The effect of copepod enriched-vegetable based diet on Giant Tiger Prawn (*Penaeus monodon*) post-larvae

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Abstract. Plankton is the primary food sources for many fish larvae as well as other organisms during their early stage of development. Zooplankton such as copepods play a major role in freshwater and marine environment as live food that offer great variety of sizes, species and nutritional value to the larvae. The aim of this study is to increase the nutritional value of copepod and its effect on *Penaeus monodon* post-larvae growth performance. The experiment was carried out 30 days and comprised with four different treatments of diets. The diets fed to copepod consisted of algal diet which is *Tetraselmis* sp. that acted as a control followed by three types of vegetable-based diet which is carrot, water spinach, and lettuce. The efficiency of the copepods enriched was further evaluate on its growth, survival and proximate composition. The outcome of the study showed that highest specific growth rate (SGR) in *P. monodon* post-larvae was obtained when fed with copepods enriched water spinach (11.28±0.38%) and the highest survival rate of *P. monodon* was obtained when being fed with copepods enriched *Tetraselmis* sp. (91.67±0.29%). Proximate analysis composition for enriched copepods and *P. monodon* fed with enriched copepods showed the water spinach produce highest protein and lipid content compared to other enrichment. The current result showed that vegetable based are able to replace the microalgae, hence it also can give an advantages to the economy in aquaculture and higher yields.

Keywords: Copepods, *P. monodon*, Enrichment, Proximate analysis, Specific growth rate

1. Introduction
Live feed is an important basic diet for newly hatched fish and shrimp larvae as they still have incomplete digestive system and are lacking in enzymes. They are still at very young stage to generate their own required nutrients or convert them from any prey-cursor obtained from their diet. They need a ready-made diet with readily available nutrient to be absorbed through their digestive system [1]. Live food organisms include all plants (phytoplankton) and animal (zooplankton) live grazed upon by economically importance fishes. Phytoplankton are generally eaten by zooplankton through the basis of the food chain. Live food are able to swim in water column and constantly available to fish and shellfish larvae are likely to stimulate larvae feeding response [2]. In an aquatic ecosystem, this live food organism constitutes the most valuable resources for aquaculture. Advances in live food enrichment technique have help to boost importance and potential of live food organism in the raising larvae aquatic species. The success in the hatchery production of fish fingerlings for stocking in grow out production
system is largely depending on the availability of suitable live food for feeding fish larvae, fry and fingerlings [3].

Furthermore, copepod are commonly feed by fish larvae in the ocean and act as natural prey for marine fish larvae and crustacean [4]. Cyclopoid copepod are the main food source for marine organism [5], it is because the nutritional profile of copepods appears to match better the nutritional requirements of marine fish larvae [6]. Copepods inhabit a huge range of salinities, from the fresh water to hypersaline conditions. They can be found virtually everywhere there is water, from subterranean cave to pool collected in bromeliad leaves or in damp leaf litter on the ground, from stream, rivers and lake to the open ocean and the sediment layer beneath. Copepod are live food that contain a high level of fatty acids required by many marine fish larvae species since it the best feeding. It is superior regarding nutritional value compared with other live food [7]. Copepod also contain high level of Docosahexaenoic acid (DHA) and poly unsaturated fatty acid (PUFA)[7].

Penaeus monodon, commonly known as the Giant Tiger Prawn or Asian tiger shrimp are commercially farmed in Malaysia. The intensity of Giant Tiger Prawn farming is related to its strength, tolerance of adverse water quality conditions and high-density culture, early maturation and relatively easy reproduction in confinement, and fast growth. The latest study has showed that HUFA are very important for many fish species [8]. Therefore, size of food particle also play a major role in food intake during larvae development of P. monodon [9]. Nowadays, the demand in aquaculture had increasing as over half of the fish and crustacean consume by world’s people are produce from aquaculture. Live feed plays a major role to produce the best quality of larvae that will promote them to enhance their growth and turns into adult in a short period. Based on previous study, the best feed to enrich live food are microalagae because it has more nutritious compare with other sources [10]. In culturing microalgae, it is required high cost and the production of good quality of microalgae are limited [11]. According to Pushparajan and Soundarapandian [12] P. monodon one of the crustacean that are high demand in world market. Its serve high in price for national and international market [13]. Lately, the difficulty of post-larvae to consume the big size of food due to their small mouth will affect growth and survival rate [14]. The growth performance of P. monodon post larvae stunted due to insufficient nutrient. Therefore, we need to enrich the nutrient content of the live feed as they are very suitable to be feed on post-larvae of P. monodon at first feeding stage. This study demonstrated (i) nutritional profile analysis of copepod enriched with vegetable based diet and (ii) the effect on post-larval giant tiger prawns (Penaeus monodon) growth performance.

