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THE STABILITY OF n-3 HIGHLY UNSATURATED FATTY ACIDS IN VARIOUS *ARTEMIA* POPULATIONS FOLLOWING ENRICHMENT AND SUBSEQUENT STARVATION

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Introduction

Research on n-3 highly unsaturated fatty acids (n-3 HUFA), such as eicosapentaenoic acid (EPA) and especially docosahexaenoic acid (DHA), demonstrated that they play a key role for the normal growth, survival, pigmentation, stress and disease resistance of many species of marine finfish (Watanabe, 1993; Rainuzzo et al., 1994).

More than 95% of the marketed cysts belong to the species *Artemia franciscana*. Contrary to other prey organisms, like rotifers, *A. franciscana* cannot obtain high DHA/EPA ratio after enrichment and it degrades DHA during starvation following enrichment (Dhert et al., 1993; Danielsen et al., 1995). Recent studies (Evjemo et al., 1995) suggested that DHA metabolism may be species and/or strain dependent. The genus *Artemia* comprises many other bisexual species and parthenogenetic populations that are not used in aquaculture so far. In this paper we screen representatives of various bisexual and parthenogenetic *Artemia* populations, following the fate of DHA, EPA and n-3 highly unsaturated fatty acids (HUFA) after enrichment with an experimental emulsion containing high levels of DHA.

Materials and methods

The studied bisexual populations (in parenthesis the abbreviations used throughout the text and the *Artemia* Reference Center -ARC- cyst bank number) were from Great Salt Lake, Utah - USA (GSL, ARC No. 1311), Lake Urmia, Iran (URM, ARC No. 1292) and Inner Mongolia, Yimeng-Taigemiao, P.R. China (YIM, ARC No. 1298) and belong to the species *A. franciscana*, *A. urmiana* and *A. sinica* respectively. Also, several parthenogenetic populations with different ploidy levels were evaluated: Megalon Embolon, Greece (MEM, tetraploid, ARC No. 1279), Citros, Greece (CIT, tetraploid, ARC No. 1280), Daban Lake, Xinjiang province, P.R. China (DAB, diploid

and tetraploid, ARC No. 1197) and Aibi Lake, Xinjiang province, P.R. China (AIB, diploid, ARC No. 1198).

The experimental emulsion Em50D (kindly provided by INVE Aquaculture NV, Belgium) was based on ethyl esters (52% of WW) and contained 7% EPA, 45.8% DHA and 56.3% total n-3 HUFA (as % of total fatty acids).

The experiments were performed in glass tubes of 1 l capacity with cylindroconical shape. Continuous aeration from the bottom ensures good water circulation, while high oxygen levels are kept above 5-6 $mg.l^{-1}$ using an additional small airstone (Fig.1).

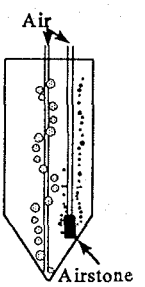

Fig.1. Schematic diagram of the experimental system.

The experimental procedure was as follows: Natural seawater was filtered through a 0.25µm cartridge filter and disinfected with 1ppm active chlorine which was removed by aeration overnight. The buffering capacity of the seawater was increased by adding $0.5g.l^{-1}$ of $NaHCO_3$, which kept pH levels between 8.3 to 8.5. The cysts ($4g.l^{-1}$ density) were disinfected for 20min in a solution of 200ppm active chlorine prior to hatching. After thorough washing with tap water to remove the NaOCl, cysts were incubated in filtered and disinfected seawater at 28°C, $2g.l^{-1}$ density and continuous light of at least 3000lux. For all strains nauplii at a developmental stage of late instar-I, beginning of instar-II were harvested and transferred to the tubes at 25°C and a density of $200ind.ml^{-1}$. The emulsion was added in two equal aliquots of $0.2g.l^{-1}$ at 0h and at 12h. After 24h, survival was determined, part of the nauplii sampled for HUFA analysis and the remaining were transferred after rinsing with seawater into new cylindroconical tubes at a density of $100ind.ml^{-1}$ and at 12°C. The starved animals were sampled after 12, 24, 48 and 72h. Fatty acid methyl esters (FAME) were prepared through direct transesterification following the method of Lepage and Roy (1984). FAME were separated on a Carlo Erba Mega Series 5160 HRGC gas chromatograph equipped with a 50m very polar capillary column BPX70, hydrogen as a carrier and the injection mode was on-column. The homogeneity of regression coefficients (slopes) was tested following the method described by Sokal and Rohlf (1981).

