Sri Lanka J. Aquat. Sci. ***18*** *(2013): 17-26*

Growth and survival of post-larvae of giant freshwater prawn (*Macrobrachium rosenbergii*) reared using feeds formulated with different sources of protein

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Abstract

Semi-intensive aquaculture of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man) in Sri Lanka is limited mainly due to unavailabilty of suitable commercial feeds. At present, the widely used commecial feed is poultry feed starter-I, which is of low nutritive value. Present study was therefore carried out to develop a suitable feed using currently unutilized material as the protein source namely, cattle intestines disacrded from slaughter houses, mussel meat discarded after removing shells for lime and poultry industries, and trash fish discarded at landing sites.

Post-larvae of *M. rosenbergii* having an initial weight of 44-78 mg were stocked in glass tanks measuring 60 cm x 30 cm x 30 cm at a stocking density of 50 individuals per tank and fed with formulated experimental feeds and the poultry feed starter-I as the control diet. Feeding experiments were carried out in triplicate in two culture cycles of 90 days each.

Specific growth rates of post-larvae when fed with experimental feeds formulated with mussel, trash fish and cattle intestines as the protein source were 2.08, 2.07 and 2.03 in the first culture cycle and 2.25, 2.27 and 2.33 in the second culture cycle respectively. These were significantly higher than those observed when fed with the control diet which were 1.36 and 1.39 in the first and second culture cycles respectively ($p < 0.05$). Higher specific growth rates resulted in significantly higher weight gains in post-larvae fed with experimental feeds (458-898 mg) than in those fed with the control diet (217-318 mg) ($p<0.05$). Survival rates of post-larvae fed with experimental feeds (42-52%) were significantly higher than those of the post-larvae fed with the control diet (16-24%) whereas food conversion ratios of the former (2.47-3.22) were significantly lower than those of the latter (4.90-5.02) ($p < 0.05$).

Present study indicates that lower amounts of feeds formulated using mussel meat, trash fish or cattle intestines as the protein source yield better growth and

survival rates of *M. rosenbergii* post-larvae than the currently used poultry feed starter-I. Since discarded material are used as the protein source, the cost of feed will also be low resulting in high profits.

Keywords: *M. rosenbergii*, giant freshwater prawn, post-larval rearing, formulated feed

Introduction

Giant freshwater prawn *Macrobrachium rosenbergii* (de Man) is one of the major contributors to the world aquaculture production. Global production of *M. rosenbergii* in 2010 was 203,211 tonnes with a total value of US$ 1.123 x 10^9 (FAO 2013). Due to its high importance in aquaculture, large amount of research had been carried out throughout the world on various aspects of its biology, ecology and aquaculture (Wowor and Ng 2007).

In Sri Lanka, research on the aquaculture of *M. rosenbergii* had mainly focussed on the rearing of larvae with the objective of getting a high production and survival. Ratnayake et al. (2011) have reported that to get the highest production of larvae from the Sri Lankan stock of *M. rosenbergii*, the best male:female ratio is 1:5 and the optimum salinity is 5 ppt. Some research had also been carried out in Sri Lanka to evaluate the performance of different larval feeds in order to reduce the use of live *Artemia* nauplii (Asoka and Hettiarachchi 2004).

In 2012, 16.75 million post-larvae of *M. rosenbergii* had been produced in Sri Lanka of which 14.53 million had been stocked. Of these, about 99% had been stocked in perennial and seasonal tanks for extensive aquaculture. Only 170,000 post-larvae had been used for semi-intensive aquaculture in estate tanks and ponds (NAQDA 2013).

Lack of suitable commercial feed has limited the development of semi-intensive aquaculture of *M. rosenbergii* as a commercial industry in Sri Lanka. Some farmers use the commercial feed of *Peneaus monodon* to feed the post-larvae of *M. rosenbergii*; however, many farmers use less expensive poultry feed starter-I, which results in low growth rates probably due to low nutritive value of the feed (Amaraweera, unpublished). Present study was carried out to develop a post-larval feed with high nutritional quality, which could be used in semi-intensive aquaculture of *M. rosenbergii* using currently unutilized protein sources.

Materials and Methods

Three types of feeds were formulated using the method descibed by Shiang (2001). The protein sources used were cattle intestine carcasses discarded from slaughter houses, mussel meat discarded after removing shells for lime and poultry industries, and trash fish discarded at landing sites. The percentages of different ingredients used in the formulation of each experimental feed are given in Table 1. The poultry feed starter-1, which is currently used to feed *M. rosenbergii* in grow-out ponds in

Sri Lanka was used as the control. Protein contents of the three formulated experimental feeds and the control diet were determined using the Kjeldhal method.

