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Variation of growth and proximate composition in *Portunus pelagicus* juveniles fed with selected feeds in recirculating aquaculture system (RAS)

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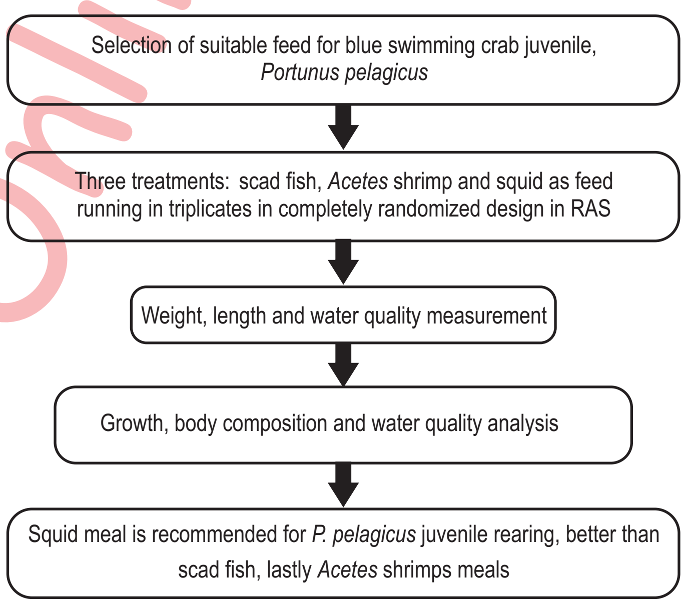
Abstract

Aim: The experiment was conducted to determine the suitability of using scad fish, *Acetes* shrimp and common squid for the direct and indirect application as diets for the juvenile rearing of the blue swimming crab *Portunus pelagicus*.

Methodology: A total of 54 crab juveniles with an initial weight of 2.46 ±0.94 g were randomly stocked in triplicate at 6 crabs per 60 l tank (0.26 m^2) experimental units and fed twice daily with three different types of feed namely scad fish, squid tissue and frozen *Acetes* shrimp for a period of 31 days.

Results: At the end of the trail, ammonia and nitrite-nitrogen compound were significantly higher in *Acetes* shrimp fed group. It is found that final body weight (g) of *P. pelagicus* was significantly higher when fed with squid, however no significant differences were noticed between scad fish and *Acetes* shrimp fed groups. The whole body proximate composition was similar in all the diet fed individuals except the level of lipid which was higher in squid compared to scad fish and *Acetes* shrimp. Significantly ($p<0.05$) better food conversion ratio (FCR) was observed in juvenile crab fed with squid even though scad fish possessed the highest crude protein of 83.33%.

Interpretation: Overall, the results suggested that squid meal could be recommended for *P. pelagicus* juvenile culture. Hence, squid meal may be incorporated in crab artificial diet as protein source to attain better survival and growth.



Introduction

Portunus pelagicus is mainly a bottom feeder carnivorous crustacean and feeds on different types of benthic fauna and flora within its habitat (Wu and Shin, 1998). They dwell in a wide range of shallow coastal habitats such as muddy and sandy bottoms, seagrass and algal beds, and show wide range of habitat complexity (Asphama *et al.,* 2015). Blue swimming crabs are considered as important species for fishers of lower income group that depends on this crab's fishery (Asphama *et al.,* 2015). In Malaysia, it is the top most popular marine crab species and fetches high price in the market. As a delicacy, they have a great demand in local and international markets, and recognized as a high-protein and low lipid food containing 21.54 -22.64% protein, 0.81-1.21% total lipid (Gokoolu and Yerlikaya, 2003) and good sources of fatty acids (Deniz, 2016). It also serves as an important member of food chain due to its relationship with various sub-trophic levels, thus playing a significant role in the ecosystem (Hall *et al.,* 2006).

