EFFECT OF DIETARY SUPPLEMENTATION OF TOCOFEROL ACETATE ALONE AND WITH VARYING COMBINATIONS ON GROWTH, SURVIVAL AND FATTY ACIDS PROFILE OF *MACROBRACHIUM ROSENBERGII* LARVAE THROUGH *MOINA MICRURA* ENRICHMENT

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ABSTRACT

A study was carried out to determine the effect of tocopherol acetate along with cod liver oil astaxanthin enriched *Moina micrura* (MC- control, M1- tocopherol acetate enriched, M2- tocopherol acetate combined with cod liver oil (CLO) enriched and M3- tocopherol acetate combined with astaxanthin enriched) on growth, survival and fatty acid composition of *M. rosenbergii* (de Man) larvae (TC- unenriched *Moina* fed larvae, T1- tocopherol acetate enriched *Moina* fed larvae, T2- tocopherol acetate + CLO enriched *Moina* fed larvae to T3 - tocopherol acetate + astaxanthin enriched *Moina* fed larvae). Growth was expressed as the time taken in to the settlement of 95% post larvae. Maximum growth *i.e.*, the lowest time taken to the 95% PL settlement (40 days) and the maximum survival percentage (61%) was observed in both T2 and T3 treatments fed with M2 and M3 *Moina* respectively. Minimum growth and survival was observed in unenriched *Moina* fed larvae (TC). In larval treatments T2, (larvae fed with (M2) vitamin E + CLO enriched *Moina*), showed a higher percentage of EPA, DHA and higher HUFA level than other treatments.

Key words: Dietary supplementation, Tocoferol acetate, *Macrobrachium rosenbergii, Moina micrura*.

INTRODUCTION

Macrobrachium rosenbergii is the most widely used species of paleimonid prawns in aquaculture. Interest in the aquaculture of fresh water prawn has increased proportionally during past few years. Economic success in any commercial culture of a species mainly depends on the food requirement. Larval feed is most important for the rapid growth of fish and/or prawn to minimize the production cost as well to maximize the production level. Most fin fish and shell fish culturists among all over the world are depending on live feed mainly *Artemia* as the larval feed during the early developmental stages. Because of the increasing demand for *Artemia* in world wide aquaculture (Bengston *et al.*, 1991), its cysts are likely to be costly and occasionally scarce, especially in developing countries. In addition, recent ecological changes in the Grate Salt Lake, USA, which produces 80% of the world *Artemia,* are likely to cause a serious limitation (Lavens and Sorgeloos, 2000). As the food and feeding in prawn hatcheries account for about 60% of total operational costs (Hagwood and Willis, 1976), enrichment of *Artemia* and its use will add additional expenses and may not be accessible to hatchery operators, particularly in developing countries (Alam *et al.,* 1995). Although live feed is identified as a

good source of nutrition, all live feeds e.g., *Artemia, Moina,* copepods, rotifers and other zooplankton may not be rich in all required nutritional qualities. Several workers have investigated the nutritional levels and the requirements of scampi seed. Unsaturated fatty acids (HUFA) specially EPA and DHA are essential dietary requirement 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 live feed seems to be a better practice to enhance their nutritional quality.

Different enrichment media for this purpose have been used by some researchers are vitamins (Kolkovski *et al.,* 2000; Tarui *et al.,* 2006), microencapsulated diets (*Southgate and Lou*, 1995), unicellular alga (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). According to He and Lawrence, 1993, tocoferol acetate has the highest vitamin E activity. The aim of the present study was to find out the effect of tocoferol acetate (vitamin E) enriched diet on the growth, survival and quality of the diet larvae according to their fatty acid profile.

MATERIALS AND METHODS

Stocking and maintenance of larvae

Experiment with ten treatments coupled with three replicates, each to follow a completely randomized design, was conducted for 45 days duration in fifty liter capacity plastic round tubs. Larvae (stage III) were kept at a density of 22 individuals per liter in 12 ppt salinity water. The salinity was gradually reduced to 8 ppt by adding fresh water as the larvae began to metamorphose to the post larvae. Growth of larvae was expressed as the number of days until the first appearance of post larvae and the number of days until 95% of the larvae metamorphosed in to post larvae. The survival (%) was taken as the final number of the animals as a percentage of the initial number of the animals.

