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Biochemical profile of shrimp larvae fed with five different micro algae and enriched *Artemia salina* under laboratory conditions

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Abstract
Shrimp farming is one of the most important aquaculture and economically lucrative practices of maritime countries. Providing a Specific Pathogen Free (SPF) shrimps to the farmers is a big challenge that must be addressed to meet the demand. In general, microalgae are utilized in aquaculture as a live feed for the shrimps. The retention time of food in the gut is lower than in mysis and postlarvae, while feces production is high. Therefore, knowledge of the optimum level of protein, lipids and carbohydrates would be effective in reducing feed costs and water pollution. The types and levels of these nutrients in the diet have been shown to affect the growth. Thus, present study focused to examine the biochemical profile of *Penaeus monodon* and *Litopenaeus vannamei* shrimp culture at different stages from Z3 to PL20. From the results, it has proven that the biochemical concentration will be vary (Protein - 16.06±0.582 to 21.98±0.81, Carbohydrate-3.82±0.576 to 8.32±0.649, Lipid-11.22±0.322 to 24.02±0.396) when fed with different microalgae in both the shrimp species.

Keywords: Aquaculture, shrimp larvae, micro algae, *Artemia salina*, biochemical profile

Introduction
During the shrimp growth after metamorphosis it is considerably affected by the gradual change from planktonic to benthic existence coinciding with changes in the gut (Karthik *et al.*, 2015 a-d) [6-9]. These changes appear to take place during early postlarval development and not abruptly at metamorphosis to post larval stage1 (Wickins, 1976) [19]. During early post larval development, high mortality was noticed due to the changes in the gut associated digestive enzyme production levels (Madhumathi and Rengasamy, 2011a, b) [13, 14]. The first feeding during the growth of any cultivable organism is the most 'critical phase' of their life cycle for their survival. Hence developing a new technology and new live feed may offer great hope for the future with a promise for blue revolution in the century to match the green revolution. Protein is the major component in the natural food of penaeids shrimps. Thus, feeding the penaids with protein rich live diets such as, phytoplankton such as microalgae (2 - 20 µm) and zooplankton such as rotifers (50 - 200 µm) and brine shrimp, *Artemia salina* (200 - 300µm) can increase the gut associated digestive enzymes (Anonymous, 2000).
Furthermore, literature has revealed that, some diatoms secrete a number of anti-bacterial substances and serve as a good food for post larvae of Penaeus shrimp. Diatoms such as *Chaetoceros* sp can rapidly reduce ammonia nitrogen. In order to maintain the good quality of pond water, scholars suggest that inoculation of diatoms to predominate the probiotic system would be a prudent approach. However, it is not easy to inoculate diatoms for they need strict growth conditions. The kind of ecological environment that is required to promote the growth of diatoms has not been reported so far (Xu, 2006) [20]. Austin *et al.*, (1992) [1] have reported a kind of microalga (*Tetraselmis suecica*), which can inhibit pathogenic bacteria such as, *Aeromonas hydrophila, A. salmonicida, Serratia liquefaciens, Vibrio anguillaram, V. salmonicida* and *Yersinia ruckeri*. The microalgae most frequently used in aquaculture include *Chlorella, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema and Thalassiosira (Spolaore et al., 2006).*

Micro algae and *Artemia salina* like zooplankton help to stabilize and improve the water quality improve the oxygen production, promote pH stabilization and regulate disease causing bacterial population and above all the probiotics and stimulate immunity in the host animal. Moreover, *Artemia salina* is biologically uncontaminated readily available and acceptable larval feed and established as a standard live feed for over 85% of marine species. Keeping this in mind, the present study focused to examine the biochemical profile of *Penaeus monodon* and *Litopenaeus vannamei* shrimp culture from zoea to postlarvae 20 stages by feeding with five different microalgae such as, *Isochrysis galbana, Chaetoceros calcitrans, Tetraselmis* sp, *Chlorella* sp and *Nannochloropsis* sp and through enriched with *Artemia salina*.

