American-Eurasian Journal of Agronomy, 4 (2): 28-33, 2011
ISSN 1995-896X

Effect of Algal Enriched *Artemia* sp. On the Growth and Digestive Enzyme Activity of *Penaeus monodon* from Zoea to Postlarval 20

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Abstract: This study was subjected to investigate the growth and digestive enzyme activity in *Penaeus monodon* from Zoea to postlarvae 20 stages fed algae and algae enriched *Artemia* sp. nauplii. Among the five different algal sources the animals at PL 20 stage showed maximum length when fed with *Artemia* sp. nauplii enriched with *D. salina*. On studying the ontogenetic changes in the digestive enzyme of protease recorded on *Penaeus monodon* at Z3 stage fed with natural diets *C. calcitrans* at pH 8 showed high activity, thereafter from M3/PL I stages the activity was decreased. The amylase activity of *P. monodon* fed with *C. calcitrans* at Z3 - M3 stages showed maximum value at 35 minutes, thereafter there was a decrement in activity from PL I to PL5. However, from PL 6 to PL 20 there was a gradual increase in amylase activity. The relationship between the digestive enzyme activity and growth performance of shrimp is unclear. Recent studies with PL in *P. monodon* indicated that growth could not be related directly to digestive enzyme activities in shrimp.

Key words: Digestive enzymes · *Artemia* · Algal diets · *Penaeus monodon*

INTRODUCTION

The intensification of prawn culture as a result of a continuous demand for prawns has been confronted with the problems of inherent variability and wide disparity in size of penaeid postlarvae (PL) at the end of nursery culture [1]. Development after metamorphosis is considerably affected by the gradual change from planktonic to benthic existence coinciding with changes in the gut. These changes appear to take place during early postlarval development and not abruptly at metamorphosis to PL1 [2,3]. Lovett and Felder [4] assumed that ontogenetic changes occur in early postlarval development to adapt to a new feeding habit in order to maximize digestion and assimilation of food. Moreover, a `critical period' in early development of postlarvae (PL1- PL14 days old) has been identified with high mortality [2,5] characterized by changes in the gut associated with digestive enzyme production levels. The study of digestive enzymes constitutes a key aspect in understanding the digestive system and the nutritional requirements of specific stages of development [6,7]. Investigations on enzyme response related to feeding and nutrition have mostly been targeted at shrimp larval stages [8,9] and at adults [10,6]. Because protein is the major component in the natural food of penaeids [11], proteolytic enzymes may play a key role in the assimilation processes of these species. Changes in larval enzyme response to diet have been explained as a physiological adaptation to availability of nutrients, in addition to ontogenetic changes [12]. Artificial diets have been widely used in postlarval culture for early stages of development. However, little is known about whether postlarval enzyme response can be adapted to feed and hence be reflected in growth, or whether physiological response is mainly controlled by ontogenetic development.

Based on this, the present study were examined the growth and digestive enzyme activity in *Penaeus monodon* from Zoea to postlarvae 20 stages fed algae and algae enriched *Artemia* sp. nauplii. Initially, growth and digestive enzyme response to the diet fed were evaluated for the postlarval development. Finally, the effects of feeding algae and *Artemia* sp. nauplii enriched algae were evaluated in terms of enzyme activity to determine whether this physiological response is reflected in growth during early postlarval development.

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MATERIALS AND METHODS

The five different microalgae such as *Isochrysis galbana, Cheatoceros calcitrans, Skletonema coastatum, Dunaliella salina* and *D. bardawil* were obtained from Center for advanced studies in Botany, University of Madras and used in this experimental study.

Experimental Animal: 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.

