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Full Length Research Paper

Bioencapsulation strategy and highly unsaturated fatty acids (HUFA) enrichment in *Artemia franciscana* nauplii by using marine trash fish *Odonus niger* liver oil

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To investigate the maximum accumulation of highly unsaturated fatty acids (HUFA) in *Artemia franciscana* nauplii through bioencapsulation process, five different concentrations (0 - 4%) of emulsified *Odonus niger* liver oil were prepared. The prepared emulsions were used to enrich *A. franciscana* at different time intervals of 6, 12, 18 and 24 h. After the enrichment period, the fatty acid composition of the nauplii were analysed and estimated individually along with freshly hatched *A. franciscana* and *O. niger* liver oil. The HUFA content such as linoleic (18: 2n-6), linolenic (18: 3n-3), arachidonic (20: 4n-3 + n-6), eicosapentaenoic (20: 5-n3) and docosahexaenoic (22: 6n-3) acids were 12.87, 0.21, 2.66, 2.86 and 2.30% dry weight (DW), respectively, in *O. niger* liver oil, and 8.60, 17.20, 1.80, 2.40 and 0.1% DW, respectively, in freshly hatched *A. franciscana*. During 6 – 24 h of enrichment period, all the above HUFA increased considerably from 8.76 to 10.84, 17.24 to 23.84, 1.16 to 3.98, 2.45 to 5.88 and 0.30 to 2.69% DW, respectively. The increase in the level of individual HUFA of *A. franciscana* enriched with various concentrations of emulsified liver oil at different time durations showed a positive linear relationship and the correlation coefficient obtained were statistically significant (P< 0.05).

Key words: *Artemia franciscana*, HUFA, linoleic, linolenic, eicosapentaenoic, docosahexaenoic.

INTRODUCTION

Growth and survival of shrimp larvae is greatly affected mainly by their nutrition as well as occurrence of infectious diseases (Watanabe et al., 1982; Kanazawa et al., 1985; Trust 1986; Alderman, 1988). Hence nutritional research on the cultivable species of aquatic animals especially shrimp has been receiving great attention all over the world. Live feed such as *A. franciscana* and rotifers (*Brachionus plicatilis*) are main food sources for larval forms of crustaceans, in particular for shrimp. Till date, *A. franciscana* are considered the best diet for feeding zoophages organisms and are provided as live food to over 85% of the aquaculture species (Bhat, 1992). These live feeds are filter feeders and have been used successfully as biological carriers for transferring essential nutrients to predator larvae, using bioencapsulation technique (Leger et al., 1986; Citarasu et al., 1998; Immanuel et al., 2004).

Considering the pre-requisites of n3 and n6 highly unsaturated fatty acids (HUFA) in live feeds, various studies have been made to improve their availability through enrichment methods. It was also reported that, supplementation of HUFA enriched *A. franciscana* improve the survival, growth, disease resistance and other environmental stress resistance in shrimp (Leger and Sorgeloos, 1994; Rees et al., 1994; Kyungmin et al., 2000; Immanuel et al., 2004).

Several studies have been carried out in the fatty acid sources of commercial diets as well as plant and animal fats or oils to the larval stages of cultivable finfish and shellfish species through bioencapsulation process in live feeds like *Artemia* and or rotifer. However, the enrichment process in live feeds using dietary sources is very important, because it is time dependent and the enriched fatty acid profile in *Artemia* is subjected to change

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Table 1. Composition of different concentrations (A-E) of emulsified lipid diets 100 ml^{-1} water.

Diets	*O. niger* liver oil (%)	Egg yolk (g)	Fat soluble vitamins (g)*	Water soluble vitamins (g)**	Bakers yeast (g)
A	0	1	2	10	0.20
B	1	1	2	10	0.20
C	2	1	2	10	0.20
D	3	1	2	10	0.20
E	4	1	2	10	0.20

*Rovigon (Fat soluble vitamin A+E). ** Becozinc (Water soluble vitamin B+C) – Roach product, Mumbai, India.

according to the enrichment duration. It was reported that during 12 to 24 h enrichment of newly hatched *A. franciscana* with lipid source, a significant increase in HUFA content may be detectable (Tamaru et al., 2003; Narciso et al., 1999).

Until recently bioenrichment studies in *Artemia* or rotifer was performed with reference to the concentrations of various nutrients, especially lipid and fatty acids. But information pertained to the enrichment time dependent variation in the concentration of lipid and accumulation of nutrients in live prey organisms is still needed. In view of this, the present work was undertaken to determine the appropriate concentration of marine trash fish *Odonus niger* liver oil to accumulate maximum level of essential fatty acids in *Artemia franciscana* nauplii at definite time of enrichment.

MATERIALS AND METHODS

Preparation of emulsified lipid diets

To start with, *O. niger* liver oil was extracted under laboratory condition as described by Immanuel et al. (2004). Five different enrichment lipid diets having 0 to 4% (A to E) *O. niger* liver oil supplemented with egg yolk and vitamins (water and fat soluble) were prepared following the method of Watanabe et al. (1983) and Immanuel et al. (2004) (Table 1). These diets were homogenized individually with 100 ml water for 5 min. to form an emulsion. The stability of the emulsion was checked before use, and the emulsions were stored at 4^{o}C until use.

