

Project Number	VIE36 IF04		
Project Title	Testing a practical artificial diet for mud crab, <i>Scylla paramamosain</i>, on grow-out in Mekong Delta, Vietnam		
Vietnamese Institution	Research Institute for Aquaculture No. 2		
Australian Institution	Charles Darwin University		
Commencement Date	May 2006	Completion Date	December 2006
Objectives			
To obtain the best practical diet (in terms growth rate, survival rate, feed conversion ratio and water quality) which can replace the Vital feed (an expensive feed for Kuruma prawn) and trash fish under laboratory conditions.			
Activities			
<ol style="list-style-type: none"> 1. Conduct Experiment 1: to test three practical diets: a) the "best" diet from Vu-anh Tuan's PhD research; b) Diet a) plus additives (e.g., vitamins and minerals); and c) Diet b) with further supplement (e.g., vitamins and minerals plus mixed enzymes). 2. Conduct Experiment 2: To compare the "best practical diet" from experiment 1, with Vital feed and trash fish (small tilapia) 			
Milestones			<i>Expected Date</i>
<i>Nbr</i>	<i>Milestone Description</i>	<i>Deliverables</i>	
1	CARD Contract Signed	<ul style="list-style-type: none"> • Research Agreement signed. Milestones and payment schedules in place 	Apr. 2006
2	Research Report	<ul style="list-style-type: none"> • A summary report of all trial results, including data of survival, growth rate, total bacteria and water quality, feed conversion ratio and financial analysis of feed treatments. 	Dec. 2006

PROJECT COMPLETION REPORT

1. Introduction

Although mud crab aquaculture has been rapidly expended throughout South East Asian countries, most culture systems depended heavily on low value fish (trash fish) as feeds (Sugama 1998). In general, trash fish has been shown to be suitable for mud crab culture but there are some disadvantages. Trash fish may not always be available, particularly in the wet

season, and there are extra costs such as electricity and a freezer necessary for storage (William & Abdullah 1999). Furthermore, utilisation of juvenile fish as trash fish, which would otherwise be consumed by the coastal local population, can impact on fisheries due to the removal of juveniles of commercially valuable species (William & FitzGerald 2002).

Some commercial mud crab feeds have been introduced to the market, but their acceptability had not been ascertained (Baliao, Dan, Santos & Muguel 1998). It has also been demonstrated that juveniles of mud crab, *S. serrata*, can utilise feeds formulated for Kuruma prawns (Vital and Ebi-star made in Higashimaru Company, Japan). However, the prices of Vital and Ebi-star feeds were considered too expensive (A\$ 11/kg and A\$6/kg respectively) to support commercial mud crab aquaculture development (Shelley 2001).

Although there have been a number of studies on formulated feeds for mud crabs, there is still lacking information to develop properly a practical diet for mud crabs. In particular, there are a few evaluations of artificial diet for mud crab, *Scylla paramamosain* in Vietnam. To gather present studies from PhD program of Vu-anh Tuan and experience of current ACIAR nutrition project produced a range of practical diet to test at laboratory scale in this study.

Spirulina is a kind of algae powder from Chuan Kuan Enterprise Co., LTD, Taiwan which is used to feed for shrimp baby. This product contains approximately 60% crude protein, 6% crude fat, 9% carbohydrate, and 2% fibre. 30% of spirulina was recommended to substitute for fish spawner. It was also suggested to use as feed additive in diets for prawn and fish spawners due to it contains natural Beta-carotene source (Akiyama, Dominy & Lawrence 1992). In this study, Spirulina was substituted at rate of 4% in diet which purposed to improve mud crab growth.

Calcium is an essential mineral required for skeletal structure, blood clotting, activation of enzymes, muscle contraction and cell permeability and its level relates to phosphorus (ratio of calcium:phosphorus of 1:1 to 1.5:1 in diet of shrimp (Akiyama *et al.* 1992). Tissue salts is a commercial product of Martin & Pleasance, VIC, Australia. Each tablet is 500 mg consisting of 0.5mcg of the Mineral Tissue Salt Calcium Phosphate (CaP) in a lactose base. In this study, CaP was used to purpose in increasing of growth of juvenile mud crabs.

The objectives of this study is to evaluate formulas of practical diets (a recommended diet formula from Vu-Anh Tuan's PhD), the recommended diet and Spirulina supplementation, the recommended diet and CaP supplementation which can replace trash fish and the vital feed (expensive feed for Kuruma prawn) in term of the growth rate, survival rate, and water quality.

