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Improving the Fatty Acid Profile of Fairy Shrimp, *Streptocephalus dichotomus*, Using a Lipid Emulsion Rich in Highly Unsaturated Fatty Acids

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Fatty acids are the largest component of lipids and have become a useful tool in the determination of live feeds to a variety of cultured species. Bioencapsulation is a technique which allows high-level incorporation of desired components (i.e., fatty acids, vitamins, antibiotics, etc.) in live feeds, which in turn can be supplemented to the consumer organisms. The procedure described in the present study serves as a platform of technology for enriching the *Streptocephalus dichotomus*. Uptake of two enrichment diets (ALGAMAC2000 and DHA-SELCO) by adult *S. dichotomus* was investigated. The fatty acid profile supports the hypothesis that the enrichment diet increases the level of essential fatty acids, such as linolic, linolenic, eicosapentenoic, and docosahexaenoic acids. The average content (percent of total fatty acids detected) of the enriched organism by different highly unsaturated fatty acid (HUFA) products were as follows: ALGAMAC2000 showed 14−22% saturated fatty acid (SFA), 17−18% monounsaturated fatty acid (MUFA), 28−41% polyunsaturated fatty acid (PUFA), 23−34% $n-3$, and 4.9−7.5% $n-6$, whereas DHA-SELCO showed about 20−23% SFA, 20−26% MUFA, 38% PUFA, 28−31% $n-3$, and 7.5−10% $n-6$. Our present investigation proves that both HUFA-rich diets appear to be an appropriate enrichment diet, and further provides an additional rationale for using fairy shrimp as a maturation diet for any cultivable freshwater organism.

KEYWORDS: Bioencapsulation; gas chromatography; enrichment; fatty acids; linolenic acid; EPA; DHA

INTRODUCTION

In aquaculture studies, highly unsaturated fatty acids (HUFAs) and a subset of polyunsaturated fatty acids (PUFAs) have been found to be critical for maintaining high growth, survival, and reproductive rates and high food conversion efficiencies for a wide variety of marine and freshwater organisms (*1*). The limited ability of crustaceans and fishes for de novo synthesis of PUFAs such as linoleic (18:2$n-6$, LOA) and linolenic (18:3$n-6$, LNA) acids or HUFAs such as eicosapentaenoic acid (20:5$n-3$, EPA) and docosahexaenoic acid (22:6$n-3$, DHA) was reported by Kanazawa et al. (*2*, *3*), suggesting that these fatty acids (FAs) are essential. However, to fulfill their physiological requirements, they need to acquire these vital FAs only through their feed/diet (*4*, *5*).

Generally, freshwater fish differ significantly from marine fish with respect to fatty acid content and requirement (*6*), and several studies have suggested that a dietary inclusion of $n-6$ fatty acids is required (*7*). Keeping the vital role of ArA, EPA, and DHA in mind, it seems very likely that a deficiency or unbalanced dietary proportion of these essential fatty acids would affect normal development. Globally, rotifers, *Artemia* nauplii, and *Moina* have been widely used as live feeds for larval fish during the past decade, but they seem to be deficient in $n-3$ HUFAs, especially EPA and DHA (*8*−*11*). Development of a suitable bioencapsulation technique for live feed resulted in a major breakthrough in hatchery production in the 1980s. Using this technique, the live feed lacks in certain nutritional elements can be remedied by an "enrichment" process which ultimately improves their nutritional composition prior to offering them to the consumer organisms (*12*). Several methods have been adopted to increase the $n-3$ HUFA content in live feeds using microalgae (*13*), ω-yeast (*14*), microparticles (*15*), self-emulsifying products (*16*, *17*), microencapsules (*10*, *15*, *18*), and fish byproducts, silages containing $n-3$ PUFAs (*19*). This proved to be effective in improving the fatty acid content.