Materials and Methods

Microalgae

The five different microalgae such as *Isochrysis galbana, Chaetoceros calcitrans, Tetraselmis* sp. *Chlorella* sp. and *Nannochloropsis* sp obtained from AMET Microbial Culture Collection Centre, Department of Marine Biotechnology, AMET University. Walne medium is used as the Nutrient medium for the culture of the micro algae (Walne 1970).

Experimental animal

The shrimp (nauplii 24 h) of *Penaeus monodon* and *Litopenaeus vannamei* obtained from a commercial shrimp hatchery located in Marakanam, Kanchipuram District, Tamil Nadu, India. 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.

Maintenance of *P. monodon* and *L. vannamei* larvae in the laboratory

The *P. monodon* and *L. vannamei* larvae transported from the hatchery, at the Nauplius stage, to the laboratory. They were carried to the lab in 10 litre plastic bags with 1/3rd Vol. filled with water and 2/3rd with oxygen, at a temperature of 25-27 ^{0}C and a concentration of 1000 larvae per litre. The bags placed inside the 50 litre tanks for acclimation to the experimental laboratory conditions. The larvae stocked at density of 150 larvae per litre in 5 l FRP tanks with 3 litres of filtered sea water. For disinfections 0.3 mL of 10% sodium hypochlorite was used. Aeration turned on for 5-10 minutes until the chlorine is fully mixed, then turned it off and let the tank stand for 12-24 hours and after that the aeration system turned on and chlorine concentration measured with a swimming pool chlorine test kit then added sodium thiosulphate crystals dissolved first in water at the rate of 1ppm (1g/m3) for every 1ppm of chlorine left in solution until no yellow color seen (FAO 2007, Pablo *et al* 2005). About 30-50% of the water is being drained daily from the 2nd day onwards and fresh filtered sea water is being added. Water siphoned out from the bottom of the tank, mesh-net strainers used to prevent the removal of the larvae along with the water and wastes. Each experiment carried out in triplicate.

Experimental design

The experiments conducted at the Department of Marine Biotechnology, AMET University, Chennai. A total of twelve 100 L glass tanks (Six tanks for *Penaeus monodon* and another six tanks for *Litopenaeus vannamei*) added with 70 L of filtered seawater at 32‰ salinity and kept at ambient temperature (28 ± 1 °C) and aerated continuously. The seeds transferred in the tank with a stocking density of 75 nauplii per litre.

Feeding schedule from zoea to post larvae

The Zoea of *Penaeus monodon* and *Litopenaeus vannamei* kept in the experimental tanks fed with five different microalgae such as *Isochrysis galbana, Chaetoceros calcitrans, Tetraselmis* sp, *Chlorella* sp and *Nannochloropsis* sp. On 1st day of the Zoea (Z) of I- III stages fed thrice with 30 × 10^4 cells/mL of algal cells. On the 2nd day of the Mysis (M) (I- III/Postlarvae 1) of *Penaeus monodon* and *Litopenaeus vannamei* fed thrice with 40 × 10^4 cells/mL of algal cells. From 3rd day up to 20th day they were fed thrice with 3-8 No/mL of *Artemia salina* nauplii enriched with different microalgae. The 24 h old *Artemia salina* nauplii enriched with *Isochrysis galbana, Chaetoceros calcitrans, Tetraselmis* sp, *Chlorella* sp and *Nannochloropsis* sp at 20, 30, 40, 50 and 60 × 10^4 cells/mL at 16 h and 9 h respectively for a period of 24 h as fed to *Penaeus monodon* and *Litopenaeus vannamei*. Filtered seawater exchanged daily and the debris settled at the bottom siphoned out without disturbing the animals. This experiment conducted up to the stages of PL20. At the end of the experiments the animals from all the experimental tanks were randomly selected and the survival rate and the average length were recorded (Karthik *et al.,* 2015) [6].

Water quality analysis

Water quality analysis has done using following standard methods. pH pen (Scan – 2- Eutech cybernetics PTE Ltd, Singapore) used to measure the water pH and handy refractometer (Atago, Japan) for estimating salinity. Dissolved oxygen and temperature together was measured with the help of handy D.O meter (YSI 55 model). Ammonia determined using the sea water method as described by Solarzano (1969) [15] and Koroleft (1969) and recorded as parts per million (ppm). Nitrate and nitrite was estimated following the methods described by Strickland and Parsons (1972) [16].