Enrichment of *Moina micrura*

Moina was first cultured in outdoor tanks using the slurry, 5 kg (of raw cow dung, 1 kg of ground nut oil cake and 0.5 kg single super phosphate mixture). Harvested *Moina* were thoroughly washed and grouped into three equal parts and the enrichment was achieved by placing them in the emulsion medium at the rate of 1ml per liter of water. The enrichment was conducted for a period of 24 hrs. Those enriched *Moina* were again harvested with strainers and rinsed with water before feeding the larvae.

Feeding schedule

M. rosenbergii larvae were fed with egg custard and live feed *Moina micrura* enriched with different enrichment media per treatment. Feeding frequency of larvae was five times a day, *i.e.,* 7.00 am egg custard , 10.30 am - *Moina*, 3.00 pm - *Moina*, 6.00 pm - *Moina* and 09.00 pm. - *Artemia*.

Lipid Extraction, Fatty Acid Methyl Ester and Fatty acid analysis

Total lipid of both enriched *Moina and* test larvae (after metamorphosis in to PL) was extracted following the Folch (1957) method. The AOAC (1995) method was followed to esterify the lipid extract and FAMEs were prepared. From that FAME, fatty acid composition of both samples were analysed

by using Gas Chromatography, Mass spectrometry (Shimadzu QP2010). The fatty acids were identified referring to the retention time and GCMS library and presented in area percentage of total identified fatty acids.

Statistical analysis

Results were presented as means ± standard error of means (S.E.M.). Difference among the control and treatment means were analyzed by one-way analysis of variance (One way ANOVA) followed by Duncan's new multiple range tests. Differences were considered statistically significant when $P < 0.05$. Statistical analysis was carried out using the SPSS statistical package (SPSS Inc., Chicago, IL, USA).

RESULTS

Growth

The quick appearance of first post larvae during the experimental period was in both T2 & T3 (fed with *Moina* enriched with tocoferol acetate + cod liver oil and tocoferol acetate+ astaxanthin) group (30.5±0.50 days). The lowest time (highest growth), significantly ($p<0.05$) lowest larval rearing period was also observed in T2 & T3 group (40.0±0.0 and 40.0±1.0 days) & the highest time period (42.5±0.5 days), taken for the appearance of 95% PL was in control. Hence, the better growth rate in this experiment was observed in T2 & T3 groups & the least growth rate was observed in the control treatment (Table 1).

Survival

The highest survival (61.0±2.0%) was also shown in T3 treatment group fed with *Moina* enriched with cod-liver oil emulsion (Table 1) whereas, the lowest survival (33.0±1.5) was obtained with the control treatment.

Fatty acid composition of enriched *Moina micrura*

The fatty acid profile of enriched *Moina micrura* is summarized in Table 3. The total saturated fatty acid level was highest (59.64±0.24) in M3 (*Moina* enriched with tocoferol acetate + astaxanthin) and was lowest (36.59±1.09) in M3 (enriched with tocoferol acetate + cod liver oil). Mono unsaturated fatty acid level was high (45.15±1.33) in M3, whereas, lowest (35.57±1.01) was found in M1. However, the highest HUFA level was found in M1 group but it was not significantly different with MC or M2 groups ($P< 0.05$). Significantly lowest HUFA level was found in M3.

Fatty acid profile of *Mcrobrachium rosenbergii larvae*

The fatty acid profile of larvae fed with enriched *Moina micrura* is summarized in Table 4. The total saturated fatty acid level was high in TC (unenriched *Moina* fed larvae) (*Moina* enriched with tocoferol acetate + astaxanthin) (44.05^{c}±0.09) and significantly ($P<0.05$) lowest (36.91^{a}±0.18) in T2 (larvae fed with tocoferol acetate + cod liver oil enriched *Moina*). Mono unsaturated fatty acid level was high (36.84^{d}±0.02) in T3, whereas, significantly lowest level (30.15^{a}±0.06) was found in T1. Linoleic acid (C18:2n-6) percentage was lowest (8.21^{a}±0.11%) in T3 and was maximum (11.20^{c}±0.18%) in T1. Composition of linolenic acid (C18:3n-3) was maximum (4.74^{c}±0.27%) in T1 and was not detected in T2. The highest arachidonic acid (C20: 4n-6) percentage was recorded (6.54±0.22%) in T1. In the control treatment, it was 5.30±0.04%. Therefore, it can be concluded that T1 is the better

treatment for obtaining high ARA level. In T2 it's level was 5.85^{bc}±0.36. Percentage of EPA (C20: 5n-3) was highest (5.78^{d}±0.46%) in T2 and was lowest (3.89^{ab}±0.27%) in T1. In the control treatment (TC), amount of EPA was 4.20±0.05%. Hence, in this study the best treatment for getting higher EPA level was T2. Among different enrichment groups, DHA content was highest (3.55^{c}±0.29%) in T2 and was lowest (0.53±0.05%) in control (TC) treatment. In this study, higher DHA level was obtained fromT2 treatment; hence, the better treatment for getting higher DHA level is T2 (larvae fed with tocopherol acetate + CLO enriched *Moina*). Significantly (P< 0.05). Highest (15.40^{d}±0.36) HUFA level was found in T2 group and significantly lowest (10.03^{a}±0.14) HUFA level was noticed in control treatment.