In recent years, the nutritional importance of fairy shrimp as a food source for fish and crustaceans has been on the foreground (*20*, *21*), because of their high individual biomass, high reproductive rate, and rapid growth. Adult *Streptocephalus dichotomus* has proved to be a palatable feed for juvenile *Carassius auratus* (*22*), although it has been shown that it is deficient in HUFAs, such as EPA, ArA, and DHA (*23*). Although there is increasing evidence showing the potentiality of fairy shrimp in freshwater aquaculture, basic information related to time, transfer, and incorporation of essential nutrients in freshwater organisms, especially fairy shrimps, is lacking.

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The objective of this study was to determine the comparative and quantitative effects of diet on the essential fatty acid profile of *S. dichotomus*. As these fairy shrimps are filter feeders, the fatty acid composition can be enhanced with a nutritional supplement, to provide a better opportunity for effective utilization of *Streptocephalus* in freshwater aquaculture.

MATERIALS AND METHODS

Samples. Adult fairy shrimp, *Streptocephalus dichotomus*, were cultured and maintained as outlined by Munuswamy et al. (*24*). For enriching the live feeds, several approaches have been followed: (1) adjustment of lipid or vitamin content of live feeds just before feeding them to other organisms (this is referred to as short-term enrichment, usually <8 h), (2) feeding of live feeds with enriched diets for more than 24 h or feeding throughout the culture period. Both enrichment techniques have been adopted by many researchers; however, each of them has its advantages and disadvantages. For the advantage of being fast and flexible, we adopted the short-term enrichment for the present study.

Enrichment Protocol. Super DHA-SELCO (INVE Aquaculture NV, Belgium) and ALGAMAC2000 (Aquamarine Biofauna, Inc., United States) were used, since these two enrichment emulsions have been used extensively in previous studies. The commercial product described in the present study is used to see their ability to enrich fairy shrimp, and the mention of them is not an endorsement. Fairy shrimps from a single batch culture were used, and the experiments were duplicated. Briefly, the adults were transferred to the enrichment tank with freshwater, to which was added the emulsion at a concentration of 0.6 g/L (*25*). The material was suspended and prehydrated by blending the appropriate quantity in freshwater for 1 min. The enrichment tank was aerated vigorously so that oxygen levels exceeded 4 ppm during enrichment and also to avoid the binding of lipid globules. Enriched adults were harvested at 3 h intervals and washed with freshwater, after which the samples were processed for further investigation.

Lipid Extraction. Total lipids were extracted from unenriched and enriched adult *Streptocephalus* according to the method of Folch et al. (*26*). A 1 g sample of homogenized muscle tissue was extracted with 5 mL of chloroform/methanol (2:1 v/v) and shaken for 20 min in an Erlenmeyer flask with a magnetic stirrer. After filtering, the liquid phases were mixed and washed with 0.5 mL of 0.9% sodium chloride solution. The lower phase was collected after the phase separation, and the solvent was evaporated. The residue was transferred to a 10 mL glass vial and maintained at −20 °C until analysis was performed. The total lipid content was determined according to Barnes and Blackstock (*27*). Briefly, an aliquot of 0.5 mL of lipid sample was taken and allowed to evaporate under nitrogen. The individual dry samples were digested with 0.5 mL of concentrated sulfuric acid in a boiling water bath for 15 min. A known volume of 0.2 mL of acid digest was taken, to which was added 5 mL of phosphovanillin reagent. The mixture was allowed to stand for 30 min, and the color intensity was determined by spectrophotometry at 520 nm. Chloroform and cholesterol were used as the blank and standard, respectively.