Experimental Design: 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 continuosuly. 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 were fed with five different microalgae: *Isochrysis galbana, Cheatoceros calcitrans, Skeletonema coastatum*, *Dunaliella salina* and *D. bardawil*. On 1st day of the Zoea (Z) of I- III stages were fed thrice with 30×10^4 cells/mL of algal cells. On the 2nd day of the Mysis (M) (I- III/Postlarve 1) of *P. monodon* were 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* sp. nauplii enriched with different microalgae. The 24 h old *Artemia* sp. 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 as 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). At the end of the experiments the animals were randomly selected and recorded for total length (Rostum to Telson) and total body fresh weight were measured The animal samples collected on 1st day (Zoea III stages) after naupliiar stage, 2nd day (Mysis 111), 5th day (PL5), 10th day (PL10), 15th day (PL15) and 20th day (PL20) were analysed for different enzyme analysis.

Digestive Enzyme Activities of *P. Monodon* Fed with Different Microalgae

Protease Assay: This assay was performed using the method followed by [13]. Fifty milligram of the sample was ground in a mortar and pestle with 750 µL of glass distilled water. After homogenization the samples were sonicated for 30 seconds and centrifuged at 12000 rpm for 10 minutes at 4°C. The supernatant was collected and kept on ice. Total protease activity was measured by the modified Casein Method using casein dissolved in 0.1 M Tris buffer at pH 8. The total protease activity was determined at different pH values at 3.0, 4.0, 6.0 and 8.0 using citrate phosphate buffer. The assay mixture consisted of 400 µL of casein solution, 400 µL buffer and 200 µL supernatant of homogenate and incubated at 30°C for 60 minutes. The reaction was then stopped by adding 0.3 M Trichloroacetic acid. After cooling on ice bath for 15 minutes, the assay mixture was centrifuged for 10 minutes at 4°C. The absorbance of the supernatant was measured at 280 nm with Beckman spectrophotometer. The results are expressed as mg tyrosine per g protein in sample per 60 minutes.

Amylase Assay: This assay was performed using the method followed by [14]. Fifty milligram of the sample was homogenised with 1500 µL of ice cold phosphate buffer (pH 6.9) using a mortar and pestle. The homogenates were centrifuged at 13,000 rpm for 6 minutes. The supernatant was collected and kept at 25°C. 0.05 mL of the above enzyme sample 1.0 mL of substrate solution (potato starch solution in 0.2 M Phosphate buffer at pH 6.9) was added. Digestion was stopped at different time intervals between 3 and 45 minutes by adding 2 mL of 1 % 3,5 dinitrosalicyclic acid. The sample mixture were kept in a boiling water bath for five minutes and the production of reducing sugars was spectrophotometerly measured at 546 nm. Maltose was used for calibration curve ranging from 10 - 100 µg/mL.

Statistical Analyses: 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 of Zoea to Postlarvae 20 of *Penaeus monodon* fed with different microalgae and *Artemia* sp. nauplii enriched with microalgae The *Penaeus monodon* of Zoea - Postlarvae 1 were fed with five different microalgae such as *Isochrysis galbana*, *Skeletonema coastatum, Cheatoceros calcitrans, Dunaliella salina* and *Dunaliella bardawil*. Among the five different algal sources the animals fed with maximum length of 17 mm (Fig. 1) was recorded at PL 20 of the animals fed with *Artemia* sp. nauplii enriched with *D. salina*.

Protease Activity: The protease activity of Z3- M3 (Table 1) stages of *P. monodon* fed with five different algae revealed that the animals fed *C. calcitrans* exhibited a maximum protease total activity of 17.28 U/mL at pH 8 and a minimum total activity of 12.4 U/mL at pH 4 was recorded with *D. salina.* The protease total activity at Z3 stage when fed with *D. salina* was 14.4 U/mL. There was a gradual increase in the enzyme activity from PL 2 to PL 5 (Table 2). Maximum protease activity of 22 U/mL recorded at PL 5 stage when fed with *A. salina* nauplii enriched *S. coastatum* at pH 8 was more than 7.0 %, 8.0 %, 9.0 % and 10 % to that of the animals fed with *C. calcitrans, I. galbana, D. salina* and *D. bardawil* respectively. There was a gradual decrease in protease activity from PL 6 to PL 20 stages of *P. monodon* larvae. The animal fed with *Artemia spp* enriched *C. coastatum* showed the protease activity of 0.54 U/mL at pH 8 which was less than 96 %, 94%, 94 % and 75 % to that of the animal fed with *D. salina, D. bardawil, I. galbana* and *S. coastatum,* respectively (Tables 3,4, 5) stages also showed decreased values.

