Recent Research in Science and Technology 2011, 3(8): 76-82
ISSN: 2076-5061
Available Online: http://recent-science.com/

Evaluation of nutritional Parameters and carotenoid pigment from *Penaeus monodon* of Zoea – PL 20 stages fed with live algal diet and *Artemia* enriched algal diet

Madhumathi Mani* **and Rengasamy Ramasamy**

Centre for advanced studies in Botany, University of Madras, Maraimalai campus, Chennai - 600025, Tamil Nadu, India

Abstract

This study was subjected to investigate the nutritional value and carotenoid content of *Penaeus monodon* from Zoea – Post larval 20 stages when fed with five different microalgae and *Artemia* sp. nauplii enriched with five different microalgae. Among the algae alone treatments, *P. monodon* fed to *Chaetoceros calcitrans* showed better growth and length at Z3-M3/PL1 stages. Whereas from PL 1 – PL 20 stages of *P. monodon* fed with *Artemia* sp. naupli enriched with *D. salina* showed better results on the tested parameters when compared to *Artemia* sp. nauplii enriched with other algal sources. The maximum accumulation of carotenoids and good colouration were observed in the PL 20 stages of the animal fed with *Artemia* sp. nauplii enriched with *Dunaliella salina*. The Polyunsaturated fatty acids (PUFA) content seems to be high in *P. monodon* at Z3- M3/PL 1 stage when fed with *C. calcitrans*, whereas at PL 20 stages profile is high when fed with *Artemia* sp. nauplii enriched with *D. salina*. This study showed that besides diatoms, *D. salina* has the potential betacarotene pigment and supports better growth and nutritional components.

Keywords: Algal sources, *Artemia* sp., *Penaeus monodon*, Nutritional evaluation

INTRODUCTION

Among the components in aquaculture practises, live feeds are the essential link between endogenous nutrition and exogenous feeding of aquatic animals that are commercially cultured. Live feeds play a crucial role in the diet of the early stages of larvae and they are considered in regulating the survival of fish and crustaceans until the animals can ingest formulated feeds. Live diets include phytoplankton such as microalgae (2 - 20 μm) and zooplankton such as rotifers (50 - 200 μm) and brine shrimp, *Artemia* sp. (200 - 300 μm) [1]. Phytoplankton comprises the base of the food chain in the marine environment and therefore, microalgae are indispensable in the commercial rearing of various species of marine animals as a food source for all growth stages of bivalve molluscs, larval stages of some crustacean species, and very early growth stages of some fish species. The most frequently used species in commercial mariculture operations are the diatoms (Bacillariophyceae) *Skeletonema costatum*, *Thalassiosira pseudonana*, *Chaetoceros gracilis* and *C. calcitrans*; the Crysophyceae *Isochrysis galbana*; Chlorophyceae *Tetraselmis suecica*, *Monochrysis lutheri* and *Chlorella* sp. [25].

The brine shrimp, *Artemia* constitutes as the most widely used feed source in the larviculture of fish, shellfish and nauplii. *Artemia* is given as live feed to over 85 % aqua cultured species around the world since it has several advantageous features such as small size, easy ingestion, high nutritional value, complete protein diet and yield better survival of the animal [13]. Apart from the major task in development of feeding regime for the larvae [23]. Bio-encapsulation method is now being developed for oral delivery of vitamins, chemotherapeutic vaccines, essential PUFAs, pigments, sterols and carotenoids through *Artemia* nauplii [24, 16]. Colour is one of the major factors in shrimp culture industries, which determines the market price especially in *Penaeus monodon* in international market and thus supplementation of carotenoid pigments in the diet has been demonstrated to yield higher pigments in shrimp [11, 22].

The green alga *Dunaliella,* produces abundant β-carotene [25] (an accessory light harvesting pigment) [7], which is recognized as the lipid antioxidant that quench singlet oxygen and the precursor for provitamin A. It is well documented that crustaceans are unable to biosynthesise carotenoids and therefore, the carotenoids are included in the feed and fed to the animals up to PL stage through *Artemia* sp. nauplii. The aim of this present study is to explore the nutritional evaluation of *Penaeus monodon* from zoea to PL20 stages that were fed with five different algal sources and *Artemia* sp. nauplii enriched with different algal sources.

