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The effect of enriched *Artemia* sp. on growth, nutritional composition, and survival performance of *Macrobrachium rosenbergii* (giant freshwater prawn)

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ABSTRACT

Artemia sp. or the brine shrimp is essential in commercial hatchery operations for young fish and prawns as live feed. *Artemia* is popular due to its availability and easy handling. However, *Artemia* is costly to enrich. This study aims to find Artemia's best enrichment by observing *Macrobrachium rosenbergii* or giant freshwater prawns' survival rate, growth rate, and nutritional composition after being fed with different enriched *Artemia*. This study was conducted for 30 days using four diets with three replicates per diet for the enriched *Artemia*. The diets of *Artemia* consisted of the microalgae *Isochrysis* sp. as the control, fish bones extract (FE), green mustard, and palm kernel cake (PKC). The survival rate and specific growth rate (SGR) of the post larvae *M. rosenbergii* fed with *Artemia* enriched with FE achieved the best performance with 92.00±1.80% and 6.19±0.02% respectively. Proximate analysis of enriched *Artemia* showed that FE resulted in the highest in protein (59.63±0.01%) and lipid contents (13.89±0.86%). Proximate analysis of post-larvae *M. rosenbergii* also showed that those fed with *Artemia* enriched with FE has the highest protein content (56.93±1.72%) and the lipid content (11.15±1.95%). Therefore, it is highly recommended to use *Artemia* enriched with FE to feed the giant freshwater prawn. This enrichment will reduce *Artemia*'s feeding costs since FE is easy to find and very cost-efficient yet nutrient-rich.

INTRODUCTION

Artemia is commonly used as live feed to giant freshwater prawn *Macrobrachium rosenbergii*. *Artemia* is a genus of aquatic crustaceans also known as 'brine shrimp'. *Artemia* is the only genus in the family Artemiidae. *Artemia* can be born from a latent

cyst in 24 hours. The cyst can be stored for a long time **(Dhont *et al*., 2013)**. *Artemia* has 40 – 60% of crude protein **(Zarei, 2013)**, 13 – 19% of fat and 3 – 14% of highly unsaturated fatty acid (HUFA) **(Kumar *et al*., 2015)**. *Artemia* is usually enriched before being used as live feed; organic by-products and green water or microalgae are used as food source for *Artemia* (**Verma *et al*., 2011; Sui *et al*., 2013; Jusoh *et al*., 2020**). The larvae of *Artemia* are omnivorous and feed primarily on zooplankton or microalgae. Zooplankton is the most widely used enrichment containing high protein and amino acids **(Vilchis, 2010)**. *Artemia* needs a high protein feed that would deliver a high nutrient *Artemia* **(Herawati *et al*., 2014)**.

Artemia have a high salinity tolerance; they can tolerate up to 50% of the concentration of salt, which is 30ppt to 50ppt **(Emslie, 2012).** On the other hand, enhancement of microalgae growth rate is difficult **(Khan *et al*., 2018)**. This is because microalgae are fragile and need to be monitored frequently. Hence, the industry needs to adjust their optimum salinity to ensure the microalgae bloom in hatchery condition. A few microalgae species need intense care during the isolation and upscale process, such as brown-golden marine microalgae *Isochrysis* sp.

The giant freshwater prawn *Macrobrachium rosenbergii* is the biggest *Macrobrachium* species. The average size of *M. rosenbergii* is 33 cm for males and 29 cm for females. *M. rosenbergii* is nocturnal, shallow and sluggish, and is territorial. Half of them will be submerged in sediments during the day, and they prefer sandy, detritus-rich and vegetated areas. *M. rosenbergii* is commonly reared in aquaculture, especially in Asian countries. According to FAO estimates, the production of *M. rosenbergii* reached 400,000 tons in 2010, 20 times higher growth relative to the beginning of the 1990s. *M. rosenbergii* is the most common species of *Macrobrachium* in the aquaculture industry **(Nhan, 2009)** due to its nutrient content. The wild *M. rosenbergii* composition is 68.27% of protein and 8.44% of lipid content **(Ferdose and Hossain, 2011)**.