2. Material and Methods

2.1. Copepod stock culture
Cyclopoid copepod was sampled from Setiu water, Terengganu, Malaysia. The sample were sustained for a few of generation after the sample were brought to Universiti Malaysia Terengganu Hatchery. Normal habitat condition for optimal temperature is at 26°C-27°C and optimal salinity is 10-15ppt. After the copepod turn into several of generation, the copepod is transferred into 1000 ml tank as a stock culture. The stock culture tank was filtered the seawater. In order to reach optimal salinity which is 12ppt demineralized water are added. Once in 2 days, 20 % of the water will be change to avoid any contamination. Also, feeding and observation on copepod will be done.

2.2. Experimental design
Three different vegetable base diet such as carrot, water spinach and lettuce are used to enrich copepod. Microalgae diet which is Tetraselmis sp. are used as a control for the enrichment. Four diet treatment that feed on copepod will be done in three replicate and each were used in this experiment. [T1-Tetraselmis sp. (control), T2- Water spinach, T3- Carrot, T4- Lettuce] Marine algae diet was cultured in the UMT hatchery by using 5000ml conical flask. Vegetable base diet feed on copepod at the concentration 500mg L-1 [15]. Through the experimental period, copepod was feed daily. 100L tank will be used to transfer the stock and respectively will be feed at least one generation of copepod [15].
2.3. Culture of Penaeus monodon’s post-larvae

100 L aquarium with 12 replicate was filled with 60 L of saltwater. 200 tiger prawn post-larvae were fed twice daily with copepod that were enriched with four different type of enrichment which is *Tetraselmis* sp. (control), carrot, water spinach and lettuce. Then the aquarium was covered with black plastic to avoid cannibalism [16]. At pH 7-8, the water quality is maintaining while the temperature was maintained at 28°C-30°C and salinity at 25ppt-30ppt [17]. Next, 50% of water will be change once a week.

2.4. Protein and lipid analysis of copepod and Penaeus monodon’s post-larvae

The ingredients that are used to do the proximate analysis are copepod that enriched with *Tetraselmis* sp. and vegetable base diet which is carrot, lettuce and water spinach. Then *P. monodon* also will be do proximate analysis. After that, the feed formulation for all diets will be done. Experimental diet will be prepared at Makmal Pemakanan Ikan.

2.4.1. Estimation protein

Protein content was determined using Kjeldahl method that involves digestion, distillation and titration. About 0.2g of sample had weighed and added into the digestion tube. Then, 5ml of sulphuric acid will be added. One catalyst also added to the digestion process and the digestion tube was heated to 420°C for one and a half hour using a scrubber. After cooling the digestion tube, distillation process was conducted on the digested sample. 30ml receiver solvent had been prepared with 30ml of 4% boric acid with 8 drops of indicator solution is added in a flask. The flask will then be placed at the end of the condenser receiver. 40ml of distilled water and 30 ml of 40% NaOH added into the digestion tube. The distillation unit Kjeldahl Buchii was activated and sample will be distilled for 5 minutes. Distilled sample had titrated using 0.1N hydrochloric acid (HCl) until the distilled solution change from blue to light pink. Blank had been prepared similarly without sample [18].

Calculation of protein content:

\[
% \text{N} = \frac{[(T - B) \times N \times 14.007]}{\text{weight of sample in mg}} \times 100
\]

\[
% \text{Protein} = \% \text{N} \times F
\]

Where:
- \(T\) = Titration volume for sample (ml)
- \(B\) = Titration volume for blank (ml)
- \(N\) = Normality of HCl
- \(F\) = Protein factor for nitrogen to protein (6.25 for animal base samples)

2.4.2. Estimation lipid

Extraction cup had been dried in an oven at 100°C for 1 hour. The extraction thimble was removed and kept in a desiccator to cool and weighed accurately and recorded as \(W1\). Extraction thimble was tightly placed at the ring of the metal holder and its rack. Place a filter paper in the extraction thimble. About 2g sample will be weighed and recorded as \(W2\) and then inserted in the thimble and covered with cotton. 40ml to 50ml petroleum ether had filled in the extraction cup and the extraction cup tightly secured on the reflux set. The process of lipid determination by using this extraction unit had taken about one hour and half to complete. After completion, the extraction cup was placed in an oven for 2 hours at 100°C, cooled in a desiccator, weighed and recorded as \(W3\).