Amylase Activity: The *P. monodon* nauplii fed with different microalgae were estimated for the amylase activity in different time intervals. Among them Z3 - M3 stages (Table 6) of *P. monodon* fed with the algal sources showed high amylase activity than PL I to PL 5 stages

Table 1: Protease activity of Z3-M3 stages of *Penaeus monodon*

	pH			
	3	4	6	8
Algal sources	Total activity (U/ml)	Total activity (U/ml)	Total activity (U/ml)	Total activity (U/ml)
Isochrysis galbana	8.82 [d]	13.58 [d]	16.10 [b]	16.52 [c]
Cheatoceros calcitrans	16.32 [a]	15.04 [b]	15.84 [c]	17.28 [a]
Skeletonema coastatum	10.92 [c]	17.03 [a]	15.86 [c]	16.64 [b]
Dunaliella bardawil	13.5 [b]	14.7 [c]	16.2 [a]	15.6 [d]
D. salina	7.07 [e]	12.4 [e]	14.23 [d]	14.4 [e]
C.D (0.05)	0.1386	0.0645	0.0304	0.0421

Table 2: Protease activity of PL 6-PL10 stages of *Penaeus monodon*

	PH			
	3	4	6	8
Algal sources	Total activity (U/ml)	Total activity (U/ml)	Total activity (U/ml)	Total activity (U/ml)
Isochrysis galbana	0.49 [a]	0.14 [c]	0.049 [d]	20.09 [d]
Cheatoceros calcitran	0.37 [b]	0.02 [e]	0.042 [d]	20.58 [b]
Skeletonema coastatum	0.32 [d]	0.24 [a]	0.08 [c]	22.00 [a]
Dunaliella bardawil	0.37 [b]	0.18 [b]	0.37 [b]	19.84 [e]
D. salina	0.33 [c]	0.056 [d]	0.44 [a]	20.16 [c]
C.D (0.05)	0.0025	0.0034	0.0073	0.0324

Table 3: Protease activity of *Penaeus monodon* in PL 6- PL10 stages of

	pH			
	3	4	6	8
Algal sources	Total activity (U/ml)	Total activity (U/ml)	Total activity (U/ml)	Total activity (U/ml)
Isochrysis galbana	0.74 [c]	0.74 [b]	0.59 [c]	0.74 [c]
Cheatoceros calcitrans	14.96 [a]	0.68 [b]	0.68 [b]	0.54 [c]
Skeletonema coastatum	9.52 [b]	12.92 [a]	0.20 [e]	3.40 [b]
Dunaliella bardawil	0.63 [c]	0.46 [c]	0.42 [d]	0.73 [c]
D. salina	0.69 [c]	0.79 [b]	0.79 [a]	13.86 [a]
C.D (0.05)	0.2491	0.2064	0.0087	0.2153

Table 4: Protease activity of PL 10- PL 15 stages of *Penaeus monodon*

	PH			
	3	4	6	8
Algal sources	Total activity (U/ml)	Total activity (U/ml)	Total activity (U/ml)	Total activity (U/ml)
Isochrysis galbana	0.26 [d]	0.13 [d]	0.13 [c]	0.45 [e]
Cheatoceroscalcitrans	0.71 [a]	0.82 [a]	0.60 [a]	1.15 [a]
Skeletonemacoastatum	0.28 [c]	0.22 [b]	0.11 [d]	0.78 [b]
Dunaliella bardawil	0.35 [b]	0.17 [c]	0.08 [e]	0.53 [d]
D. salina	0.24 [e]	0.08 [e]	0.56 [b]	0.72 [c]
C.D (0.05)	0.0074	0.0114	0.0098	0.0103