Hatching of *Artemia* cyst

A. franciscana (Great Salt Lake strain, UT, USA) cysts were disinfected with hypochlorite solution (200 µg L^{-1}) for 15 – 20 min before hatching. After washing with water to remove the trace of hypochlorite, the cysts were incubated in seawater in a cylindrical glass container with continuous aeration and light. After hatching, the second instar stage *A. franciscana* were separated from the hatching container through a 120 µm sieve and were used for enrichment study.

Enrichment procedure

The separated nauplii were transferred to 5 l enrichment container at a density of 100 nauplii ml^{-1} of seawater (35 ppt) at room temperature (28 ± 1^{o}C). The five different diets were prepared with varying concentrations of *O. niger* liver oil (0 - 4%; A to E) mixed with 200 mg mL^{-1} baker's yeast. Baker's yeast was added to prevent starvation of control *A. franciscana*, which is known to reduce their lipid content (Benjits et al., 1976; Immanuel et al., 2004). Aeration was provided to the rearing containers to keep the oxygen level at 5 ppm. Thus five sets (A to E) of enrichment containers with four numbers each were maintained for different time intervals of enrichment (6, 12, 18 and 24 h). After the enrichment period, the enriched *A. franciscana* were harvested from the respective containers and rinsed with filtered seawater. Each enrichment experiment was performed in triplicate.

Fatty acid analysis

Fatty acid composition of *O. niger* liver oil, freshly hatched *A. franciscana* and enriched *A. franciscana* at different time intervals were analysed and estimated individually by the method of Miller and Berger (1985) and expressed as area present fatty acid methyl esters (FAME).

Statistical analysis

The results obtained in the present experiment were interpreted through Students t-test and regression analysis following Zar (1999)

RESULTS

The fatty acid composition of *A. franciscana* enriched with emulsified *O. niger* liver oil had considerable variation with respect to concentration and enrichment duration (Tables 2 - 5). In *O. niger* liver oil the total amount of saturated, monounsaturated and HUFA were 47.05, 30.41 and 22.54% of dry weight (DW), respectively. In freshly hatched *A. franciscana*, the values were 25.41, 27.02 and 30.10% of dry weight (DW), respectively. The HUFA contents such as linoleic (18: 2n-6), linolenic (18: 3n-3), arachidonic (20: 4n-3 + n-6), eicosapentaenoic (20:5-n3) and docosahexaenoic (22: 6n-3) acids were 12.87, 0.21, 2.66, 2.86 and 2.30% DW respectively in *O. niger* liver oil and 8.60, 17.20, 1.80, 2.40 and 0.1% DW respectively in freshly hatched *A. franciscana* (Table 2).

After 6 h of enrichment, the saturated, monounsaturated and HUFA contents in *A. franciscana* enriched with different concentrations (A to E) of lipid ranged from 23.04 to 31.00, 26.30 to 30.23 and 31.03 to 44.18% of

Table 2. Fatty acid composition of *O. niger* liver oil, freshly hatched *A. franciscana* and *A. franciscana* enriched for 6 h with increasing concentrations (A-E) of emulsified *O. niger* lipid.

Carbon No.	*O. niger* liver oil	Freshly hatched nauplii	A	B	C	D	E
9 : 0	0.39	nd	nd	nd	nd	nd	nd
10 : 0	1.43	nd	nd	nd	nd	nd	nd
11 : 0	1.14	nd	nd	0.32	0.7	0.73	nd
12 : 0	nd	0.14	0.28	0.4	0.83	0.90	0.78
13 : 0	1.16	0.12	0.24	0.57	0.63	0.64	0.83
14 : 0	3.05	1.38	1.78	2.67	1.93	1.14	1.69
15 : 0	2.06	0.4	0.9	1.48	1.82	1.26	1.38
16 : 0	18.5	14.32	14.48	13.4	10.1	8.43	10.4
17 : 0	1.03	1.0	1.5	1.05	0.85	0.93	1.02
18 : 0	14.7	7.70	9.62	10.5	10.5	8.46	9.76
19 : 0	nd	nd	nd	nd	nd	0.05	0.03
20 : 0	2.42	0.35	0.54	0.5	0.92	0.36	0.6
21 : 0	0.12	nd	nd	nd	nd	nd	nd
22 : 0	0.78	nd	nd	0.19	0.1	0.14	nd
23 : 0	nd	nd	nd	nd	nd	nd	nd
24 : 0	0.27	nd	nd	nd	nd	nd	nd
14 : 1	0.43	0.38	0.45	0.47	0.32	0.41	0.4
16 : 1	4.32	3.52	3.64	3.65	4.92	4.3	3.3
18 : 1	17.84	22.20	24.96	23.3	22.9	22.7	21.2
20 : 1	4.02	0.58	0.65	0.79	1.3	1.15	1.0
22 : 1	3.8	0.34	0.53	0.37	0.65	0.5	0.35
18 :2n-6	12.87	8.60	8.90^{a}	8.76^{a}	8.87^{a}	10.14^{b}	9.50^{c}
18 :3n-3	0.21	17.20	17.24^{a}	18.70^{b}	20.40^{c}	22.90^{d}	19.80^{ce}
20:4n-3 + n-6	2.66	1.80	1.90^{a}	1.16^{b}	2.12^{c}	3.31^{d}	3.60^{e}
20:5n-3	2.86	2.40	2.45^{a}	2.75^{b}	4.32^{c}	5.10^{d}	4.65^{ce}
22:5n-3	1.64	nd	0.24	0.16	0.46	0.83	1.05
22:6n-3	2.30	0.10	0.30^{a}	0.60^{b}	1.23^{c}	1.90^{d}	0.95^{e}
Σ saturated	47.05	25.41	29.34	31.00	28.40	23.04	26.5
Σ monoenes	30.41	27.02	30.23	28.60	30.10	29.06	26.30
Σ PUFA	22.54	30.10	31.03	32.10	37.40	44.18	39.6

Diets A, B, C, D and E contain 0, 1, 2, 3 and 4% O. niger liver oil, respectively. nd: Not detected. Fatty acid content is expressed as area present FAME and % (by dry weight). Each value is a mean of triplicate samples. Value in a row with different superscript alphabets is statistically significant ($P< 0.05$; t-test).

