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EFFECT OF INCUBATION TIME WITH DOCOSAHEXAENOIC ACID (DHA, 22:6N-3) ENRICHMENT ON FATTY ACID(S) PROFILE OF THE EGYPTIAN *ARTEMIA* NAUPLII.

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SUMMERY

Freshly-hatched asexual *Artemia* nauplii collected from Qarun lake Egypt and bisexual *Artemia franciscana* nauplii from Great Salt Lake, USA were enriched with 22:6-3 docosahexaenoic acid (DHA) by using lipid emulsion containing 95%of DHA ethyl esters (% total fatty acid). Temperature and salinity during the 24 h enrichment and subsequent 24h starvation were 28°C and 34 gl^{-1}, which are the standard condition for *Artemia franciscana.* The initial DHA values after the hatching were 0 and 0.2 mg/g^{-1} DW for *Artemia franciscana* and Qarun, respectively. Initial 20:5n-3 (eicosapentaenoic acid EPA) values were high in the *A. franciscana* nauplii (8.4 mg/g^{-1}DW as compared to 0.2 mg/g^{-1}DW in the Qarun). During the first enrichment period (24 h), the DHA content increased to 22.56 mg/g^{-1}DW and 27.3 mg/g^{-1}DW *A. franciscana* and Qarun, respectively. After 24h enrichment the respective DHA values were 25.26 mg/g^{-1}DW and 29.73 mg/g^{-1}DW. During the enrichment, increasing the content of EPA, which suggests the bioconversion of DHA to EPA in Qarun strain. Subsequent starvation (24-48h), DHA and EPA levels in both strains decreased (DHA 8.16 and 11.4 mg/g^{-1}DW, EPA 13.73 and 3.93 mg/g^{-1}DW for *A. franciscana* and Qarun strains, respectively. This is according to the previous findings showing an importance and rapid catabolism of DHA in Qarun population. This finding increase the potential of use Qarun strain in the marine hatcheries for larval rearing.

Keywords: *Egyptian Artemia, qarun, DHA enrichment, EPA, parthenogenetic.*

INTRODUCTION

The economic importance of *Artemia* for shellfish and marine larviculture is substantial (Bengtson *et al.*, 1991; Lavens and Sorgeloos, 2000; Dhont and Sorgeloos, 2002). The size range of *Artemia* and their different physical forms make them very versatile to the growth and development of fish and shellfish fry. Over the years, more than 90 % of the world 's commercial harvest of brine shrimp cysts derived from Great Salt Lake (GSL) (Laven and Sorgeloos. 2000). GSL proved –however big –to remain a natural ecosystem subject to climatic and other influences and has illustrated this by inflicting unpredictable and fluctuating cyst harvest, which in return affect the cyst price.

Egypt, as a part of the Mediterranean area, has several saline lakes, lagoons and solar saltworks. These environments are suited for the development of natural populations of brine shrimp. In the South West of the Nile delta *Artemia* was reported in Qarun lake in the El-Fayum (Imesal Saline Company). The population from Qarun has a mean cyst diameter of 246 µm (Amat, 1980; Triantaphyllidis *et al.*, 1996), it may be among the smallest values that have ever been recorded for parthenogenetic *Artemia*. The size of cysts from Qarun seems to be comparable to the Great Salt Lake (GSL) *A. franciscana* cysts, which is one of the main sources of brine shrimp cysts for use in aquaculture (Triantaphyllidis *et al.*, 1994, El-Bermawi and Omer, 2008). This feature makes the population from Qarun very attractive for commercial use. The levels of the essential fatty acids 20:5ω3 and 22:6ω3 in Qarun are considered to be not sufficient to secure good survival and growth of the marine larval organisms (Levine and Sulkin, 1984; Léger *et al.*, 1986; 1987; El-Bermawi, 2003). In addition the samples have been collected from salt ponds where the salinity was around 120 to 200 g/l. In such conditions of high salinity, microalgal species such as diatoms (Haptophyceae and Prymnesiophytes) and most Cryptophytes, that contain significant amounts of EPA and DHA, cannot exist in the medium. The existence of these diatoms can improve the nutritional value of *Artemia* (Triantaphyllidis *et al.*, 1996).