2. Materials and Methods

The experiment was carried out at the Bac Lieu Experimental Research Station for Aquaculture, Nha Mat ward, Bac Lieu town, Bac Lieu province, Vietnam (belongs to the RIA 2) using juvenile mud crabs (*Scylla paramamosain*) (crablets) of 1g (crab 8). The crablets were produced from the same batch at hatchery.

Culture system and water preparation

A static water system was used for this experiment. Fifteen 500 L fibreglass tanks, containing 400 L of treated water (see below) were used in this experiment. Each tank held 14 plastic containers inside, representing one replicate. One crablet was kept in each plastic container to prevent cannibalism.

Water was treated as for shrimp hatchery. At the beginning, water was settled for 3 days and 1.5 ppm KMnO_4 was used to oxidise organic matter present in the water. Four days after, water was chlorinated with 30 ppm of chlorine for 3 days. After that water was neutralized with thiosulphate (20 ppm) and then filtered through a sand filter into a reservoir tank.

Water was filled into trial tanks and remained at 400L each. During the course of the experiment, salinity was maintained at 24 ppt. Values of water temperature at 7:30 and 14:00 hours were 23.2 ± 0.9 °C and 26.3 ± 0.7 °C respectively.

Experimental diets

Three practical diets were tested. The first one was the recommended diet from Vu-anh Tuan's PhD (T1). The second diet (T2) was added with Spirulina algae powder (4%), and the third diet (T3) was substituted with 0.05% of Tissue Salt (CaP). Control diets were vital feed for kuruma prawn (T4) and marine low value trash fish (T5). The breakdown of the ingredients for the diets is listed below.

Table 1 Composition of experimental diets

Ingredients	T1	T2	T3	T4	T5
Fishmeal	52.00	52.00	52.00	-	-
Soybean	13.00	13.00	13.00	-	-
Squid meal	5.00	5.00	5.00	-	-
Wheat flour	7.98	4.70	8.65	-	-
Flax seed oil	1.50	1.50	1.50	-	-
Fish oil	4.50	4.50	4.50	-	-
Soy lecithin	2.00	2.00	2.00	-	-
Cholesterol (80%)	0.20	0.20	0.20	-	-

Acetes meal	2.00	2.00	2.00	-	-
Fish source	0.50	0.50	0.50	-	-
Monosodium glutamate	0.80	0.80	0.80	-	-
Alginic acid	4.00	4.00	4.00	-	-
Vitamin mixture	2.50	2.50	2.50	-	-
Mineral mixture	1.00	1.00	1.00	-	-
Astaxanthin 10%	0.10	0.10	0.10	-	-
EAA's	1.20	1.20	1.20	-	-
Glucosamine	1.00	1.00	1.00	-	-
CaP	0.00	0.00	0.05	-	-
Spirulina	0.00	4.00	0.00	-	-
Vitamin C (36%)	0.70	0.70	0.70	-	-
Antioxidant (Bianox)	0.02500	0.02500	0.02500	-	-
Antifugus (Mtox)	0.25000	0.25000	0.25000	-	-
Vital fish	-	-	-	100	-
Marine trash fish	-	-	-	-	100

EAA's: essential amino acid mixture

'-': zero

CaP is from Martin & Pleasance, VIC, Australia

Spirulina is from Omega Company, Taiwan

Feeding

The formulated feed was fed with 5 % of average body weight while the trash fish was fed 10% because of trash fish was wet. The crabs were fed twice a day at 7:00 and 17:00. Two hours from feeding, the uneaten feed was removed using siphoning.

Experimental design and data collection

All treatments were done in triplicates using a randomised complete design (RCD). Survival rate, growth rate, carapace width, total bacterial population, and water quality parameters: water temperature, dissolved oxygen, pH, salinity, alkalinity, biological oxygen demand (BOD), chemical oxygen demand (COD), nitrite (NO₂⁻), phosphate (PO₄⁻²), ammonia (NH₃) were monitored.

Survival rate, weight gain (%), and specific growth rate (g/day) were obtained for every 15 days.

- Weight gain, specific growth rate, and feed conversion ratio were calculated according to Tacon (1990).

$$\text{Weight gain (WG, \%)} = \frac{W_f - W_i}{W_i} \times 100$$

$$\text{Specific growth rate (SGR, \% day}^{-1}\text{)} = 100 * \frac{\text{Ln}W_f - \text{Ln}W_i}{T}$$

$$\text{Feed conversion ratio (FCR, g g}^{-1}\text{)} = \frac{D_f}{WG}$$

where W_i is the initial wet weight (g), W_f is the final wet weight (g), D_f is the real dry feed intake (g), D_f is total feed intake (g) minus uneaten feed (g).