DW, respectively. The maximum amount of PUFA (44.18%) was registered in *A. franciscana* enriched with 3% emulsified *O. niger* liver oil (Table 2). Prolonged enrichment beyond 6 h, that is, up to 24 h, significantly increased the level of tested fatty acids. For instance, after 12 h enrichment, the level of saturated, monounsaturated and PUFA increased from 23.27 to 31.39, 26.95 to 30.98 and 32.41 to 46.16% DW, respectively, with the maximum values in 1, 0 and 3% emulsified *O. niger* liver oil enriched *A. franciscana*. The results on

Table 3. Fatty acid composition of *A. franciscana* enriched for 12 h with increasing concentrations (A-E) of emulsified *O. niger* lipid.

Carbon No.	A	B	C	D	E
9 : 0	nd	nd	nd	nd	nd
10 : 0	nd	nd	nd	nd	nd
11 : 0	nd	0.37	0.74	0.75	nd
12 : 0	0.30	0.44	0.85	0.93	0.80
13 : 0	0.27	0.60	0.66	0.67	0.83
14 : 0	1.82	2.72	1.98	1.16	1.73
15 : 0	1.00	1.51	1.86	1.25	1.40
16 : 0	14.51	13.42	10.15	8.45	10.48
17 : 0	1.54	1.08	0.87	0.95	1.06
18 : 0	9.64	10.53	10.52	8.44	9.78
19 : 0	nd	nd	nd	0.08	0.05
20 : 0	0.56	0.52	0.93	0.40	0.63
21 : 0	nd	nd	nd	nd	nd
22 : 0	nd	0.20	0.1	0.19	nd
23 : 0	nd	nd	nd	nd	nd
24 : 0	nd	nd	nd	nd	nd
14 : 1	0.62	0.67	0.48	0.43	0.45
16 : 1	3.81	3.85	4.45	4.34	3.4
18 : 1	25.06	23.51	23.00	22.3	21.4
20 : 1	0.77	0.84	1.30	1.22	1.25
22 : 1	0.72	0.44	0.62	0.49	0.45
18 :2n-6	9.13 [a]	9.16 [a]	9.15 [a]	10.47 [b]	9.79 [c]
18 :3n-3	17.47 [a]	19.10 [b]	20.68 [c]	23.23 [d]	20.09 [ce]
20:4n-3 + n-6	2.13 [a]	1.56 [a]	2.40 [b]	3.64 [c]	3.89 [d]
20:5n-3	2.68 [a]	3.15 [b]	4.60 [c]	5.43 [d]	4.94 [ce]
22:5n-3	0.47	0.56	0.74	1.16	1.34
22:6n-3	0.53 [a]	1.00 [b]	1.51 [c]	2.23 [d]	1.24 [e]
Σ saturated	29.64	31.39	28.66	23.27	26.76
Σ monoenes	30.98	29.31	29.85	28.78	26.95
Σ PUFA	32.41	34.53	39.08	46.16	41.29

Diets A, B, C, D and E contain 0, 1, 2, 3 and 4% *O. niger* liver oil, respectively. nd: Not detected. Fatty acid content is expressed as area present FAME and % (by dry weight). Each value is a mean of triplicate samples. Value in a row with different superscript alphabets is statistically significant ($P < 0.05$; t-test).

enhancement of individual HUFA in *A. franciscana* during 12 h enrichment indicated an increase from 9.13 to 10.47% of linoleic acid, 17.47 to 23.23% linolenic acid, 1.56 to 3.89% arachidonic acid, 2.68 to 5.43% eicosa-

Table 4. Fatty acid composition of *A. franciscana* enriched for 18 h with increasing concentrations (A-E) of emulsified *O. niger* lipid.

Carbon No.	A	B	C	D	E
9 : 0	nd	nd	nd	nd	nd
10 : 0	nd	nd	nd	0.16	0.19
11 : 0	0.18	0.42	0.78	0.8	0.74
12 : 0	0.34	0.48	0.89	0.95	0.85
13 : 0	1.18	0.30	0.63	0.70	0.81
14 : 0	1.86	2.74	1.96	1.10	1.78
15 : 0	0.60	0.58	0.96	0.42	0.65
16 : 0	14.50	13.60	10.36	8.46	10.55
17 : 0	1.50	1.12	0.92	0.96	1.07
18 : 0	9.60	10.58	10.71	8.56	9.76
19 : 0	nd	0.02	0.04	0.06	0.08
20 : 0	0.60	0.56	0.95	0.38	0.62
21 : 0	nd	nd	nd	nd	nd
22 : 0	0.10	0.24	0.16	0.18	0.14
23 : 0	nd	nd	nd	nd	nd
24 : 0	nd	nd	nd	nd	nd
14 : 1	0.66	0.72	0.50	0.48	0.46
16 : 1	3.90	3.92	4.47	4.40	3.48
18 : 1	25.26	23.86	23.23	22.43	21.73
20 : 1	0.79	0.90	1.38	1.27	1.29
22 : 1	0.78	0.50	0.68	0.52	0.47
18 :2n-6	9.24^{a}	9.22^{a}	9.23^{a}	10.61^{b}	9.83^{bc}
18 :3n-3	17.53^{a}	19.31^{b}	20.76^{c}	23.30^{d}	20.21^{ce}
20:4n-3 + n-6	2.21^{a}	1.62^{b}	2.46^{c}	3.81^{d}	3.92^{de}
20:5n-3	2.70^{a}	3.20^{b}	4.64^{c}	5.60^{d}	4.98^{e}
22:5n-3	0.50	0.60	0.75	1.23	1.36
22:6n-3	0.58^{a}	1.10^{b}	1.72^{c}	2.44^{d}	1.30^{be}
Σ saturated	30.46	30.64	28.36	22.73	27.24
Σ monoenes	31.39	29.90	30.26	29.10	27.43
Σ PUFA	32.78	35.05	39.56	46.99	41.60