Post-larvae of *M. rosenbergii* were stocked in glass tanks measuring 60 cm × 30 cm × 30 cm under normal laboratory conditions at the Regional Research Centre of National Aquatic Resources Research and Devlopment Agency (NARA) at Rekawa. Fully randomized design was used in allocating the feed types to different tanks. Feeding trials were conducted in triplicate. Each tank was filled with aged tap water and was continuously aerated throughout the experimental period. Post-larvae were 30 days old at the time of stocking. In each tank, 50 individuals were stocked. The experiment was carried out in two culture cycles each with a duration of 3 months (from 1st May to 31st July 2004 and from 1st August to 31st October 2004). The initial mean length and mean weight of the post-larvae in the 1st culture cycle were 13 mm and 44 mg while in the 2nd cycle these were 27 mm and 78 mg respectively. Post-larvae were fed twice a day, in the morning and evening at a rate of 10% of the body weight. Unconsumed feed were siphoned out immediately after feeding to avoid mixing with faeces and weighed after oven drying at 85°C to a constant weight (Pillay 1990) to determine the exact amount of feed consumed by post-larvae. At every fortnight, 25 post-larvae were collected from each tank and and weighed. The proximal crude protein levels of the post-larvae at the end of each culture cycle were determined using the Kjeldhal method.

Water temperature, pH, dissolved oxygen content, alkalinity, water hardness, nitrite content and dissolved ammonia content in each tank were recorded at every fortnight interval. Water temperature was measured using a glass thermometer. Alkalinity and water hardness were measured using digital test kits (Model: 16900 HACH – USA) and pH was measuted using a pH meter (Model: MTW –pH 330/seto). Dissoved oxygen content was measured using the Winkler method and the dissolved nitrite and ammonia contents were measured using the UV/visible spectrophotometer (Model: DR 4000-USA) as described by Silva et al. (1996).

The number of post-larvae survived in each tank was recorded at the end of the each culture cycle to determine the survival rate.

Table 1. The percentages (by weight) of different ingredients used in the formulation of each experimental feed.

Ingredients	M_1	M_2	M_3
Rice bran	37%	37%	37%
Peanut cake	30%	30%	30%
Mussel shell powder	3%	3%	3%
Vitamins and growth hormones	1%	1%	1%
Mussel meat	30%	-	-
Trash fish	-	30%	-
Cattle intestines	-	-	30%

Specific growth rates and food conversion ratios were determined using the following equations (Priestley et al. 2006).

$$\text{Specific growth rate} = \frac{\text{Ln (Final weight – Initial weight)}}{\text{Duration of the experiment}}$$

$$\text{Food conversion ratio} = \frac{\text{Amount of food consumed}}{\text{Increase in body weight}}$$

Results were statistically analysed using one way ANOVA followed by Tukey's pairwise comparisons (Zar 1996). MINITAB (Version 15.0) software package was used for the statistical analysis.

Results

Mean values for water quality parameters during the two culture cycles are given in Table 2. During the experimental period, the water temperature ranged from 28^{o}C to 29.5^{o}C and pH ranged from 7.92 to 8.82. Dissolved oxygen contents varied from 4.48 mg l^{-1} to 12.00 mg l^{-1}. The ranges for alkalinity, hardness, nitrite content and dissolved ammonia content were 108-152 mg $CaCO_3$ l^{-1}, 171-274 mg $CaCO_3$ l^{-1}, 0.008-0.225 mg l^{-1} and 0.018-0.994 mg l^{-1} respectively. There were no significant differences in the water quality parameters among different treatments ($p > 0.05$). Similarly, there were no significant diferences in the mean values for water temperature, pH, alkalinity and total hardness between the treatments and the control ($p > 0.05$). The dissolved oxygen content was significantly higher and dissolved ammonia and nitrite contents were significantly lower in the control tanks than in the treatment tanks during both culture cycles ($p < 0.05$) (Table 2).

Mean values for % survival and specific growth rates of post-larvae when fed with formulated experimental feeds (M_1, M_2 and M_3) were not significantly different from each other ($p > 0.05$) but were significantly higher than that of the post larvae fed with the control diet (M_4) in both culture cycles ($p < 0.05$) (Table 3). The mean values for food conversion ratio were not significanly different from each other when fed with formulated experimental feeds ($p > 0.05$) but were significantly lower than that when fed with the control diet in both culture cycles ($p < 0.05$) (Table 3).