Post-larvae and adult *M. rosenbergii* are omnivorous benthivores based exclusively on algae mollusks and another aquatic organism. They rely on live feed like *Artemia* during their early stage of live. This is because live feeds are easily detected and captured due to their swimming movement in water, and easy to digest **(Conceicao *et al*., 2010)**. When there is no live food among them, they may eat a small portion of organic food.

Incomplete and inconsistent nutrient composition in *Artemia* is one of the problems in feeding management **(Carter, 2015)**. *Artemia* has low nutrients for marine species, especially in highly unsaturated fatty acids (HUFA). Nonetheless, high HUFA diet for their enrichment may lead to excess of lipid and suboptimal dietary content for *M. rosenbergii*. So, the enrichment technique for *Artemia* is vital to have the correct levels of lipid, protein, vitamins, and minerals.

The use of live feed in hatchery rearing for almost all fish and shellfish species still seems to be essential and going to continue in the immediate future. *Artemia*, microalgae, rotifer, and copepod can be used as live feed. *Artemia* is the most widely used and popular because it is convenient and readily available in the market. *Artemia* feed and fertilizer costs are already in the range of 30% of overall total cost. For example, the enrichment often used in industry, Super Selco, cost about RM125 for only 125 ml.

Therefore, this study aims to find *Artemia'*s best enrichment by observing *M. rosenbergii*'s survival rate, growth rate, and nutritional composition, after being fed with different enriched *Artemia*.

MATERIALS AND METHODS

The brine shrimp, *Artemia* cysts were purchased from Bio Marine brand. The cyst were decapsulated in 15 L aquarium filled with saltwater at 26ºC to 27ºC with vigorous aeration **(Treece, 2000)**. The nauplii i.e. the first larval stage having an unsegmented body and a single eye, were enriched with four types of diet for 24 hours **(Sorgeloos *et al*., 2001)**.

The enrichment was prepared according to feeding treatments. Four enrichments were prepared: the microalgae *Isochrysis* sp. as control, fish bone extract (FE), mustard green, and palm kernel cake (PKC) (**Al Azad and Lal, 2018; Amitha *et al*., 2019; Rasdi *et al*., 2020**). Microalgae *Isochrysis* sp. diet was cultured in 5 L conical flask at the university's hatchery. The concentration for marine algal was 500 mg L^{-1}. This is equivalent to 2 x 10^7 ml^{-1} algal cells **(Zaleha and Busra, 2012)**. The palm kernel cake (PKC) concentration was 500 mg L^{-1} **(Paray and Al Sadoon, 2016)**.

Each enrichment was given to three replicates of *Artemia*. After that, the enriched *Artemia* were fed to giant freshwater prawn *M. rosenbergii*.

1. *Culture of Post-Larvae M. rosenbergii*

Four 15 L aquariums were prepared. 100 post-larvae *M. rosenbergii* were put in each aquarium and cultured with aeration. The post-larvae were fed twice daily with *Artemia* enriched with *Isochrysis* sp. as control, fish bone extract (FE), mustard green, and palm kernel cake (PKC) for 30 days **(Paulraj and Altaff, 1999)**. 50% water was changed every week and the water quality were maintained at 26 – 27ºC, while for salinity at 0 – 6 ppt, and for the pH 6-7.5 which means the water parameter were checked daily **(Yuslan *et al*., 2021)**.

2. *Survival Rate and Growth Rate of Post-Larvae M. rosenbergii*

The survival and specific growth rates (SGR) of *M. rosenbergii* were calculated using the formulae:

Survival rate = (Surviving fish / number of initial density) x 100

Specific growth rate (SGR) = $(\ln W_t - \text{In } W_i \times 100) / t$

3. Protein and Lipid Analysis of Artemia and M. rosenbergii

Enriched *Artemia* with the diet treatments were subjected to proximate analysis. Post-larvae *M. rosenbergii* fed with enriched *Artemia* were also subjected to the proximate analysis.