Diets A, B, C, D and E contain 0, 1, 2, 3 and 4% *O. niger* liver oil, respectively. nd: Not detected. Fatty acid content is expressed as area present FAME and % (by dry weight). Each value is a mean of triplicate samples. Value in a row with different superscript alphabets is statistically significant ($P< 0.05$; t-test).

pentaenoic acid and 0.53 to 2.23% doco-sahexaenoic acid in 0 – 4% emulsified O. *niger* liver oil (Table 3). Similarly during 18 and 24 h of enrichment, the range of saturated fatty acids recorded was from 22.73 to 30.64 and 24.01 to 31.18% DW respectively. The range of monounsaturated fatty acids was 27.43 to 31.39 and 27.76 to 31.63% DW, respectively. Likewise, the poly-unsaturated fatty acids (PUFA) ranged from 32.78 to

Table 5. Fatty acid composition of *A. franciscana* enriched for 24 h with increasing concentrations (A-E) of emulsified *O. niger* lipid.

Carbon No.	A	B	C	D	E
9 : 0	nd	nd	nd	nd	nd
10 : 0	nd	nd	nd	0.14	0.16
11 : 0	0.16	0.40	0.74	0.82	0.76
12 : 0	0.32	0.44	0.86	0.98	0.86
13 : 0	1.18	0.32	0.64	0.72	0.84
14 : 0	1.90	2.79	1.90	1.24	1.80
15 : 0	0.62	0.60	0.98	0.45	0.70
16 : 0	14.60	13.72	10.38	8.78	10.62
17 : 0	1.62	1.34	1.08	1.10	1.16
18 : 0	9.75	10.63	10.73	8.84	9.87
19 : 0	0.04	0.08	0.08	0.14	0.10
20 : 0	0.65	0.60	1.00	0.42	0.69
21 : 0	nd	nd	10.02	0.06	0.08
22 : 0	0.15	0.26	0.18	0.20	0.18
23 : 0	nd	nd	nd	0.12	0.14
24 : 0	nd	nd	nd	nd	nd
14 : 1	0.72	0.78	0.54	0.54	0.56
16 : 1	3.98	4.02	4.50	4.48	3.52
18 : 1	25.28	23.90	23.25	22.52	21.84
20 : 1	0.81	0.94	1.38	1.30	1.32
22 : 1	0.84	0.56	0.72	0.56	0.52
18 :2n-6	9.39 ^{a}	9.44 ^{a}	9.48 ^{b}	10.84 ^{c}	9.92 ^{bd}
18 :3n-3	17.64 ^{a}	19.84 ^{b}	20.92 ^{c}	23.84 ^{d}	20.42 ^{ce}
20:4n-3 + n-6	2.32 ^{a}	1.74 ^{b}	2.52 ^{c}	3.94 ^{d}	3.98 ^{de}
20:5n-3	2.78 ^{a}	3.29 ^{b}	4.70 ^{c}	5.88 ^{d}	5.02 ^{e}
22:5n-3	0.60	0.74	0.82	1.29	1.39
22:6n-3	0.62 ^{a}	1.18 ^{b}	1.90 ^{c}	2.69 ^{d}	1.44 ^{be}
Σ saturated	30.99	31.18	28.85	24.01	27.96
Σ monoenes	31.63	30.20	30.50	29.40	27.76
Σ PUFA	33.41	36.23	40.34	48.48	42.17

Diets A, B, C, D and E contain 0, 1, 2, 3 and 4% *O. niger* liver oil, respectively. nd: Not detected. Fatty acid content is expressed as area present FAME and % (by dry weight). Each value is a mean of triplicate samples. Value in a row with different superscript alphabets is statistically significant ($P< 0.05$; t-test).

46.99 and 33.41 to 48.48%. The individual HUFA such as linoleic, linolenic, arachidonic, eicosapentaenoic and docosahexaenoic acids were slightly increased from 9.22 to 10.84, 17.53 to 23.84, 1.62 to 3.98, 2.70 to 5.88 and 0.58 to 2.69% DW, respectively, during 18 and 24 h enrichment (Tables 4 and 5).