Table 1 : Composition of the *e*mulsions Used for *Moina e*nrichment

Composition	Tocopheryl Acetate emulsion	CLO + TA emulsion	Astaxanthin+ TA emulsion
Water (ml)	40	40	40
Egg yolk (ml)	20	20	20
Gelatin (g)	4.5	4.5	4.5
Tocopheryl Acetate (g)	6.0	3.0	3.0
Vitamin D*	-	-	-
Cod liver oil (ml)	-	20	-
Astaxanthin (mg)	-	-	4

TA (Tocopheryl Acetate), D* (1α - Hydroxyvitamin D3), CLO = Cod Liver Oil

Table 2 : Average cumulative mortality of larvae subjected to different salinity levels

Treatment	1st PL appearance (Days)	95% PLs appearance (Days)	Survival (%)
TC	**32.0** ^{b}±1.00	42.5 ^{c}±0.50	33.0 ^{a}±1.50
T1	32.0 ^{a}±2.00	41.0 ^{ba}±1.00	38.5 ^{a}±1.50
T2	30.5 ^{a}±0.50	40.0 ^{a}±1.00	61.0 ^{b}±1.00
T3	30.5 ^{a}±0.50	40.0 ^{a}±0.00	61.0 ^{b}±1.00

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

Table 3 : Fatty acid profile of *Moina micrura* enriched with tocoferol acetate and its combinations

Fatty acid	Control	M1	M2	M3
C12:0	0.42^{b}±0.03	0.39^{b}±0.03	0.36^{b}±0.09	0.07^{a}±0.02
C13:0	-	-	0.22^{b}±0.07	-
C14:0	3.45^{b}±0.20	3.24^{b}±0.18	4.05^{c}±0.49	2.79^{a}±0.265
C15:0	1.41^{b}±0.15	1.22^{b}±0.23	3.75^{c}±0.19	0.60^{a}±0.10
C16:0	25.37^{ab}±0.18	28.26^{bc}±0.85	24.66^{a}±0.51	31.04^{d}±0.89
C17:0	1.60c^{a}±0.01	1.10^{a}±0.14	3.54^{b}±0.08	1.38^{a}±0.21
C18:0	10.41^{d}±0.12	8.89^{b}±0.14	-	10.09^{cd}±0.17
C20:0	-	-	0.34^{b}±0.21	0.29^{b}±0.07
SAFA	**^{b}±012**	**43.11^{b}±0.48**	**36.59^{a}±1.09**	**46.27^{c}±0.79**
C16:1n-9	1.48^{c}±0.13	-	15.05^{d}±0.26	0.22^{b}±0.05
C16:1n-7	-	7.29^{d}±0.02	0.89^{b}±0.01	4.99^{c}±0.02
C18:1n-9	26.24^{c}±0.09	24.14^{b}±0.58	10.72^{a}±0.19	30.83^{d}±0.72
C18:1n-6	8.60^{c}±0.045	4.14^{a}±0.45	9.45^{d}±0.11	4.94^{b}±0.48
C18:1n-5	-	-	-	0.28^{b}±0.02
C20:1n-9	-	-	0.47^{b}±0.11	2.96^{c}±0.14
C22:1n-9	-	-	-	0.92^{b}±0.065
MUFA	**36.33^{a}±0.27**	**35.57^{a}±1.01**	**36.59^{a}±0.61**	**45.15^{b}±1.33**
C16:2n-6	-	1.59^{b}± 0.07	-	-
C18:2n-6	9.19^{b}±0.04	12.94^{c}±0.31	16.14^{d}±0.75	5.75^{a}±0.12
C18:3n-3	4.70^{c}±0.11	3.28^{b}±0.15	7.80^{d}±0.21	0.49^{a}±0.09
C20:2n-7	-	-	-	0.37^{b} ± 0.09
C20:3n-7	0.80^{b}± 0.15	-	-	-
PUFA	**14.69^{b}±0.30**	**17.81^{c}±0.53**	**23.94^{d}±0.96**	**6.62^{a}±0.13**
C20:4n-6	2.90^{d}±0.22	2.11^{c}±0.02	1.76^{b}±0.10	0.24^{a}±0.03
C20:5n-3	0.40^{a}± 0.16	1.38^{d}± 0.02	1.11^{c}± 0.06	0.76^{b} ±0.12
C22:6n-3	-	-	-	0.68^{b}± 0.00
HUFA	**3.30^{b}±0.06**	**3.50^{b}±0.00**	**2.87^{b}±0.04**	**1.69^{a}±0.15**
n-3 PUFA	4.70^{c}±0.11	3.28^{b}±0.15	7.80^{d}±0.21	0.49^{a}±0.09
n-3 HUFA	0.40^{a}± 0.16	1.38^{c}± 0.02	1.11^{bc}± 0.06	1.44^{c} ±0.11
n-6 PUFA	9.19^{b}±0.04	14.53^{c}±0.38	16.14^{d}±0.75	5.75^{a}±0.12
n-6 HUFA	2.90^{c}± 0.22	2.11^{b}± 0.02	1.76^{b}± 0.10	0.24^{a}±0.03
n-3/n-6	0.42^{c}±0.02	0.28^{ab}±0.00	0.49^{c}±0.01	0.32^{b}±0.02
n-6/n-3	2.38^{b}±0.16	3.56^{c}±0.00	2.00^{a}±0.06	3.12^{c}±0.25

