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Nutritional Enhancement of Total Lipid, n-3 and n-6 Fatty Acids in *Artemia urmiana* Nauplii by Enriching with ICES/30/4

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Abstract: *Artemia urmiana* nauplii were enriched with three different concentrations (100, 200 and 300 ppm) of commercial emulsion, ICES/30/4 during two periods (12 and 24 h) to evaluate the enhancement of its Highly Unsaturated Fatty Acids (HUFAs). This source was selected because of its high concentration of the longest chain HUFA's in the n-3 and n-6 series. When 24-h-old *Artemia* nauplii were enriched with 100 ppm concentration of ICES30/4 during 12 h enriching period, the docosahexanoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) contents of the nauplii increased to 0.77, 1.22 and 0.34 and when enriched with 300 ppm during 24 h increased to 5.99, 4.97 and 0.73 mg g^{-1} dry weight, respectively. DHA, EPA and ARA in control nauplii were 0.00, 0.82 and 0.61 mg g^{-1} dryweight, respectively. Total lipid increased from 16.79% in control group to 20.87% in the treatment ICES30/4 24-300. The results suggest that high amount of emulsion and prolong the enriching period are effective in enriching *Artemia* nauplii in both DHA and EPA increasingly ($p<0.05$) but in other fatty acids, there are differences only among period treatments ($p<0.05$) and concentration are not any increasing effective. There are only differences among concentration treatments in total lipid $p<0.05$) and enriching period do not show any differences.

Key words: Enhancement HUFA, emulsion level, enrichment periods

INTRODUCTION

Enrichment of *Artemia* nauplii with n-3 HUFAs prior to feeding the nauplii to larval fish and shrimp is a common procedure in the aquaculture industry (Jones *et al*., 1993). These fatty acids are essential for the normal development of larval fish and shrimp, but most of the commonly available strains of *Artemia* used as food for these larvae have only a very small amount of these HUFAs (Watanabe *et al*., 1978).

Immanuel *et al*. (2007) enriched *A. franciscana* with marine trash fish liver oil for enhancement of HUFA.

Rekha *et al*. (2007) enriched *Artemia salina* nauplii with microalgae and baker's yeast for use in larviculture.

The importance of DHA, 22:6(n-3), facilitating the normal development of larval fish and oyster spat have been noted by many investigators including (Watanabe, 1993; Ozkizilcik and Chu, 1994). However, few of the existing enrichment methods produce significant increases in the DHA content of *Artemia* nauplii. Menhaden oil, the fish oil most commonly used in microencapsulated or emulsified oils, generally has a DHA content of less than 12% total fatty acids (Ozkizilcik and Chu 1994).

Much focus has been placed on the essential role of long-chain n-3 fatty acids especially in early nervous system development of fish and shrimp, but the n-6 HUFA, arachidonic acid is also important as the precursor of some prostaglandins and other biologically active compounds which regulate growth and reproductive functions (Stanley-Samuelson, 1987; De Petrocellis and Di Marzo, 1994).

The purpose of this study was to determine the effectiveness of enriching *Artemia* nauplii with long chain fatty acids by using different concentrations of ICES30/4 as a commercial emulsion, rich in both n-3 and n-6 long chain fatty acids.

MATERIALS AND METHODS

This study was conducted in 2007 in Artemia Research Center, Urmia University. *Artemia urmiana* cysts were hatched and divided into twenty one 25 L

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tanks filled with filtered 30 g L^{-1} salinity sea water at a density of approximately 300 individuals mL^{-1} and enriched by the direct method described by Watanabe *et al*. (1982). The culture was strongly aerated and the water temperature ranged between 27-29°C.

Lipid emulsions were prepared by mixing 100 mL of sea water, 1 g of raw egg yolk, 0.5 g of coconut lecithin and sufficient oil to obtain concentrations of 100, 200 and 300 ppm ICES30/4 in *Artemia* medium.

For each concentration of oil, samples of *Artemia* were taken after 12 and 24 h. A sample of *Artemia* exclusively fed on ICES/0/0 as control, was also taken. *Artemia* were collected with a 100 ppm scoop mesh, thoroughly washed with freshwater, carefully dried with filter paper and stored at-40°C until analysis.