- Survival rate (%) = $\frac{\text{Final number}}{\text{Initial number}} \times 100$
- The carapace width increment was estimated as below

$$\text{Carapace width increment (CWI, \%)} = \frac{CW_f - CW_i}{CW_i} \times 100$$

where CW_i is initial carapace width, CW_f is the final carapace width,

Statistical analysis

The data of survival, growth rate, total bacteria, and water quality parameter were analysed using ANOVA and LSD 95% (SPSS 10 for Window, SPSS Inc., USA, 1999).

3. Results

Water quality

pH and dissolved oxygen

Average values of pH and dissolved oxygen concentrations at 7:30 were similar between treatments. These values were presented in the Table 2.

Table 2. Average values of pH and dissolved oxygen of water during 70 days

Parameters	Diets				
	T1	T2	T3	T4	T5
pH	7.99±0.06	8.00±0.07	7.99±0.06	7.99±0.06	7.99±0.08
DO (mg/L)	5.03±0.16	5.02±0.17	5.03±0.17	5.04±0.15	5.06±0.15

There is no difference in pH and DO between treatments during a course of experiment ($P > 0.05$). The stable pH values at all treatments were as a result of the same water supply source. In particular, the experiment was carried out indoor and thus there was little or no

algae growing in these tanks, resulting in a small variation in pH. Similarly, dissolved oxygen (DO) values were high and were similar at all treatments because of constant aeration.

Table 3. Average values of alkalinity, phosphate (PO_4^{3-}), biological oxygen demand (BOD), chemical oxygen demand (COD), ammonia (NH_3), and nitrite (NO_2^-), and total bacteria (T-bac)(mean \pm SE) during 70 days of experiment

Diets	Alkalinity (ppm)	PO_4^{3-} (ppm)	BOD (ppm)	COD (ppM)	NH_3 (ppm)	NO_2^- (ppm)	T- bac (cell/mL)
T1	102.9 \pm 1.3 ^a	0.3 \pm 0.0 ^b	2.3 \pm 0.0 ^a	7.3 \pm 0.6 ^a	0.0011 \pm 0.0003 ^a	0.2 \pm 0.0 ^a	3474.4 \pm 606.1 ^a
T2	101.7 \pm 0.9 ^a	0.4 \pm 0.0 ^c	2.4 \pm 0.0 ^b	7.6 \pm 0.4 ^a	0.0013 \pm 0.0004 ^a	0.2 \pm 0.0 ^a	2560.9 \pm 752.3 ^a
T3	104.4 \pm 1.4 ^a	0.3 \pm 0.01 ^b	2.7 \pm 0.1 ^{cd}	8.4 \pm 0.1 ^a	0.0009 \pm 0.0001 ^a	0.2 \pm 0.0 ^a	2837.0 \pm 529.6 ^a
T4	93.1 \pm 1.8 ^b	0.5 \pm 0.0 ^d	2.5 \pm 0.1 ^{bc}	6.9 \pm 0.6 ^a	0.0011 \pm 0.0001 ^a	0.2 \pm 0.0 ^a	3543.0 \pm 324.3 ^a
T5	105.4 \pm 1.5 ^a	0.2 \pm 0.0 ^a	2.2 \pm 0.1 ^a	6.8 \pm 0.0 ^a	0.0007 \pm 0.0001 ^a	0.2 \pm 0.0 ^a	3869.4 \pm 637.6 ^a

Means within the same column having a similar superscript letter are not significantly different at 5% of significance (P>0.05).

Alkalinity

Alkalinity content tended to decrease from the beginning to the end of experiment. At the last weeks, the alkalinity from the treatment fed with T4 (Vital feed) was significantly lower than that from other treatments (Table 3, Figure 1).

The lower alkalinity content of the water in the Vital feed treatment. This could be due to Vital feed had lower Ca^{2+} ion than that of other diets.

Phosphate

In general, the Phosphate contents increased for all treatments. Although the T4 treatment had low alkalinity, its water had the highest content of phosphate (Table 3 and Figure 1 & 2). This result could be affected from diet. The Vital feed may contain higher phosphate content than other diets.

