5. ACTIVITIES OF DIGESTIVE ENZYMES

5.1. Introduction

Digestion is a key process in the metabolism of decapods crustaceans since it determines the availability of the nutrients needed for all their biological functions. The study of the digestive physiology is of great importance, since net efficiency of the whole process relies on the type and function of the available enzymes (Vonk, 1960). Digestive enzymes are of particular interest, since the rate of digestion in the intestinal system limits the uptake of nutrients to the hemolymph and can potentially limit the growth of the whole organism. Therefore, the study of digestive enzymes is major to when studying the adaptations of any organism to fluctuations in food availability, the nutritional status of individuals, the adaptation to circadian rhythms, and the moulting cycle or developmental changes (Conklin, 1995; Carrillo-Farnes et al., 2007). These aspects are especially important during larval development, when substantial changes in the structure, physiology, size, and body shape are produced and the individuals are exposed to changes in food quantity and quality. Therefore, a great number of studies have focused on the assessment of the ontogeny of digestive enzyme activities of prawns (Lemos et al., 1999, 2000; Saborowski et al., 2006).

Crustaceans digest a variety of food protein materials and possess high concentration of digestive serine proteinases, especially trypsin and chymotrypsin, with the hepatopancreas responsible for synthesis, storage of zymogens, and secretion. Decapod digestive enzymes are synthesized and secreted by the hepatopancreatic F cells (Skinner, 1985).
In most cases, the enzyme secretion is holocrine (Ceccaldi, 1997). Studies on the physiology of the hepatopancreas (Bunt, 1968; Loizzi, 1971; Gibson and Barker, 1979; Nakamura, 1987) have defined the role of hydrolytic enzymes in the digestive processes of crustaceans. The properties of the enzymes associated with the tract will determine the digestive capabilities of the organisms. The nutrition of aquatic organisms is essential to their profitable aquaculture, and the formulation of effective feed depends on our knowledge of the nutritional biochemistry and physiology of the cultured species (Galgani, 1983). A knowledge of digestive enzymes in an organism helps to determine its digestive capabilities, which in turn helps the selection of ingredients to be included in a diet (Lee et al., 1980; Divakaran and Ostrowsky, 1990). The digestive enzymes of crustaceans have been studied over the last century for applications in physiology, biochemistry and food science (Jiang et al., 1991; Garcia-Carrefio, 1992). One important and expensive component for the penaeid shrimp diet is the proteic fraction. To assess the catalytic abilities of crustacean protein, it is important to understand the properties of their digestive proteases (Galgani, 1988).

Protein is one of the most important components of food because, after digestion, it supplies amino acids needed to construct the organism’s proteins. Construction of proteins is requisite for maintenance of tissues and growth. Crustaceans also use amino acids as a source of energy (Fox et al., 1994; Shiau, 1998). Studies in aquaculture have already shown that the rate of digestion and absorption of essential amino acids in dietary protein can be determined from the knowledge of the functional properties of proteases in the digestive tract (Eshel et al., 1993). Several studies have also been conducted to identify and characterize digestive enzymes from the larval to grow-out stages of marine
prawns (Maugle et al., 1982; Galgani and Nagayama, 1987; Celis-Guerrero et al., 2004), and most of the digestive enzymes identified in these crustaceans were serine proteases and some metallo proteases (Garcia-Carreno and Haard, 1993). Enzymes collectively called proteases accomplish digestion of protein in food by protein hydrolysis. The penaeid digestive gland is known to contain high proteolytic activity (Tsai et al., 1986; Van Wormhoudt et al., 1992).

Pancreatic α-amylase is an important enzyme needed for starch hydrolysis in the small intestine of both non ruminants and ruminants. α-amylase, an endoenzyme, hydrolyzes α-1, 4-glucosidic bonds in polyglucosans. The locations of the bonds in the molecule to be hydrolyzed are selected at random; each cleavage by α-amylase produces a reducing end. Several methods have been used to determine α-amylase activity and (or) concentration (Walker and Harmon, 1996).