Food and feeding is one of the most essential factors influencing the growth performance of the blue swimming crabs. Several studies on feeding and diet of *P. pelagicus* have been conducted in various countries of the world such as natural diets (Hyslop, 1980; Ikhwanuddin *et al.,* 2014), diet changes (Arshad *et al.,* 2002), food habits (Chande and Mgaya, 2004), food and feeding habits (Josileen, 2011), feeding ecology (Kunsook *et al.,* 2014) and feed acceptability (Tina and Darumas, 2014). However, there is very little information available regarding the effect of different selected fresh diets on variation of growth and proximate composition in *P. pelagicus* juveniles in RAS system. Thus, the study was conducted to evaluate the growth performance of *P. pelagicus* juvenile using different types of fresh diets.

Materials and Methods

Experimental site: This experiment was carried out at the hatchery complex in the Marine Science Center of UPM, Port Dickson, Negeri Sembilan, Malaysia.

Source and rearing of experimental crabs: Hatchery-produced healthy crab instar (C1) with intact appendages was used for the present experiment; and nursery rearing was performed in three rectangular tanks (3 m^3). In order to minimize cannibalism between crabs, sandy substratum were used at the bottom and tanks were filled with aerated seawater up to a level of 50 cm depth. The animals were nursery reared for 30 days. The crabs received UV treated seawater (32 – 33 ppt) with a daily water exchange of 20%. Water pH was maintained at a range between 7.5 and 8.0 using calcium carbonate whenever necessary. Diffused aeration was provided to maintain the dissolved oxygen (DO) level in each nursery tank. During the culture period, the first crab instars were fed at 30% body weight with a mixed diet containing scad fish, squid and shrimp meal (Feed No. 5002, C.P. Aquaculture Private Limited) twice a day in feeding trays. At the end of 30 days period of nursing, the crabs attained nearly 25 mm carapace width. The healthy juvenile with intact appendages was harvested for further growth performance trial.

Design of experiment: The feeding trial was performed in 9 plastic basins (0.26 m^2) with a water volume of approximately 60 l. Each basin was attached to a recirculation bio-filtering system. Each basin contained a 10 cm layer of sea sand at the bottom and 15 cm of free water surface above the sand layer. The sandy substratum was adjusted to the culture condition for 15 days before starting the experiment.

Stocking: In this study, a total of 54 juvenile (C30) crabs were used and each basin was stocked with six healthy intact juvenile crabs (24/ m^2) with mean initial wet weight of 2.46 g, carapace width 31.05 mm and carapace length 15.74 mm.

Experimental feeds: In this experiment, three types of foods such as scad fish, squid (*Loligo* sp.) and *Acetes* shrimps were used and these diets were chopped into approximately 1mm particle size. Three replicates for each treatment were randomly assigned. All the feeds were stored in freeze and thawed before being fed to the juveniles. Association of Official Analytical Chemists (AOAC, 1997) method was followed to analyze the proximate composition of the used foods (Table 1).

Feeding: The feeding amount was applied for 3 days (before starting the experiment) at 15%, 20% and 25% of the total body weight of wet weight basis to determine the exact feeding required per day. Twenty percent was shown the best feed consumed performance than those of other two percentages. Subsequently, during the experimental period, the selected fresh frozen diets were fed for the first 15 days at 20% of their total wet body weight and adjusted by 10% until the end of the culture period (Baliao *et al.,* 1999). The blue crabs were fed manually at early morning (9.00 a.m.) and late afternoon (6.00 p.m.) daily.

Water quality parameter: The water quality parameters taken for analysis are salinity, temperature, pH and dissolved oxygen, they were measured once a week with YSI 556 Multi-probe (USA); ammonia-nitrogen and nitrite-nitrogen were measured using a commercial kit (API pharmaceutical test kit).

Growth trials: Before stocking the crabs in the culture tank, the initial average body weight (g), carapace length (mm) and width (mm) were recorded. Body weight, carapace width and length of *P. pelagicus* were measured fortnightly until the end of culture period using a digital balance (0.01 g) and a digital caliper (0.01 mm; Mitutoyo, Japan), respectively. At the end of experiment, the final body weight gain (%), specific growth rate (SGR) (%), survival rate (%), feed conversion ratio (FCR), feed efficiency (FE) (%) and protein efficiency ratio (PER) of the *P. pelagicus* were calculated for different treatments. An the end of the experiment, few crabs were randomly selected and stored at -20 °C for the whole-body proximate composition analysis.