MATERIALS AND METHODS

The algal cultures such as the diatoms *Chaetoceros calcitrans* and *Skeletonema coastaum* and the green algae *Dunalliella salina* and *D. bardawil* were obtained from the Algal Culture Collection at the Center for Advanced Studies in Botany, University of Madras. The *Isochrysis galbana* were obtained from Central Institure of Brackish Aquaculture (CIBA). The diatoms, *Isochrysis galbana* were maintained in F/2 medium [9] and the green algae were maintained in DeWalne's medium. All the five different microalgae stock culture were carried out in 500m L respective basal medium inoculated with 50 mL of optimally grown cultures and kept under 30 μE/m²/s¹/ light intensity provided with cool white fluorescent lamps at 24 ± 1ºC with 12/12 photoperiod.

Received: May 21, 2011; Revised July 29, 2011; Accepted July 29, 2011.

*Corresponding Author

Madhumathi Mani
Centre for advanced studies in Botany, University of Madras, Maraimalai campus, Chennai - 600025, Tamil Nadu, India.

Tel: +91-4422202752
Email: madhumathi83@gmail.com

Hatching of *Artemia* cysts

Five hundred gram of the cysts of *Artemia* sp. obtained from the Department of Zoology, University of Madras, was used for this investigation. *Artemia* cysts were hatched as per the standard procedure outlined by [21]. One gram of cysts was taken and hydrated in sterile fresh water for 1 h in a conical flask with vigorous aeration. After 1 h, cysts were collected, rinsed with fresh water and transferred to decapsulation solution (sodium hypo chloride 200 µg/l) for 10 minutes. The orange – pink cysts were collected through 100 µm mesh, thoroughly washed with freshwater and removed hypochlorite. The treated decapsulated cysts were then transferred to 1L seawater and kept under 1000 lux light intensity, vigorously aerated at room temperature for 24 h. The hatched nauplii from the decapsulated cysts (more than 90 % Instar – I) were siphoned out by exposing them to light by wrapping with dark cloth at the basal region of the flask and discarded unhatched and empty shells. The nauplii were washed thoroughly in seawater and used for the enrichment experiments.

Enrichment of *Artemia* nauplii

The 24 h old 600 nauplii in 1L of seawater were fed separately with the microalgae *viz*; *Cheatoceros calcitrans* (22nd day), *Skeletonema coastatum* (20th day), *Dunaliella salina* (15th day) and *D. bardawil* (21st day) (obtained from the Centre for Advanced studies in Botany, University of Madras) and *Isochrysis galbana* (obtained from Central Institute of Brackish aquaculture, Chennai) at different cell concentrations viz; 10×10^4 cells/mL, 20×10^4 cells/mL, 30×10^4 cells/mL, 40×10^4 cells/mL, 50×10^4 cells/mL and 60×10^4 cells/mL respectively. The experiment was conducted for a period of 24 h. Every 3 h interval the animals were observed under microscope and recorded their gut region as well as recorded for different biochemical characteristics. *Artemia* sp. nauplii were separated from the enrichment container using 120 µm sieve, where nauplii alone collected at the top of the sieve and the algae sources are washed off. The collected nauplii are washed in fresh water and used for the below feeding experiment.

Experimental design

The experiment was designed to test the effect of different algal species and *Artemia* sp. nauplii enriched with different algae to the growth, body weight, survival and biochemical profile of prawn larvae. Eighty thousand seeds (nauplii 24 h) of *Penaeus monodon* obtained from the Central Institute of Brackish Aquaculture (CIBA), Muttukadu, near Chennai were used in the experiments. They were kept in seawater with aeration for a period of 6 h in order to avoid any stress to the animals and then used for the experiments. The experiments were conducted at the CIBA, Chennai. The experimental tanks were added with 70 L of filtered seawater at 32 % salinity and kept at ambient temperature (28 ± 1°C) and aerated continuously. The seeds were transferred in the tank with a stocking density of 75 nauplii per litre.

