within 12 hours after hatching (Table 3). At the later stages the lack of income feed proteins against the background of reserve nutrients exhaustion in cysts causes a decrease in protein content in the starved *Artemia* organism. Similar trends were observed to those nauplii who received the supplement once. The two-staged introduction at the least test concentration of a supplement obvious provides better adaptation and reduction of protein energy needs.

Considering that the studied supplement has a lipid nature, an increase in the content of total lipids at the final stages of saturation procedure compared with unenriched *Artemia* is clear evidence of Easy DHA Selco assimilation by brine shrimp nauplii. The lack of the traditional feeds for nauplii, namely microalgae, has led to rapid loss of carotenoids. Besides the loss of carotenoids as one of the main antioxidants may be associated with the intensification of oxidative processes, for which polyunsaturated fatty acids serve as substrates.

The proof of this is the results obtained by the accumulation of TBARS in nauplii enriched with polyunsaturated fatty acids (Fig. 1). As is known, an intensive oxygenation of the medium may be one of the reasons for strengthening the process of free radical oxidation. A sufficient amount of the substrate for lipid peroxidation – polyunsaturated fatty acids, which are introduced with a supplement, and a constant aeration leads to increased of lipid peroxidation (LPO) and intensive formation of its products. The primary products of lipid peroxidation (lipid hydroperoxide) are unstable and decompose to form of the secondary products. Among all the TBARS the malonic dialdehyde is the most famous, which is the main component of TBARS that reacts with thiobarbituric acid. Under such circumstances, the introduction of PUFA-containing supplement in large doses causes maximizing of free radical oxidation of lipids (Fig. 1). It should be marked that the lowest level of TBARS accumulation was observed in *Artemia*, that received Easy DHA Selco in two stages.

It is known that the consumption of live feed positively effects on the general enzymatic acitivity in fuvenile fish intestines. Therefore it is important wherever using of bioencapsulation technology has not resulted in the inhibition of hydrolytic activity in feed organisms. Among proteolytic enzymes presented in *Artemia* organism trypsin, leucine, valine and cystine aminopeptidases and thiol protease, that in the active center contains SH-groups of cysteine residues, are the most common (Warner & Shridhar 1985, Moraiti-Ioannidou et al. 2009). The obtained results about formation of *Artemia* highest values of proteolytic activity at the alkaline pH are consistent with findings of other researchers (Garcia-Ortega et al. 1998). Inhibition of proteolytic activity as of 24 h of the study was observed in the the majority of experimental groups with *Artemia*. Using of live feed with reduced hydrolytic activity could adversely affect on the digestive tract of fish larvae during their transition to exogenous feeding. The solution of this problem is to reduce the saturation time up to 18 h as the lipolytic and proteolytic activities of *Artemia* nauplii are similar to that of intact brine shrimp or even exceeds it at this period.

It should be noted that after 24 h of enrichment period the brine shrimps were placed into the automatic feeder from where they were transfered to a basins with fish. As a rule, the maximum stay period in fish feeders is one day. Accordingly, the maximum age of *Artemia*, that fish larvae were fed with was 48 hours. Both during the period of enrichment procedures and while keeping in the feeders the nutritional value of *Artemia* nauplii can undergo changes that must be considered when using live feeds. To minimize the negative effects of the extended keeping of nauplii after hatching and loss of their nutritional value it is appropriate to shorten the bioencapsulation procedure.

Due to obtained results, the procedure *Artemia* nauplii saturation with a supplement Easy DHA Selco should be reduced to 18 hours, that will help to shorten the loss of basic nutrients and maintain a sufficient level of hydrolytic activity in feed organisms. On the other hand, the joint

use of the freshly hatched and enriched *Artemia* nauplii as live feed for fish larvae might have a positive result.

CONCLUSIONS

According to the research results, the use of PUFA-containing supplement Easy DHA Selco provides an increase in the share of ω3 polyunsaturated fatty acids, particularly DHA, in *Artemia* nauplii. However, apart of accumulation the desired product in the brine shrimp, changes in the nutritional composition, hydrolytic activity and mortality of live feed also occur during bioencapsulation. Given to this, the enrichment procedure should be performed by supplement introduction in a total dose of 0.6 g / l in 2 stages and must be shortened to 18 h. The use of such a scheme minimizes the side effects of bioencapsulation procedure.

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Received: 10.09.2017.
Accepted: 14.10.2017