Calculation of lipid content:

\[
% \text{Lipid} = \frac{(W3 - W1)}{(W2)} \times 100
\]

Where:
- \(W1\) = Extraction cup weight (g)
- \(W2\) = Sample weight (g)
- \(W3\) = Extraction cup weight + lipid (g)
2.5. Growth and survival rate of *Penaeus monodon*’s post-larvae

The study was conducted for 30 days. During the experiment, 3 subsamples of tiger prawn post-larvae were taken from the 10L culture tank from each replicate corresponding to each treatments to calculate and record the growth and survival rate for larvae of *P. monodon*. Gentle aeration was provided and water quality will monitor to encourage good growth and better survival rate of the post-larvae [19]. Growth and survival rate of post-larvae are taken once a week using the formula:

\[
SGR = \frac{(\ln W_t - \ln W_i \times 100)}{t}
\]

Where:
- \(W_t\) = final weight (g)
- \(W_i\) = initial weight (g)
- \(t\) = time (days)

Survival rate was calculated with:

\[
\text{Survival rate} \% = \frac{(\text{Total number of final post larvae} - \text{Total number of initial post larvae})}{t}
\]

Where:
- \(t\) = time (days)

2.6. Data analysis

Data were presented as the mean standard deviation (SD). All the data collected through experiment were analysed by using one-way analysis of variance (ANOVA). The differences of the treatments for each feeding trials are considered significant at the \(P<0.05\) level. The post-hoc test were tested using Tukey’s test. All the data were tested for normality, homogeneity, and independence to satisfy the assumption for ANOVA.

3. Results and Discussion

3.1. Specific growth rate and survival rate of Tiger prawn post-larvae

The highest specific growth rate of *P. monodon* were achieved when feed on copepod that were enriched with water spinach (11.28±0.38%) compared with other feeding treatments such as *Tetraselmis* sp. (10.93±0.02%), carrot (10.22±0.25%) and lettuce (10.19±0.07%).

| Diets              | Specific growth rate (%) (mean ± SD) | Survival rate (%) (mean ± SD) |
|--------------------|--------------------------------------|-------------------------------|
| Water spinach      | 11.28±0.38<sup>a</sup>              | 89.67±0.29<sup>a</sup>        |
| Carrot             | 10.22±0.25<sup>b</sup>              | 80.00±0.50<sup>b</sup>        |
| *Tetraselmis* sp.  | 10.19±0.07<sup>b</sup>              | 91.67±0.29<sup>a</sup>        |
| Lettuce            | 10.93±0.02<sup>a</sup>              | 83.67±7.83<sup>c</sup>        |

*All value is mean ± standard deviation (n = 4). The different small letters indicate a significant difference between different treatments (\(P<0.05\)).

For survival rate shows that The highest *P. monodon* post-larvae obtained fed on copepod enriched *Tetraselmis* sp. (91.67±0.29%; \(P<0.05\)) compared with other feeding treatments such as water spinach (89.67±0.29%). They are significantly effect within the treatments (\(P<0.01\)).

3.2. Protein and lipid analysis of copepod

The highest percentage for protein of cyclopoid copepod were attained when copepod we enriched with water spinach (89.18±0.95%), compared with other feeding treatments such as, *Tetraselmis* sp.
(84.78±0.96%; P<0.05). There is significant between treatments. Percentage of protein in copepod enriched carrot (64.18±0.72%) and lettuce (77.91±2.53%) has shown the lowest percentage in term of protein. Next, the highest percentage for lipid in copepod were attained when enriched with water spinach (60.09±6.80%) compared with other feeding treatments such as Tetraselmis sp. (84.78±0.96%), carrot (64.18±0.72%) and lettuce (77.91±2.53%).

**Table 2**: The percentage of protein and lipid for Cylopoid copepod that enriched with three different type of vegetable-based diets and *Tetraselmis sp.* as a control.

| Diets       | Protein in copepod (%)(mean ± SD) | Lipid in copepod (%)(mean ± SD) |
|-------------|-----------------------------------|---------------------------------|
| Water spinach | (89.18±0.95%)a                    | (60.09±6.80%)a                  |
| *Tetraselmis sp* | (84.78±0.96%)b                    | (62.04±24.18%)c                 |
| Carrot      | (64.18±0.72%)d                    | (92.10±1.24%)a, b               |
| Lettuce     | (77.91±2.53%)c                    | (26.13±18.47%)b, c              |