Feeding schedule from Zoea to Post Larvae of *Penaeus monodon*

The Zoea of *Penaeus monodon* kept in the experimental tanks was fed with five different microalgae: *Isochrysis galbana, Cheatoceros calcitrans, Skeletonema coastatum*, *Dunaliella salina* and *D. bardawil*. The Zoea (Z) of I- III stages were fed thrice with 30×10^4 cells/mL of algal cells. The Mysis (M) (I-III/Postlarve1) of *P. monodon* were fed thrice with 40×10^4 cells/mL of algal cells. Thereafter from PL 2- PL 20 stage they were fed thrice with 3-8 no/ml of *Artemia* sp. nauplii enriched with different microalgae. The 24 h old *Artemia* nauplii enriched with *I. galbana*, *C. calcitrans, S. coastaum, D. salina* and *D. bardawil* at 20, 30, 40, 50 and 60×10^4 cells/mL at 16 h and 9 h respectively for a period of 24 h were fed to *P. monodon*. Filtered seawater was exchanged daily and the debris settled at the bottom was siphoned out without disturbing the animals. This experiment was conducted for a period of 20 days (PL20).

Growth, Body weight and survival of *P. monodon*

The growth, total body fresh weight and survival of *P. monodon* were recorded from the animal fed with different microalgae and microalgae enriched with *Artemia* sp. nauplii and fed to *P. monodon*. At the end of the experiments the animals were randomly selected and recorded for the above parameters.

Biochemical analysis

The *P. monodon* of Zoea – M3/PL 1 stage were fed with different microalgae and from PL 2- PL 20 stage were fed with *Artemia* sp. nauplii enriched microalgae individually were taken for biochemical analysis. Fifty mg fresh weight of the animal samples from Zoea to Postlarvae 20 were taken and estimated for different biochemical constituents. The total protein was quantified following the method of [3]. The total carbohydrate was quantified as per the method of [6]. The total lipid was quantified as per the method of [12]. The carotenoid content of the PL 20 stage of *P. monodon* animals fed with *Artemia* sp. nauplii enriched five different microalgae were studied for its carotenoid content by following the method of [20]. The amount of carotenoids extracted from *P. monodon* was scanned under UV visible spectrophotometer at 450 nm and further confirmed by TLC. All the values were given on wet weight basis. All the biochemical analyses were carried out with three replications. The values are expressed as µg/ml. The fatty acid profile were analysed by following the method of [17] using Gas Chromatography.

Statistical analysis

The experimental data were tabulated and analyzed using one-way ANOVA by the Agres statistical software package. The least significant difference (LSD) analysis was performed to group the treatment mean values.

RESULTS

Growth in length

Better growth in length was observed in *Penaeus monodon* fed with *C. calcitrans* at M3/PL1 stage followed by *S. coastatum, I. galbana, D. bardawil* and *D. salina.* Whereas in PL 5 stage of *P. monodon* fed with *Artemia* sp. nauplii enriched *D. salina* showed a maximum growth in length when compared to *Artemia* nauplii enriched with *I. galbana* (Fig. 1).

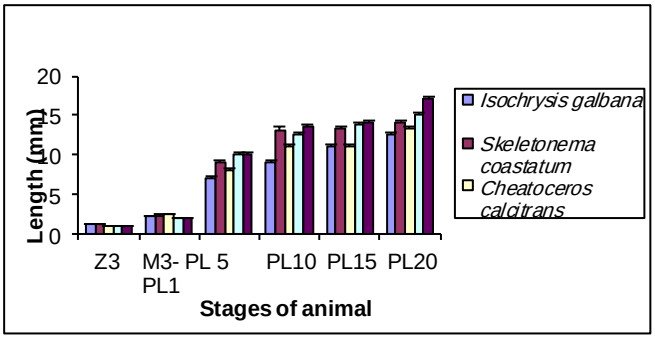


Fig. 1. Length of *P. monodon* Z3- PL I fed with different microalgae and PL 5 - PL 20 fed with *Artemia* nauplii enriched microalgae.

Fresh weight

The animal fed with five different microalgae showed on par similar value of fresh weight but there was a significant increase in fresh weight of the animals at PL 5 stage of *P. monodon* fed with *Artemia* sp. nauplii enriched with *D. salina* and minimum in *I. galbana* (Fig. 2).