Results and discussion

Table I shows the increase of DHA, EPA and n-3 HUFA levels after enrichment and the alteration during the starvation period. The n-3 HUFA levels that were incorporated in the nauplii were different between the various strains. YIM, URM, GSL and CIT incorporated more fatty acids compared to MEM, DAB and AIB. For similar enrichment levels there were differences in the DHA/EPA ratio. The URM population had the highest DHA/EPA ratio (2.3) after enrichment that gradually decreased to 0.8 after 72h starvation. GSL had a DHA/EPA ratio of 1.4 after

enrichment and 0.4 after 72h starvation. The changes of DHA level as a function of time fitted for all strains to a linear model with bisexual populations showing r^2 values close to 1 and parthenogenetic populations CIT, MEM, DAB and AIB had r^2 values close to 0.9. There are no statistically significant differences between the slopes of the DHA regression lines for the various strains ($F_s=1.20<F_{.01[6,20]}3.87$).

Contrary to DHA levels that decreased linearly in function of time, EPA levels slightly increased even after the 24h enrichment period (Table I). Although a linear decrease of the total n-3 HUFA was observed (there are no statistically significant differences between the slopes of the various strains [$F_s=1.44<F_{.01[6,20]}3.87$]) EPA remained constant and relatively stable during the first 48h of starvation while it started decreasing when the DHA levels were very low.

Table I. DHA, EPA and total n-3 HUFA levels expressed as $mg.g^{-1}$ of dry weight. T0: nauplii before enrichment; T24: nauplii after 24h enrichment at 25°C; S12, S24, S48, S72: nauplii after 12, 24, 48 and 72h starvation at 12°C. s represents the slopes derived from the regression lines of DHA and n-3 HUFA as a function of the duration of starvation (r^2 is their determination coefficient)

	YIM			URM			GSL			CIT		
	DHA	EPA	Σw-3	DHA	EPA	Σw-3	DHA	EPA	Σw-3	DHA	EPA	Σw-3
T0	0	7.5	7.9	0.4	2.7	4.3	0.5	6.4	8.8	0	3.6	3.8
T24	24	14.1	40.8	24.2	10.5	38.1	25.1	17.7	44.1	23.4	12.8	38.2
S12	21.2	15.6	39.4	-	-	-	22.3	20.6	45.4	14.7	13.5	29.9
S24	19.1	14.3	36.1	20.3	14.4	38.7	17.5	20.2	39.9	14.4	16.3	32.4
S48	15	15.5	33	13.5	12.3	28.5	11.8	17.7	32.4	5.6	12.8	19.3
S72	9.7	11.8	23.7	10.6	12.6	26.3	6.6	15.2	23.8	3.2	11	14.9
s	-0.193		-0.230	-0.198		-0.190	-0.260		-0.305	-0.267		-0.319
r^2	0.997		0.954	0.978		0.841	0.991		0.958	0.911		0.924

Table I (continued)

	MEM			DAB			AIB		
	DHA	EPA	Σw-3	DHA	EPA	Σw-3	DHA	EPA	Σw-3
T0	0	12.3	12.6	0.4	3.5	4.4	0	14.7	15.5
T24	16.3	16.8	34.7	17.4	10.9	30.7	13.7	17.4	32.3
S12	10	16.9	28	12.8	11.8	26.4	9.6	16.7	27.8
S24	8.3	16.5	26.2	7.3	9.3	18.1	8.5	17.9	27.6
S48	3.6	15.3	20.3	5.2	11.2	17.7	5.2	11.2	17.7
S72	1.5	11.8	13.9	2.9	5.6	9.2	3.5	11.9	16
s	-0.191		-0.270	-0.190		-0.276	-0.132		-0.236
r^2	0.907		0.975	0.867		0.909	0.926		0.936

Since *de novo* synthesis of HUFA in *Artemia* is not known to occur, this could be an indication that the DHA, apart from being catabolized, is partially converted to EPA. Further research using radioactively labelled molecules of DHA is required to confirm this.

The differences between the various strains in the incorporation of the emulsion can be attributed to the fact that the different developmental stages (i.e. the duration of instar-I, II and III stages) last for a different period and thus the physiological rates of feeding are different within an enrichment period of 24h.

Evjemo et al. (1995) demonstrated differences in DHA stability during starvation following enrichment between a Chinese bisexual strain (Yimeng 1188) and an *A. franciscana* (GSL 1311) strain. Although the YIM strain that was used in this study and the Chinese strain used by Evjemo et al. (1995) are from the province of Inner Mongolia (but from different habitats) they do not show the same DHA stability. In this study, all the populations showed the same response as the GSL strain of the latter study. Since there is cross fertility and hence conspecificity, between the bisexual populations in the P.R. China (Triantaphyllidis et al., 1994), it might be possible that DHA catabolism is strain specific. Further study is needed to understand the factors and the mechanisms that influence DHA catabolism in *Artemia*.

Conclusions

There are no differences between the studied *Artemia* populations in terms of DHA stability during starvation following enrichment. DHA as well as (n-3) HUFA's are decreasing linearly in function of time. EPA is more stable than DHA, retaining high levels that are comparable to the values obtained after enrichment for a period up to 48h starvation.

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