*All values are mean ± standard deviation (n = 4). The different small letters indicate a significant difference between different treatments (P<0.05).*

3.3. Protein and lipid analysis of *Penaeus monodon* post-larvae

The highest percentage for protein of *P. monodon* enriched cyclopoid copepod were attained when *P. monodon* post-larvae fed with copepod enriched with water spinach (134.11±1.94%), compared with other feeding treatments such as, carrot (119.43±0.0.72%; P<0.05). There is significant between treatments. Percentage of protein in copepod enriched *Tetraselmis sp.* (113.41±1.65%) and lettuce (128.2±6.86%) has shown the lowest percentage in term of protein. Next, the highest percentage for lipid in copepod were attained when enriched with water spinach higher (9.64±0.10%) compared with other feeding treatments such as *Tetraselmis sp.* (2.51±0.34%), carrot (6.63±0.57%) and lettuce (3.32±0.29%).

**Table 3**: The percentage of protein and lipid for *Penaeus monodon* post-larvae fed with Cylopoid copepod that enriched with three different type of vegetable-based diets and *Tetraselmis sp.* as a control.

| Diets       | Protein in *Penaeus monodon* (%) (mean ± SD) | Lipid in *Penaeus monodon* (%) (mean ± SD) |
|-------------|-----------------------------------------------|-------------------------------------------|
| Water spinach | (134.11±1.94%)a                              | (9.64±0.10%)a                             |
| *Tetraselmis sp* | (113.41±0.72%)c                              | (2.51±0.34%)b                             |
| Carrot      | (119.43±0.0.72%)b                            | (6.63±0.57%)c                            |
| Lettuce     | (128.2±6.85%)b                               | (3.32±0.29%)c                            |

*All values are mean ± standard deviation (n = 4). The different small letters indicate a significant difference between different treatments (P<0.05).*

Result from the present study proven that the nutritional value for vegetable that contained grain of wheat had seemed to be an excellent food for copepod [20]. The population growth was not significantly affected by the copepod fed with different diets, increasing the population growth depended on the food types [21]. However, in this study had showed that the growth rate of *P. monodon* post-larvae was impacted by the diet used. Among all the diet used, *P. monodon* post-larvae fed with copepod enriched water spinach has the highest number of specific growth rate (11.28±0.38%) compared with others diets. The specific growth rate of *P. monodon* post-larvae was significantly affected by the copepods enriched with water spinach. Therefore, the other enriched copepod was also applicable to be used to increased and sustain the copepod production since there are only slight difference in terms of number of specific growth between the control diet used.
According to Yousif et al. [22] the essential amino acid content in water spinach are high. The profile of saturated, monounsaturated and PUFA contents on cyclopoid copepod was significantly affected by the quality of food types that had been consumed [23]. In this study had showed that the using of vegetable-based diets also can affected the nutrient content for copepod that had feed on *P. monodon* post-larvae. Prior studies have noted that in order to increase and enriched zooplankton’s live foods such as *Artemia*, copepods and rotifers, it is advisable to use microalgae such as *Tetraselmis* sp. [24] to feed larvae and shrimps. However, the result from this study had showed that vegetable-based diets as an enrichment for copepod are able to be replace the microalgae as it has only slightly significant difference with the microalgae treatments. *P. monodon* post-larvae that fed with copepod enriched water spinach have showed the highest result of proximate protein and lipid and also highest result in specific growth performance. The lowest protein and lipid content is *P. monodon* post-larvae that fed copepod enriched vegetable-based of carrot.

4. Conclusion

In conclusion, our result indicated that the specific growth rate, survival rate and proximate composition of protein and lipid analysis of *P. monodon* was affected by different enriched copepod. The efficiency of the copepod enriched vegetable-based diet was further evaluated on its growth, survival and composition of lipid and protein. The food source from organic material had gave a sustainable production for copepod. This result shown that the highest specific growth rate in *P. monodon* was obtained when fed on copepod enriched with water spinach (*Ipomea aquatica*) (11.28±0.38%). Besides, the highest survival rate achieved by *P. monodon* fed with copepod enriched *Tetraselmis* sp. (91.67±0.29%) which is the control for this study. Even though the highest survival rate achieved by *P. monodon* fed with control treatment, but it has only a slight significant difference with copepod enriched water spinach (89.67±0.29%). The current result showed that water spinach (*Ipomea aquatica*) can be replaced the microalgae as the enrichment for copepod in order to improve the nutrient content in its. At the same time, it also can reduce the hatchery cost usage for microalgae type base of enrichment.

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