Whole-body proximate composition: Biochemical analysis was carried out following the standard procedures of AOAC (1997). Crude protein (N×6.25) was determined using the Kjeldahl method after an acid digestion using protein analyzer Unit (2300 Kjeltec Analyzer Unit, Foss Tecator). Crude lipid was determined by petroleum ether extraction using a Soxtec system (Soxte System 2050 Foss Tecator). Crude fiber was determined by utilizing fibertec system. Moisture was determined by oven drying at 105 ºC for 24 hr; and ash was determined by muffle furnace at 600°C for 4 hr.

Calculations and statistical analysis : Finally, data analyses were performed using the following standard formula:

$$\text{Weight gain (\%)} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100$$

(Tina and Darumas, 2014)

$$\text{SGR (\%)} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Culture period (day)}} \times 100$$

(Ikhwanuddin *et al.*, 2012)

$$\text{FCR} = \frac{\text{Total quantity of feed consumed (gm)}}{\text{Total weight (gm)}}$$

(Maheswardue *et al.*, 2008)

$$\text{PER} = \frac{\text{Final wet weight (gm)} - \text{Initial wet weight (gm)}}{\text{Ingested protein}}$$

(Estefanell *et al.*, 2011)

$$\text{FE (\%)} = \frac{\text{Final wet weight (gm)} - \text{Initial wet weight (gm)}}{\text{Feed intake per crab (g)}} \times 100$$

(Estefanell *et al.*, 2011)

$$\text{Survival rate (\%)} = \frac{\text{Total number of survival crab}}{\text{Initial number of stocked crab}} \times 100$$

(Talpur *et al.*, 2011)

Protein retention % = (% final body protein content × final body weight) - (%initial body protein content × initial body weight) / total feed x % protein diet × 100 (Ishak *et al.*, 2016)

Lipid retention % = (% final body lipid content × final body weight)- (% initial body lipid content × initial body weight) / total feed x % lipid diet × 100 (Ismail *et al.*, 2016)

Table 1 : Proximate composition of different diets given to *Portunus pelagicus* juvenile (% on a dry basis)

Proximate composition	T1 (Scad fish)	T2 (Squid)	T3 (*Acetes* shrimp)
Moisture (%)	72.70±0.47	74.47±2.02	77.92±1.63
Protein (%)	83.33±1.01	75.41±0.46	56.74±0.94
Lipid (%)	4.76±0.35	3.14±0.15	3.27±0.02
Fiber (%)	6.72±0.19	8.33±0.14	4.69±0.11
Ash (%)	4.52±0.09	4.56±0.03	9.92±0.41

*Moisture (%) on as weight basis

Table 2 : Water quality data taken on weekly basis for culturing blue swimming (*Portunus pelagicus*) crab juvenile fed with selected feed for 31days

	Water quality parameters		
Treatment	**T1 (Scad fish)**	**T2 (Squid)**	**T3(*Acetes* shrimp)**
Water temperature (°C)	26.58±0.35[a]	26.75±0.50[a]	26.46±0.40[a]
Dissolved oxygen (mg l^{-1})	6.17±0.26[a]	6.24±0.35[a]	6.16±0.30[a]
Salinity (ppt)	31.23±0.13[a]	31.19±0.11[a]	31.17±0.12[a]
pH	7.88±0.03[a]	7.91±0.04[a]	7.90±0.4[a]
Ammonia-nitrogen(mg l^{-1})	0.33±0.12[b]	0.27±0.13[b]	1.21±0.50[a]
Nitrite-nitrogen (mg l^{-1})	0.02±0.07[b]	0.04±0.10[b]	0.63±0.50[a]

All values represent mean ±SD. Mean values bearing the different letters in the same rows are significantly different (P<0.05)

