Supply of Astaxanthin and its combinations through live feed (*Moina micrura*) enrichment affects the growth, survival and fatty acid profile of *Macrobrachium rosenbergii* larvae

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Abstract

A study was carried out with three replicates to determine the effects of feeding *Moina micrura* enriched with astaxanthin alone (M1) or astaxanthin in combination with either vitamin E (M2), vitamin D (M3) or Cod Liver oil (M4) on the growth, survival and fatty acid composition of giant fresh water prawn *Macrobrachium rosenbergii* (de Man) larvae. Growth rate was expressed as the time taken to the settlement of 95% post larvae. Maximum growth, the lowest time taken to the 95% PL settlement (38.5±0.50 days), was observed in larvae fed with M3 *Moina*. The highest survival rate (66.0±1.00%) was observed in those fed with M4 *Moina* and the second highest survival (61.0±1.00%) and growth rates (40.0±0.00 days) were shown with M2 *Moina*. The minimum values for both growth (42.5±0.50 days) and survival (33.0±1.50%) were observed in the group fed un-enriched *Moina*. Results also showed that the survival of prawn larvae increased as the quantities of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased in the dietary *Moina*. The highest levels of EPA (5.57±0.21%), DHA (3.50±0.21%) and highest total Highly Unsaturated Fatty Acids (HUFA) (13.87±0.68%) were seen in the Moina fed on astaxanthin and Cod Liver Oil (CLO).

The results of the study showed that the nutritive quality of *Moina*, with respect to important fatty acids, can be increased by enrichment and will influence the growth, survival and the fatty acid composition of fresh water prawn larvae fed on them.

Keywords: *Macrobrachium rosenbergii*, *Moina micrura* enrichment

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Introduction

Among paleimonid prawns, *Macrobrachium rosenbergii* is the most widely used species in aquaculture. Culture practices of this species have considerably expanded in recent years, especially in Asian countries (New, 2005). Each prawn grows to a weight of about 100-200 g in about 7-9 months with a wide divergence in growth rates (New, 1995). In most aquaculture systems, more than half the total expenditure is for nutrition as the feed requirement of culture organisms is often the most important factor influencing the economic success of the system.

Good quality larval feed is important for the rapid growth of the young fish or prawn in order to minimize the production cost as well as to maximize the production level. Live feed organisms are more favoured than artificial feed in larval and early post-larval stages of different fish and shell fish species (Das *et al.*, 2007); indeed, the larval stages of *M. rosenbergii* prefer live feed organisms, and at present *Artemia* is the most widely used larval feed. Seasonal variation in their availability and the high cost due to ecological changes in the natural habitats (Lavens and Sorgeloos, 2000), however, often limit the use of *Artemia* in hatcheries. According to Hagwood and Willis (1976), the feeding cost in hatcheries reach around 60% of the total cost and could increase further with enrichment practices for *Artemia*. For these reasons, it is necessary to develop a locally available low cost live feed organism which is nutritionally sound in order to minimize the risk in aquaculture practice. *Moina micrura*, a fresh water cladoceran, has shown promise as a supplement to *Artemia* in improving the production of *M. rosenbergii* larvae (Alam, 1992, Alam *et al.*, 1991, 1993). It is widely distributed all over the world and can be harvested throughout the year and culture is relatively inexpensive. Highly Unsaturated fatty acid (HUFA) especially EPA and DHA are essential dietary requirements for fish and prawn larvae during their early developmental stages (Owen *et al.*, 1975; Kanazawa *et al.*, 1979a, b; Watanabe, 1982; Devrese *et al.*, 1990). Enrichment of the live feed appears to be a good practice to enhance their nutritive value, especially with regard to these essential fatty acids. Like *Artemia, Moina* does not provide all the nutrients required for larval development of freshwater prawn (Das *et al.*, 2007). Researchers, therefore, have investigated the use of vitamins (Kollkovski *et al.*, 2000; Tarui *et al.*, 2006), microencapsulated diets (Sauthgate and Lou, 1995), unicellular algae (Watanabe *et al.*, 1980; Cho *et al.*, 2001), various emulsions (McEvoy *et al.*, 1995; Immanuel *et al.*, 2001; Liddy *et al.*, 2005; Moren *et al.*, 2006; Das *et al.*, 2007; Prabitha, 2007), liposomes (Monroig *et al.*, 2006), antibiotics (Gil *et al.*, 2001) and amino acids (Tonheim *et al.*, 2000) as enrichment media to enhance the nutritive value of the live feed.