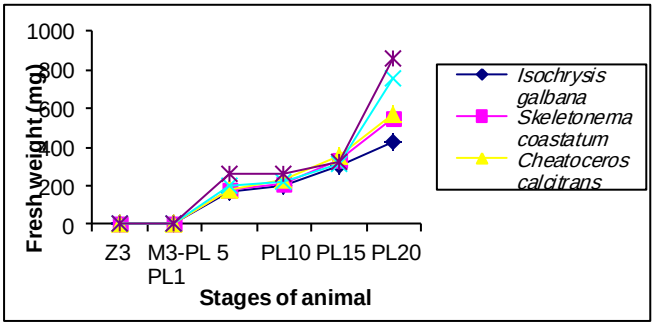


Fig. 2. Fresh weight of *P. monodon* Z3- PL I fed with different microalgae and PL 5 - PL 20 fed with *Artemia* nauplii enriched microalgae.

Survival

Among the different microalgae, significant increase in survival rate 75 % was observed at M3/PL 1 stage of *P. monodon* fed with *C. calcitrans* and low survival rates were observed in the animal fed with *D. salina* (Fig. 3). Further at PL 20 stage 80 % survival of *P. monodon* fed with *Artemia* sp. nauplii enriched with *D. salina* was observed and least survival of 70 % observed in the animals fed with *Artemia* sp. nauplii enriched with *I. galbana* (Fig. 4).

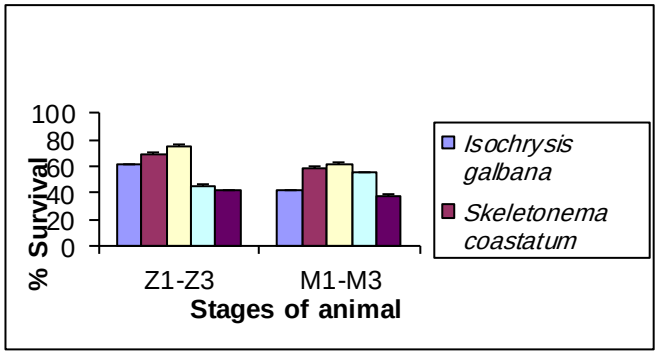


Fig. 3. Survival of *Penaeus monodon* fed with different microalgae.

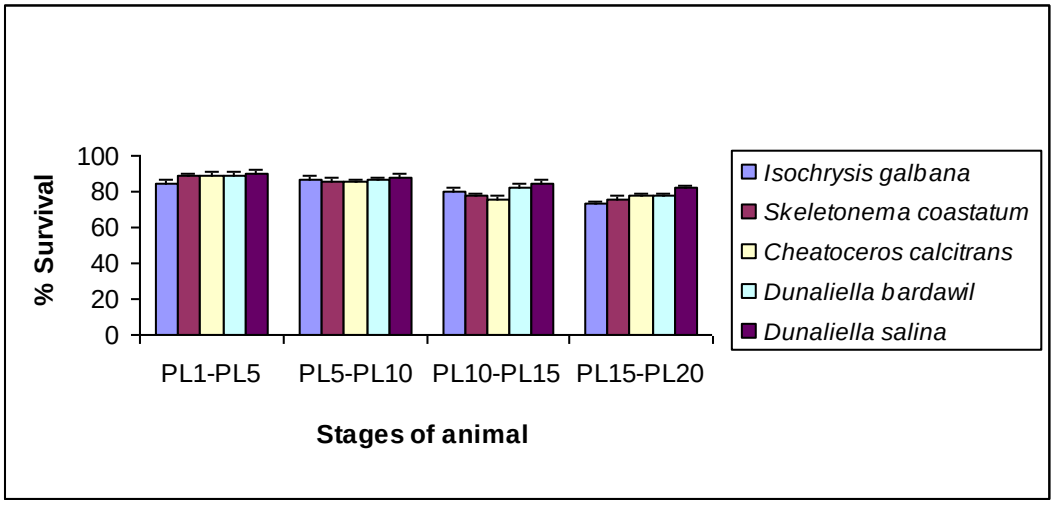


Fig. 4. Survival of *Penaeus monodon* fed with *Artemia* sp. nauplii enriched microalgae.