Fatty Acid Analyses. Fatty acid compositions from the total lipids were analyzed as methyl ester derivates (FAME) according to the method of Morrison and Smith (*28*). Briefly, 100 mg of lipid was saponified with 2 mL of sodium methoxide in methanol (0.5 N) and incubated for 10 min in a boiling water bath. The solution was then cooled at room temperature, and to this was added 2 mL of boron trifluoride/methanol complex (14%). The solution was then heated for 20 min in a boiling water bath (80 °C). After cooling, 1 mL of hexane was added, and the mixture was heated for another 2 min and cooled at room temperature. To this was added 1.25 mL of saturated sodium chloride. The mixture was shaken vigorously, and after phase separation, the organic layer was suctioned with a Pasteur pipet and transferred to a 2 mL vial containing 1 mm of anhydrous sodium sulfate. The fatty acid methyl esters were analyzed using a Hewlett-Packard 5890 gas chromatograph, equipped with a flame ionization detector and a diethylene glycol succinate (DEGS) column. Nitrogen was used as the carrier gas at a flow rate of 1.3 mL min^{-1}. The injector and detector temperatures were 200 and 230 °C, respectively. The oven temperature was programmed from an initial temperature and time of 160 °C for 10 min to 180 °C at 1.5 °C min^{-1}, which was maintained for 5 min. Integrated peak areas of the fatty acid methyl esters were identified by comparison with known standards (Nu-Check-Prep, GLC-68A). Results are expressed as the percent (w/w) of total fatty acids detected.

Statistical Analysis. Data on the lipid content and fatty acid profiles of experimental animals were analyzed statistically using one-way analysis of variance (ANOVA) (*29*). All statistical analyses were carried out using the STATISTICA (*30*) program.

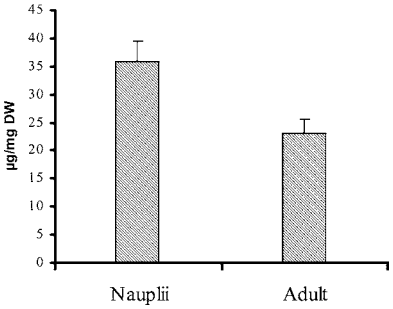

Figure 1. Total lipid content in fairy shrimp, *Streptocephalus dichotomus.*

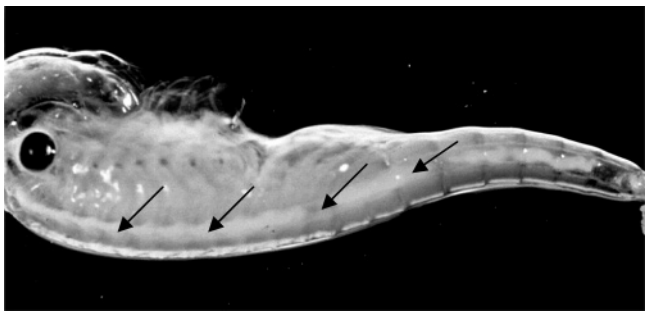

Figure 2. Adult fairy shrimp showing the effective ingestion (arrows) of exogenous HUFA products

RESULTS

Analysis of total lipid in various stages of fairy shrimp showed nauplii have a higher lipid content than the adults ($P < 0.05$) (Figure 1). Our previous study showed the use of adult fairy shrimp in ornamental fish culture. In the present investigation we focused on the utilization of adult fairy shrimp as a maturation diet. From the results it is evident that adult *Streptocephalus* can be enriched with an exogenous source of fatty acids. Our investigation shows that short-term enrichment is effective and the enrichment is directly proportional in relation to time as proved by the fatty acid analysis.

The fatty acid composition of fairy shrimp enriched with ALGAMAC2000 and DHA-SELCO is presented in Figures **3** and **4**. The fatty acid content of unenriched fairy shrimp showed a high level of linolenic acid and 18 series acids, but the absence of DHA (Figure 3). Fairy shrimp enriched with ALGAMAC2000 showed an overall increase in DHA (0.35%) and EPA (8.52%) contents within 3 h of incubation in the enrichment medium. Linoleic (4.22%), linolenic (19.78%), eicosopentanoic (13.29%), and docasahexenoic acid (0.94%) contents were increased at 6 h of incubation (Figure 3).

DHA-SELCO-enriched fairy shrimp also showed an increase in EPA (11.29%) and DHA (1.92%) contents in 3 h, which is comparatively higher than that of ALGAMAC. At 6 h of enrichment, further accumulation of DHA (3.19%) in the body tissue of fairy shrimp was observed. High profiles of 18 series fatty acids, EPA, and DHA were observed at 6 h of enrichment (Figure 4).

