Asian Fisheries Science **14**(2001): 377-388 ISSN: 0116-6514
https://doi.org/10.33997/j.afs.2001.14.4.003

Asian Fisheries Society, Manila, Philippines

Effects of Feeding Lipid Enriched *Artemia* nauplii on Survival, Growth, Fatty Acids and Stress Resistance of Postlarvae *Penaeus indicus*

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Abstract

A study was carried out to determine the influence of emulsified fish *Odonus niger* liver oil (0 to 4%) enriched *Artemia* nauplii (A to E) on survival, growth, stress resistance and highly unsaturated fatty acid (HUFA) content of postlarvae of *Penaeus indicus* (PL5 to PL20). Maximum specific growth rate of 26.5% was recorded in diet D and a minimum of 24.2% in diet A fed individuals. Survival of *P. indicus* fed with lipid enriched *Artemia* naupli was higher than the control diet.The salinity stress resistance (0 ppt) of *P. indicus* revealed that, the individuals fed with diet D were more stress resistant (45 min) than those that received other diets (35 min). At high salinity (50 ppt), diets A and B fed individuals survived up to 360 min while in other diets (C, D and E) the larvae survived up to 420 min. In both low and high salinities a cumulative mortality index (CMI) of 106.0 and 84.0 was recorded for diet A fed groups and reduced by 26.41 and 34.52% for those receiving diet D. The fatty acid profile of the *Artemia* nauplii enriched with selected concentrations (0, 1, 2, 3 and 4%) of *O. niger* oil was varied remarkably and accordingly. The polyunsaturated fatty acid (PUFA) showed an increasing trend in enriched *Artemia* nauplii, ranging from 31.03% (by weight) in control (A) to 44.18% (by weight) in 3% (D) oil. Similarly the PUFA of *P. indicus* postlarvae (PL5 to PL20) fed with the lipid enriched *Artemia* nauplii increased from 26.61% (by weight) in control (A) to 42.33% (by weight) in postlarvae fed with 3% (D) oil enriched *Artemia* nauplii. The overall performance of growth enhancement, stress tolerance and fatty acid profile of *P. indicus* revealed that, those fed with diet D were more efficient than the other groups.

Introduction

Growth and survival of marine fish or crustacean larvae are often influenced by the size and dietary value of the organisms used as food (Sorgeloos 1981). Nutritional research on the cultivable species of aquatic animals has been receiving great attention all over the world. Investigations on the nutritional quality of live feeds revealed the importance of essential fatty acids (EFA) in larval nutrition. As a consequence, *Artemia* nauplii with maximum

EFA have been mass produced for larviculture. However, frequent feeding of *Artemia* alone resulted in high mortalities in various marine fishes (Fujita 1973 and Kitajima 1978).

Considering the prerequisites of n-3 HUFA in live feeds, various studies have been made to improve their availability through enrichment methods. Supplementation of HUFA enriched *Artemia* nauplii improved the survival and growth in shrimp larval stages (Chamberlain 1988, Leger and Sorgeloos 1994). Recently several authors have investigated the requirements of n-3 HUFA by feeding various levels of HUFA enriched *Artemia* nauplii to finfish and crustacean and the effect of this supplementation on growth and stress resistance. Rees et al. (1994) evaluated the resistance to osmotic shocks of *P. monodon* postlarvae fed with HUFA enriched *Artemia* nauplii and also analyzed the fatty acid changes of experimental animals. Tackaert et al. (1989), Dhert et al. (1992), Kraul et al. (1993) and Ako et al. (1994) studied the stress resistance in *Mugil cephalus* and larval mahimahi (*Coryphaena hipparus*) respectively. The aim of the present work is to study the effect of feeding lipid enriched *Artemia* nauplii to *P. indicus* postlarvae (PL5-PL20) on the survival, growth, stress resistance and fatty acid changes.

Materials and Method

Four-day old *P. indicus* postlarvae (PL4) were purchased from a local shrimp hatchery, M.M. Aquapark, Chettikulam, Tamilnadu, India and stocked in a cylindrical fiber glass tank (150 l capacity) filled with chlorine disinfected (5 ppm) sea water (35 ppt). The larvae were starved for 24 h before the commencement of the feeding experiment. The feeding experiment was conducted for 15 days (PL5 to PL20).