Biochemical composition of larvae

Protein

Protein was measured by the method of Lowry *et al* (1951) [12] using bovine serum albumin as standard. The intensity of the purple blue colour formed proportional to the amount of protein which read in Spectrophotometer at 660nm.

Carbohydrate

The carbohydrate in the sample extracted and determined according to Anthrone method (Hedge, 1962). Crude extracts used for glycogen analysis mixing 0.1 mL of each component with 1 mL anthrone reagent (0.1% dissolved in 76% sulfuric acid). Absorbance measured at 630 nm against a reagent blank. Carbohydrate quantified using dextrose solution as the standard.

Lipid

Lipids extracted from samples, by homogenization in chloroform/ methanol (2:1,v/v), containing 0.01% (w/v) butylated hydroxytoluene (BHT) as an antioxidant, according to Folch *et al,* (1957) [3] and determined according to the Bligh and Dyer (1959) [2] method. Absorbance recorded at 560 nm.

Results and Discussion

In general, traditional and non-scientific shrimp farms depend upon the shrimp seeds caught from the wild or those entered

with the tides for stocking ((Karthik *et al.,* 2013 & 2014) [10, 11]. Such seeds collected naturally from tides may have fortuitous infection with microbes and bound to affect shrimp culture. There is a demand for healthy and quality seeds throughout the year. Thus, the successful shrimp culture is dependent upon stocking disease free, healthy seeds in the hatcheries (Soundarapandian and Babu, 2010) [17]. The applications of microalgae for aquaculture are associated with nutrition and other biological activities (Hemaiswarya *et al.,* 2011).

In general, the retention time of food in the gut is lower than in mysis and postlarvae, whereas feces production is high. Therefore, knowledge of the optimum level of protein, lipids and carbohydrates would be effective in reducing feed costs and water pollution. The types and levels of these nutrients in the diet have been shown to affect the growth. Hence an attempt was done to evaluate whether the gross protein, carbohydrate and lipid compositions of the algal diets do not explain the observed differences in growth of the larvae nor did they correspond to the gross composition of the larvae (*P. monodon* and *L. vannamei*) and the obtained biochemical profile (Protein - 16.06±0.582 to 21.98±0.81, Carbohydrate - 3.82±0.576 to 8.32±0.649, Lipid - 11.22±0.322 to 24.02±0.396) were illustrated (Table 1 & 2). In general, for both larvae and juvenile diet containing *C. calcitrans* (live or concentrated) were frequently amongst the better performing diets. Brown and Robert (2002) have found that the control oysters had superior growth and survival and these were not improved by supplementing with any of the *T. Isochrysis* live or concentrated diets. High nutritional performances with mollusc larvae including *C. gigas* have been related by Utting and Millican (1997) [18]. Madhumathi and Rengasamy, (2011a, b) [13, 14], also observed the similar results, like high survival rate, length and weight of zoea-PL I of *P. monodon* fed with *C. calcitrans* and PL I- PL20 stages fed with *C. calcitrans* enriched *Artemia* nauplii diets. Similarly, they also stated 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*. Since, the demand for shrimp products in world markets continues to increase, the continuous supply of healthy, inexpensive and robust shrimp seed stocks to the farmers is important to maintain production of adult shrimps. As the same microalgae also play a vital role in aqua ponds therefore using commercially available micro algae enriched feed supplements (Spilac, Guybro Chemical Pvt Ltd) and plankton booster (Grocape, Guybro Chemical Pvt Ltd) will enhance the production in aquaculture sector. From the results, it is concluded that, the use of *Chaetoceros calcitrans* and enriched *Artemia salina* will promote the successful production of shrimp larvae in hatcheries and reduce the potential negative impact of shrimp farming on the environment like, organic matter accumulation, ammonification, eutrophication and water toxicity and increase the productivity of the farms to the benefit of local economies.