Egyptian aquaculture has been expanding rapidly with the production of fish and shrimp, especially after these activities attracted the interest of the private sector (GAFRD, 2007). Important investments have been made in the setting up of fish and shrimp hatcheries, which need substantial quantities of *Artemia* cysts and biomass. High import taxes and high dollar exchange rate are considered to be an additional load to the fry price (El-Bermawi, 2003). The present study examines the metabolic fate of the major n-3 HUFA in two genetically different strains to evaluate the potential value of the Qarun strain for application in marine hatcheries.

MATERIALS AND METHODS

Cyst processing:

Parthenogenetic *Artemia* cysts collected from saltwork of Qarun lake, (El-Fayum, Imesal Saline Company) were processed according to the methods of Lavens and Sorgeloos (1996); different sieves sizes were used to remove all foreign materials. Density separation in brine (>200 g/l) with strong aeration for one day was then done using Instant Ocean® (synthetic sea salt, Aquarium Systems, Inc, Mentor, Ohio, USA) salt mixture. The aeration was stopped for 2 hrs in order to allow heavy debris to sink and full cysts, empty cysts and light debris to float. Cysts and light debris were collected and washed with fresh water for 10 min using a 150 µm mesh sieve to remove all the salt. For full cysts separation, cysts were washed in fresh water with strong aeration for 10 min, then full cysts were collected from the bottom of the cone. Fast and homogeneous drying of the cysts in oven at a temperature of <40°C was provided until a 10 % moisture content was reached. This level of moisture has been forund to o stop the metabolic activity in the cysts. Dried cysts were vacuum packed and stored at 4°C. Processed GLS (*Artemia franciscana*) strain from *Artemia* Reference Center Cyst Bank was directly used.

Cyst hatching and enrichment:

Artemia cyst from two different locations, *A. franciscana* (Great salt lake, USA) and *Artemia parthenogenetica* from Qarun lake (a new population described by El-Bermawi, 2003 from Qarun lake, Egypt), were used in this experiment. The Cysts (12 g l^{-1} because of the poor hatching %) from Qarun and (4 g l^{-1}) from *Artemia franciscana* cysts were incubated in filtered seawater (34 g l^{-1} salinity) at 28°C under continuous aeration and light for hatching. After hatching, the nauplii were separated from the cyst shells and transferred to 2 liter cylindroconical glass tubes at 28°C and a density of 200 individual /ml^{-1} with continuous aeration from the bottom of the cone using an additional air-stone to keep oxygen levels above 5-6 mg l^{-1} for enrichment. Freshly hatched *Artemia* nauplii were enriched with 22:6n-3 by using emulsion containing 95 % of DHA ethyl esters (% of the total fatty acids). The emulsion (0.4 g l^{-1}) was added at the beginning of enrichment (t =0 h) and after 12 h (t =12 h). After 24 h enrichment (t =24 h), surviving nauplii were transferred into glass tubes at a density of 100 individual /ml^{-1} and at 28°C for a subsequent fastedof 24 h (s = 24 h). Triplocate samples from each tube were taken at t_0, t_6, t_{12} and t_{24} as well as 24 s for lipid analysis.

Fatty acid analysis:

Fatty acids were determined by gas chromatography. Fatty Acid Methyl Ester (FAME) were prepared via a modified procedure of Lepage and Roy (1984). This method implicates a direct acid catalyzed transesterification without prior extraction of total fat, on dry sample amount ranging from 10 to 150 mg. Ten percent of an internal standard (20:2n-6) was added prior to the reaction. Fatty acid methyl esters were extracted with hexane. After evaporation of the solvent the FAME were prepared for injection by redissolving them in iso-octane (2 mg/ml). Quantitative determination was done by a Chrompack CP9001 gaschromatograph equipped with an autosampler and a TPOCI (temperature programmable on-column injector). Injections (0.2 µl) were performed on-column into a polar 50 m capillary column, BPX70 (SGE Australia), with a diameter of 0.32 mm and a layer thickness of 0.25 µm connected to a 2.5 mm methyl deactivated pre-column. The carrier gas was H_2, at a pressure of 100 kPa and the detection mode FID. The oven was programmed to rise from the initial temperature of 85°C to 150 °C at rate of 30°C/ min, from 150°C to 152°C at 0.1°C/min, from 152°C to 172°Cat 0.65 °C/min, from 172°C to 187°C at 25°C/min and to stay at 187°C for 7 min. The injector was heated from 85°C to 190°C at 5°C/sec and stayed at 190°C for 30 min. Identification was based on standard reference mixtures (Nu-Chek-Prep, Inc., U.S.A.). Integration and calculations were done on computer with the software program: Maestro (Chrompack) (Han, 2001). Decapsulated cysts for all the tested strains have been used for the FAME test, since the hatching percentage was poor after 24 hrs (Abatzopoulos *et al.*, 1989).