Biochemical studies

The *P. monodon* larvae fed with *Cheatoceros calcitrans* at M3/PL I stage showed maximum of total protein, carbohydrate and lipid. Whereas, PL1 to PL 20 stages revealed that the PL 10 stage of *P. monodon* attained maximum amount of total protein was 10 % maximum to that of the animal fed with *C. calcitrans*, whereas the accumulation of lipid content also seems to be maximum when it was fed with *Artemia* sp. nauplii enriched *D. salina* and total carbohydrate was maximum in PL 5 stages (Fig. 5, 6, 7).

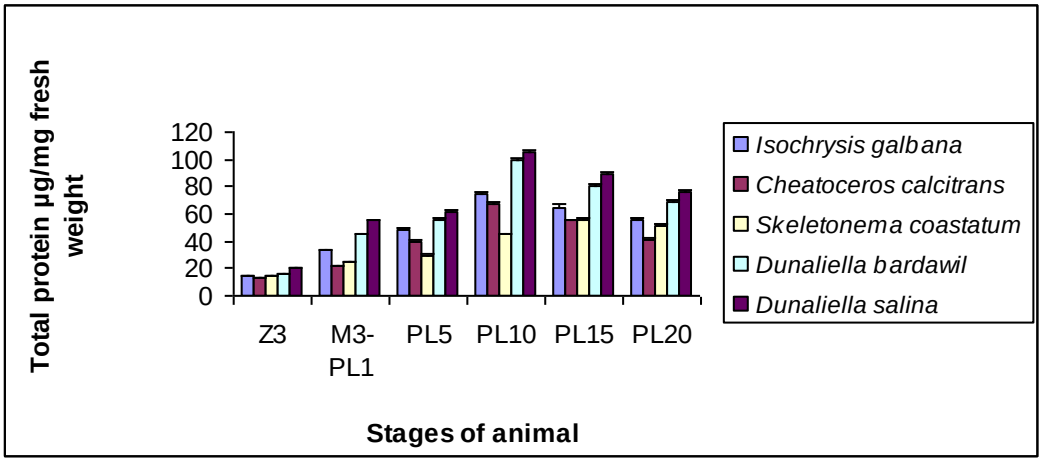


Fig. 5. Total protein of *Penaeus monodon* Z3- PL I fed with different microalgae and PL 5- PL 20 fed with *Artemia* sp. nauplii enriched microalgae

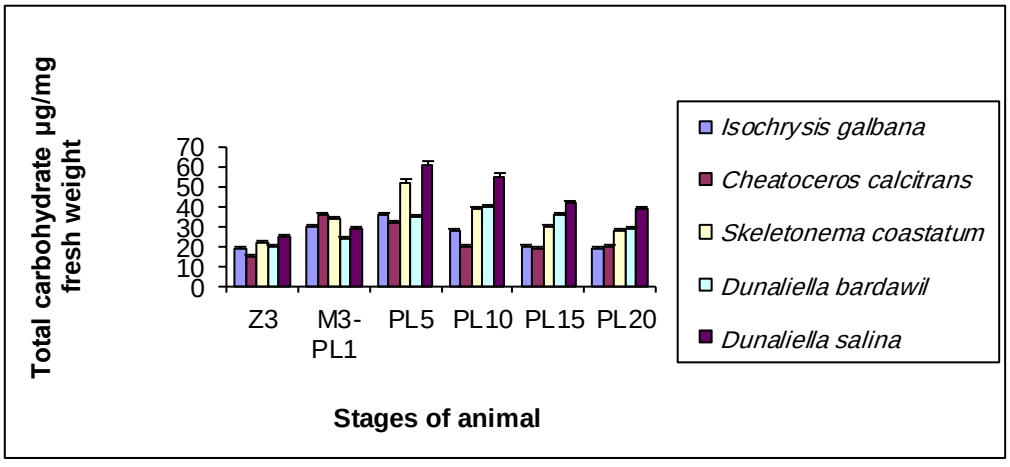


Fig. 6. Total carbohydrate of *Penaeus monodon* Z3- PL I fed with different microalgae and PL 5- PL 20 fed with *Artemia* sp. nauplii enriched microalgae