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

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

Fatty acid	Control	T1	T2	T3
C12:0	0.25^{b}±0.01	-	0.18^{b}±0.05	0.21^{b}±0.03
C14:0	3.52^{c}±0.06	2.79^{b}±0.22	1.34^{a}±0.02	1.52^{a}±0.04
C15:0	0.71^{c}±0.08	0.88^{d}±0.02	0.25^{a}±0.01	0.35^{b}±0.01
C16:0	24.93^{ab}±0.38	25.01^{ab}±0.15	25.19^{b}±0.17	27.39^{d}±0.45
C17:0	1.58^{d}±0.04	1.11^{c}±0.05	0.48^{a}±0.025	0.72^{b}±0.13
C18:0	13.05^{c}±0.51	10.37^{ab}±0.08	9.32^{a}±0.33	10.17^{ab}±0.09
C20:0	-	-	0.14^{b}±0.025	-
SAFA	**44.05^{c}±0.09**	**40.16^{b}±0.07**	**36.91^{a}±0.18**	**40.37^{b}±0.22**
C16:1n-9	1.48^{c}±0.13	1.96^{d}±0.02	0.82^{b}±0.06	0.67^{ab}±0.11
C16:1n-7	-	-	2.17^{bc}±0.15	2.48^{c}±0.20
C16:1n-6	2.12^{b}±0.00	3.33^{c}±0.31	-	-
C18:1n-9	28.14^{b}±0.04	24.86^{a}±0.27	28.05^{b}±0.05	29.27^{c}±0.31
C18:1n-7	2.50^{b}±0.05	-	-	4.40^{c}±0.24
C18:1n-6	-	-	4.84^{b}±0.26	-
C20:1n-9	-	-	0.24^{b}±0.01	-
MUFA	**34.25^{b}±0.14**	**30.15^{a}±0.06**	**36.13^{cd}±0.52**	**36.84^{d}±0.02**
C18:2n-6	8.96^{ab}±0.05	11.20^{c}±0.18	8.9^{ab}±0.25	8.21^{a}±0.11
C18:3n-3	2.47^{b}±0.11	4.74^{c}±0.27	-	2.66^{b}±0.04
C20:3n-7	0.21^{b}±0.07	0.31^{b}±0.04	-	-
C20:3n-3	-	-	2.52^{b}±0.16	-
PUFA	**11.65^{ab}±0.09**	**16.26^{c}±0.13**	**11.49^{ab}±0.09**	**10.88^{a}±0.16**
C20:4n-6	5.30^{ab}±0.04	6.54^{c}±0.22	5.85^{bc}±0.36	4.75^{ab}±0.10
C20:4n-3	-	-	0.22^{b}±0.03	-
C20:5n-3	4.20^{abc}±0.05	3.89^{ab}±0.27	5.78^{d}±0.46	4.54^{bc}±0.07
C22:6n-3	0.53^{a}±0.05	2.98^{bc}±0.22	3.55^{c}±0.29	2.62^{b}±0.39
HUFA	**10.03^{a}±0.14**	**13.41^{c}±0.27**	**15.40^{d}±0.36**	**11.91^{b}±0.36**
n-3PUFA	2.47^{ab}±0.11	4.74^{c}±0.27	2.52^{ab}±0.16	2.66^{b}±0.04
n-3HUFA	4.73^{a}±0.105	6.87^{b}±0.05	9.55^{d}±0.72	7.16^{bc}±0.46
n-6PUFA	8.96^{ab}±0.05	11.20^{c}±0.18	8.97^{abc}±0.25	8.21^{a}±0.11
n-6HUFA	5.30^{ab}±0.04	6.54^{c}±0.22	5.85^{bc}±0.36	4.75^{ab}±0.10
n-3/n-6	0.50^{a}±0.02	0.65^{b}±0.02	0.81^{c}±0.09	0.75^{bc}±0.04
n-6/n-3	1.97^{c}±0.00	1.52^{b}±0.06	1.23^{ab}±0.14	1.32^{ab}±0.07