Preparation of emulsified lipid diets

The enrichment diet was prepared in our laboratory following the method of Watanabe et al. (1982 and 1983). These emulsified diets were prepared using 0 to 4% *O. niger* (Trigger fish) liver oil produced in our laboratory (Immanuel 1996), supplemented with egg yolk, vitamins (water and fat soluble) (Table 1) and were taken in a homogenizer along with 100 ml water and homogenized for 2 to 3 min to form an emulsion. The stability of the emulsion was checked before use and was refrigerated.

Enrichment procedures

The second instar stage *Artemia* nauplii (*Artemia franciscan*a-Great Salt Lake) were separated from the hatching container through a 120 µm sieve, rinsed with filtered sea water and transferred to 5 l enrichment containers at a density of 100 nauplii ml^{-1} of sea water (35 ppt) at room temperature (28 ± 10°C). The five different diets were prepared with varying concentrations of *O. niger* liver oil (A, B, C, D and E) mixed with 200 $mg \cdot l^{-1}$ baker's

yeast. Strong aeration was provided to the rearing containers to keep the oxygen level at 5 ppm. Baker's yeast was added to prevent starvation of control nauplii, which is known to reduce their lipid content (Benjits et al. 1976). The enrichment period was 6 h, after which the *Artemia* nauplii were harvested and rinsed with sea water.

Feeding experiment

After measuring the length and weight, healthy postlarvae (PL5) of *P. indicus* were transferred to experimental tanks containing 10 l of filtered sea water with ambient temperature (28 ± 10^0C) and salinity (35 ppt). The larvae were maintained at a stocking density of 15 1^{-1}. Mild aeration was given continuously to keep the dissolved oxygen at the optimum level (> 5.0 mg·l). The entire experiment was carried out with five replicates.

Feeding schedule

During the experimental period *P. indicus* larvae were fed at *ad libitum* with lipid enriched *Artemia* nauplii, offered four times per day (6, 10, 14 and 18 h) at the rate of 10, 10, 20 and 60%, respectively. The unfed *Artemia* nauplii were removed from the *P. indicus* larval rearing tanks after the respective hours of feeding using an appropriate sieve. Ten percent of the daily ration was provided at the first feeding when *Artemia* nauplii were freshly enriched. To maintain the nutritional quality of the *Artemia* nauplii, the remaining enriched *Artemia* nauplii were kept in cold storage at 2 to 100^0C with gentle aeration (Leger et al. 1983 and 1986).

Stress study

In an attempt to evaluate the physiological condition and possibly the quality of the shrimp postlarvae, the resistance of the postlarvae fed for 15 days was exposed to salinities of 0 and 50 ppt in 5 l beaker with 2.5 l water (25 animals each with 3 replicates), respectively. Survival was monitored at

Table 1. The composition of five different emulsified lipid diets per 100 ml water.

Diets	*O. niger* liver oil (%)	Egg yolk (g)	Fat soluble vitamins* (g)	Water soluble vitamins** (g)	Baker's 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

*Becozinc (Water Soluble Vitamin B+C)
(Capsule of Zinc, B complex fortified with Vitamin C)
**Rovigon (Fat Soluble Vitamin A+E)
(Roch products Ltd. Bombay)

regular intervals of 5 min. each for all the stress studies except for the stress performance at 50 ppt (60 min. intervals) until all the animals succumbed. Postlarvae not reacting to gentle mechanical stimulation using a soft paintbrush were considered dead. The nonreacting animals were not removed from the beakers until the end of the test.

Cumulative mortality index (CMI)

The CMI was calculated by summing up the mortality counts measured at each time interval over the experimental period as follows.

CMI = DX1 + DX2 + DX3 + ...+DXn,

where D is the number of dead individuals at time X1, X2, X3,.....Xn. The higher the CMI value, the lower the resistance to salinity shock.

Fatty acid analysis

Fatty acid composition of both *Artemia* nauplii diets and test animals (*P. indicus*, PL20) were analyzed and estimated following the method of Miller and Berger (1985) using Gas Chromatography. The results are expressed as area percent fatty acid methyl esters (FAME).

Statistical analysis

The data obtained in this experiment were subjected to students t-test and analysis of variance following Snedecor and Cochran (1973).