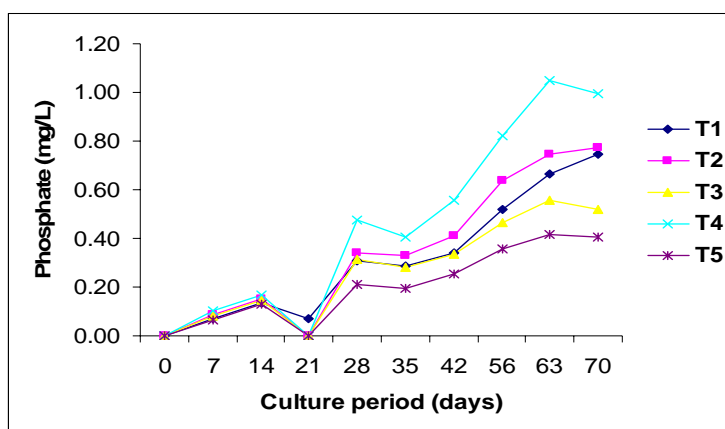


Figure 2 Phosphate fluctuated during the course of experiment

Chemical oxygen demand and biological oxygen demand

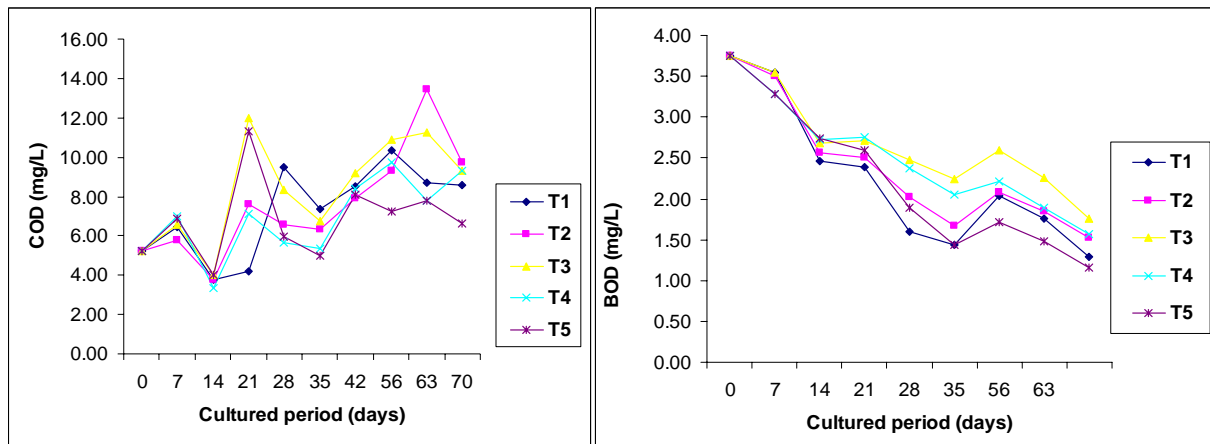


Figure 3. Chemical oxygen demand and biological oxygen demand during of experimental period

COD increased while BOD decreased during the experiment period. The values of COD and BOD was less than 14 ppm and 4 ppm, respectively).