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

DISCUSSION

Establishing a feeding regime for optimal growth, survival and health of the fin fish and shell fish larvae is one of the main objectives of developing larval rearing strategies (Immanuel, *et al.*, 2001). Enrichment of live and/or artificial feed is an alternative to improve its food value and therefore the growth and survival of fish and shell fish larvae. Various enrichment media lead to the enhancement of nutritive quality and it directly affect the growth, survival and quality of the preyer.

In this experiment, the best growth was obtained in treatment T2 and T3, which also showed the best survival. The enrichment with tocoferol acetate in combination with CLO and astaxanthin enhanced the growth and survival of *M. rosenbergii* larvae than enrichment with tocoferol acetate alone. Tocoferol acetate has the highest vitamin E activity (He and Lawrence, 1993) mainly the membrane lipid protection. Astaxanthin has the higher ability to protect the membrane lipid from peroxidation than tocoferol acetate. EFA levels in the body tissue positively affect the growth and survival of animals (Kanasawa *et al.*, 1979). The best growth in this experiment can be due to the effect of essential fatty acid levels of CLO and the antioxidant effect of astaxanthin, which might have involved protecting the fatty acid levels of both diet and bodying tissue of larvae. Although in *Moina* enrichment practices successful enhancement of fatty acid levels could not be observed yet the larval treatment groups showed the successful enhancement through the CLO included dietary supplementation. The T2 treatment showed the high levels of EPA and DHA. The highly unsaturated fatty acid might have positively contributed in the increment of growth and survival. Further, the antioxidant effect of astaxanthin, which is more powerful than vitamin E has helped the protection of lipids.

In this experiment, use of CLO as a HUFA source was practiced. HUFA source as an enrichment media was examined by some authors. Rani *et al.* (2006) has revealed a 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 cod liver oil and probiotic emulsion enriched *Artemia*. The study results are in agreement with them as the larvae showed 2 and 2.5 days earlier first and 95% PL settlement respectively. A faster growth of walleye juveniles (*Stizostedion vitreum*) fed with *Artemia* enriched with a combination of CLO and n-3 HUFA concentrate, has been observed by Czesny *et al.*, (1999). In our study, similar results in both T2 and T3 were observed. Importance of HUFA is studied by Read (1981) for *Penaeus indicus*. According to Sheen and D'Abramo (1991), supplementation of dietary n-3 HUFA increased the growth of *Macrobrachium rosenbergii* juveniles and survival of *Penaeus* spp and *Macrobrachium rosenbergii* (Bengston *et al.*,1991). Murthy (1998) revealed that, *Artemia* nauplii enriched with HUFA rich cod liver oil improved the metamorphosis of *Macrobrachium rosenbergii* larvae. According to Nevejan *et al.* (2003), Scallop (*Argopecten purpuratus*) larvae fed with alga supplemented with DHA and EPA, had a better daily growth rate as compared to non supplemented algae fed larvae. Present study results are in agreement with their findings. However, high levels of both EPA and DHA above a certain level resulted in a lower growth (Glencross et al, 2001). According to them, increasing the level of both EPA and DHA affect the n-3:n-6 balance and consequently it adversely affect the growth. In our study, the highest survival was shown by both T2 and T3 group fed with CLO enriched *Moina* (61.0±1.0%). *Macrobrachium rosenbergii* larvae fed with

HUFA enriched with 75% *Artemia* + *Isochrysis galbana* showed a higher survival rate (70.7%) (Pillai *et al.,* 2003). However, relatively long larval rearing period of the species resulted a decrease of survival as they showed the cannibalistic behavior due to differential growth rate. Results indicated that feeding with CLO enriched feed enhances the survival percentage of *Macrobrachium rosenbergii*. Kolkovski *et al.* (2000), observed negligible effect of supplementation of vitamin E on fish survival. In our study, it was observed that enrichment of *Moina* with tocoferol acetate alone had significantly low survival (38.5± 1.5) when compared to its combinations. Lowest survival in this study was observed in the control treatment.