3.1. Estimation of Protein Content

Protein content was determined using the Kjeldahl method (**Zhang *et al*., 2020**) which involves three processes: digestion, distillation, and titration. For the digestion process, 0.2 g of sample was weighed then put into digestion tube. 5 ml of sulphuric acid (H_2SO_4) was added. One Kjeldhal tablet was put inside the tube, where it acted as the catalyst to help the digestion process. The digestion tube was heated to 420ºC for one and

a half hour using the scrubber. After cooling, the product of digestion process underwent the distillation process. 30 ml receiver solvent was prepared including 20 ml of 4% boric acid (S_3BO_3) in the flask. 8 drops of the indicator were added. The flask was placed at the end of the condenser receiver. 40 ml of distilled water and 30 ml of 40% sodium hydroxide (NaOH) were added into the digestion tube. The distillation unit Kjeldhal Buchii was activated and distilled the sample for five minutes. The sample that had been distilled were titrated using 0.1 N hydrochloric acid (HCl) until the distillation solution color changed from blue to light pink. Blank sample was also prepared but without the digestion product.

The protein content was calculated using the formula:

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

$$\% \text{ protein} = \%N \times F$$

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 sample)

(**Zhang *et al.*, 2020**)

3.2. Estimation of Lipid Content

Extraction cup was put in an oven at 100ºC for an hour. After removing the extraction thimble, the extraction cup was kept in desiccator to cool it down. The extraction cup was weighed and labeled as W_1. The extraction thimble was put tightly at the ring metal of bolder and its rack. Filter paper was placed in the extraction thimble. 2 g of sample was weighed and labeled as W_2. The thimble was inserted and covered with cotton. 40 ml to 50 ml of petroleum ether was poured into extraction cup and the cup was secured on the reflux set. The lipid was extracted using extraction unit and took about one hour and a half to complete. After that, the extraction cup was placed in the oven for two hours at 100ºC, and cooled in desiccator. The extraction cup was weighed and recorded as W_3.

The lipid content was calculated using the formula:

$$\% \text{ lipid} = (W_3 - W_1) / (W2) \times 100$$

Where:

$\mathbf{W_1}$ = extraction cup weight (g)

$\mathbf{W_2}$ = Sample weight (g)

$\mathbf{W_3}$ = extraction cup weight + lipid (g)

RESULTS

1. Survival Rate of *Macrobrachium rosenbergii*

Table (1) shows that post-larvae *M. rosenbergii* fed with *Artemia* enriched with fish bone extract (FE) had the highest survival rate (92.00 ± 1.80%), followed by microalgae *Isochrysis* sp. (86.33 ± 0.58%), green mustard (85.33 ± 5.39%), and palm kernel cake (PKC) (78.17 ± 1.61 %).

Table 1. Survival rate of post-larvae *M. rosenbergii* fed with four different enriched *Artemia*. All values mean ± standard deviation.

Artemia Enrichments	Survival Rate of *M. rosenbergii* (%)
***Isochrysis* sp.**	86.33 ± 0.57^{c}
Fish bone extract (FE)	92.00 ± 1.80^{b}
Green mustard	85.33 ± 5.39^{b}
Palm kernel cake (PKC)	78.17 ± 1.61^{a}

Small letters indicate significant difference between treatments.