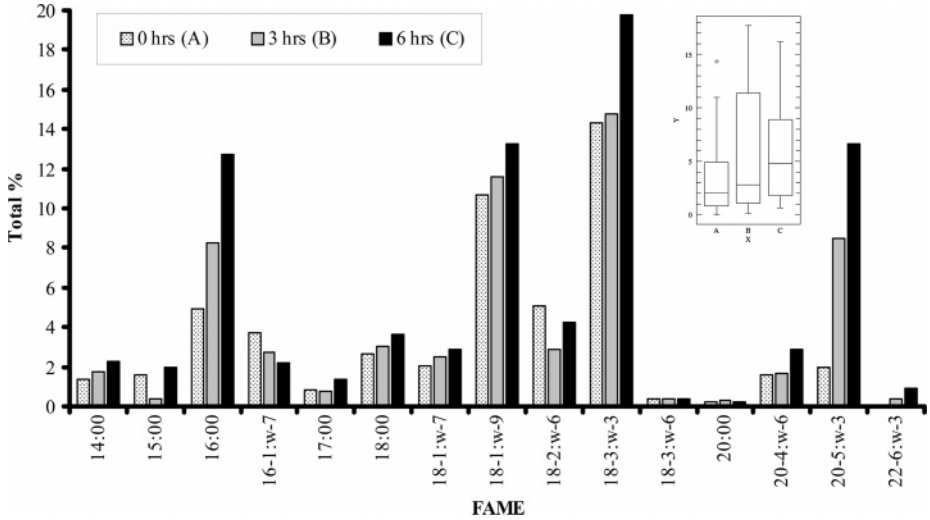

Figure 3. Fatty acid profile of the fairy shrimp enriched with ALGAMAC2000 (expressed as percent of total fatty acids). The line through the middle of the box shows the median, and the outer edge of the box corresponds to the 25th and 75th percentiles.

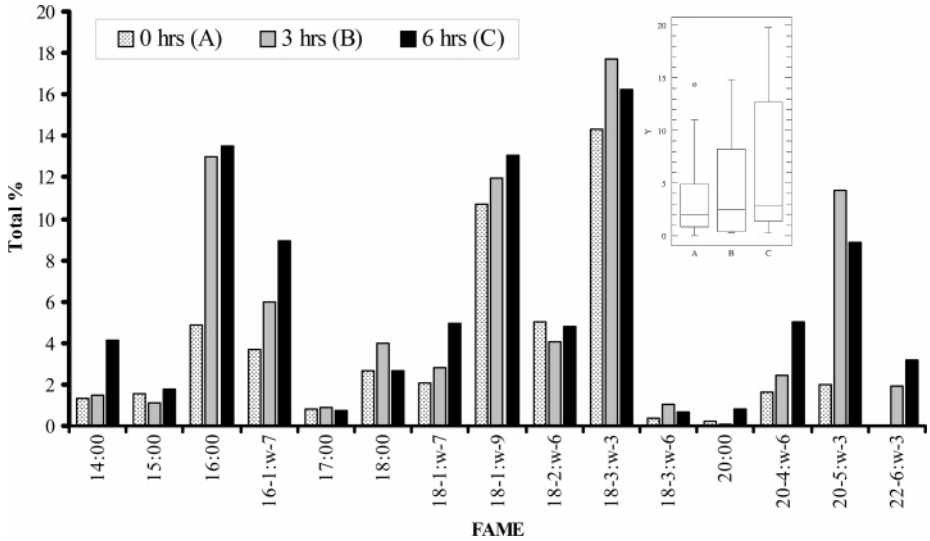

Figure 4. Measured fatty acid content of the fairy shrimp enriched with DHA-SELCO (expressed as percent of total fatty acids). The line through the middle of the box shows the median, and the outer edge of the box corresponds to the 25th and 75th percentiles.