Results

Survival

The highest survival rate of 88.93% was recorded for the *P. indicus* postlarvae (PL20) fed with diet C. Shrimp larvae reared on control diet exhibited the lowest survival rate of 61.79%. Shrimps fed with diets B, D and E registered survival rates between 71.19 and 74.66% (Table 2). Analysis of variance revealed that variation in the survival rate of *P. indicus* fed with control and experimental diets was statistically significant ($F_{1, 20}$ to 4, 5.08; $P<0.05$).

Specific growth rate

The specific growth rate was almost the same for all the test diets fluctuating from 26.5% in diet D to a minimum of 24.2% in the control diet (Table 2). However, statistical analysis of the specific growth rate by “t” test

showed significant differences (P<0.05) among diets A, C and D fed *P. indicus* postlarvae.

Stress resistance, salinity tolerance, treatment at 0 ppt salinity

P. indicus postlarvae (PL20) exposed to a salinity stress from 35 ppt to 0 ppt and fed on control diet A and diet B died within 35 min. Animals fed on diet D had a higher stress tolerance up to 45 min. The postlarvae fed on diets C and E had an intermediate stress tolerance (Fig.1). The CMI calculated for the *P. indicus* postlarvae are provided in table 3. Compared to the control group (A), the CMI was reduced by 11.32, 16.98, 26.41 and 14.15% in diets B, C, D and E, respectively. Statistical analysis revealed that the CMI of the control diet fed group was significantly different (P<0.05) from those of the other diets except diets B and E.

Treatment at 50 ppt salinity

The results obtained for the stress test conducted at 50 ppt salinity are illustrated in figure 2. *P. indicus* postlarvae fed with control diet A and diet

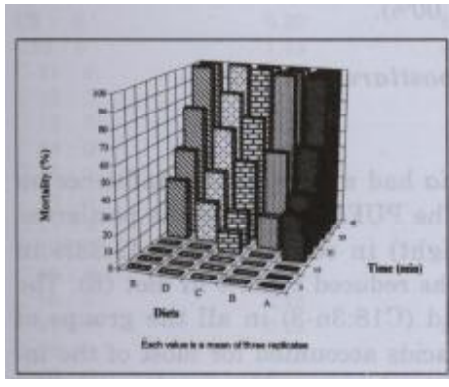

Fig. 1. Mortality (%) of *P. indicus* postlarvae (PL20) exposed at 0 ppt salinity stress.

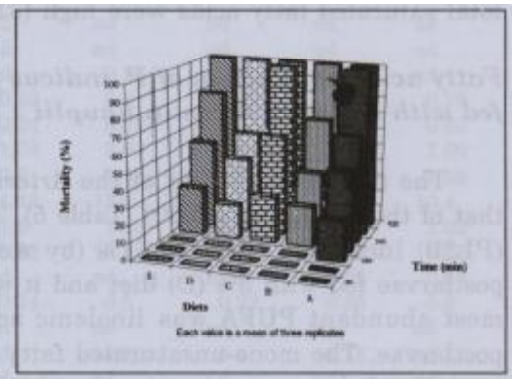

Fig. 2. Mortality (%) of *P. indicus* postlarvae (PL20) exposed at 50 ppt salinity stress.

Table 2. Survival (%) and specific growth rate (%) of *P. indicus* postlarvae fed with different levels of lipid enriched *Artemia* nauplii. Each value (X ± S.D) is the mean of 50 individuals estimate.

Diets	Initial length (mm)[1]	Initial wet weight (mg)[1]	Final length (mm)[2]	Final wet weight (mg)[3]	Survival (%)	Specific growth rate (%)[4]
A	7.0 ± 0.61	1.2 ± 0.07	21.8 ± 3.41	49.1 ± 1.01	61.79 ± 4.03	24.26 ± 0.73[a]
B	7.2 ± 0.32	1.2 ± 0.06	23.4 ± 2.69	53.3 ± 2.48	72.13 ± 6.44	24.94 ± 0.18[a]
C	7.3 ± 0.40	1.2 ± 0.06	23.9 ± 2.94	59.0 ± 2.60	88.93 ± 3.54	25.52 ± 0.35[b]
D	7.7 ± 0.65	1.3 ± 0.02	24.7 ± 3.50	71.0 ± 4.08	74.66 ± 3.52	26.56 ± 0.44[c]
E	7.8 ± 0.35	1.3 ± 0.03	23.6 ± 2.90	55.9 ± 1.95	71.19 ± 3.58	24.90 ± 0.23[a]

[1]Measured on 50 individuals / treatment
[2]Data from individual tanks (n=5)
[3]One way ANOVA - Statistically significant (p<0.05)
[4]Values in a column superscript with different alphabets are statistically significant (p<0.05; "t" test).