Table 3 : Mean (±SD) carapace length and width, specific growth rate, feeding rate and survival of *P. pelagicus* juvenile fed with different fresh diets for 31 days

	Treatment		
Parameters	**T1 (Scad fish)**	**T2 (Squid)**	**T3 (*Acetes* shrimp)**
Final carapace width (mm)	53..69±3.32^{b}	68.02±3.51^{a}	51.19±3.76^{b}
Final carapace length (mm)	26.93±1.69^{b}	34.11±1.78^{a}	25.70±1.89^{b}
Initial weight (g)	2.46±0.01^{a}	2.46±0.01^{a}	2.46±0.01^{a}
Final weight (g)	10.38±1.66^{b}	19.77±1.05^{a}	10.10±2.29^{b}
Weight gain (g)	7.92±1.66^{b}	17.31±1.05^{a}	7.64±2.29^{b}
Weight gain (%)	321.95±67.49^{b}	703.52±42.87^{a}	310.43±93.10^{b}
SGR	25.55±5.36^{b}	55.83±3.40^{a}	24.63±7.38^{b}
FCR	3.94 ±0.89^{b}	2.79±0.64^{b}	6.22±0.89^{a}
PER	0.25±0.05^{b}	0.55±0.03^{a}	0.24±0.07^{b}
FE (%)	26.74±6.94^{a}	36.11±10.74^{a}	16.29±2.42^{b}
Survival (%)	44.44±9.62^{a}	38.89±9.62^{a}	27.78±9.62^{b}

All values represent mean ± SD. Different superscript letters within a row denote significant differences (p < 0.05); SGR= specific growth rate; FCR = feed conversion ratio; PER = protein efficiency ratio; FE (%) = feed efficiency

Table 4 : Whole body composition (% on as dry basis) of *P. pelagicus* crab juvenile fed with three selected diets

Proximate composition	**Initial crab juvenile**	**T1 (Scad fish)**	**T2 (Squid)**	**T3 (*Acetes* shrimp)**
Moisture (%)	58.04±0.33	58.08±0.34^{a}	58.76±0.09^{a}	58.12±0.17^{a}
Protein (%)	31.06±0.55	31.12±0.27^{a}	31.52±0.10^{a}	31.09±0.20^{a}
Lipid (%)	1.59±0.08	1.64±0.18^{a}	1.89±0.13^{a}	1.06±0.18^{b}
Fiber (%)	7.33±1.06	6.45±0.19^{a}	6.40±0.63^{a}	7.24±0.21^{a}
Ash (%)	39.59±1.44	35.72±0.89^{a}	35.12±0.22^{a}	35.82±0.53^{a}
PR (%)		3.78±0.83^{a}	6.66±0.19^{a}	5.88±2.44^{a}
LR (%)		3.51±1.16^{b}	4.77±0.50^{a}	3.15±2.29^{b}

All values represent mean ± SD. Mean values bearing the same superscripts in the same row are not significantly different (p > 0.05); PR (%) = protein retention; LR (%)= lipid retention; Moisture (%) on as weight basis

Data analysis: All statistical analysis were conducted using one way ANOVA. The statistically significant among the treatments were considered if the P value was ≤ 0.05 (5% significant level) using post-hoc Duncan's test.

Results and Discussion

The water quality parameters measured throughout the experimental period are presented in Table 2. However, a significant amount of ammonia-N and nitrite-N level was observed in *Acetes* shrimp. The other parameters such as dissolved oxygen (DO), water temperature, pH and salinity were not significantly affected by different diet treatments.

Growth performance, feed utilization and survival rate of *P. pelagicus* are presented in Table 3. Carapace width and length, weight gain, SGR (%) and PER of *P. pelagicus* juvenile fed with squid were observed significantly higher (P < 0.05) than those fed the scad fish and *Acetes* shrimps. The best FCR and FE (%) were found significantly higher in squid diet, followed by fed with scad fish and *Acetes* shrimp.