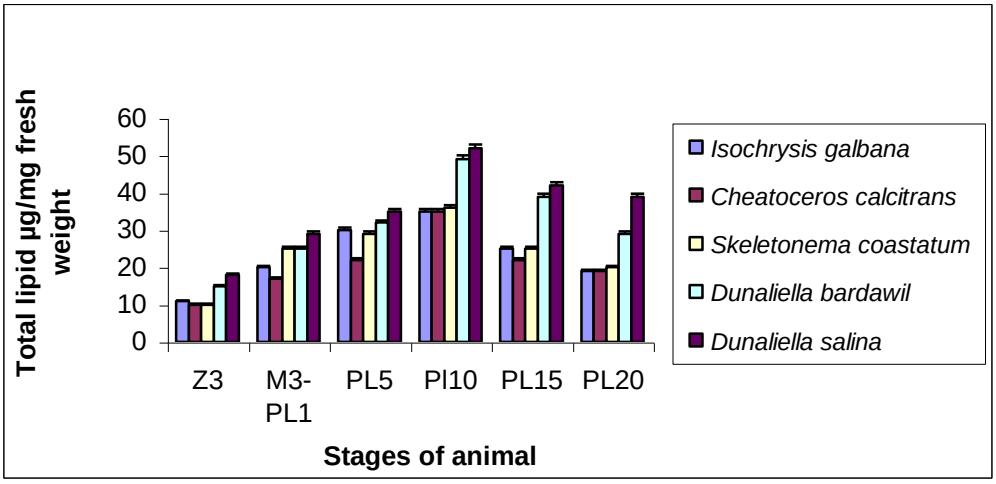
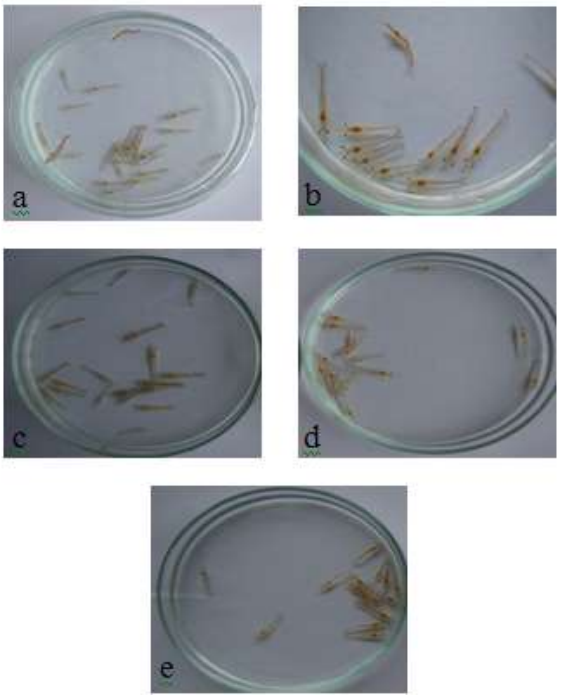


Fig. 7. Total lipid content of *Penaeus monodon* Z3- PL I fed with different microalgae and PL 5- PL 20 fed with *Artemia* sp. nauplii enriched microalgae

Carotenoid content of *P. monodon* fed with *Dunaliella salina* and *D. bardawil*

Total carotenoids observed from PL 20 stage of *P. monodon* (Fig. 8) fed with *Artemia* sp. nauplii enriched with *D. salina* and *D. baradwil*, showed good coloration.



a: *Penaeus monodon* fed with *Artemia* sp.nauplii enriched with *Isochrysis galbana*
b: *P. monodon* fed with *Artemia* sp.nauplii enriched with *Cheatoceros calcitrans*
c: *P. monodon* fed with *Artemia* sp.nauplii enriched with *Skletonema coastatum*
d: *P. monodon* fed with *Artemia* sp.nauplii enriched with *Dunaliella salina*
e: *P. monodon* fed with *Artemia* sp.nauplii enriched with *D. bardawil*

Fig. 8. PL20 stage of *Penaeus monodon* fed with *Artemia* sp.nauplii enriched with different algal sources

Qualitative and Quantitative analysis of carotenoids

The extracted carotenoids from *P. monodon* fed with *Artemia* sp. nauplii enriched *D. salina* when scanned under UV visible spectrophotometer showed maximum content of carotenoids when compared to *D. bardawil* . Futher when subjected to Thin Layer Chromtaography, carotenoids from *P. monodon* fed *Artemia* sp. nauplii enriched *D. salina* showed the Rf value 0.85 corresponded to the standard β-carotene. Whereas the carotenoids from the animal fed with *Artemia* sp. nauplii enriched with *D. bardawil* showed Rf value of 0.77 which may be astaxanthine diester (Fig. 9).