Ammonia and Nitrite

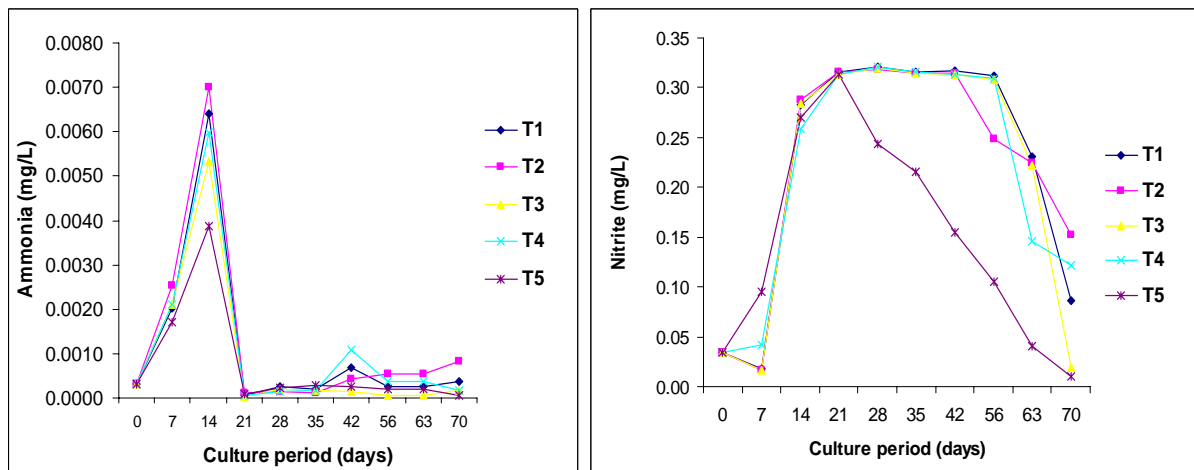


Figure 4. Ammonia and nitrite concentrations during the experimental period

During a course of the experiment, the contents of ammonia from all treatment were similar and very low except during a period of 7th and 14th. The values of ammonia were found from 0.004 to 0.007(mg/L). The peak of ammonia content at day 14th can be explained that after being fed, a part of feed was diluted into water while there was little bacteria population developed at the beginning of the experimental course. This caused to increase ammonia content at days 7th and 14th. After that time ammonia was transformed to nitrite as a result the ammonia content decreased while nitrite content increased sharply (Figure 4).

With respect to nitrite, three weeks from the start of the experiment, the nitrite contents from all treatments increased rapidly to about 0.3 (mg/L). The high and stable nitrite contents were found in all treatments from the 21st days to 63rd days, except in the trash fish treatment. However, after day 63rd, all treatments were observed as the decline in nitrite content.

Total bacteria

The total bacterium population density in the water of all treatments was described in Figure 5. The density of bacteria increased from at the beginning and decreased at the end of experiment. The lower bacteria density at the end of the experiment was the result of the water exchange at day 60th. The variations in the mean values of bacterial population densities were not significantly different from one another (Table 3).

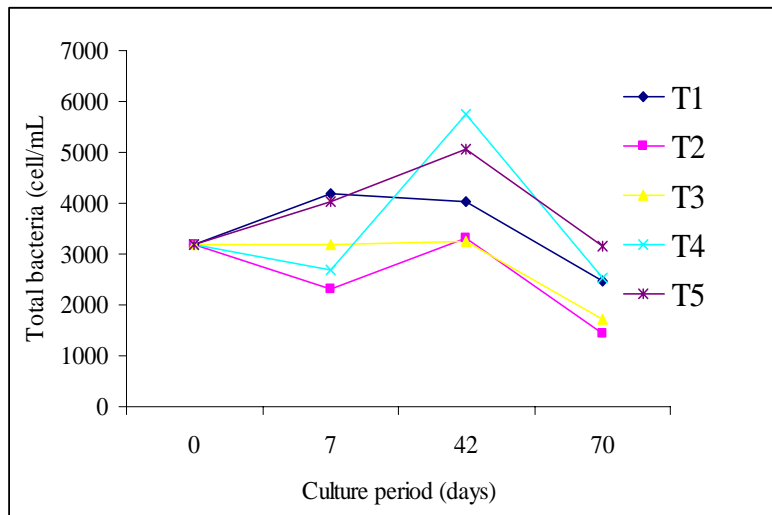


Figure 5 Total bacteria fluctuation during the experimental period

Body weight and Carapace width

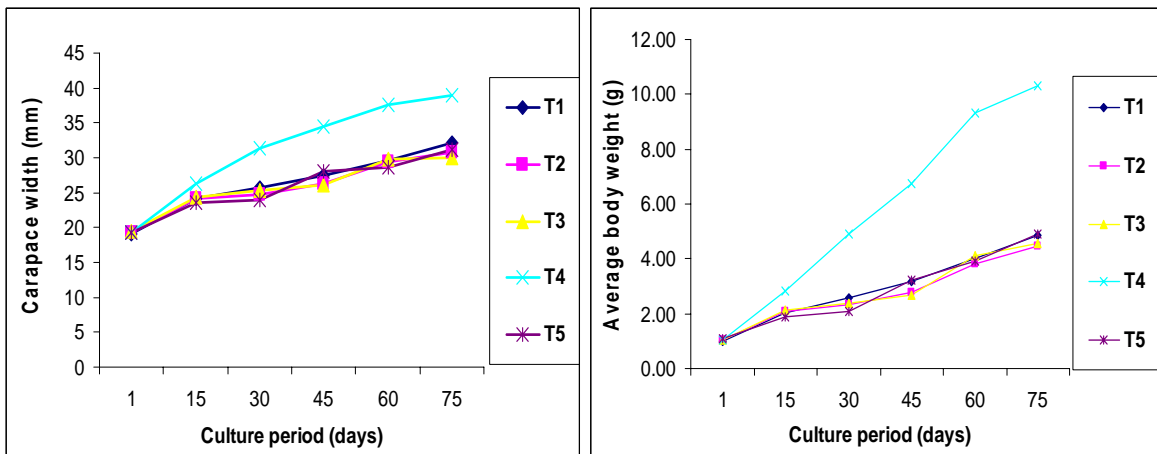


Figure 6 Carapace width and body weight of mud crabs during the course of experiment