Lipases are enzymes that hydrolyze ester bonds of aliphatic acids in triglycerides. Lipases are physiologically important because they digest fats into fatty acids for absorption across a cell membrane, and transform triglycerides in to more polar molecules (Lopez-Lopez et al., 2003). Lipases have been found in many species (Berner and Hammond, 1970), but few studies have reported on lipases in decapods crustaceans. The first report on the presence of a true lipase (glycerol-ester hydrolase E.C.3.1.1.3) in decapods was in Homarus americanus adults (Brockeroff et al., 1970), confirming the digestion of triolein in the gastric juice. Hoyle (1973) reported lipase activity in stomach juice of H. americanus using the same substrate. Lee and Lawrence (1985) found lipase activity in the digestive system of Litopenaeus setiferus using tributyrin as substrate. Biesot and Capuzzo (1990a, b) used triole in to determine the activity of lipase in

Fiber, which includes cellulose, is becoming increasingly recognized as a direct or indirect nutrient, and its utilization may differ among species of prawn (D’Abramo and Sheen, 1994). Cellulose may contribute to efficient utilization of dietary proteins (Gomez Diaz and Nakagawa, 1990). Although Fair *et al.* (1980) reported that dietary cellulose levels up to 30% did not affect growth adversely, Gomez *et al.* (1988) found that dietary cellulose levels from 15% to 20% resulted in growth retardation. The Cellulose digesting activity has been detected in *M. rosenbergii* (Noborikawa, 1978; Lee *et al.*, 1980; D’Abramo and Sheen, 1994). It has been detected in a number of other crustaceans: *Carrcinus maenas* and *Cranyon cranyon* (Kristensen, 1972), *Gammarus* (Chamier, 1991), snow crab (*Chionoecetes opilio*) (Brethes *et al.*, 1994), crayfish (Brown, 1995), northern krill (Donachie *et al.*, 1995), Slipper lobster (Johnston and Yellowlees, 1998). Xue *et al.* (1999) demonstrated the presence of endogenous cellulase activity in the red claw crayfish (*Cherax quadriscanus*), and also reviewed the enzymatic requirements for cellulose digestion and absorption. In the present study, digestive enzymes activities, such as protease, amylase, lipase and cellulase were assayed in the hepatopancreas of *M. rosenbergii* PL fed with feeds prepared by replacing fishmeal with *S. platensis*, *C. vulgaris* and *A. pinnata*.
5.2. Materials and Methods

Feeding experiment

*Macrobrachium rosenbergii* (PL-30) with the length and weight range of 1.56±0.29 cm and 0.22± 0.039 g respectively were used for feeding experiment. Thirty PL for each diet in triplicate were maintained in plastic tanks with 40 L water. One group served as control. The experimental groups were fed with the respective concentration of FM replaced with *S. platensis, C. vulgaris* and *A. pinnata* inclusion level of 25%, 50%, 75% and 100% incorporated diets. The feeding was adjusted to two times a day (6:00 am and 6:00 pm). The daily ration was given at the rate of 10% of the body weight of PL with two equal half throughout the experimental period. The feeding experiment was prolonged for 90 days; mild aeration was given continuously in order to maintain the optimal oxygen level.

Isolation and homogenization of digestive tract

The whole digestive tract was isolated and homogenized at 4°C using ice cold distilled water (1:5 ration w/v). The contents were centrifuged at 4°C at 10,000 rpm for 20 min and the supernatant was used for the estimation of digestive enzymes. Soluble protein concentration was determined by the Lowry et al. (1951) method, using bovine serum albumin as the standard.

Estimation of digestive enzymes

Protease activity

Total protease activity was determined by the casein-hydrolysis method (Furne et al., 2005). Buffers for each pH assays were: 0.1 M glycine-NaOH pH 10.0.
A reaction mixture contained casein at 1% (w/v) (0.25 ml), buffer (0.25 ml) and supernatant from the homogenates (0.1 ml) was incubated for 1 h at 37°C. The reaction was stopped by addition of 0.6 ml 8% (w/v) trichloroacetic acid solution; kept for 1 h at 2°C; centrifuged at 1800 g for 10 min and the absorbance of supernatant was measured at 280 nm against blank. For the blank preparation, the supernatant from the homogenates was added at the end of the incubation period, just before addition of trichloro acetic acid. Tyrosine solution was used as standard. One unit of enzyme was defined as the amount of enzyme required to catalyze the formation of 1.0 µmol of tyrosine per min.