The increase in level of individual HUFA of *A. franciscana* enriched with various concentrations of emulsi-

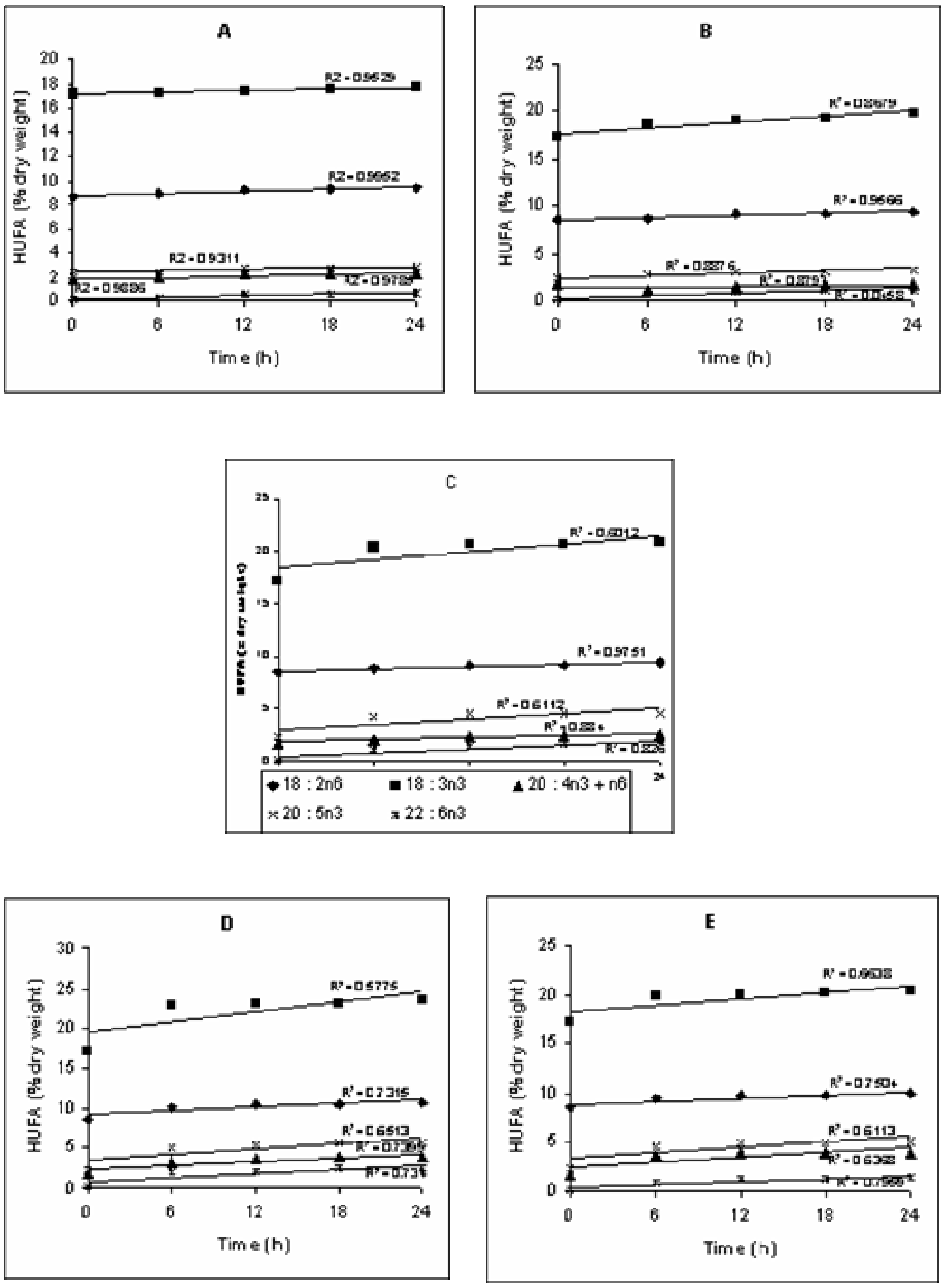

Figure 1. Changes in the contents of selected HUFA (% dry weight) in *A. franciscana* during different hours (6-24 h) of enrichment in relation with different concentrations. (A-E) of *O. niger* lipid. Diets A, B, C, D and E contain 0, 1, 2, 3 and 4% *O. niger* liver oil, respectively.

sified liver oil at different time durations showed a positive linear relationship. The HUFA such as linoleic (R^2 = 0.7315 to 0.9952), linolenic (R^2 = 0.5775 to 0.9529), arachidonic (R^2 = 0.6368 to 0.9785), eicosapentanoic (R^2 = 0.6112 to 0.9311) and docosahexaenoic (R^2 = 0.0458 to 0.9886) acids were positively correlated ($P < 0.05$) (Figure 1).

The percentage increase in individual monounsaturated fatty acids of *A. franciscana* enriched with various concentrations (0 – 4%) of emulsified *O. niger* liver oil