B exposed at 35 ppt could survive up to 360 min. At the same time *P. indicus* (PL20) fed with diets C, D and E exhibited 68, 56 and 80% mortality. Compared to the control diet A fed shrimps, the CMI was reduced by 30.95, 14.28, 34.52 and 22.61% in diets B, C, D and E respectively (Table 3). These differences in stress reduction percentage were statistically significant at $P<0.05$.

Fatty acid composition of enriched artemia nauplii

The fatty acid composition of the *Artemia* nauplii is summarized in table (4). The polyunsaturated fatty acids (PUFA) such as linoleic acid (C18: 2n-6), linolenic acid (C18:3n-3), arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) showed the highest increment in the nauplii, ranging from 31.03% (by weight) in control (A) to 44.18% in the *Artemia* enriched with 3% oil emulsion (D). The most abundant PUFA was linolenic acid. The detected mono-unsaturated fatty acids in all enriched *Artemia* nauplii showed only a slight variation ranging between 26.25 to 30.23%. The total saturated fatty acids of the *Artemia* nauplii enriched with 3% (D) oil was low (23.04%). In 1% (B) oil enriched nauplii, the total saturated fatty acids were high (31.00%).

Fatty acid composition of P. indicus postlarvae (PL20) fed with enriched Artemia nauplii

The fatty acid content of the *Artemia* had a considerable influence on that of the postlarvae (PL20) (Table 5). The PUFA content of the postlarvae (PL20) increased from 26.61% (by weight) in control (A) to 42.23% in postlarvae fed with 3% (D) diet and it was reduced to 32% in diet (E). The most abundant PUFA was linolenic acid (C18:3n-3) in all the groups of postlarvae. The mono-unsaturated fatty acids accounted for most of the increase and their concentration was high in the control group (A) (27.45%) and low (23.11%) in the 3% (D) oil enriched *Artemia* nauplii fed postlarvae. The total saturated fatty acids of the different groups of postlarvae were

Table 3. Cumulative Mortality Index (CMI) value of *P. indicus* postlarvae reared on *Artemia* nauplii enriched with different lipid diets exposed at low and high salinities (0 and 50 ppt).

Diets	0 ppt (up to 45 min.)		50 ppt (up to 420 min.)	
	CMI	Reduction of Stress (%)	CMI	Reduction of Stress (%)
A	106^{a}	0	84^{a}	0
B	94^{ab}	11.32	58^{b}	30.95
C	88^{cb}	16.98	72^{c}	14.28
D	78^{ab}	26.41	55^{dc}	34.52
E	91^{ab}	14.15	65^{+E11bc}	22.61

Postlarvae were transferred from full strength seawater (35 ppt) to freshwater (0 ppt) and high saline water (50 ppt). Each value is the mean of 3 replicates. Within each column, means with the same superscript are not statistically significant ($P<0.05$).

37.04, 36.19, 32.33, 29.31 and 33.21% in control (A), 1% (B), 2%(C), 3%(D) and 4%(E) oil enriched *Artemia* nauplii fed *P. indicus* postlarvae (PL20), respectively.

Discussion

One of the main objectives of developing larval rearing strategies is the establishment of a feeding regime that results in optimal growth, survival and health of the finfish and shellfish larvae. Several studies have demonstrated that the need for essential fatty acids (EFA), differs considerably from species to species as well as within the species. Rainbow trout requires fatty acids of the linolenic family (n-3) as EFA (Castell et al. 1972), whereas carp, eel and chum salmon require not only linolenic acid but also linoleic acid for good growth (Watanabe et al. 1982). Similarly with *P. japonicus*, 1% linoleic or linolenic acid supported their best growth and survival (Kanazawa

Table 4. Fatty acid composition *O. niger* liver oil and *Artemia* nauplii enriched with increasing concentration (A - E) of emulsified *O. niger* liver oil.