**Amylase activity**

Amylase activity was determined by the starch-hydrolysis method of Hidalgo *et al.* (1999). A reaction mixture containing 2% (w/v) starch solution (0.125 ml), 0.1 M citrate-phosphate buffer at pH 7.5 (0.125 ml) and supernatant from the homogenates (0.05 ml) were incubated for 1 h at 37°C. After the incubation period, the Somogyi-Nelson colorimetric method was followed and the absorbance was measured at 600 nm against reagent blank. The reagent blank was made by the addition of supernatant from the homogenate immediately after the incubation period. Maltose solution was used as standard. One unit of amylase was defined as the amount of enzyme required to produce 1.0 µmol of maltose per min.

**Lipase activity**

Lipase activity was determined following the method described by Furne *et al.* (2005), consisting of degrading triacylglycerol to free fatty acids. A solution of 1% polyvinyl alcohol (PVA) and 5 ml of 0.1 N HCl in 1 L of distilled water was heated to 75-85°C, cooled, filtered and adjusted to pH 8.0 with 0.1 N NaOH. Virgin olive oil was
added to an aliquot of the previous solution to obtain a substrate concentration of 0.1 M. This mixture was emulsified for 5 min. In addition, McIlvaine buffer was prepared in 0.1 M citric acid and 0.2 M disodium phosphate. A reaction mixture containing PVA solution-emulsified substrate (1 ml), McIlvaine buffer at pH 8.0 (0.5 ml), and supernatant from the homogenates (0.5 ml) was incubated for 4 h at 37°C. Afterwards, 3 ml of 1:1 ethanol-acetone solution was added to stop the reaction and break the emulsion. Phenolphthalein in ethanol 1% (w/v) was added to the reaction mixture and titrated with 0.01 M NaOH. For the blanks, the same procedure was followed but boiled enzyme was used. Porcine type-II pancreatic lipase was used as standard. One unit of lipase was defined as the amount of enzyme required to hydrolyse 1.0 micro equivalent of fatty acids from triacylglycerols in 1 h at pH 8 and 37°C.

**Ccellulase activity**

Cellulase activity was determined by the method of Gonzalez-Pena et al. 2002. The gastric fluid from individual prawns was transferred to 1.5ml centrifuge tubes, homogenized in 1 volume of 10 mM ice cold sodium citrate at pH 7.0 with a motorized homogenizer at maximum speed for 1 min and centrifuged at 4°C at 10,000 g for 3 min. The supernatant was then transferred to a new tube and stored at -20°C until analysis. Each prawn’s hepatopancreas was homogenized in 1 volume of 10 mM sodium citrate at pH 7.0 with a motorized homogenizer at maximum speed for 1 min. After this, the homogenate was centrifuged at 10,000 g for 5 min at 4°C. The supernatant was then treated as for gastric fluid.
Take 1 ml of 1% microcrystalline cellulose, 1 ml of 0.1 M phosphate buffer and 1 ml of enzyme extract solution in a test tube. Incubate the test tubes for 1 h at 37°C. After 1 h, stop the reaction by the addition of 0.5 ml of dinitrosalicylic acid reagent. Note the absorbance at 540 nm. Deduce the value from the standard curve prepared using glucose. One unit of cellulase is defined as the amount of enzyme per ml which releases one µg of glucose per minute. All enzyme activities are expressed as specific activity (units per milligram of soluble protein).
5.3. Results

Protease

Table 5.3.1 shows the protease enzyme activity of control and experimental diets fed PL groups. The protease enzyme was found to be higher in PL fed with 50% *C. vulgaris* inclusion diet fed group followed by the 50% *S. platensis* and 50% *A. pinnata* inclusion diet. The protease activity was observed minimum level in 75%, 25% and 100% *C. vulgaris* and *S. platensis* diets as compared with control group. But the same enzyme activity was comparatively less in 75% and 100% of *A. pinnata* inclusion diets fed prawn PL. The statistical analysis (DMRT) revealed that the protease enzyme activity between control and experimental diets fed prawns were statistically significant (P<0.05).

Amylase

The results on amylase enzyme activity of PL groups offered with different dietary sources are given in Table 5.3.1. The amylase activity was significantly higher in FM replaced with 50% *C. vulgaris* inclusion diets followed by the 50% *S. platensis* and 50% *A. pinnata* inclusion diets fed *M. rosenbergii* PL. whereas the amylase enzyme activity was found to be minimum in PL fed with 25%, 75% and 100% of *C. vulgaris S. platensis* and *A. pinnata* diets. Compared with control diet fed group the variation between amylase enzyme activity of prawns fed with experimental diets was statistically significant (P<0.05).