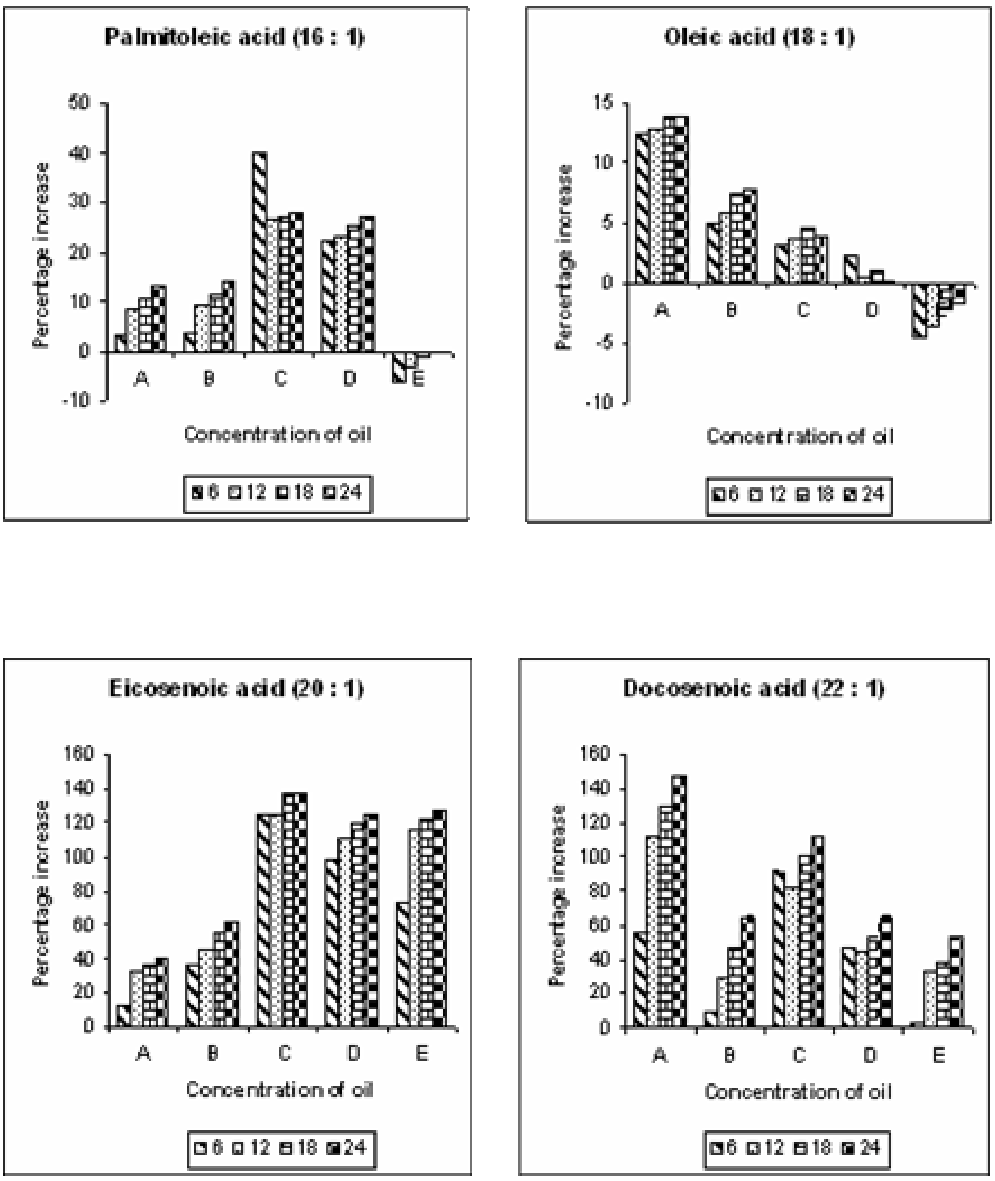

Figure 2. Percentage increase in individual monounsaturated fatty acids of *A. franciscana* enriched with various concentrations (A - E) of emulsified *O. niger* lipid over the control (freshly hatched *A. franciscana*) at various time duration (6 to 24 h). Diets A, B, C, D and E contain 0, 1, 2, 3 and 4% *O. niger* liver oil, respectively.

over the control (freshly hatched *A. franciscana*) at various time duration (6 to 24 h) of enrichment showed that plamitolic (16: 1) and oleic (18: 1) acids were found to increase in *Artemia* enriched with 0 to 3% *O. niger* liver oil in all the enrichment duration over control. But at 4% concentration, these two fatty acids were found to decrease over control in all the enrichment duration (0 to –6.25% and –1.62 to –4.50%). But at the tested enrichment concentrations and durations, the eicosenoic (20:1) and docosonic (22:1) acids were established increasing trend (12.10 to 137.93% and 2.94 to 147.06%) over control (Figure 2).

The individual HUFA like linoleic, linolenic, eicosapentanoic and docosahexaenoic acids were found to increase in all the concentrations and also during the enrichment durations over the control with the maximum accumulation in 3% concentration with respect of 17.9 to 24.65, 33.14 to 36.86, 112.5 to 136.67% and 1800 to 2460% in different enrichment durations (6 – 24 h). But the arachidonic acid level was increased over control in all the tested concentrations except 1% and at this level, it showed declining trend over the control (-3.33 to –35.6%) (Figure 3).