Carbon No	*O.niger* liver oil	A	B	C	D	E
C9 : 0	0.39	nd	nd	nd	nd	nd
C10 : 0	1.43	nd	nd	nd	nd	nd
C 11 : 0	1.14	nd	0.32	0.7	0.73	nd
C 12 : 0	nd	0.28	0.4	0.83	0.9	0.78
C 13 : 0	1.16	0.24	0.57	0.63	0.64	0.83
C 14 : 0	3.05	1.78	2.67	1.93	1.14	1.69
C 15 : 0	2.06	0.9	1.48	1.82	1.26	1.38
C 16 : 0	18.5	14.48	13.4	10.1	8.43	10.4
C 17 : 0	1.03	1.5	1.05	0.85	0.93	1.02
C 18 : 0	14.7	9.62	10.5	10.5	8.46	9.76
C 19 : 0	nd	nd	nd	nd	0.05	0.03
C 20 : 0	2.42	0.54	0.5	0.92	0.36	0.6
C 21 : 0	0.12	nd	nd	nd	nd	nd
C 22 : 0	0.78	nd	0.19	0.1	0.14	nd
C 23 : 0	nd	nd	nd	nd	nd	nd
C 24 : 0	0.27	nd	nd	nd	nd	nd
C 14 : 1	0.43	0.45	0.47	0.32	0.41	0.4
C 16 : 1	4.32	3.64	3.65	4.92	4.3	3.3
C 18 : 1	17.84	24.96	23.3	22.9	22.7	21.2
C 20 : 1	4.02	0.65	0.79	1.3	1.15	1
C 22 : 1	3.8	0.53	0.37	0.65	0.5	0.35
C 18 : 2w6	12.87	8.9	8.76	8.87	10.14	9.5
C 18 : 3w3	0.21	17.24	18.7	20.4	22.9	19.8
C 20 : 4w3+w6	2.66	1.9	1.16	2.12	3.31	3.6
C 20 : 5w3	2.86	2.45	2.75	4.32	5.1	4.65
C 22 : 5w3	1.64	0.24	0.16	0.46	0.83	1.05
C 22 : 6w3	2.3	0.3	0.6	1.23	1.9	0.95
Total saturated fatty acids	47.05	29.34	31.00	28.4	23.04	26.5
Total monounsaturated fatty acids	30.41	30.23	28.6	30.1	29.06	26.3
Total polyunsaturated fatty acids	22.54	31.03	32.10	37.4	44.18	39.6

nd : not detected

Fatty acid content is expressed as area present FAME and % (by dry weight). Each value is a mean of two replicate samples.

et al. 1979). Moreover, EFA has a role in contributing to a better immune system as well as disease resistance in several finfish and shellfish species. In the present study, EFA enriched *Artemia* nauplii fed *P. indicus* postlarvae displayed higher survival rate, specific growth rate and stress resistance.

The observation suggests that lipids are important for *P. indicus* postlarvae especially for survival. In the present study the overall survival of the control group fed with enriched *Artemia* nauplii devoid of lipid registered the lowest survival (61.79%) as compared with those reared other lipid enriched diets (>70%). Xu et al. (1993) showed that Chinese prawn (*P. chinensis*) fed the control diet (EFA deficient diet containing 16:0 and 18:1n-9 as the supplemented fatty acid sources) experienced low survival and growth; feeding with a diet with 1% 18:2n-6 has improved the survival of the prawns. Rees et al. (1994) also observed that survival increased when shrimps were fed with HUFA enriched *Artemia* nauplii. Enrichment with 200 to 300 ppm SELCO resulted in a significantly better survival (>60%) than the control (newly hatched *Artemia* nauplii).

Table 5. Fatty acid composition of *P. indicus* postlarvae (PL20) fed with *Artemia* nauplii enriched with increasing concentration (A - E) of emulsified *O. niger* liver oil.