Lipase

Table 5.3.1 shows the result on lipase enzyme activity of control and experimental groups of prawn PL. After the feeding trial experiment of 90th day, the lipase enzyme activity was significantly increase (P<0.05) in 50% *C. vulgaris* inclusion diet, followed by
the 50% of *S. platensis* and *A. pinnata* diets. The 25%, 75% and 100% of *C. vulgaris* and *S. platensis* diets fed group showed minimum level of lipase enzyme activity when compared with control group. The 75% and 100% of *A. pinnata* inclusion diet fed group lipase enzyme activity showed comparatively lower when compared with control group. The DMRT test made on the lipase enzyme activity between control and the experimental diets revealed that the variation between them was statistically significant (P<0.05).

**Cellulase**

The cellulase activity of *M. rosenbergii* PL fed with control and experimental groups was showed in Table 5.3.1. After the feeding trial experiment of 90th days, the cellulase enzyme activity was significantly increased in experimental diets fed PL groups. Cellulase activity was significantly increased in the order of 25%>50%>75% and 100% of *S. platensis*, *C. vulgaris* and *A. pinnata* inclusion diets fed PL when compared with control group PL. The increase was compared with control experimental diets which revealed that the variation between them was statistically significant (P<0.05).
Table 5.3.1. Digestive enzyme activity of *M. rosenbergii* PL fed with different formulated diet

<table>
<thead>
<tr>
<th>Diets</th>
<th>Aspects</th>
<th>Initial</th>
<th>Control</th>
<th>Experimental diets</th>
<th>% of Replacement</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(B1+FM75+R25)</td>
<td>(B1+FM50+R50)</td>
<td>(B1+FM25+R75)</td>
</tr>
<tr>
<td><em>S. platensis</em></td>
<td>Protease</td>
<td>0.39 ± 0.09</td>
<td>1.10 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.19 ± 0.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.27 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.22 ± 0.07&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td></td>
<td>Amylase</td>
<td>0.27 ± 0.12</td>
<td>0.78 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.01 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.18 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.92 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Lipase</td>
<td>0.28 ± 0.07</td>
<td>0.61 ± 0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.70 ± 0.11&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.83 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.76 ± 0.07&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cellulase</td>
<td>1.99 ± 0.06</td>
<td>4.41 ± 0.33&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>4.76 ± 0.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.04 ± 0.22&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>5.40 ± 0.22&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. vulgaris</em></td>
<td>Protease</td>
<td>0.39 ± 0.09</td>
<td>1.10 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.26 ± 0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.31 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.21 ± 0.07&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Amylase</td>
<td>0.27 ± 0.12</td>
<td>0.78 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.94 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.04 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Lipase</td>
<td>0.28 ± 0.07</td>
<td>0.61 ± 0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.75 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.86 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80 ± 0.04&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cellulase</td>
<td>1.99 ± 0.06</td>
<td>4.41 ± 0.33&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>5.11 ± 0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.41 ± 0.28&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>5.97 ± 0.31&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. pinnata</em></td>
<td>Protease</td>
<td>0.39 ± 0.09</td>
<td>1.10 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.14 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.18 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.06 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Amylase</td>
<td>0.27 ± 0.12</td>
<td>0.78 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.04 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.14 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.99 ± 0.09&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Lipase</td>
<td>0.28 ± 0.07</td>
<td>0.61 ± 0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.66 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.74 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cellulase</td>
<td>1.99 ± 0.06</td>
<td>4.41 ± 0.33&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>5.47 ± 0.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.15 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.50 ± 0.19&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represented for protease, amylase and cellulase in Unit/mg protein; lipase represented in Unit×10<sup>3</sup>. Each value is a mean ± SD of three replicate analyses, within each row means with different superscripts letters are statistically significant P<0.05 (one way ANOVA and subsequently post hoc multiple comparison with DMRT, paired sample ‘t’ test also applied).