The aim of the present study was to investigate the effects of enriching *Moina micrura* using astaxanthin - alone or in combination with vitamin E, D and cod liver oil - on the growth, survival and the fatty acid profile of prawn larvae fed on them.

Materials and Methods

Procurement of test animals

Healthy broodstock of *M. rosenbergii*, collected from a natural source, were maintained in hatchery brood stock tanks (1500 l) on both natural and artificial feed. After attaining maturity, berried females were transferred to 300 litre circular fiberglass tanks containing water with 12% salinity. The larvae obtained from those mother prawns were reared in 300 litre capacity round
tanks and were fed initially with *Artemia* nauplii.

Experimental design and maintenance of M. rosenbergii larvae

The experiment was conducted in fifty liter capacity plastic round tubs. The five treatments consisted of the following:
TC- unenriched *Moina micrura* (MC) fed larvae (Control)
T1- astaxanthin enriched *Moina* (M1) fed larvae
T2- astaxanthin and vitamin E enriched *Moina* (M2) fed larvae
T3- astaxanthin and vitamin D enriched *Moina* (M3) fed larvae
T4- astaxanthin and Cod Liver Oil (CLO) enriched *Moina* (M4) fed larvae

Each treatment had three replicates assigned to the 15 tanks in a completely randomized design.

The *M. rosenbergii* larvae (stage III) were introduced at a density of 80 individuals per liter of 12 g/l salinity water. They were fed with egg custard and the experimental live feed *Moina micrura* enriched with different enrichment media according to the design. The larvae were fed five times a day, namely, 7.00 am - egg custard, 10.30 am - *Moina*, 3.00 pm - *Moina*, 6.00 pm - *Moina* and 09.00 pm - *Artemia*. The salinity was gradually reduced to 8 g/l by adding freshwater as the larvae began to metamorphose to the post larvae (PL). Growth of larvae was expressed as the number of days until the first appearance of post-larvae and the number of days taken for 95% of the larvae to metamorphose into post-larvae. The survival rate was calculated as the number of larvae at the end of the experiment as a percentage of the original number introduced.

Enrichment of Moina micrura

Mass culture of *Moina:* Nutrient medium (slurry) for the culture tanks was prepared by adding 5 kg of raw cow dung, 1 kg of groundnut oil cake and 0.5 kg single super phosphate. It was kept for 5 days to allow for mineralization, fermentation of the slurry and for escape of obnoxious gases, mainly ammonia and hydrogen sulphide. *Moina* obtained from the Aquaculture Division, Central Institute of Fisheries Education, Fisheries University, was cultured in outdoor tanks containing this nutrient medium. The slurry was first added to the water filled tanks at the rate of 4 ml/litre for 2-3 days. On the third day *Moina micrura* was inoculated at around 100 individuals per litre and slurry application was continued at 2 ml per litre at intervals of 2 days. Mild aeration was

carried out to prevent anoxia. *Moina* were harvested early in the morning and at sunset with the help of a scoop net made of 250 micron cloth and thoroughly washed before the enrichment procedure. The Moina was divided into equal groups and enriched by placing them in each of the treatment media (Table 1) containing 1 ml of the emulsion per liter of water for a period of 24 hrs. Those enriched *Moina* were again harvested with strainers and rinsed with water before feeding the larvae.

Table 1. Composition of the emulsions used for *Moina* enrichment

Composition	**Astaxanthin emulsion (M1)**	**Cod liver oil and Astaxathin emulsion (M2)**	**Astaxanthin and Vitamin E emulsion (M3)**	**Astaxanthin and Vitamin D emulsion (M4)**
Water	40 ml	40 ml	40 ml	40 ml
Egg yolk	20 ml	20 ml	20 ml	20 ml
Gelatin	4.5 g	4.5 g	4.5 g	4.5 g
Vitamin E	-	-	3.0 g	-
Vitamin D	-	-	-	2.5 mcg
Cod liver oil	-	20 ml	-	-
Astaxanthin	6 mg	4 mg	4 mg	4 mg

Lipid extraction, fatty acid methyl ester and fatty acid analysis

The total lipid from the enriched *Moina* and the test larvae (after metamorphosis in to PL) was extracted following the Folch (1957) method. Esterification of the lipid extract and preparation of Fatty Acid Methyl Esters (FAMEs) followed the AOAC (1995) method. Gas Chromatography coupled with Mass spectrometry (Shimadzu QP2010) was used to separate and quantify the FAME, which were identified by the retention time using a GCMS library.