In both culture cycles, the weight gains of post-larvae during the first 4 weeks were more or less similar and then rapid increases in weight were recorded in those fed with the formulated experimental feeds (Figure 1). The highest final weight as well as the highest weight gain was recorded in the post-larvae fed with the formulated experimental feed where cattle intestine was used as the protein source (M_3). The lowest final weight as well as the lowest weight gain was recorded in the post larvae fed with the control diet (M_4) (Table 3). The final weights of the post-larvae fed with the formulated exprimental feeds were not significantly different

form each other ($p > 0.05$) but were significantly higher than that of the post-larvae fed with the control deit ($p < 0.05$) in both culture cycles (Table 3).

Table 2. Mean ± SD values for the water quality parameters in the rearing tanks of post-larvae of *M. rosenbergii* fed with three formulated experimental feeds and control diet during the two culture cycles (M_1= Experimental feed formulated with mussel meat as the protein source, M_2 = Experimental feed formulated with trash fish as the protein source, M_3 = Experimental feed formulated with cattle intestine as the protein source, M_4 = Poultry feed starter-1).

Parameter	Culture Cycle	Diet			
		M_1	M_2	M_3	M_4
Water temperature (^oC)	I	$28.4^a \pm 0.05$	$28.4^a \pm 0.05$	$28.4^a \pm 0.08$	$28.4^a \pm 0.08$
	II	$28.6^a \pm 0.05$	$28.6^a \pm 0.05$	$28.6^a \pm 0.08$	$28.6^a \pm 0.08$
pH	I	$8.3^a \pm 0.02$	$8.3^a \pm 0.02$	$8.3^a \pm 0.05$	$8.4^a \pm 0.04$
	II	$8.3^a \pm 0.06$	$8.3^a \pm 0.05$	$8.3^a \pm 0.04$	$8.4^a \pm 0.05$
Dissolved Oxygen (mg l^{-1})	I	$6.71^a \pm 0.11$	$6.67^a \pm 0.09$	$6.90^a \pm 0.08$	$7.50^b \pm 0.04$
	II	$6.70^a \pm 0.12$	$6.74^a \pm 0.27$	$6.59^a \pm 0.12$	$7.49^b \pm 0.07$
Alkalinity (mg l^{-1})	I	$130.76^a \pm 1.43$	$129.66^a \pm 2.37$	$130.33^a \pm 1.45$	$131.90^a \pm 1.50$
	II	$141.66^a \pm 2.89$	$140.05^a \pm 1.06$	$138.47^a \pm 1.11$	$140.04^a \pm 0.70$
Hardness (mg l^{-1})	I	$209.52^a \pm 1.90$	$207.47^a \pm 3.08$	$209.95^a \pm 2.97$	$231.71^a \pm 1.43$
	II	$209.53^a \pm 1.93$	$207.47^a \pm 3.08$	$209.95^a \pm 2.97$	$209.52^a \pm 1.90$
Nitrite (mg l^{-1})	I	$0.0463^a \pm 0.01$	$0.0566^a \pm 0.01$	$0.0479^a \pm 0.01$	$0.0153^b \pm 0.01$
	II	$0.0368^a \pm 0.06$	$0.0470^a \pm 0.02$	$0.0579^a \pm 0.01$	$0.0109^b \pm 0.02$
Ammonia (mg l^{-1})	I	$0.2593^a \pm 0.22$	$0.4972^a \pm 0.12$	$0.3197^a \pm 0.30$	$0.0466^b \pm 0.08$
	II	$0.2455^a \pm 0.05$	$0.3828^a \pm 0.06$	$0.4721^a \pm 0.01$	$0.140^b \pm 0.02$

Mean values with different superscripts in the same row are significantly different from each other ($p < 0.05$).

The mean ± SD values for proximate protein levels (as %) in the formulated experimental feeds (M_1, M_2 and M_3) and the control diet (M_4) were 33.13 ± 0.30, 34.26 ± 0.20, 35.23 ± 0.25 and 20.82 ± 0.31 respectively. These values for M_1, M_2 and M_3 were not significantl different from each other ($p > 0.05$) but were significantly higher than that of M_4 ($p < 0.05$).

Proximate protein levels of post-larvae before and after the feeding experiments are given in Table 4. The proximate protein levels have not changed significantly when fed with the control diet of M_4 ($p > 0.05$) in both culture cycles. The proximate protein levels of post-larvae were not significantly different from each other when fed with M_1, M_2 and M_3 ($p > 0.05$) but were significantly higher than the initial level and the level when fed with the control diet M_4 ($p < 0.05$).