B1- Basal ingredients; FM- Fishmeal; R- Replacement
5.4. Discussion

Feed is the major operational cost for most aquaculture enterprises (D’Abramo and Sheen, 1994). Formulation of well-balanced diets and their adequate feeding are the most important for successful aquaculture. The nutritive value of formulated feeds depends on the digestibility of the individual components (Gonzalez-Pena et al., 2002). Though alternative protein ingredients have also been considered for feed formulation (Sudaryono et al., 1999; Mendoza et al., 2001; Forster et al., 2003), protein digestibility of feedstuffs mostly depend on the type and quality of the raw material and processing strategy (Pike and Hardy, 1997; Francis et al., 2001), that may oscillate considerably in regional terms (Garcia-Carreno et al., 1997; Swick, 2002). A formulated diet can be well balanced and contain all the essential dietary nutrients but still not produce desired results if the various nutrients are not readily available. The true nutritive value of a formulated diet therefore ultimately depends on the bioavailability of the ingredients, and not purely on diet composition (Lee and Lawrence, 1997). Animals rely on a functional digestive system to efficiently utilize the nutrients present in the food (Anderson and DeSilva, 2003), and the morphology of the digestive tract, the physiological conditions of the larvae and the rearing environment all play major roles in determining the digestibility of foods (Lee and Lawrence, 1997).

In the present study postlarvae of M. rosenbergii were fed with compounded diet containing fishmeal gradually replaced with various protein ingredients S. platensis, C. vulgaris and A. pinnata at various concentrations and the effect of these protein levels on digestive enzyme activity, such as protease, amylase, lipase and cellulose level in the hepatopancreas of M. rosenbergii was studied. In these studies it was found that the
highest enzyme activity was attained at partial replacement of fishmeal with *S. platensis* (50%), *C. vulgaris* (50%) and *A. pinnata* (50%), 37 to 41% protein level. Similarly, Nandeesha *et al.* (1998) reported that, *Spirulina* inclusion diets fed *C. carpio* had significantly increased hepatopancreas protease, amylase and lipase activity when compared with control. Also, Umesh *et al.* (1994) obtained the 50% of *S. platensis* inclusion diet was significantly improve the protein digestibility in common carp *C. carpio*. Nandeesha *et al.* (1994), reported that 25%, 50%, 75% and 100% level of fishmeal replace with *S. platensis* diets significantly increase the digestibility activity in catla, rohu and common carp mixed culture. Mustafa and Nagakawa (1995) suggested that the Algae contributes to an increase in protein assimilation and feed utilization. *Undaria finnatifida* and *Ascophyllum nodosum* contributed to the absorption of dietary carbohydrate and protein as an energy source, and stimulated assimilation of nutrients in to body constituents.

**Protease**

Several authors have reported the changes in the proteolytic enzyme activity in different fishes and crustaceans. Smith *et al.* (1992) observed that the size of the shrimp, the source and the level of protein concentration in the diet, all affected the proteolytic activity to a certain degree in *P. vannamei*. Crustacean penaeids adapt quite well to changes in diet composition by induction of digestive enzymes synthesized and secreted in the hepatopancreas (Le Moullac *et al*., 1997). These digestive enzymes are able to hydrolyze a variety of substrates and various factors are involved in their regulation. Among them are diet (Le Moullac *et al*., 1997; Guzman *et al*., 2001), ontogenic changes (Lovett and Felder, 1990), body size (Lee and Lawrence, 1985), circadian rhythms
(Molina et al., 2000), molting stage (Molina et al., 2000) and even a stimulant effect from the pond water has been reported (Moss et al., 2001). The larval and postlarval stages of penaeid shrimp go through a series of metamorphic changes that affect the enzymatic activity (Lovett and Felder, 1990). Nevertheless, changes in the digestive enzyme activities are also found in juveniles and adults. It appears that these changes are related to growth and feed digestibility (Lee and Lawrence, 1985). Changes in digestive enzyme activity may indicate physiological responses to different nutritional conditions (Le Moullac et al., 1997) and it has been hypothesized that the enzyme activity is high for those substrates that are more common in the diet (Moss et al., 2001). The present study, protease activity showed significantly increasing in partial replacement of FM with S. platensis, C. vulgaris and A. pinnata feed fed groups. The results indicated that the supplementary ingredients protein source is well utilized by the prawn M. rosenbergii PL.