Statistical analysis

Results are presented as means ± standard error of means (S.E.M.). Differences between the control and treatment means were analyzed by one-way analysis of variance (one way ANOVA); means were compared using Duncan's new multiple range test and considered significant at the 5% level. A statistical package (SPSS) was used for all these analyses.

Results

Growth and survival

The earliest appearance of post-larvae (28.0±2.00 days), the highest growth rate and the least larval rearing period (38.5±0.50 days) were observed in the group (T3) fed on *Moina* enriched with astaxanthin and vitamin D. The longest time taken for the appearance of 95% PL (42.5±0.5 days) was for the control group. Hence, the better growth rate in this experiment was observed in T3 group and the least growth rate was observed in the control treatment (TC). The highest survival rate (66.0±1.00%) was shown by the treatment group (T4) fed with *Moina* enriched with cod-liver oil and astaxanthin emulsion and the lowest survival rate (33.0±1.5%) in the control group (Table 2).

Table 2. Growth and survival rates of *Macrobrachium rosenbergii* larvae fed with *Moina micrura* enriched with astaxanthin and its combinations

Treatment	Days taken for 1st Post-Larva to appear	Days taken for 95% PLs to appear	Survival rate %
TC	32.0^{b}±1.00	42.5^{c}±0.50	33.0^{a}±1.50
T1	32.0^{b}±0.00	40.5^{abc}±0.50	57.0^{b}±2.00
T2	30.5^{ab}±0.50	40.0^{ab}±0.00	61.0^{bc}±1.00
T3	28.0^{a}±2.00	38.5^{a}±0.50	58.0^{b}±1.00
T4	29.0^{ab}±0.00	40.5^{abc}±0.50	66.0^{cd}±1.00

* Values in the same column with different superscripts differ significantly ($p<0.05$)

The Fatty acid composition of enriched Moina micrura

The fatty acid profiles of enriched *Moina micrura* are summarized in Table 3, in which fatty acids with 2-3 double bonds have been categorized as Poly Unsaturated Fatty Acid (PUFA) and those with more than 3 double bonds are categorized as Highly Unsaturated Fatty Acids (HUFA). The total saturated fatty acid level (SAFA) was highest (59.64±0.24) in (M1) *Moina* enriched with astaxanthin and least (40.49 ±0.45) in (M4) Moina enriched with astaxanthin and cod liver oil. Mono-unsaturated fatty acid (MUFA) levels were high (45.15±1.33) in M2 and lowest (30.94^{a}±0.36) in M1. The highest total HUFA levels (4.79±0.32), significantly different ($P<0.05$) from others, was found in the M4 group, with the lowest ($P<0.05$) HUFA levels (0.19±0.00) in group M1. The highest level (3.29± 0.43) of n-3 HUFA was also found in the M4 group.

Table 3. Fatty acid profile of *Moina micrura* un-enriched (MC) and enriched with astaxanthin (M1) and its combinations (M2 to M4)