Analytical procedures: Total lipid and fatty acid composition of each sample were determined. Total lipid was extracted after homogenization in chloroform-methanol as described by Folch *et al*. (1957) and determined according to standard AOAC method (AOAC, 1997). Fatty acid mixtures were prepared from the crude lipids by saponification with KOH. Unsaponifiable matter was determined gravimetrically. The fatty acid were analyzed in a Varian GC-14A gas chromatograph (Shimadzu) equipped with a flame ionization detector (250°C) and a Supelcowax-I0 fused-silica capillary column (30 m× 0.32 mm I.D., Supelco, Bellefonte, PA, USA) using helium as carrier gas. The initial temperature was 180°C for 10 min followed by a thermal gradient to 215°C at 2.5 C min and then maintained for 12 min. Individual fatty acid was identified by reference to authentic standards and to well characterize emulsion oil.

Statistical analysis: All biochemical results are given as means and were subjected to a two factor factorial analysis of univariate, SPSS Ver. 14. Differences between means were studied using Tukey's multiple range test ($p<0.05$).

RESULTS

ICES0/0 and ICES30/0 were analyzed with GC and showed in Table 1. Although the total lipid content of *Artemia* increased, in general terms, over time and with the elevation of the amount of oil present in the culture medium, but statistical analysis showed there are no any differences among enrichment periods and also among combination between concentrations and periods ($p>0.05$), Only there are significant differences among concentrations in lipid percentage of *Artemia* nauplii ($p<0.05$).

Comparing the fatty acid composition of the treatments and control (Table 2), the content of C18:0 increased to high level in treatment ICES30/4 with 200 ppm concentration and 24 h enriching period. According to statistical analysis, there are no any differences among concentrations ($p>0.05$) but there are significant differences among enrichment period in C18:0 of *Artemia* nauplii ($p<0.05$).

The content of C18:1n7 increased to high level in treatment ICES30/4 with 100 ppm concentration and 24 h enriching period. Although, there are significant differences between 12 and 24 h treatments ($p<0.05$), but there are no any differences between treatments of 100, 200 and 300 ppm in 24 h enriching periods ($p>0.05$) . The content of C18:2n6-cis decreased after all enrichment. It seems ICES30/4 could not improved this fatty acid in the nauplii compare to control. Regarding to the content of C20:4n6 (ARA), there were an increasing trend in 100 ppm to 300 ppm concentration treatments and analysis of variance shows, there are differences between concentration and period treatments ($p<0.05$) but their combinations have not any differences ($p>0.05$).

Table 1: Ingredients of the ICES0/0 and ICES30/4 emulsion

Sample	ICES 30/4	ICES 0/0
C18:0	6.26	1.99
C18:1n7	2.70	0.00
C18:2n6	4.03	2.75
C20:4n6	0.78	0.00
C20:5n3 (EPA)	6.29	0.00
C22:6n3 (DHA)	20.90	0.00

Table 2: Total lipid (DW %) and fatty acid composition (mg g^{-1} DW) of *Artemia* nauplii enriched with ICES30/4 in different concentrations and periods Average±SD

Sample (Hour/Level)	Naplius	ICES (12-100)	ICES (12-200)	ICES (12-300)	ICES (24-100)	ICES (24-200)	ICES (24-300)
Total lipid	16.79%	18.13%	19.67%	19.89%	18.64%	19.83%	20.87%
Fatty acids mg g^{-1} DW							
C18:0	4.90 (0.19)	5.30 (0.10)	5.43 (0.06)	5.41 (0.11)	6.09 (0.22)	6.51 (0.23)	5.95(0.16)
C18:1n7	2.86 (0.13)	3.30 (0.38)	3.45 (0.04)	3.52 (0.18)	4.63 (0.19)	4.50 (0.17)	3.79(0.12)
C18:2n6-ci	8.25 (0.15)	7.63 (0.09)	7.36 (0.08)	7.41 (0.43)	7.04 (0.27)	7.08 (0.27)	6.97(0.11)
C20:4n6	0.61 (0.07)	0.34 (0.09)	0.51 (0.08)	0.55 (0.06)	0.66 (0.05)	0.69 (0.06)	0.73(0.05)
C20:5n3 (EPA)	0.82 (0.11)	1.22 (0.04)	2.36 (0.09)	3.41 (0.19)	2.19 (0.16)	3.93 (0.11)	4.97(0.26)
C22:6n3 (DHA)	0.00	0.77 (0.21)	1.97 (0.27)	2.88 (0.13)	1.74 (0.13)	4.23 (0.07)	5.99(0.06)
DHA/EPA	0.00	0.64	0.84	0.82	0.79	1.09	1.22