Carbon No	A	B	C	D	E
C 11 : 0	nd	0.16	0.35	0.35	0.37
C 12 : 0	nd	nd	0.24	0.2	nd
C 13 : 0	nd	nd	0.42	0.51	0.03
C 14 : 0	1.56	0.9	1.04	1.78	1.38
C 15 : 0	1.20	0.86	0.53	0.58	0.47
C 16 : 0	17.30	17.44	14.05	12.62	15.35
C 17 : 0	1.94	1.41	1.21	1.71	1.25
C 18 : 0	14.66	15.34	13.43	10.82	14.32
C 19 : 0	nd	nd	0.08	0.02	nd
C 20 : 0	0.38	0.24	0.88	0.67	0.04
C 21 : 0	nd	nd	0.06	0.02	nd
C 22 : 0	nd	nd	0.04	0.03	nd
C 23 : 0	nd	nd	nd	nd	nd
C 24 : 0	nd	nd	nd	nd	nd
C 14 : 1	0.1	0.32	0.23	0.25	0.36
C 16 : 1	2.9	2.54	1.88	1.97	2.75
C 18 : 1	23.83	22.5	20.16	19.35	21.04
C 20 : 1	0.46	0.67	0.76	1.04	0.9
C 22 : 1	0.16	0.46	0.25	0.5	0.27
C 18 : 2w 6	5.98	7.31	8.96	11.4	8.17
C 18 : 3w3	13.28	14.1	16.38	16.84	13.26
C 20 : 4w3+w6	2.63	2.49	5.61	5.73	4.35
C 20 : 5w3	3.84	4.42	5.22	6.1	4.15
C 22 : 5w3	0.06	0.1	0.15	0.42	0.61
C 22 : 6w3	0.82	1.9	1.85	1.74	1.46
Total saturated fatty acids	37.04	36.19	32.33	29.31	33.21
Total monounsaturated fatty acids	27.45	26.49	23.28	23.11	25.32
Total polyunsaturated fatty acids	26.61	30.32	38.17	42.23	32.00

nd : not detected
Fatty acid content is expressed as area present FAME and % (by dry weight). Each value is a mean of two replicate samples.

Millamena et al. (1988) compared the culture performance of *P. monodon* postlarvae fed *Artemia* enriched with either ricebran (HUFA deficient) or two algal species containing different levels of HUFA. The PL10 postlarvae, reared for 20 days on *Artemia* enriched with algal had better growth than those fed with unenriched *Artemia*. The fact is that the growth enhancement of *Artemia* enriched with algae might supply the vital components (vitamins and minerals) other than HUFA. Moreover the influence of HUFA on the culture performance of postlarvae of penaeid shrimp also varied from species to species. In *P. vannamei*, Leger et al. (1987) reported that despite improved growth performance of late larval and postlarval stages (Mysis 3 to PL8), the survival was not greatly different between control and HUFA enriched (600 ppm SELCO for 24h) diet fed groups. Similarly in *P. monodon*, Rees et al. (1994) reported marked variation in stress resistance between control and HUFA enriched *Artemia* fed groups, even though the growth difference did not vary that much. Tackaert et al. (1989) also observed that HUFA affects postlarvae stress resistance more during the early stages (PL5 to PL10) than in later stages.

Fatty acid composition of control and lipid enriched *Artemia* nauplii varied much in the present study and in particular PUFA content increased from 31.03% in the unenriched *Artemia* nauplii to 44.18% in those receiving the 3% oil enrichment. A similar increase in PUFA (8%) over the non-enriched *Artemia* nauplii (Great Salt Lake) within 24 h of enrichment using different concentrations of booster diets with emulsified vegetable and fish oils was also reported by Buzzi (1989). Leger et al. (1986) reported that *Artemia* nauplii (Great Salt Lake strain) enriched with mixed lipid enrichment booster for about 24 h had PUFA content with 9.9% of 20:5 n-3 and 5.9% of 22:6 n-3 as a percentage of total fatty acids. In the present study, after 6h enrichment period, these two fatty acids considerably increased (2.45 to 5.1% and 0.3 to 1.9%) in the different levels of lipid enriched *Artemia* nauplii. As reported by Rees et al. (1994) 22:5n-3 was increased from 0.04% in control *Artemia* nauplii to 1.15% in 400 ppm 12 h SELCO enriched nauplii; 22:6n-3 also increased from 0.30 to 5.10%, respectively.

The linolenic acid (18:3n-3) which was very abundant in control *Artemia* nauplii (17.24%) had increased from 1.5 to 5.5% during the 6 h enrichment in all the lipid enriched *Artemia* nauplii in the present study. In contrast, Rees et al. (1994) observed a higher level of linolenic acid (18:3n-3) in control *Artemia* nauplii and it was gradually decreased (approximately 4 to 6%) when the concentration of the SELCO product increased.