Table 1: Biochemical profile (Mean ± SD) of *P. monodon*

Algal Source	Biochemical Profile	Different stages of *Penaeus monodon*				
		Z3-M3	PL1-PL5	PL6-PL10	PL11-PL15	PL15-PL-20
Isochrysis sp	Protein	16.06±0.582	17.08±0.858	18.08±0.486	19.18±0.303	18.18±0.465
	Carbohydrate	6.12±0.681	17.86±0.709	5.52±0.576	14.1±0.674	8.32±0.649
	Lipid	11.22±0.322	13.32±0.834	15.62±0.931	12.74±0.983	23.22±0.614
Chaetoceros sp	Protein	20.78±0.736	16.38±0.589	21.36±0.602	18.66±0.427	19.28±0.432
	Carbohydrate	8.44±0.63	15.56±0.472	7.88±0.668	15.56±0.472	7.2±0.787
	Lipid	24.02±0.396	15.42±1.21	20.88±0.887	18.42±0.646	19.08±0.54
Tetraselmis sp	Protein	18.32±0.471	16.66±0.422	17.32±0.471	17.08±0.858	18.634±0.758
	Carbohydrate	3.82±0.576	14.64±0.789	5.92±0.614	17.86±0.709	7.68±0.672
	Lipid	14.56±0.931	17.24±0.646	17.94±0.427	13.32±0.834	20.98±4.39
Chlorella sp	Protein	19.28±0.432	19.18±0.303	20.78±0.736	16.38±0.589	21.98±0.81
	Carbohydrate	7.2±0.787	14.1±0.674	8.44±0.63	15.56±0.472	7.94±0.421
	Lipid	19.08±0.54	12.74±0.983	24.02±0.396	15.42±1.21	20.54±0.879
Nannochloropsis sp	Protein	18.634±0.758	18.66±0.427	18.32±0.471	16.66±0.422	21.94±0.594
	Carbohydrate	7.68±0.672	15.56±0.472	3.82±0.576	14.64±0.789	8.14±0.378
	Lipid	20.98±4.39	18.42±0.646	14.56±0.931	17.24±0.646	21.5±0.552

Table 2: Biochemical profile (Mean ± SD) of *L. vannamei*

Algal Source	Biochemical Profile	Different stages of *L. vannamei*				
		Z3-M3	PL1-PL5	PL6-PL10	PL11-PL15	PL15-PL-20
Isochrysis sp	Protein	18.32±0.471	16.66±0.422	17.32±0.471	17.08±0.858	18.634±0.758
	Carbohydrate	3.82±0.576	14.64±0.789	5.92±0.614	17.86±0.709	7.68±0.672
	Lipid	14.56±0.931	17.24±0.646	17.94±0.427	13.32±0.834	20.98±4.39
Chaetoceros sp	Protein	18.634±0.758	18.66±0.427	18.32±0.471	16.66±0.422	21.94±0.594
	Carbohydrate	7.68±0.672	15.56±0.472	3.82±0.576	14.64±0.789	8.14±0.378
	Lipid	20.98±4.39	18.42±0.646	14.56±0.931	17.24±0.646	21.5±0.552
Tetraselmis sp	Protein	19.28±0.432	19.18±0.303	20.78±0.736	16.38±0.589	21.98±0.81
	Carbohydrate	7.2±0.787	14.1±0.674	8.44±0.63	15.56±0.472	7.94±0.421
	Lipid	19.08±0.54	12.74±0.983	24.02±0.396	15.42±1.21	20.54±0.879
Chlorella sp	Protein	16.06±0.582	17.08±0.858	18.08±0.486	19.18±0.303	18.18±0.465
	Carbohydrate	6.12±0.681	17.86±0.709	5.52±0.576	14.1±0.674	8.32±0.649
	Lipid	11.22±0.322	13.32±0.834	15.62±0.931	12.74±0.983	23.22±0.614
Nannochloropsis sp	Protein	20.78±0.736	16.38±0.589	21.36±0.602	18.66±0.427	19.28±0.432
	Carbohydrate	8.44±0.63	15.56±0.472	7.88±0.668	15.56±0.472	7.2±0.787
	Lipid	24.02±0.396	15.42±1.21	20.88±0.887	18.42±0.646	19.08±0.54

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