Survival and growth in shrimp larval stages improved by supplementation of HUFA enriched *Artemia* nauplii (Leger and Sorgeloos, 1994, Chamberlain, 1988). Accoding to Cavelli *et al.,* (2000), significant differences were observed in larval size and survival, fed with three isolipidic diets containing varying levels of phospholipids (0.8, 2.4 and 4.6%) to *M. rossenbergii* females. Present results in the study are in agreement with their suggestions. Rees *et al.* (1994) suggested that higher the n-3 HUFA mainly EPA and DHA level may not be higher the growth and survival of *Penaeus monodon* post larvae. Verreth *et al.* (1994) suggested that *Clarias gariepinus* larvae fed with low HUFA and high n-3 HUFA enriched *Artemia* did not affect their growth or survival.

Palmitic acid is biosynthesized sufficiently in crustaceans from shorter fatty acids (Guary *et al.*, 1976) and that the acid could be converted to other fatty acids by desaturation and chain elongation (Morris and Sargent, 1973, Jones *et al.*, 1979). In the present study, palmitic acid (C16:0) and oleic acid (C18:1n-9) appeared in larger quantities than other fatty acids in *M. rosenbergii* larval tissue (Table 3). Relatively less proportions of linoleic acid were in their body tissue (except T3) compared to the relevant *Moina* groups. It indicated that C18: 2n-6 was apparently not synthesized *de novo* by *M. rosenbergii*. Reigh and Stickney (1989) have mentioned this earlier. However, linolenic acid could not be detected in T2. Guary *et al.* (1976) reported that EPA and DHA were more effective in promoting growth of *P. japonicus* than linoleic and linolenic acids.

Arachidonic acid, the elongation and desaturation product of C18:2n-6, was in slightly equal levels in all treatment groups and their levels were much higher than the respective *Moina* groups. D'Abramo and Sheen (1993) reported increased levels of arachidonic acid in tissues of freshwater prawns that were provided with a dietary supply. The EPA level was highest in T2 (5.78^{d}±0.46%). The lowest EPA was found in T1 (3.89±0.27%) but the DHA level was much higher than the control level. The study results show that the feeding of larvae with enriched *Moina* would enhance the EPA/DHA level of body tissue.

Despite the absence of DHA in the *Moina* in some groups, larvae in the relevant treatment groups (control, T1 and T2) (Table 3) contained DHA. It may be either due to fatty acid came from their parents, or desaturation and/or conversion or from the dietary levels. However, enriched *Moina*, when fed to *Macrobrachium* larvae, influences the highly unsaturated fatty acids (20:4n-6, 20:5n-3 and 22:6n-3) of larval tissues. Generally, the dietary lipid levels through the fatty acid composition will reflect in the body tissue fatty acids in animals (Roustaian *et al.*, 1999). Further, they reported on the bio conversion ability of *M. rosenbergii* larvae and post larvae in chain elongation and desaturation of 16:0, to stearic acid 18:0. According to them, *M. rosenbergii* is capable of converting 18:2n-

6 and 18:3n-3, to long chain highly unsaturated fatty acids such as 20:4n-6 and 20:5n-3.

The highest total HUFA level (15.40±0.36%) was observed in treatment T2, which clearly showed the successful enrichment practice with tocoferol acetate+ cod liver oil as an emulsion and the reflection of dietary fatty acids in larvae body tissue. Normally, fatty acid retention percentage revealed the capacity to synthesize HUFA (Verreth *et al.*, 1994). In nutshell, the present study demonstrated that the nutritional quality of *Moina* can be improved by enriching them with different substances. Dietary supplementation of tocoferol acetate through enrichment of *Moina* increased the growth and survival as well as the EPA and DHA level in the fatty acid profile, which showed nutritional enhancement and the quality of the giant fresh water prawn. Both T2 and T3 treatments had a high growth and best survival in this study showing the use of tocoferol acetate in combination with CLO and astaxanthin which is positively effective for them.

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