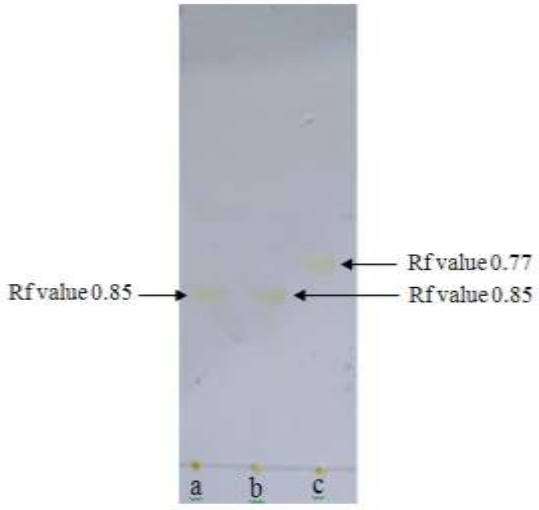


Fig. 9. Thin Layer Chromatography

Fatty acid profile of *P. monodon* fed with different microalage and *Artemia* sp. nauplii enriched with microalgae

The fatty acid profile of Z3-M3 stage of *P. monodon* revealed that the animal fed with *C. calcitrans* (Fig. 10) showed maximum levels of PUFA's 16.26 % Eicosapentanoic acid (EPA) and 6.68 % Docosahexanoic acid. The maximum level of EPA (PUFA) recorded in the animal fed with *C. calcitrans* was more than 13 %, 18 %, 31 %, 37 % when compared to the animals fed with *S. coastatum, I. galbana, D. salina* and *D. bardawil,* respectively.

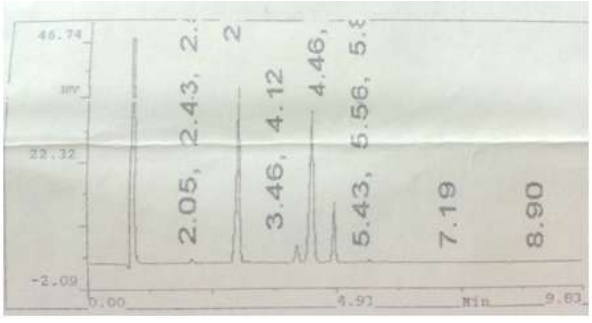


Fig. 10. Fatty acid profile of Z3-M3 stages *Penaeus monodon* fed with *Cheatoceros calcitrans.*

Whereas in PL 20 stage of *P. monodon* fed with *Artemia* sp. nauplii enriched with *D. salina* (Fig. 11) had the maximum accumulation of PUFA of 29.85 % EPA and 13.40 % DHA when compared to the animals fed with *A. salina* enriched *D. bardawil* of 23.32 % EPA and 10.53 % DHA. The maximum levels of EPA and DHA recorded in the animals fed with *Artemia* sp. nauplii enriched with *D. salina* were more than 42 % and 19 % when compared to the animals fed with *Artemia* sp. nauplii enriched with *C. calcitrans*

respectively.

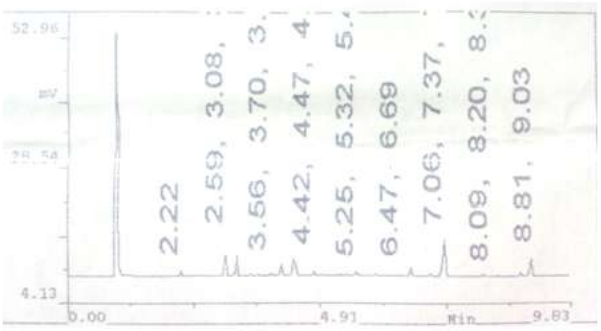


Fig. 11. Fatty acid profile of PL 20 stage of *Penaeus monodon* fed with *Artemia* sp. nauplii enriched *Dunaliella salina*