Table 1. Nutritional Indices[a] for the Unenriched and Enriched Fairy Shrimp with HUFA Products[b]

			SFA	MUFA	PUFA	*n*-3	*n*-6	*n*3:*n*6	DHA:EPA	SFA:PUFA	SFA:(MUFA + PUFA)
ALGAMAC2000			57.1	11.0	30.6	20.8	9.8		34.7	1.87	1.373
DHA-SELCO			6.5	18.8	68.2	61.1	7.1		1.3	0.095	0.075
unenriched *S. dichotomus*			11.49	16.49	23.4	16.34	7.06	16.34	0	0.491	0.288
enriched *S. dichotomus*	ALGAMAC2000	3 h	14.47	16.8	28.64	23.67	4.97	23.67	0.041	0.505	0.318
		6 h	22.19	18.36	41.55	34.01	7.54	34.01	0.071	0.534	0.370
	DHA-SELCO	3 h	20.52	20.78	38.57	31.03	7.54	31.03	0.169	0.532	0.346
		6 h	23.71	26.93	48.62	38.21	10.41	38.21	0.170	0.488	0.362

[a] Expressed as percent of total fatty acid. [b] SFA, saturated fatty acid: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0. MUFA, monounsaturated fatty acid: 16:1, 18:1. PUFA, polyunsaturated fatty acid: 18:2, 18:3.

The $n-3$:$n-6$ fatty acid ratio is an important indicator in selecting the optimal feed. Fatty acid analyses of enriched fairy shrimp showed an increased ratio of $n-3$ to $n-6$ fatty acids (Table 1) compared to that of unenriched adults. PUFA signature SFA, PUFA and $n-3$ signature DHA:EPA, SFA:PUFA underwent significant dietary modification after 3−6 h of enrichment in both ALGAMAC2000 and DHA-SELCO treatments. Statistical analysis of the enrichment also showed a positive significance between the animals fed with different treatments in regard to the fatty acid profile and time ($P < 0.001$).

DISCUSSION

Bioencapsulation is a technique where live-feed organisms are enriched with required nutrients. Various researchers have shown that the nutritional differences among *Artemia* strains (*31*, *32*), *Chirocephalus* spp. (*33*), and *Streptocephalus* spp. (*23*)

Table 2. Essential Fatty Acid Profiles of Maturation Feeds Used for Freshwater Ornamental Fish (mg/100 mg of dry weight) (*55*)

	fatty acid				
	18:2*n*6	18:3*n*3	20:4*n*6	20:5*n*3	22:6*n*3
beef heart liver	1.71	0.20	0.51	0.11	0.33
beef liver	1.56	nd[b]	0.22	nd	nd
black tubifex	1.68	0.51	0.90	0.61	nd
red tubifex	1.43	0.19	0.64	0.33	nd
daphnia	0.11	0.04	0.16	0.07	nd
earthworm	0.11	0.10	0.22	0.09	nd
mosquito worms	0.48	0.31	0.33	0.23	nd
nematodes (%)	9.91	9.28	4.64	7.35	3.25
Streptocephalus proboscideus	8.8	10.7	nd	2.4	0.7
Chirocephalus	4.57	14.3	nd	12.9	4.14
S. dichotomus	5.05	14.35	1.61	1.99	nd
Artemia	5.05	7.4	nd	8.9	nd
Moina	0.11	0.04	0.16	0.07	nd
freshwater rotifer	1.12	2.64	0.05	0.11	nd
enriched *Moina*, DOCOSA gold	0.55	1.22	0.40	1.12	4.47
freshwater rotifer	1.54	0.93	0.08	0.52	0.42
S. dichotomus, ALGAMAC[a]	4.22	19.78	2.91	13.29	0.94
S. dichotomus, DHA[a]	4.79	16.20	4.99	8.82	3.19

[a] Present study (expressed as percent of total fatty acid). [b] nd = not detected.

constrained their use in aquaculture and emphasized the necessity to improve their nutritional value. The present investigation focused on the enrichment of adult *Streptocephalus* since prawns, shrimps, and fishes requires $n-3$ HUFAs. It is clear that the medium examined in the present study can significantly elevate the total and essential fatty acids of the fairy shrimp in a short period of time. This procedure seems to be a better alternative as suggested by Sandifer and Joseph (*34*) and Reigh and Stickney (*35*) than offering these $n-3$ HUFA-enriched diets throughout the culture period. The results indicate that convenience and the length of the enrichment process should also be considered when *S. dichotomus* is prepared as a food for ornamental fish. Apart from using these commercial diets it is possible to use homemade preparations, such as cod liver oil, squid coil, sardine oil, and menhaden oil for enrichnent depending on the availability and convenience.