Table 5: Protease activity of PL 15- PL 20 stages of *Penaeus monodon*

	pH			
	3	4	6	8
Algal sources	Total activity (U/ml)	Total activity (U/ml)	Total activity (U/ml)	Total activity (U/ml)
Isochrysis galbana	0.16 [b]	0.055 [d]	0.055 [d]	0.22 [e]
Cheatoceros calcitrans	0.20 [a]	0.61 [b]	0.28 [b]	0.82 [a]
Skeletonema coastatum	0.10 [e]	0.15 [c]	0.10 [c]	0.61 [b]
Dunaliella bardawil	0.15 [c]	0.15 [c]	0.053 [d]	0.30 [d]
D. salina	0.13 [d]	0.69 [a]	0.41 [a]	0.41 [c]
C.D (0.05)	0.0014	0.0111	0.0060	0.0092

Column values followed by the same letters are not significantly different at P = 0.05

Table 6: Amylase activity of Z3 - M3 stages of *Penaeus monodon*

	Different time interval (min)								
Algal sources	5	10	15	20	25	30	35	40	45
Isochrysis galbana	0.72 [d]	0.72 [c]	0.79 [c]	0.82 [c]	0.95 [a]	0.92 [b]	1.02 [b]	1.02 [a]	0.85 [b]
Cheatoceros calcitrans	0.76 [b]	0.94 [a]	0.90 [b]	0.76 [d]	0.76 [d]	0.94 [a]	1.12 [a]	0.90 [c]	0.99 [a]
Skeletonema coastatum	0.74 [c]	0.94 [a]	0.92 [a]	0.88 [b]	0.77 [c]	0.68 [d]	0.77 [d]	0.77 [d]	0.74 [c]
Dunaliella salina	0.27 [e]	0.44 [d]	0.55 [d]	0.60 [e]	0.66 [e]	0.55 [e]	0.38 [e]	0.27 [e]	0.22 [d]
D. bardawil	0.84 [a]	0.84 [b]	0.92 [a]	0.92 [a]	0.80 [b]	0.80 [c]	0.92 [c]	0.92 [b]	0.84b
C.D (0.05)	0.0085	0.0079	0.0060	0.0047	0.0039	0.0062	0.0109	0.0112	0.0112

Table 7: Total amylase activity (U/ml) of PLI - PL 5 stages of *Penaeus monodon*

	Different time interval (min)								
Algal sources	5	10	15	20	25	30	35	40	45
Isochrysis galbana	0.75 [c]	0.77 [c]	0.78 [c]	0.77 [c]	0.75 [a]	0.74 [b]	0.70 [b]	1 [a]	0.69 [b]
Cheatoceros calcitrans	0.79 [c]	0.80 [a]	0.81 [a]	0.83 [b]	0.78 [c]	0.78 [d]	0.74 [d]	0.72 [d]	0.71 [c]
Skeletonema coastatum	0.88 [a]	1.09 [b]	1.11 [a]	0.97 [a]	0.79 [b]	0.79 [c]	0.72 [c]	0.64 [b]	0.59 [b]
Dunaliella salina	0.77 [b]	0.94 [a]	0.87 [b]	0.77 [d]	0.76 [d]	0.74 [a]	0.72 [a]	0.64 [c]	0.58 [a]
D. bardawil	0.18 [d]	0.24 [d]	0.31 [d]	0.37 [e]	0.49 [e]	0.62 [e]	0.55 [e]	0.37 [e]	0.31 [d]
C.D (0.05)	0.0103	0.0079	0.0060	0.0047	0.0039	0.0062	0.0109	0.0112	0.0112