Leger et al. (1986) emphasized the advantage of enrichment and highlighted the inevitable energy loss in newly hatched nauplii, during the absorption of yolk. Previous trails on rearing *M. rosenbergii* larvae carried out using the same broodstock, showed that the *Artemia* nauplii containing 3.0% of 20:5n-3 and 1.0% of 22:6 n-3 (n-3 PUFA content = 25.7%) were superior to artificial diets, promoting a survival rate of 38.8% (Maclean 1986). The present result suggested that the dietary efficiency promoting development and survival is more related to the concentration of all the n-3 HUFA and this result is in consistence with an earlier report by Sandifer and Joseph (1976). They

demonstrated that a diet supplemented with 3% marine shrimp oil (containing 7% of 20:5n-3 and 6% of 22:6 n-3) promoted a remarkable growth rate in juvenile prawn, attesting the importance of n-3 fatty acids in *Macrobrachium* nutrition.

Further nutritional trials on the marine shrimp *P. japonicus* demonstrated that the n-3 PUFA's such as eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) were more effective in promoting growth than linoleic (18:2n-6) and linolenic (18:3n-3) acids (Guary et al. 1976, Kanazawa et al. 1979 and Kayama et al. 1980). Although in the present study the concentration of linolenic acid (18:3n-3) practically increased from 17.24% in control to 22.90 in 3% diet (D) and the low concentration resulted in poor performance in terms of survival and development rate up to a certain level, beyond this level (4% Diet E) the developmental rate decreased further in *P. indicus* postlarvae. The poor performance of higher lipid enrichment may be attributed to the formation of defective emulsion, which ultimately led to the non availability of n-3 HUFA for the *Artemia* nauplii. It was well evidenced by the low concentration of n-3 HUFA accumulation in higher lipid enrichment in the present study.

The result of the fatty acid analysis carried out in the control and experimental diets fed *P. indicus* postlarvae (PL20) showed that the individuals receiving enriched *Artemia* nauplii with 3% (diet D) emulsified lipid reflected higher percentage of total PUFA (42.23%), whereas in control diet it was very low (26.61%). Working on fresh water prawn *M. rosenbergii* larvae, Buzzi (1989) reported an enhanced PUFA level for those individuals fed with n-3 booster diet enriched *Artemia* nauplii. A similar observation was also found in *P. monodon* (PL20) fed with different levels of SELCO enriched *Artemia* nauplii (Rees et al. 1994).

The better stress resistance was noticed in the present study for *P. indicus* postlarvae (PL20) fed on 3% lipid enriched *Artemia* nauplii at low and high salinities (0 and 50 ppt) than the other diets. Studying the effect of n-3 HUFA, Horstmark et al. (1987) demonstrated that erythrocytes of rats fed HUFA rich cod liver oil achieved a higher resistance to hypo osmotic shock, an effect that probably resulted from a higher incorporation of n-3 HUFA in cell membranes. If a similar phenomenon also occurs in crustaceans, the better resistance of shrimp fed HUFA enriched *Artemia* may be attributed to the increased osmotic resistance of their cells, delaying the onset of irreversible damage in some essential tissues (Rees et al. 1994). It was observed that the gills of HUFA enriched postlarvae of *P. stylirostris* and *P. vannamei* of the same stage displayed a more ramified structure. Since the gills are the site of essential osmoregulatory mechanisms in circumstances, such an increase in the area of exchange might have resulted in better resistance to osmotic shocks. The possibility of the occurrence of said phenomenon in *P. indicus* postlarvae in the present study was not ruled out; but it may not be the sole mechanism for the better performance of individuals fed HUFA enriched diets (See also Rees et al. 1994). Besides providing additional information on shrimp nutrition research in early post larval stages, the salinity stress test could be of great help to farmers and hatchery operators. Some commercial hatcheries in South America and South East

Asia have already applied similar stress tests to determine the appropriate time for stocking postlarvae.

Conclusion

The present study concludes that, the less commercial value *O. niger* liver oil (3%) can also be utilized as dietary fatty acid source for optimum physiological performance in *P. indicus* postlarvae.

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Manuscript received 15 February 2000; Accepted 18 April 2001