In aquaculture, *Artemia* biomass is well accepted; the organism fed with adult *Artemia* grew significantly faster compared to organisms fed with other diets (*36*). *Artemia* juveniles can be enriched in the same way as nauplii (*37*), and used as nursery diet or as a vehicle for antibiotic delivery (*38*) for fish and shrimp. Like *Artemia* juveniles, the fairy shrimp $n-3$ fatty acid levels increased after enrichment, more particularly, linolenic acid, EPA, ArA, and DHA. In tilapia, feeding with enriched $n-3$ fatty acid diets is reflected in their body composition with prominence of α-linolenic acid (*39*). Oie and Olsen (*40*) showed that short-term enrichment with lipid-rich diets significantly increased the lipid content. Similarly, this short-term enrichment results in higher final DHA:EPA ratios in adults compared to nauplii, since there is insufficient time to break down the DHA as the case in nauplii (*41*). We therefore utilized adult fairy shrimp rather than nauplii as the maturation diet. Studies on $n-3$ HUFAs have demonstrated that EPA and DHA play a key role in normal growth, survival, pigmentation, stress, and disease resistance in many species (*42*−*44*). In brood stock nutrition (maturation diet), lipids are important sources of metabolic energy in gonad formation (*45*−*47*). More particularly, a diet high in 20:5$n-5$ and 22:6$n-3$ promotes fecundity and larval survival thereafter (*48*−*51*).

A summary of essential fatty acids found in various feeds is presented in Table 2. These live feeds have been invariably used by most fish breeders, and it has been observed that they are effective in maturation and spawning of various ornamental fish. As the *S. dichotomus* contains high levels of carotenoids, it has been proposed to use it as a maturation diet (*52*). In addition, the EFAs observed in *S. dichotomus* might also imply their critical role in reproduction. In contrast to $n-3$ HUFAs, $n-6$ PUFAs have been largely neglected in studies in fish nutrition. However, the role of ArA in the maturation and spawning process in goldfish is to be scrutinized further. Particularly, the importance of arachidonic acid (20:4$n-6$, ArA), which is a precursor of thromboxanse, prostaglandins, and leukotrienes in many species, including humans (*53*), plays an integral part in the reproductive mechanisms of a large number of freshwater ornamental fish (*54*, *55*). Furthermore, DHA enrichment enhances immunocompetence in fish larvae (*56*). A key step to optimizing the essential fatty acid in nutritional studies is to determine the $n-3$:$n-6$ fatty acid ratio. In the present study, it is observed that both self-emulsifying diets increase the $n-3$:$n-6$ fatty acid ratio in fairy shrimp. This $n-3$:$n-6$ fatty acid ratio has a profound effect on normal metabolic function and is an important indicator of fatty acid nutrition (*57*). As the LOA, LNA, EPA, and DHA are required by prawns (*58*, *59*), the enriched fairy shrimp can be used as diets for adult prawns. Apart from this, enriched adult fairy shrimp can also be frozen or freeze-dried for later use or made into flakes or other forms of formulated feeds.

Our present investigation gives valuable information on the nutritive value of *Streptocephalus*. This study highlights the importance of dietary supplementation; especially PUFAs and HUFAs significantly enhance the EFA profile of the fairy shrimp. It would be interesting to assess whether other vitamins, key nutrients, or antibiotics can be transferred through *Streptocephalus*. The current study further adduces that enriched fairy shrimp can be used as an efficient maturation diet in freshwater aquaculture.

ABBREVATIONS USED

ArA, arachidonic acid; DHA, docosahexaenoic acid; EFA, essential fatty acid; EPA, eicosapentaenoic acid; FA, fatty acid; HUFA, highly unsaturated fatty acid; LOA, linoleic acid; LNA, linolenic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

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