From the results, it is evident that, among the five tested

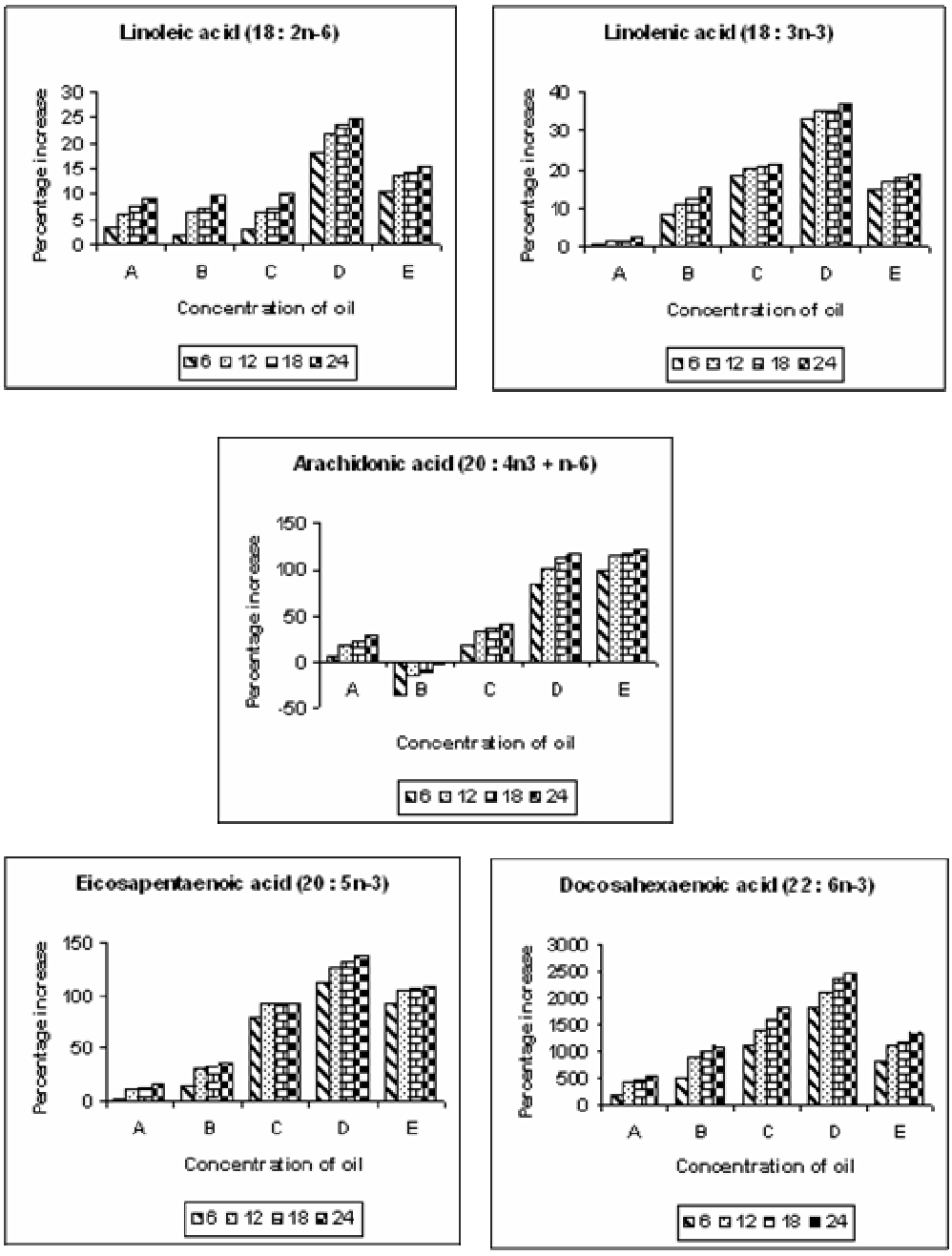

Figure 3. Percentage increase in individual PUFA of *A. franciscana* enriched with various concentrations (A - E) of emulsified *O. niger* lipid over the control (freshly hatched *A. franciscana*) at various time duration (6 to 24 h). Diets A, B, C, D and E contain 0, 1, 2, 3 and 4% *O. niger* liver oil, respectively.

emulsified concentrations of *O. niger* liver oil, 3% was found to be optimum for maximizing the PUFA content in *A. franciscana* at the enrichment durations of 6 to 24 h.

DISCUSSION

Fish oils contain the most important essential fatty acids for larval forms of cultivable organisms. These fatty acids are required not only for membrane formation and osmoregulation but also play an active role in immune systems. Variability in essential fatty acid content and low fatty acid levels in traditional live feed used in shrimp larviculture stimulated research on the commercial development of n-3 HUFA rich supplemented diets. Several studies have demonstrated the need of essential fatty acids (EFA) and have shown how it differs considerably

from species to species as well as within the species (Castell et al., 1972; Watanabe et al., 1982; Millamena et al., 1988; Abelin, 1991). Rees et al. (1994); Citarasu et al. (1998) and Immanuel et al. (2004) have reported the beneficial effect of HUFA on the survival of shrimp species like *Penaeus monodon* and *Penaeus indicus*.

To determine the n-3 and n-6 HUFA requirements of various marine finfish and shellfish species, several researches have been carried out world wide to develop standard lipid emulsion. In the present study also, standard emulsified lipids providing different n-3 and n-6 HUFA contents were prepared for assessing the level of the HUFA incorporation in *A. franciscana* nauplii.

The geographical origin of the *Artemia* species, the enrichment diet and the enrichment conditions (initial developmental stages of nauplii, enrichment time, dose and type of emulsion) are the most obvious factors known to influence the enrichment results (Leger et al. 1987). In the present study, *A. franciscana* (Great Salt Lake strain, USA) second instar stage nauplii were enriched with five different concentrations (0 – 4%) of *O. niger* lipid for four different enrichment period with 6 h interval (t6 – t24).

According to Tamaru et al. (2003), the enrichment process is time dependent. As the *Artemia* takes up the fatty acids, their fatty acid profile changes according to the duration of the enrichment period. Newly hatched *Artemia* had 7.0 mg total fatty acids/100 mg dry weight *Artemia* with no detectable levels of DHA (docosahexaenoic acid). After enrichment for 12 h, the total fatty acids increased significantly to 10.3 mg/100 mg and the *Artemia* had significantly higher amounts of essential fatty acids in the n-3 and n-6 families. After 24 h of enrichment, significantly higher levels of essential fatty acids and total fatty acids were achieved. Their study indicated that the duration of the enrichment process should be considered when preparing *A. franciscana* as a food for larvae of the ornamental fish.