RESULTS AND DISCUSSION

The fatty acid composition of the freshly-hatched nauplii of both *Artemia* strains are presented in Table (1). The initial values of docosahexaenoic acid after hatching (DHA, 22:6n-3) were 0 and 0.2 mg /g^{-1} DW for *A. franciscana* and Qarun strains respectively. The initial 20:5n-3 (eicosapentaenoic acid, EPA) value was 8.4 mg /g^{-1} DW for *A. franciscana* while it was 0.2 mg /g^{-1} DW for Qarun strain. Total ω-3 HUFA in the freshly hatched *Artemia* nauplii were 9.7 and 0.9 mg /g $^{-1}$ DW. Table 1 and Fig.1 and 2 show the modification in the DHA and EPA profiles following the enrichment with docosahexaenoic acid. During enrichment (0 to12 hrs), the DHA content increased to 22.56 and 27.3 mg./g^{-1} DW in *A. franciscana* and Qarun strain, respectively. After 24 hrs

enrichment DHA contents in both strains were elevated (25.26 and 29.33 mg/g^{-1} DW, respectively). Both strains rapidly catabolized, DHA during the starvation period (24 h), in accordance with the finding in *A. farnciscana* (Triantaphyllidis *et al.*, 1995, Han *et al.*, 1998). The content of EPA increased slightly from 8.4 to 14.66 mg/g^{-1} DW for *A. franciscana* and 0.2 to 6.53 mg/g^{-1} DW for Qarun. During the subsequent starvation (24-48 h), the DHA and EPA levels were decreased in both species to 8.16 and 11.4 mg /g^{-1}DW 13.73 and 3.93 mg /g^{-1}DW, respectively (Table 1). EPA levels were varied between the two strains during the starvation period (Qarun strain was catabolizing EPA faster than *A. franciscana*). The former value represents, to our knowledge, the lowest DHA and EPA in Qarun freshly hatched nauplii (Table1). The fatty acid composition of *Artemia* is determined by its natural habitat condition (Lavens *et al.*, 1989). Therefore, the saltwork from which

Table (1): Effect of incubation time with DHA on the fatty acid composition (mg g^{-1} dry weight) of freshly-hatched (t = 0h) *A. franciscana* (Great Salt Lake, USA) and *Artemia* parthenogenetica Qarun (Qarun Lake, Egypt). Nd: not detected.