Fatty Acid	MC (Control)	M1	M2	M3	M4
C12:0	0.42^{bc}+0.03	-	0.07^{a} + 0.02	0.49^{c}+0.065	0.28^{b}+ 0.05
C14:0	3.45^{c}±0.20	1.77^{a}±0.24	2.79^{b}±0.265	2.82^{b}±0.235	4.47^{d}±0.08
C15:0	1.41^{bc}±0.15	0.66^{ab}±0.07	0.60^{ab}±0.10	1.70^{c}±0.25	1.69^{c}±0.06
C16:0	25.37^{bc}±0.18	35.19^{e}±0.25	31.04^{d}±0.89	27.71^{cb}±0.3	26.03^{a}±0.01
C17:0	1.60c^{a}±0.01	1.53^{ba}±0.12	1.38^{a}±0.21	1.13^{a}±0.12	2.17^{c}±0.18
C18:0	10.41^{c}±0.12	20.48^{c}±0.20	10.09^{bc}±0.17	8.74^{a}±0.28	5.83^{d}±0.18
C20:0	-	-	0.29^{b}±0.07	-	-
SAFA	**42.67^{ba}±012**	**59.64^{d}±0.24**	**46.27^{c}±0.79**	**42.61^{ba}±0.02**	**40.49^{a}±0.45**
C16:1n-9	1.48^{b}±0.13	3.80^{c}±0.11	0.22^{a}±0.05	-	3.91^{c}±0.14
C16:1n-7	-	-	4.99^{b}±0.02	7.84^{c}±0.57	0.62^{a}±0.01
C18:1n-9	26.24^{b}±0.09	24.66^{a}±0.43	30.83^{d}±0.72	24.87^{a}±0.14	29.51^{c}±0.52
C18:1n-6	8.60^{d}±0.045	-	4.94^{c}±0.48	4.42^{b}±0.16	-
C18:1n-5	-	-	0.28^{b}±0.02	-	-
C20:1n-9	-	1.72^{c}±0.03	2.96^{d}±0.14	-	0.76^{b}±0.1
C22:1n-9	-	0.74^{a}±0.15	0.92^{b}±0.065	-	-
MUFA	**36.33^{b}±0.27**	**30.94^{a}±0.36**	**45.15^{c}±1.33**	**37.14^{b}±0.55**	**34.80^{b}±0.61**
C16:2n-6	-	-	-	1.76^{b}± 0.24	-
C18:2n-6	9.19^{b}±0.04	8.63^{b}±0.58	5.75^{a}±0.12	12.09^{c}±0.54	15.28^{d}±0.27
C18:3n-3	4.70^{c}±0.11	0.59^{a}±0.01	0.49^{a}±0.09	2.79^{b}±0.34	0.51^{a}±0.15
C20:2n-7	-	-	0.37^{b} + 0.09	-	-
C20:3n-7	0.80^{b}± 0.15	-	-	-	-
C20:3n-3	-	-	-	-	4.13^{b}± 0.04
PUFA	**14.69^{c}±0.30**	**9.22^{b}±0.59**	**6.62^{a}±0.13**	**16.64^{d}±0.64**	**19.92^{c}±0.16**

C20:4n-6	2.90^{d}±0.22	-	0.24^{a}±0.03	2.12^{c}±0.03	1.50^{b}±0.11
C20:5n-3	0.40^{b}± 0.16	-	0.76^{c} ±0.12	1.49^{d}± 0.08	2.45^{e}± 0.18
C20:6n-3	-	0.19^{b}± 0.00	-	-	-
C22:6n-3	-	-	0.68^{b}± 0.00	-	0.84^{b}± 0.25
HUFA	**3.30^{c}±0.06**	**0.19^{a}±0.00**	**1.69^{b}±0.15**	**3.61^{c}±0.11**	**4.79^{d}±0.32**
n-3 PUFA	4.70^{c}±0.11	0.59^{a}±0.01	0.49^{a}±0.09	2.79^{b}±0.34	4.64^{c}±0.11
n-3 HUFA	0.40^{a}± 0.16	0.19^{a}± 0.00	1.44^{b} ±0.11	1.49^{b}±0.08	3.29^{c}± 0.43
n-6 PUFA	9.19^{b}±0.04	8.63^{b}±0.58	5.75^{a}±0.12	13.85^{c}±0.30	15.28^{d}±0.27
n-6 HUFA	2.90^{d}± 0.22	-	0.24^{b}±0.03	2.12^{c}± 0.03	1.50^{c}± 0.11
n-3/n-6	0.42^{d}±0.02	0.09^{a}±0.00	0.32^{c}±0.02	0.26^{bc}±0.01	0.47^{d}±0.04

*Values in the same rows with different superscripts differ significantly ($p<0.05$)

Fatty acid profile of M. rosenbergii larvae

The fatty acid profile of larvae fed with enriched *Moina micrura* is summarized in Table 4. The total saturated fatty acid levels (SAFA) were significantly ($p<0.05$) higher in (TC) larvae fed with unenriched *Moina* and (T1) larvae fed with astaxanthin enriched *Moina* (44.05±0.09, 44.08±0.32) and least (38.56±0.54) in (T4) larvae fed with astaxanthin and cod liver oil enriched *Moina*.