Table 8: Amylase activity (U/ml) of PL5 - PL 10 stages of *Penaeus monodon*

	Different time interval (min)								
Algal sources	5	10	15	20	25	30	35	40	45
Isochrysis galbana	1.99 [c]	2.69 [c]	2.72 [d]	3.47 [c]	3.91 [c]	1.97 [e]	1.70 [e]	1.36 [e]	1.36 [e]
Cheatoceros calcitrans	2.66 [b]	2.73 [b]	2.88 [c]	3.10 [d]	3.25 [d]	3.84 [c]	2.88 [d]	2.88 [c]	2.73 [c]
Skeletonema coastatum	3.26 [a]	2.17 [d]	3.46 [b]	3.87 [a]	4.14 [b]	4.08 [a]	3.74 [a]	2.44 [d]	2.10 [d]
Dunaliella salina	2.67 [b]	3.16 [a]	3.76 [a]	3.66 [b]	4.85 [a]	3.96 [b]	3.66 [b]	3.96 [a]	3.06 [b]
D. bardawil	1.26 [d]	1.78 [e]	2.12 [e]	2..31 [e]	2.94 [e]	3.67 [d]	3.57 [c]	3.15 [b]	3.15 [a]
C.D (0.05)	0.0288	0.0202	0.0242	0.0231	0.0284	0.0328	0.0362	0.0361	0.0282

Table 9: Amylase activity (U/ml) of PL10- PL15 stages of *Penaeus monodon*

	Different time interval (min)								
Algal sources	5	10	15	20	25	30	35	40	45
Isochrysis galbana	0.1 [d]	0.3 [e]	0.57 [e]	0.62 [e]	0.67 [e]	0.78 [c]	1.35 [c]	1.71 [a]	1.56 [a]
Cheatoceros calcitrans	0.71 [c]	0.84 [d]	0.84 [d]	0.97 [d]	1.10 [c]	0.78 [c]	1.43 [b]	1.3 [b]	0.84 [d]
Skeletonema coastatum	1.17 [a]	1.48 [a]	1.79 [a]	1.96 [a]	1.79 [a]	1.34 [a]	1.45 [a]	1.28 [c]	0.89 [c]
Dunaliella salina	0.97 [b]	1.05 [b]	1.05 [c]	1.13 [c]	1.21 [b]	1.13 [b]	1.05 [e]	0.97 [e]	0.89 [c]
D. bardawil	0.97 [b]	0.97 [c]	1.15 [b]	1.24 [b]	0.89 [d]	1.33 [a]	1.06 [d]	1.06 [d]	1.06 [b]
C.D (0.05)	0.0157	0.0160	0.0171	0.0186	0.0159	0.0105	0.0075	0.0108	0.0112

Table 10: Amylase activity (U/ml) of PL15- PL 20 stages of *Penaeus monodon*

	Different time interval (min)								
Algal sources	5	10	15	20	25	30	35	40	45
Isochrysis galbana	2.17 [a]	3.69 [c]	5.04 [c]	6.88 [c]	11.89 [c]	14.59 [b]	13.93 [a]	12.95 [a]	14.35 [b]
Cheatoceros calcitrans	0.99 [e]	1.04 [e]	1.09 [e]	1.09 [e]	1.12 [e]	1.21 [d]	0.77 [d]	0.60 [e]	0.55 [e]
Skeletonema coastatum	1.22 [d]	1.58 [d]	1.53 [d]	1.69 [d]	2.09 [d]	2.53 [c]	1.63 [c]	1.42 [d]	1.17 [d]
Dunaliella salina	1.58 [b]	4.34 [a]	7.17 [b]	9.45 [a]	14.62 [a]	19.66 [a]	16.97 [a]	9.17 [c]	6.21 [c]
D. bardawil	1.44 [c]	4.18 [b]	6.15 [a]	8.66 [b]	13.14 [b]	15.85 [b]	15.65 [b]	13.02 [b]	13.01 [a]
C.D (0.05)	0.0168	0.0838	0.0996	0.1424	0.2404	0.3219	0.3168	0.3106	0.3477