Fatty Acid Methyl Ester (mg/gDW)	Incubation Time (hrs)									
	Enrichment with DHA (22:6n-3)								Without DHA (22:6n-3)	
	0		6		12		24		48	
	GSL	Qarun	GSL	Qarun	GSL	Qarun	GSL	Qarun	GSL	Qarun
14:0	2.0	0.9	2.0	0.9	2.0	0.9	2.0	0.9	2.0	0.9
14:1n-5	1.8	2.1	1.8	2.1	1.8	2.1	1.8	2.1	1.8	2.1
15:0	1.8	2.1	1.8	2.1	1.8	2.1	1.8	2.1	1.8	2.1
15:1n-5	Nd	1.1	Nd	1.1	Nd	1.1	Nd	1.1	Nd	1.1
16:0	20.6	12.0	20.6	12.0	20.6	12.0	20.6	12.0	20.6	12.0
16:1n-7	7.9	2.7	7.9	2.7	7.9	2.7	7.9	2.7	7.9	2.7
17:0	0.8	1.1	0.8	1.1	0.8	1.1	0.8	1.1	0.8	1.1
17:1n-7	2.1	Nd	2.1	Nd	2.1	Nd	2.1	Nd	2.1	Nd
18:0	6.9	6.1	6.9	6.1	6.9	6.1	6.9	6.1	6.9	6.1
18:1n-9	34.9	14.4	34.9	14.4	34.9	14.4	34.9	14.4	34.9	14.4
18:1n-7	13.6	5.9	13.6	5.9	13.6	5.9	13.6	5.9	13.6	5.9
18:2n-6^t	10.1	0.1	10.1	0.1	10.1	0.1	10.1	0.1	10.1	0.1
18:2n-6^a	0.5	4.1	0.5	4.1	0.5	4.1	0.5	4.1	0.5	4.1
18:3n-6	Nd	0.3	Nd	0.3	Nd	0.3	Nd	0.3	Nd	0.3
19:1n-9	Nd	0.5	Nd	0.5	Nd	0.5	Nd	0.5	Nd	0.5
18:3n-3	39.4	8.2	39.4	8.2	39.4	8.2	39.4	8.2	39.4	8.2
18:4n-3	4.8	1.5	4.8	1.5	4.8	1.5	4.8	1.5	4.8	1.5
19:0	Nd	0.1	Nd	0.1	Nd	0.1	Nd	0.1	Nd	0.1
19:1n-9	0.5	Nd	0.5	Nd	0.5	Nd	0.5	Nd	0.5	Nd
20:1n-9	Nd	0.4	Nd	0.4	Nd	0.4	Nd	0.4	Nd	0.4
20:1n-7	Nd	0.7	Nd	0.7	Nd	0.7	Nd	0.7	Nd	0.7
20:3n-6	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
20:4n-6	1.7	0	1.7	0	1.6	0.1	1.7	0	1.6	0
20:3n-3	0.4	0.1	0.4	0.1	0.4	0.1	0.4	0.1	0.4	0.1
20:4n-3	0.6	0.1	0.6	0.1	0.6	0.1	0.6	0.1	0.6	0.1
22:0	Nd	0.3	Nd	0.3	Nd	0.3	Nd	0.3	Nd	0.3
20:5n-3	8.4	0.2	9.36	2.6	11.33	4.63	14.66	6.53	13.73	3.93
21:5n-3	0.2	Nd	0.2	Nd	0.2	Nd	0.2	Nd	0.2	Nd
22:6n-3	Nd	0.2	10.83	14.3	22.56	27.3	25.26	29.33	8.16	11.4
Total (n-3) HUFA	9.7	0.9	21.5	16.4	43.5	32.9	49.6	36.7	31.6	16.2
Total FAME	163.8	64.2	184.3	80.6	207.3	97.1	213.4	100.9	195.3	80.4

t_0: freshly-hatched, t_6h, t_{12}h and t_{24}h: enriched and 24 h. fasted (28°C). Data represented means (n=3), except for the Qarun strain (n=2).

The Qarun Cysts were collected does not contains the microalgal species with very high DHA and EPA level. Fast and stable existence of high salinity (which is presented in tropical saltworks such Qarun salt work), microalgal species such as diatoms (Haptophyceae and Prymnesiophytes) and most Cryptophytes, that contain significant amounts of EPA and DHA, do not exist in the medium. The presence of these diatoms can improve the nutritional value of *Artemia* (Triantaphyllidis *et al.*, 1996). The standing phytoplankton density of Qarun Lake in 1995 was 1771×10^3 cells. l^{-1}. Diatoms (*Nizschia closterium, Cyclotella* spp. and *Melosira* spp.) proved to be the dominant group in the lake water (45 g/l) (El-Shabrawy, 2001) however, no data has been found related to the phytoplankton status in the saltworks in Qarun.

Fig. (1): Change of DHA (22:6n-3) level (mg/g^{-1} dry weight) of *A. franciscana* and *Artemia* parthenogenetica (Qarun) during enrichment with DHA (docosahexaenoic acid, 22:6n-3).