2. Specific Growth Rate of *Macrobrachium rosenbergii*

Table (2) shows the specific growth rate (SGR) of post-larvae *M. rosenbergii* in each treatment after 30 days. The highest growth rate is seen in *M. rosenbergii* fed with *Artemia* enriched with fish bone extract (FE) (6.19 ± 0.02%), followed by *Isochrysis* sp. (4.72 ± 0.60%) and green mustard (3.93 ± 0.03%). Those fed on *Artemia* enriched with PKC showed the lowest specific growth rate (3.47 ± 0.31%). The growth rate of *M. rosenbergii* significantly depended on different dietary enrichment ($P < 0.05$).

Table 2. Specific growth rate (SGR) of post-larvae *M. rosenbergii* fed with four different enriched *Artemia*.

Artemia Enrichments	Specific Growth Rate of *M. rosenbergii* (%)
***Isochrysis* sp.**	4.7200 ± 0.60^{c}
Fish bone extract (FE)	6.1967 ± 0.02^{b}
Green mustard	3.9300 ± 0.03^{ab}
Palm kernel cake (PKC)	3.4767 ± 0.31^{a}

Small letters indicate significant difference between different treatments.

3. Proximate Analysis

3.1. Protein and Lipid Content of M. rosenbergii

Table (3) shows the protein and lipid content in *M. rosenbergii* fed on *Artemia* enriched with fish bone extract (FE) were the highest compared to other enrichments (56.93 ± 1.72% and 11.15 ± 1.94%, respectively) followed by *Isochrysis* sp. and green mustard. There was a significant difference in *Artemia* enrichments ($P < 0.05$).

Table 3. Body composition (%) of post-larvae *M.rosenbergii* fed with four different enriched *Artemia*. All values mean ± standard deviation.

Body composition	Artemia Enrichments	%
Protein	***Isochrysis* sp.**	50.76±0.28[b]
	Fish bone extract (FE)	56.93±1.72[c]
	Green mustard	42.55±0.44[a]
	Palm kernel cake (PKC)	41.19±0.38[a]
Lipid	***Isochrysis* sp.**	6.19±0.076[b]
	Fish bone extract	11.15±1.95[c]
	Green mustard	5.27±0.01[ab]
	Palm kernel cake (PKC)	4.19±0.01[a]

Small letters indicate significant difference between treatments.

3.2. Protein and Lipid Content of*Artemia*

Table (4) shows *Artemia* enriched with fish bone extract (FE) contains the highest protein and lipid content (59.63 ± 0.00% and 13.89 ± 0.09%, respectively), followed by *Isochrysis* sp. (57.19 ± 0.09% and 8.95 ± 0.11%). PKC had the lowest protein and lipid content (45.49 ± 0.84% and 8.95 ± 0.11%).

Table 4. The body composition (%) of *Artemia* with four different enrichments.

Body composition	Enrichments	%
Protein	***Isochrysis* sp.**	57.19±0.09[d]
	Fish bone extract (FE)	59.63±0.00[c]
	Green mustard	49.60±0.64[b]
	Palm kernel cake (PKC)	45.49±0.84[a]
Lipid	***Isochrysis* sp.**	8.95±0.11[d]
	Fish bone extract (FE)	13.89±0.09[c]
	Green mustard	6.31±0.26[b]
	Palm kernel cake (PKC)	5.09±0.05[a]

Small letter indicates the significant different between treatment.

DISCUSSION

Fish and crustacean nutritional properties are important for human health **(Usydus *et al*., 2011)**. They have a lot of food potential and provide the same quality of protein like milk, meat, and egg **(Islam *et al*., 2017)**. The post-larvae stages of many shrimp and prawn species require high nutrition for development. It is essential to produce live food with high nutritional value for feeding. To produce high quality feed requires high cost; this is the reason aquacultures of freshwater giant prawn *M. rosenbergii* are difficult to expand like carp in Bangladesh, because of the high cost in producing feed **(Aarumugam *et al*., 2013)**.