Column values followed by the same letters are not significantly different at P = 0.05

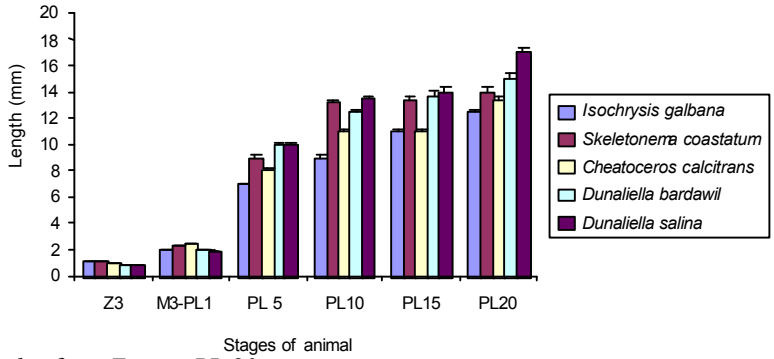

Fig. 1: Length of *P. monodon* from Zoea to PL 20 stages

(Table 7). However, there was a gradual increase in the enzyme activity from PL 6 to PL I0 stages (Table 8). Whereas the enzyme activity from PL I0 to PL 15 (Tables 9) showed decrease activity and further from Pl 15 to PL 20 there (Tables 10) showed a steady increase in values. The Z3 - M3/PL I of *P. monodon* nauplii fed with *C. calcitrans* showed maximum activity of TA 1.125 U/mL at 35 minutes and less activity of TA 0.22 U/mL with *D. salina* at 45 minutes.

DISCUSSION

The study of digestive enzymes constitutes a key aspect in understanding the nutritional requirements of specific stages of development in *P. monodon* [7]. Investigation on enzyme response related to feeding and nutrition has mostly been targeted at shrimp larval stages and adults [15]. In recent times, artificial diets and live diets have been widely used in post larval culture for early stages of larval development. However, little is known about the enzyme response of the feed in post larvae and reflected in growth, or the physiological response is mainly controlled by ontogenetic development.

On studying the ontogenetic changes in the digestive enzyme of protease recorded on *Penaeus monodon* at Z3 stage fed with natural diets *C. calcitrans* at pH 8 showed high activity, thereafter from M3/PL I stages the activity was decreased. But from PL2 to PL5 stages there was a gradual increase in the activity in *P. monodon*. Similar pattern was observed by [12] in the shrimp *P. setiferus*, *P. indicus* and *P. monodon.* They showed poor performance of protease activity in early postlarval development stages. Lee *et al.* [6] have shown that the protein source (animal and plant protein) as well as size of the shrimp influences the level of protease activities. Animal protein induces a high level of enzyme activity in small shrimps but not in large shrimps.

The amylase activity of *P. monodon* fed with *C. calcitrans* at Z3 - M3 stages showed maximum value at 35 minutes, thereafter there was a decrement in activity

from PL I to PL5. However, from PL 6 to PL 20 there was a gradual increase in amylase activity as similar to the observations made on *P. japonicus* [8]. Le Moullac *et al.* [7] reported that a maximum peak in digestive enzyme production was observed from Z3 to M3 stages of penaeid species. Pedroza *et al.* [16] recorded high amylase activity in all mysis stages of penaeid sp. when fed with live diets.

It is unlikely that the increase of enzyme activity at PL 20 was exclusively due to ontogenetic events since the complete reconstruction of digestive system seems to occur after more than 4 weeks from metamorphosis [3,17]. The relationship between the digestive enzyme activity and growth performance of shrimp is unclear. Recent studies with PL in *P. monodon* indicated that growth could not be related directly to digestive enzyme activities in shrimp.

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