**Amylase**

The usual strategy in formulating diets is to reduce costs by maximizing the inclusion of carbohydrate while simultaneously sparing expensive protein. The digestibility of various forms of dietary carbohydrates and their nutritive value for prawns remains unclear, as few studies on carbohydrate utilization have been conducted with this species. Utilization and metabolism of dietary carbohydrates appear to vary among crustacean species (New, 1976). The ability of prawns to use a particular carbohydrate source may have both a digestive and metabolic origin. Prawns appear to be able to utilize complex carbohydrates better than simple ones such as glucose (New, 1976; 1990). Dietary monosaccharide are rapidly absorbed, but are poorly utilized. Soluble starch appears to be the most suitable carbohydrate source for the giant freshwater prawn M. rosenbergii
(Gomez Diaz and Nakagawa, 1990). In the present study, the experimental group
M. rosenbergii PL showed elevation of amylase activity revealed that the S. platensis,
C. vulgaris and A. pinnata carbohydrate source utilized by the prawn PL.

**Lipase**

The lipid reserves available in crustaceans for energy is small, with only 8% of
body lipids stored as triacylglycerides in the mid gut gland (Chandumpai et al., 1991).
This could explain the lowest values of lipase activity in different animals, including
shrimp, such as P. setiferus (Lovett and Felder, 1990). In crustaceans, neutral lipids are
preferentially catabolized during fasting, while polar lipids are conserved to fulfill
structural roles (Stuck et al., 1996). Catabolism of triacylglycerides achieved by lipases is
scarcely studied in crustaceans; however, there is a variation of digestive enzymes, such
as proteinases (trypsin and chymotrypsin) during starvation conditions in shrimp
(Muhlia-Almazan and Garcia Carreno, 2002). Penaeid shrimps are ideal crustacean
models to study sequential changes of digestive enzymes during development because all
larval stages are free swimming rather than embryonated and metamorphosis to adult
morphology and habits takes several weeks (Gonzalez-Baro et al., 2000; Ying et al., 2009).
In crustaceans, lipases have been detected in crab Carcinus mediterraneus (Slim et al., 2007)
and shrimps such as, P. setiferus (Lovett and Felder, 1990) in P. monodan (Deering et al.,
1996) in M. borelli (Gonzalez-Baro et al., 2000) in L. vannamei (Gamboa-Delgado et al., 2003)
in lobster Panulirus argus (Perera et al., 2008). They are required in many biochemical
reactions, but differ in their primary structure, tissue specificity, and cellular
compartmentalization. Lipases include digestive lipases, which facilitate extracellular
cleavage of alimentary lipids (Vogt, 2002); while intracellular lipases are involved in the
mobilization of triacylglycerides in adipose tissue and have a critical role in regulating fatty acids metabolism and energy supply (Viharvaara and Puig, 2008). In the present study, the increased trends of lipase activity were identified in partial replacement of FM replaced with *S. platensis*, *C. vulgaris* and *A. pinnata* inclusion feed fed *M. rosenbergii* PL.

**Cellulase**

In the present study, the cellulase activity was significantly increased in all experimental diets when compared with control group. Particularly the cellulase activity was higher in *A. pinnata* incorporated diets followed by the *C. vulgaris* and *S. platensis* inclusion diets. The similar result was observed in *M. rosenbergii* fed with cellulose based diets (Gonzalez-Pena *et al.*, 2002). A reduction in dry matter and crude protein digestibility with increasing levels of cellulose on diets has been observed in other species (Koshio *et al.*, 1992; Brunson *et al.*, 1997). Similar results have been reported for *P. monodon*. The high digestibility of cellulose, coupled with the low digestibility of dry matter, suggests that the dietary cellulose is digested to a form which is no longer measured in the neutral detergent fiber assay used for cellulase determination. However, it appears that much of this digested cellulose is not absorbed and so remains in the gastro intestinal tract and contributes to dry matter in the feces (Gonzalez-Pena *et al.*, 2002). The present study showed, the cellulase activity was increased in all experimental groups when compared with fishmeal feed. The result revealed that the ingredients contain higher concentration of dietary cellulose and was utilized by the prawn PL.

5.5. Conclusion

Based on the findings in this study, the *M. rosenbergii* PL was able to digest the formulated feeds with *C. vulgaris*, *S. platensis* and *A. pinnata* by enhancing the secretion of digestive enzymes.