Table 4. Fatty acid profile of *Macrobrachium rosenbergii* larvae fed with *Moina micrura* enriched with astaxanthin and its combinations

Fatty acid	TC	T1	T2	T3	T4
C12:0	0.25^{bc}±0.01	-	0.21^{bc}±0.03	0.29^{c}±0.06	-
C14:0	3.52^{c}±0.06	1.4^{b}±0.14	1.52^{b}±0.04	1.68^{b}±0.00	-
C15:0	0.71^{d}±0.08	-	0.35^{bc}±0.01	0.46^{c}±0.10	1.44^{e}±0.09
C16:0	24.93^{ab}±0.38	31.99^{e}±0.02	27.39^{cd}±0.45	28.63^{d}±0.36	26.08^{cb}±0.27
C17:0	1.58^{f}±0.04	-	0.72^{e}±0.13	0.62^{dc}±0.02	0.47^{cb}±0.11
C18:0	13.05^{d}±0.51	10.69^{bc}±0.44	10.17^{ab}±0.09	11.94^{dc}±0.7	10.56^{ab}±0.47
C20:0	-	-	-	0.18^{b}±0.07	-
SAFA	**44.05^{c}±0.09**	**44.08^{c}±0.32**	**40.37^{b}±0.22**	**43.81^{c}±1.32**	**38.56^{a}±0.54**

C16:1n-9	1.48^{c}±0.13	-	0.67^{b}±0.11	0.59^{b}±0.01	-
C16:1n-7	-	1.68^{b}±0.12	2.48^{c}±0.20	2.41^{c}±0.23	2.39^{c}±0.04
C16:1n-6	2.12^{b}±0.00	-	-	-	-
C18:1n-9	28.14^{b}±0.04	32.93^{e}±0.18	29.27^{c}±0.31	30.76^{d}±0.54	28.14^{b}±0.17
C18:1n-7	2.50^{b}±0.05	-	4.40^{c}±0.24	-	5.19^{d}±0.16
C20:1n-9	-	-	-	0.46^{b}±0.09	-
MUFA	**34.25^{a}±0.14**	**34.61^{a}±0.30**	**36.84^{c}±0.02**	**34.23^{a}±0.22**	**35.72^{b}±0.29**
C18:2n-6	8.96^{ab}±0.05	9.70^{b}±0.14	8.21^{a}±0.11	8.7^{ab}±0.10	9.02^{ab}±0.30
C18:3n-3	2.47^{b}±0.11	-	2.66^{b}±0.04	2.28^{b}±0.08	2.82^{b}±0.46
C20:2n-7	-	-	-	0.18^{b} ±0.03	-
C20:3n-7	0.21^{b}±0.07	-	-	-	-
C20:3n-3	-	1.77^{b}±0.08	-	-	-
PUFA	**11.65^{ab}±0.09**	**11.48^{ab}±0.23**	**10.88^{a}±0.16**	**11.16^{ab}±0.05**	**11.84^{ab}±0.16**
C20:4n-6	5.30^{ab}±0.04	4.77^{ab}±0.34	4.75^{ab}±0.10	4.21^{a}±0.56	4.79^{ab}±0.25
C20:5n-3	4.20^{abc}±0.05	3.54^{a}±0.22	4.54^{bc}±0.07	3.90^{ab}±0.38	5.57^{d}±0.21
C22:6n-3	0.53^{a}±0.05	1.50^{b}±0.09	2.62^{c}±0.39	2.60^{c}±0.02	3.50^{d}±0.21
HUFA	**10.03^{a}±0.14**	**9.82^{a}±0.21**	**11.91^{b}±0.36**	**10.72^{ab}±0.97**	**13.87^{c}±0.68**
n-3PUFA	2.47^{ab}±0.11	1.77^{a}±0.08	2.66^{b}±0.04	2.28^{ab}±0.08	2.82^{b}±0.46
n-3HUFA	4.73^{a}±0.105	5.04^{a}±0.13	7.16^{bc}±0.46	6.51^{b}±0.41	9.07^{c}±0.42
n-6PUFA	8.96^{ab}±0.05	9.70^{b}±0.14	8.21^{a}±0.11	8.70^{ab}±0.10	9.02^{ab}±0.30
n-6HUFA	5.30^{ab}±0.04	4.77^{ab}±0.34	4.75^{ab}±0.10	4.21^{a}±0.56	4.79^{ab}±0.25
n-3/n-6	0.50^{a}±0.02	0.47^{a}±0.02	0.75^{bc}±0.04	0.68^{bc}±0.00	0.86^{c}±0.06
n-6/n-3	1.97^{d}±0.00	2.12^{d}±0.09	1.32^{abc}±0.07	1.46^{bc}±0.02	1.16^{a}±0.09