DISCUSSION

Shrimps are among the most intensively cultivated species and fetch high demand internationally. In the present study, the survival, length and weight of Zoea – PL I of *P. monodon* fed with five different microalgae and PL I- PL20 stages fed with *Artemia* nauplii enriched microalgae diets elucidate certain interesting findings such as the Zoea – Mysis/PL1 of the shrimp fed with *C. calcitrans* showed good growth and high survival rate than other microalgae. Similarly, D'souza, [4] in his work stated that *Cheatoceros* sp. showed higher survival rate and growth in Penaeid shrimp. Interestingly *P. monodon* when fed with *D. salina* showed less growth and the survival rate was only 45 % observed in the present study. Similarly, Kurmaley *et al*. [15] justified that the poorest algal diet in terms of survival and development of the larvae of shrimp fed with *Dunaliella* sp could be due to larger cell size of the alga when compared to the diatom. In the present study it has been observed that growth, fresh weight and survival is enhanced in *P. monodon* from PL 1 – PL 20 stage fed with *Artemia* nauplii enriched with *D. salina*. Similarly, Kuban *et al.* [14] observed that the survival rates , growth is superior in larvae fed with *Artemia* nauplii than microalgae alone. Pedroza *et al*. [18] substantiated that the microencapsulated live diets enhanced the growth and survival of shrimp.

The biochemical studies in the present investigation revealed that the Zoea to PLI of shrimps had high protein content of 51 % followed by carbohydrate 6 % and lipid 50 % when it was fed with *C. calcitrans*. Similar findings were reported by D'souza *et al*. [5] that Penaeid larvae fed with *Cheatoceros* sp. showed more protein, lipid and carbohydrate than those fed with other algal sources. In the present study, the *P. monodon* animal fed with *A. salina* enriched with *D. salina* cells showed high nutritive values of total protein, carbohydrate and lipid than other algal sources. The art of rearing *P. monodon* mainly depends on the natural diet that produces consistently faster growth and higher survival than the artificial diet.

Penaeids are unable to synthesize carotenoids *de novo* [8]. Astaxanthin and β-carotene are the predominant carotenoids in penaeids and therefore appropriate precursors must be supplied in the diet to obtain normal coloration. In the present study, the carotenoids extracted from *P. monodon* fed with *Artemia* nauplii enriched *D. salina* at PL 20 stage contained β-carotene as similar to that of standard. Boonyaratpalin *et al*. [2] also recorded that coloration in shrimp was due to the feed of *Artemia* nauplii enriched *D. salina* and contained β-carotene and the absence of astaxanthin. Interestingly, the *P. monodon* fed with *Artemia* sp. nauplii enriched *D. bardawil* showed the presence of astaxanthin pigment. The most important fatty acids especially arachidonic acid, eicosapentanoic acid and docosahexanoic acid are essential for the normal functioning of various physiological systems and activating immune system [10]. Taking into general consideration, *P. monodon* exclusively shows that the fatty acid content of the larvae reflects the content of the feeds [19]. The PUFA content of *P. monodon* at Z3 to PL I stages was maximum of 37 % EPA and 20 % DHA when it was fed with *C. calcitrans* followed by the animal fed with *D. salina, D. bardawil, S. coastatum* and *I. galbana*. Similarly, D'souza [7] observed that Penaeid shrimp fed with *Cheatoceros* sp. had high amount of PUFA when compared to *Dunaliella* sp. fed animals. Shrimps are cold blooded animals that need a greater amount of PUFA in the tissues to maintain membrane fluidity. In the present study, the shrimp fed with *A. salina* enriched with *D. salina* had maximum of 42 % EPA and 19 % DHA.

On the basis of the present results, it has been concluded that *Artemia* sp. nauplii enriched *D. salina* was found to be the best among the microalgae tested when fed to shrimp upto the post larval stages in terms of nutritional requirements and also coloration of shrimp. Several other research works are carried out day by day in the nutritional aspects of the shrimp. From the above result it shows that *P. monodon* in postlarval stages shows good coloration when fed with *Dunaliella salina*. Hence it can be used in the hatchery by farmers, as the external appearance of the shrimp will be attracted when exporting to other places. Rather than coloration it also has better nutritional components. Further studies should be made to learn the antioxidant property of the shrimp when fed with *D. salina*.

ACKNOWLEDGEMENTS

We are thankful to Dr. S. Sivagnanam, CIBA, Chennai, Tamilnadu, India for his valuable suggestion and help with the experiments.

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