In the present study, the fatty acid composition of *A. franciscana* enriched with emulsified *O. niger* liver oil differed considerably as a function of enrichment duration as well as enrichment concentrations. In freshly hatched *A. franciscana*, the HUFA content was 30.10%, whereas it seemed to increase from 31.03 to 44.18% during 6 h enrichment in respective concentrations (0 – 4%). Again, when the enrichment duration increased, the level of these HUFA were also found to increase considerably that is, 32.41 to 46.16, 32.78 to 46.99 and 33.41 to 48.48%DW during 18 and 24 h enrichment respectively in respective concentrations.

According to Kyungmin et al. (2000), the highest DHA value detected after 24 h enrichment was 29.9 mg g^{-1} dry weight using the 50% n-3 HUFA emulsion at 0.3 gL^{-1}. This is intermediate between the values of 36 mg docosohexaenoic acid g^{-1} dry weight reported by Evjemo et al. (1997) and the value of 21.0 mg docosohexaenoic acid g^{-1} dry weight of 24 h enriched *A. franciscana* reported by Coutteau and Mourente (1997) both using an ICES emulsion containing 30% n-3 HUFA (0.3 and 0.25 L^{-1}, respectively). In the present study also, the level of EPA (Ecosapentaenoic acid) and DHA (docosahexaenoic acid) were 2.40 and 0.1% DW in freshly hatched *A. franciscana*, but this level increased from 2.45 to 5.1% DW for EPA and 0.3 to1.9% DW for DHA during 6 h enrichment in all the tested concentrations, whereas again these two fatty acids were found to increase marginally that is, 2.78 to 5.88% DW (EPA) and 0.62 to 2.69% DW (DHA) during 24 h of enrichment. Moreover, the other important HUFA like linoleic, linolenic and arachidonic acids were found to increase to 8.60, 17.20 and 1.80% DW, respectively, in freshly hatched *A. franciscana* to the maximum of 10.84, 23.84 and 3.98% DW, respectively, in 24 h enriched *A. franciscana*.

The fatty acid level in the *A. franciscana* is the result of rapid and complex metabolic process of absorption, incorporation into body lipids and catabolism. It is well documented in marine vertebrates such as fish that n-3 HUFA levels correlate well with dietary n-3 HUFA supply (Sargent et al., 1993). Kyungmin et al. (2000) suggested that, within each enrichment trial, differences in the amount of exogenously supplied DHA were reflected in the nauplii whole body. The n-3 HUFA metabolism in *A. franciscana* contrasts, however, with that in most marine vertebrates and even marine invertebrates such as copepods is that they appear to store DHA principally in the triglyceride fraction from where it is very rapidly categorized during starvation (Barclay and Zeller, 1996; Coutteau and Mourente, 1997; Estevez et al. 1998; Navarro et al. 1999). In the present study similar or even higher DHA level was recorded when different concentrations of *O. niger* lipid enrichment in *A. franciscana* nauplii in different intervals of enrichment.

Fatty acid composition of lipid enriched *A. franciscana* depends on the concentration of lipid in the enrichment diet (Immanuel et al. 2004). The former referred authors reported that the fatty acid composition of control and lipid enriched *A. franciscana* was altered much and in particular PUFA content increased from 32.41% in the unenriched *A. franciscana* to 46.16% DW in those receiving the 3% lipid enrichment. A similar increase in PUFA (8%) over the non enriched *A. franciscana* was observed within 24 h of enrichment using different concentrations of booster diets with vegetable and fish oils was also reported by Buzzi (1989). Rees et al. (1994) reported that the fatty acids 20:5n-3 and 22:6n-3 increased from 0.04 to 0.03% respectively, in the control *A. franciscana* to 1.15 and 5.10% respectively in 12 h, 400 ppm Selco enriched *A. franciscana*. Immanuel et al. (2004) reported the *A. franciscana* enriched with different levels of lipid after 6 – 12 h of enrichment period, these two fatty acids were increased considerably (2.45 to 5.43% and 0.3 to 2.23% DW respectively). In the present study also, after 24 h enrichment period, these two fatty acids increased from 2.78 to 5.88 and 0.62 to 2.69% DW,

respectively.

The linolenic acid (18: 3n-3), which was very abundant in freshly hatched *A. franciscana* (17.20%) increased from 0.04 to 5.7% during 6 h enrichment and 0.27 to 6.03% in 12 h enrichment in various levels of lipid enriched *A. franciscana* (Immanuel et al., 2004). In the present study also, the level of the same fatty acid was 17.64 in 0% lipid enriched *A. franciscana*, however, it increased from 2.24 to 5.9% during 24 h enrichment period when the concentration of lipid increased in enriched *A. franciscana*. In contrast to this, Rees et al. (1994) observed a higher level of linolenic acid (18: 3n-3) in control *A. franciscana* and it was gradually decreased (approximately 4 – 6%) when the concentration of the Selco product increased.

The enrichment process for *A. franciscana* nauplii significantly elevate all of the fatty acids found in the nauplii. Most noble was the elevation of DHA and EPA. These two fatty acids are essential for larval growth and development in number of finfish and shellfish species. It was seen that higher DHA and EPA values were obtained with 3% *O. niger* lipid emulsion and also these values were increased manifold times during 24 h enrichment.

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