*Values in the same rows with different superscripts differ significantly ($p<0.05$)

The mono unsaturated fatty acid (MUFA) level was high (36.84±0.02) in (T2) larvae fed with astaxanthin and vitamin D enriched *Moina*. Proportion of EPA (C20: 5n-3) was highest (5.57±0.21%) in T4, and was lowest (3.54±0.22%) in T1; in the control group (TC), the amount of EPA was 4.20±0.05%. Among the different groups, the DHA (C22:6n-3) content was highest (3.50±0.21%) in T4 and was lowest (0.53±0.05%) in the control (TC). A significantly ($P<0.05$) highest n-3 HUFA level (9.07±0.42%) was found in the T4 group and significantly lowest (4.73±0.105%) n-3 HUFA level was found in the control.

Discussion

In aquaculture, feeding early larval stages of both fin fish and crustaceans is a nutritional challenge. Many larval feeds are not rich in all essential nutrients and do not fulfill their requirements. Enrichment of larval feeds, therefore, with various nutritive media is a common practice in aquaculture for enhancing their quality. Many researchers have worked on lipid sources as enrichment media because of the importance of essential unsaturated fatty acids in early larval stages. The need for n-3 HUFA has clearly been demonstrated for the larval stages of the freshwater prawn *M. rosenbergii* (Devrese *et al.*, 1990). For the complete larval cycle, these needs have been estimated at a daily dose of maximum 35 mg g^{-1} food dry weight (Romdhane *et al.*, 1995).

In the present study, the first appearance of PLs and the best growth rate was obtained in (treatment T3) the group fed with astaxanthin and vitamin D enriched *Moina*. Vitamin D is important to regulate calcium metabolism in animals. It is possible that improved regulation of calcium metabolism has helped to increase the rate of metamorphosis and influenced the rapid growth of *Macrobrachium* larvae. Vitamin D3 levels were related to calcium shifts (Meyran *et al.*, 1991, 1993) within tissues of terrestrial crustacean *Orchestia cavimana*. Lack of dietary vitamin D decreased weight gain (Shiau and Hwang, 1994) and increased feed conversion. In this study the growth results showed that addition of vitamin D along with astaxanthin led to better growth. Cheng *et al.*, (2006) have suggested that dietary calcium, phosphorus and Ca/P ratio significantly affect the weight gain in *Litopenaeus vannemai* reared in low salinity water. It can, therefore, be likely that enrichment of vitamin D with astaxanthin has helped in the absorption and metabolism of Ca and P and has resulted in the early growth of *Macrobrachium rosenbergii* larvae. Furthermore, astaxanthin is nearly five hundred more times effective than vitamin E in protecting the cell membrane as it has been shown that astaxanthin is a powerful antioxidant and can serve as a potent free-radical scavenger. Astaxanthin has been found to perform many essential biological functions, including protection against lipid-membrane peroxidation of essential polyunsaturated fatty acids and proteins. Results of this study suggest that some of those factors also could have influenced the better growth and survival of these larvae fed with astaxanthin and vitamin D enriched *Moina micrura*. Wang *et al.*, (2007) observed a higher growth rate (23.47- SGR) of larval loach (*Misqurnus anqillicaudatus*) fed with *Moina micrura* for 20 days. According to the results it can be suggested that feeding of astaxanthin and vitamin D combination enriched *Moina* can enhance the growth rate of *M. rosenbergii* larvae.