From the results in Table (1), the post-larvae *M. rosenbergii* fed with *Artemia* enriched with fish bone extract (FE) has the highest survival rate compared to other enrichments. Nutritionists have frequently used fish bone extract as an alternative protein in fish meals **(Arshad and Farzana, 2007)**. *M. rosenbergii* fed with *Artemia* enriched with palm kernel cake (PKC) has the lowest survival rate. The slightly lower survival rate

may be because of the *M. rosenbergii*'s cannibalistic behavior. The post-larvae were known to be attacked by the bigger size prawn during the molting **(Arshad and Farzana, 2007)**. A group of researchers have stated that the survival rate of *M. rosenbergii* is better with live feed enriched with algae **(Rasdi *et al.*, 2021)**. They stated that several reports showed that *P. monodon* larvae fed with a mixed diet of *Chaetoceros muelleri* and *Thalassiosira suecica* performed well.

Based on Table (2), it was observed that the specific growth rate (SGR) of *M. rosenbergii* fed with *Artemia* enriched with FE is the highest. In commercial production of *M. rosenbergii*, diet already costs around 50 to 60% of total cost in pellet production. The raw material that is usually used is the costly fish meal. The general approach to reducing the cost was by substituting the fish meal with other ingredient. **Jannathulla *et al.* (2019)** stated that bone meal is the most suitable one. Beside a good protein source, bone meal is also a good source of calcium, phosphorus, and trace minerals.

Proximate analysis was done to compare the body composition of post-larvae *M. rosenbergii* after fed with *Artemia* with different enrichments. Post-larvae *M. rosenbergii* fed with *Artemia* enriched with FE have the highest protein and lipid contents. Conversely, post-larvae fed with Artemia enriched with PKC showed the lowest of protein and lipid contents. Previous studies have shown that reared *M. rosenbergii*'s protein level ranged from 15 to 80% **(Hasanuzzaman *et al.*, 2009)**. Our finding is supported by the study on Japanese tiger prawn *Panaeus japonicus* **(Ashokkumar *et al.*, 2011)**. A study concluded that diet that has less than 10% protein has low high unsaturated fatty acid (HUFA) **(Rasdi and Qin, 2018)**. HUFA supported the survival and growth of *P. japonicus*. Freshwater prawn only needs 6 to 10% lipid. Diet that contains more than 10% lipid can cause the reduction in growth of *M. rosenbergii*. Post-larvae fed with *Artemia* enriched with PKC showed the lowest lipid content.

Proximate analysis was also done on enriched *Artemia*. Based on the Table (4), *Artemia* enriched with FE has the highest protein and lipid contents, while *Artemia* enriched with PKC has the lowest. Protein content lower than 15% can cause mortality during the rearing; protein is the most important element in formulating fish feed. On the other hand, too high protein content can inhibit the growth of the prawn **(Lee *et al.*, 2018)**. The prawn will metabolize the extra protein as energy source, and nitrogen will be excreted as ammonia. 60% protein content in body composition in *M. rosenbergii* is the result of *M. rosenbergii* fed with *Artemia* enriched with FE and *Isochrysis* sp. Notably, among many microalgae, *Isochrysis galbana* is most suitable to be used in mariculture to feed larval fish, crustaceans, and mollusks. These microalgae are high in lipid that can be used in aquaculture and biofuel **(Mishra and Mishra, 2018)**.

CONCLUSION

Macrobrachium rosenbergii fed with *Artemia* enriched with fish bone extract (FE) has the highest nutrient in body composition, survival rate, and specific growth rate (SGR). While the lowest is *M. rosenbergii* fed with *Artemia* enriched with PKC. Both FE and PKC do not need higher cost. In general, all the treatments can be used in culturing the freshwater prawn as the lower survival rates might be because of their cannibalistic behavior. But some ingredients might cause slower growth and did not have enough nutrient content for the prawn. So, it is crucial to find the enrichment that can improve the

nutritional value of prawn and the live feed. Exploration in other sources for improving nutritional value in aquaculture is recommended, especially in protein, to help researchers find the most suitable protein source with lower cost.

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