The average body weight and carapace width of crabs were highest in Vital feed (T4) treatment, while those of other treatments (T1, T2, T3, and T5) were similar (Table 4).

Growth, survival rate, and feed consumption

The highest weight gain (885.4%) and carapace width (101.85%) were detected in the crabs fed with Vital feed (T4) while these values were similar from other crabs fed T1, T2, T3, and T5 ($P>0.05$) (Table 4).

Table 4. Weight gain (WG), carapace width increment (CWI), specific growth rate (SGR), survival rate (SUR), and feed conversion ratio (FCR) (mean \pm SE)

Diet	WG (%)	CWI (%)	SGR (%/day)	SUR (%)	FCR
T1	383.5 \pm 61.7 ^a	68.2 \pm 6.7 ^a	2.1 \pm 0.8 ^a	88.1 \pm 2.4 ^a	2.0 \pm 0.0 ^b
T2	335.2 \pm 23.2 ^a	58.8 \pm 3.7 ^a	2.0 \pm 0.1 ^a	88.1 \pm 6.3 ^a	2.3 \pm 0.1 ^{cd}
T3	334.6 \pm 16.7 ^a	54.6 \pm 5.1 ^a	2.0 \pm 0.0 ^a	85.7 \pm 0.0 ^a	2.3 \pm 0.1 ^c
T4	889.5 \pm 126.0 ^b	101.9 \pm 9.2 ^b	3.0 \pm 0.2 ^b	100.0 \pm 0.0 ^b	0.8 \pm 0.1 ^a
T5	362.5 \pm 36.4 ^a	62.0 \pm 4.7 ^a	2.0 \pm 0.1 ^a	83.4 \pm 4.8 ^a	2.5 \pm 0.1 ^d

Means within the same column having a similar superscript letter are not significantly different at 5% of significance ($P>0.05$).

Similarly to the carapace width increment and weight gain, the specific growth rate (SGR) and survival rate were highest in crabs fed T4 diet while these crabs had the lowest feed conversion ratio (FCR). The crabs fed T1, T2, T3, T5 had the same SGR, SUR and FCR values.

4. Discussion

The main aim of this study was to find a formulated feed that can replace trash fish and Vital feed in the rearing of juvenile mud crabs, *Scylla paramamosain*. It was found that trash fish can be replaced by any of the formulated feed studied here. However, Vital feed still showed the best feed available in terms of growth, survival and feed conversion ratio. This study confirms the results obtained by Tuan (2006) using smaller crablets (0.03g). Further work still needs to be done to find ways of replacing Vital feed since it is expensive (A\$ 6- 11/kg) (Shelley 2001). In addition, Vital feed is formulated for shrimp and it will not be suitable for larger crabs.

Supplementation of Spirulina 4% and 0.05% CaP in diets (T2, and T3 respectively) did not improve the growth, survival rate, FCR, and water quality against the diet T1. This can be explained as follow: the addition of 4% of Spirulina may upset the balance of amino acids of diet, resulting in a decrease in weight gain. It has been shown that a balance of essential amino acids of a diet is important in promoting growth of mud crabs (Tuan, 2006). The

addition of CaP did not improve the nutritional quality of the diet. This suggests that the level of Ca in the diet was sufficient for the synthesis of the exoskeleton. However, the unbalance ratio of calcium and phosphorus may negate the effect of the added Ca. Therefore, a future study should be conducted in a range of ratio between Calcium and Phosphate in diet. For example, ratio of calcium:phosphorus of 1:1 to 1.5:1 was recommended in diet of shrimp (Akiyama *et al.* 1992).

The different results obtained in this study may also be due to the different techniques used in the production of the pellets. The three diets T1, T2, and T3 were pelleted using a small mincer while the Vital feed was a commercial product, the pellets of were produced by a better machine. Thus, the water stability of Vital feed was better than that of T1, T2, T3. Further research should be focused in this aspect.

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