The best survival was shown by the T4 group (astaxanthin and CLO enriched *Moina* fed larvae). The higher survival rate (70.7%) has been observed by Pillai *et al.*, (2003) for *M. rosenbergii* larvae fed with HUFA enriched 75% enriched *Artemia* and enriched *Isochrysis galbana*. The best survival rates in this experiment (T4) can be due to the combined effects of the essential fatty acid levels in CLO and the antioxidant effect of astaxanthin, which would

play a role in protecting the fatty acid levels of both diet and body tissue of larvae. In *Moina,* enrichment practices did not result in enhancement of their fatty acid levels, but nevertheless the larval treatment groups showed the successful enhancement using CLO as a dietary supplement. The treatment with CLO showed the highest levels of EPA and DHA and the availability of these highly unsaturated fatty acids might have positively contributed to the increase of growth and survival. The antioxidant effect of astaxanthin would have also contributed by protecting these HUFAs. According to the results of the present study, it seems that astaxanthin in combination with CLO enhances the percentage survival of *M. rosenbergii.*

In this experiment, use of CLO as a HUFA source was practiced. A faster growth of walleye juveniles (*Stizostedion vitreum*) has been observed by Czesny *et al.*, (1999) when fed with *Artemia* enriched with a combination of CLO and n-3 HUFA concentrate. In the present study, similar results were observed in T4. Considerable reduction of larval rearing period (7-8 days earlier 1st post larval settlement and 11-12 days earlier 95% PL settlement) of *M. rosenbergii* larvae fed with CLO and probiotic emulsion enriched *Artemia* has been reported by Rani *et al.*, (2006). Results of the present study have also shown that the larvae had 95% PL settlement 2.5 days earlier.

According to Sheen and D'Abramo (1991), supplementation of dietary n-3 HUFA increased the growth of *M. rosenbergii* juveniles and survival of *Penaeus* spp. and *M. rosenbergii* (Bengston *et al.*, 1991). Murthy (1998) revealed that, *Artemia* nauplii enriched with HUFA rich cod liver oil improved the metamorphosis of *M. rosenbergii* larvae. In our study, the highest survival was shown by T4 group fed with CLO and astaxanthin enriched *Moina* (66.0±1.0%). However, the relatively long larval rearing period of the species resulted in a decrease of survival as they showed the cannibalistic behaviour due to differential growth rate. Results indicated that feeding with CLO enriched feed enhanced the percentage survival of *M. rosenbergii.*

The fatty acid profile of the feeding larvae was reflected in their diet. In *M. rosenbergii* larval tissue, it was evident that palmitic acid (C16:0) and oleic acid (C18:1n-9) appeared in larger quantities than other fatty acids (Table 4). It has been shown that palmitic acid is biosynthesized sufficiently in crustaceans from shorter fatty acids (Guary *et al.*, 1976) and that these fatty acids could be converted to other fatty acids by desaturation and chain elongation (Morris and Sargent, 1973, Jones *et al.*, 1979).

EPA (C20: 5n-3) and DHA (C22: 6n-3) are two of the most abundant polyenoic fatty acids in fresh water prawn *M. rosenbergii.* The EPA level was highest in T4 (5.57± 0.20) (Table 4) and least in T1 (3.54±0.22). The results of the present study showed that the feeding of larvae with CLO enriched *Moina* enhanced the EPA level of body tissue. Losses of nutrients may take place if the live feed is not fed to the fish or crustacean larvae immediately after enrichment, or if the retention time for the prey in the fish tanks is too long (Olsen *et al.*, 2000).

Despite the absence of DHA in the *Moina* tissues in some groups (MC. M1 and M3), larvae in the relevant treatment groups (TC, T1 and T3) contained DHA. The enriched *Moina*, when fed to *Macrobrachium* larvae, influenced the HUFAs (20:4n-6, 20:5n-3 and 22:6n-3) of larval tissues. In the present study both n-3 and n-6 HUFA contents were lowest in T1 group (9.82 ± 0.21), which was fed with Moina with the lowest HUFA level (0.19± 0.00). Furthermore, T1 also showed the lowest n-3 PUFA level and the lowest n3:n6 ratio too. Generally, the dietary lipid levels, i.e. fatty acid composition, are reflected in the body tissue fatty acids in the larvae (Roustaian *et al.*, 1999).

It is evident that, from the results of this study, the nutritional quality of *Moina* can be improved by enriching with different nutritional supplements, not only increasing the growth and survival but also enhancing the EPA and DHA levels.

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