The content of C20:5n3 (EPA) and C22:6n3 (DHA) increased to high level in treatment ICES30/4 with 300 ppm concentration and 24 h enriching period. The results of statistical analysis showed that there are significant differences among concentration ($p<0.05$), period ($p<0.05$) and their combinations ($p<0.05$) treatments in both EPA and DHA contents. Table 2 showed the highest ration of DHA/EPA obtain when *Artemia* nauplii enrich with 300 ppm concentration of ICES30/4 during 24 h period (1.22) while this ratio in control which is 0.00.

DISCUSSION

The rapid increase in total lipid content of *Artemia* observed during the enrichment process was similar to that reported by other authors in experiments carried out with lipid emulsions (Rainuzzo *et al*., 1994).

This study is in agreement with Rodriguez *et al*. (1996), which studied the improvement of rotifer nutritional value with varying the type and concentration of oil and the enrichment period, suggested it is more effective to increase the enrichment time than the amount of oil present in the culture medium.

Certain patterns can be observed in the incorporation of the different groups of fatty acids, probably due to their metabolic activities or enzymatic affinities. The behavior of *Artemia* with respect to n-6 and n-3 fatty acids seemed to differ from the saturated and monoenes. In this case, after 24 h of enrichment, DHA attained values more than 5 times and EPA less than 2 times greater than that of the initial *Artemia* and, as other authors have pointed out, n-6 and n-3 HUFA fatty acids were also assimilated to a great extent in *Artemia* after 24 h of enrichment (Rainuzzo *et al*., 1994).

To achieve increments of n-6 and n-3 HUFA in *Artemia*, not only it is more effective to increase the enrichment time but also the amount of oil present in the culture medium.

Walford and Lam (1987) used microcapsules as enrichment materials, recommended enrichment times over 5 h and observed maximum assimilation of n-3 HUFA after 12 h. Watanabe (1993) considered the optimum enrichment period to be 12 h for rotifers and nauplii of *Artemia* fed on lipid emulsions. Mcevoy *et al*. (1995) showed that enrichment periods of 24 h increased the risk of perioxidation of polyunsaturated fatty acids in *Artemia* enrichment medium. It seems that the difference between the present works with the results of above authors is due to differences in species of *Artemia*.

This study in agreement with other researchers, Leger *et al*. (1987), Rodríguez *et al*. (1996) and Watanabe (1993) suggested 300 ppm concentration of the enrichment oil for *Artemia* nauplii. Oil concentrations above 300 ppm are not recommended for the well being of the *Artemia* from an economic standpoint. This could be due to the fact that an excess of oil in the medium could lead to water quality degradation and to enhanced bacterial growth (Dabrowski and Poczycznski, 1988).

In the present study, ICES containing 300 ppm oil generated increased DHA/EPA ratio after 24 h of enrichment (DHA/EPA =1.2). The literature indicates that this amount should be sufficient to meet the requirements of marine fish larvae (Snther and Jobling, 2001). Previous studies have shown that the EPA/DHA ratio in the diet is important for larval development (Mourente *et al*., 1993; Reitan *et al*., 1994).

In summary, the results of the present study indicate that ICES30/4 can improve the nutritional quality of lipid and some fatty acid in *Artemia urmiana* nauplii and in order to obtain increased n-3 and n-6 HUFA content in *Artemia*, prolong the enrichment period and increase the amount of oil present in the culture medium have the same effective value but for saturated and monoen fatty acid, prolong the time is more effectiveness than high concentration. The best results in terms of nutritive value were obtained with ICES 30/4, with concentration 300 ppm and an enrichment period of no longer than 24 h.

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