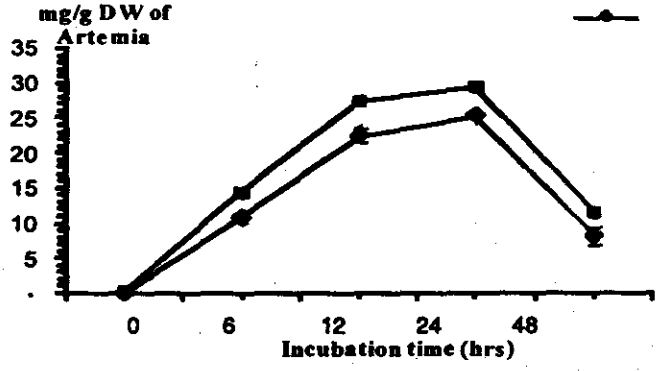
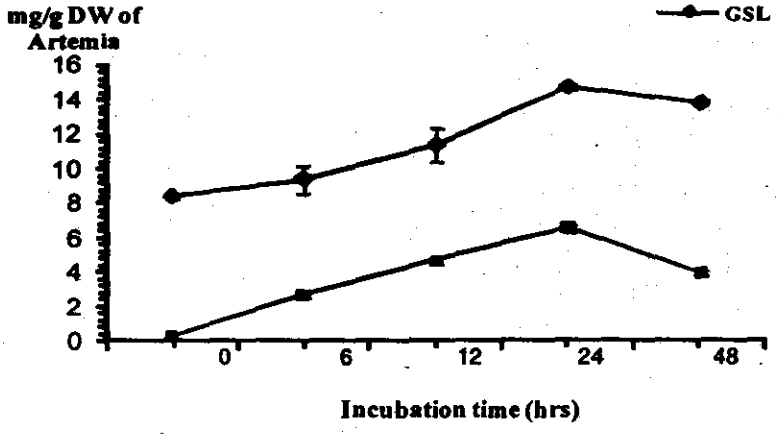


Fig. (2): Change of EPA (eicosapentaenoic, 20:5n-3) level (mg/g^{-1} dry weight) of *A. franciscana* and *Artemia* parthenogenetica (Qarun) during enrichment with DHA (docosahexaenoic acid, 22:6n-3).



The low DHA and EPA in Qarun strain during the first enrichment period might be due to a slower development of the nauplii, possibly related to sub-optimal culture conditions (e.g. temperature, salinity). During the enrichment, increasing the content of EPA in both strains, which suggests the bioconversion of DHA to EPA (Han *et al.*, 1998) and increase the total ω3 (HUFA).

Further enrichment study is required to examine the metabolic fate of other HUFA such EPA and ARA (arachidonic acid, 20:4n-6) in highly purified lipid emulsions with Qarun *Artemia* nauplii.

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دراسة تأثير مدة التحضين لاثراء الحامض الدهنى DHA على تركيب الاحماض الدهنية لأحد انواع الارتيميا المصرية

ناجى منصور البرماوى

قسم الإنتاج الحيوانى والسمكى - كلية الزراعة – سابا باشا – جامعة الإسكندرية – الإسكندرية – مصر.

تم اثراء نوعين من الارتيميا حديثة الفقس احدهما جنس من ارتيميا سان فرانسيسكو وارتيميا خنثى من بحيرة قارون بالحامض الدهنى DHA وذلك باستخدام مستحلب ٩٥٪ من DHA ودرجة الحرارة والملوحة كانت ٢٤ مئوية و ٣٤ جزء فى الألف على الترتيب، وكانت خلال فترة التجويع ٢٨ درجة مئوية والتى تعتبر مثلى لارتيميا فرنسيسكانا .

القيم الأولية لـ DHA بعد الفقس كانت صفر ، ٠.٢ ملليجرام/ جرام وزن جاف لارتيميا فرنسيسكانا وقارون على التوالى والمحتوى الاساسى من EPA كان مرتفع فى ارتيميا فرنسيسكانا ٨.٤ ملليجرام/جرام مقارنة ٠.٢ ملليجرام/جرام وزن جاف فى قارون. خلال الاثراء ٢٤ ساعة كان المحتوى من DHA ارتفع الى ٢٢.٥٦ ملليجرا/جرام وزن جاف و ٢٧.٧ ملليجرام /جرام وزن جاف لكل من ارتيميا فرنسيسكانا وقارون وخلال الاثراء إرتفع محتوى النوعين من الارتيميا من EPA حيث ان الارتيميا تحول جزء من DHA الى EPA.

انخفاض تركيز كلا من EPA , DHA فى كلا من النوعين (فرنسيسكانا وقارون) وذلك خلال مرحلة التجويع(DHA ٨.١٦ و ١١.٤ ملليجرام /جرام وزن جاف و ١٣.٧٣ و ٣.٩٣ ملليجرام/جرام وزن جاف فى كلا النوعين).

هذا البحث يبين اهمية تحويل DHA فى ارتيميا بحيرة قارون ويزيد من قابلية استخدام ارتيميا بحيرة قارون فى مفرخات أسماك المياه المالحة.