Proximate composition (moisture, protein, lipid, fiber and ash) of *P. pelagicus* juvenile fed with different diets are shown in Table 4. There were no significant differences (P > 0.05) in moisture, fiber and ash of juveniles fed with selected diets. Lipid and lipid retention were found highest (p<0.05) in juvenile crab fed with squid and lowest in crab fed with *Acetes* shrimp. The highest protein (p>0.05) was also observed in squid fed group, whereas the lowest in *Acetes* shrimp diet treatment.

The recirculating system can be potentially used for intensive culture with limited pollutant discharge, thereby increasing fish or crustaceans production and reducing water usage as well as adverse environmental impacts (Davis and Arnold, 1998). In the present study, the average values of the physico-chemical parameters were found within suitable range reported by Seeman *et al.* (2015). After the administration of different feed, the level of ammonia and nitrite-nitrogen compound (p<0.05) were slightly different in *Acetes* shrimp diet treatment; it remained within acceptable ranges for growth and survival of *P. pelagicus* juvenile.

Several researchers have given their efforts on developing artificial diets on growth and survival rate of aquatic organisms (Catacutan, 1997; Soundarapandian, 2008). It was found that feeding efficiency, weight gain, SGR (%) and growth of carapace width of P. pelagicus were significantly higher fed with squid diet. Soundarapandian *et al.* (2013) reported that dietary protein is required at 20 to 60% for optimum growth of crustaceans. Since all feeds used in this study contained more than minimum protein requirement of crab diet, for instance scad fish 83.33%, squid 75.41% and *Acetes* shrimp 56.74%, therefore, other factors like digestibility has also a lead role in the feeding trial (Kanazawa *et al.*, 1970). Squid meal is recognized as highly digestible diet for many of the crustacean (Reigh, Braden and Craig, 1990; Catacutan, 1997). For this reason it has been used as a feed ingredient in this study .

In this study, the crabs weight gain was directly not only influenced by protein content of the feeds but also it digestibility. This is in agreement with the previous reports of Chayawat *et al.*, (2008) who revealed that the crabs fed with minced fish exhibited considerably higher weight gain and specific rate than crabs fed with wet pellets and alga (seaweed) diet. They also observed that the highest and lowest growth of crabs fed with fish and seaweed, respectively. This might be for low protein and fat content of seaweed. Crustacea (2015) also found similar results in different size groups of *S. serrata*. More or less similar growth rate was also obtained in *S. tranquebarica* fed with *Acetes* sp. incorporated pellet feed and clam meat (Soundarapandian and Murugesan, 2010). Chayawat *et al*. (2009) in an another study reported higher specific growth rate and carapace width in blue swimming crabs fed with mixed fish and blue mussel compared to only red seaweed. No significant differences were observed between the crabs fed with diet containing 48% protein and control diet; however maximum growth was observed with the diets that contained 50% total crude protein (Sheen and Wu, 1999).

Lowest survival was recorded in crab fed *Acetes* shrimp; cannibalism is a major cause of low survival of crustaceans, so this trail is also not exempted from the case. . However, survival was improved by maintaining individual crabs in darkness per cultured unit in the present study as reported by several researchers (Celada *et al.*, 1989; Minagawa, 1994; Kevrekidis, 1996). The proximate composition of juvenile in the study were not affected by rearing techniques, except the crude lipid level which were significantly higher in squid compared to scad fish and *Acetes* shrimp. Although the whole body protein was still high in squid fed group but it was not a significant amount, which is in agreement with studies conducted on *Portunus* sp. (Gokoolu and Yerlikaya, 2003), *P. sanguinolentus* (Siddiquie *et al*., 1987), *Scylla serrata* (Sheen, 2000) and *Callinectes sapidus* (Farragut, 1965).

The overall results showed that the better water quality can be maintained in the aquaculture system by RAS set up. The growth of carapace length, weight gain, SGR (%), PER and FE (%) were found higher in crabs, while they fed with squid diet than the crabs fed with scad fish and *Acetes* shrimp, thus suggesting that incorporation of squid meal in juvenile blue swimming crab meal may produce better survival and growth.

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