Table 3. Mean ± SD values for survival rates, specific growth rates, intial weights, final weights, weight gains and food conversion ratios of post-larvae of *M. rosenbergii* fed with the formulated experimental feed ad control diet during the two culture cycles. (M_1, M_2, M_3 and M_4 are same as those stipulated in Table 2).

	Culture cycle	Diet			
		M_1	M_2	M_3	M_4
Percentage survival rate	I	$50.00^a \pm 2.0$	$48.33^a \pm 3.1$	$49.00^a \pm 2.0$	$18.00^b \pm 2.0$
	II	$47.00^a \pm 3.2$	$51.33^a \pm 4.2$	$46.33^a \pm 3.1$	$20.66^b \pm 4.7$
Specific growth rate (% day^{-1})	I	$2.08^a \pm 0.05$	$2.07^a \pm 0.04$	$2.09^a \pm 0.04$	$1.36^b \pm 0.06$
	II	$2.25^a \pm 0.02$	$2.27^a \pm 0.02$	$2.33^a \pm 0.07$	$1.39^b \pm 0.03$
Initial weight (g)	I	0.44	0.44	0.44	0.44
	II	0.78	0.78	0.78	0.78
Final weight (g)	I	$0.50^a \pm 0.08$	$0.56^b \pm 0.04$	$0.69^c \pm 0.03$	$0.26^d \pm 0.01$
	II	$0.68^a \pm 0.04$	$0.81^b \pm 0.06$	$0.98^c \pm 0.02$	$0.40^d \pm 0.03$
Weight gain (g)	I	0.458	0.513	0.643	0.217
	II	0.609	0.732	0.898	0.318
Food conversion ratio	I	$3.22^a \pm 0.27$	$3.18^a \pm 0.09$	$2.84^a \pm 0.12$	$4.90^b \pm 0.57$
	II	$3.15^a \pm 0.31$	$2.99^a \pm 0.16$	$2.47^a \pm 0.13$	$5.02^b \pm 0.18$

Mean values with different superscripts in the same row are significantly different from each other ($p < 0.05$).

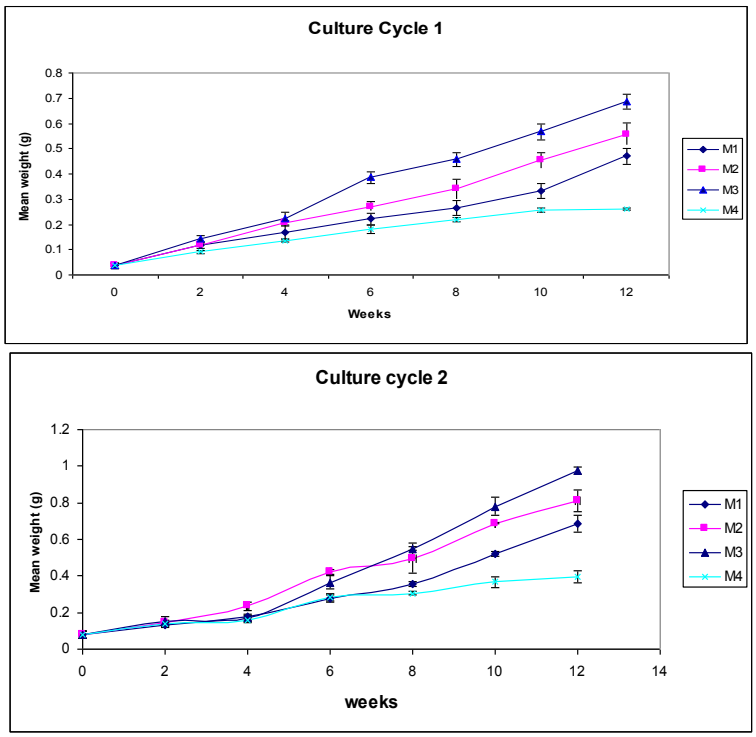

Figure 1. Increase in weight of post-larvae fed with formulated experimental feeds and the control diet. (M_1, M_2, M_3 and M_4 are same as those stipulated in Table 2).

Table 4. Mean ± SD values for proximate protein levels (%) of post-larvae of *M. rosenbergii* at the begining of the experiment and after feeding with three formulated experimental feeds and the control diet during the two culture cycles. (M_1, M_2, M_3 and M_4 are same as those stipulated in Table 2).

Culture cycle	Initial	Diet			
		M_1	M_2	M_3	M_4
I	$20.1^{b} \pm 0.16$	$22.41^{a} \pm 0.35$	$23.61^{a} \pm 0.17$	$23.78^{a} \pm 0.42$	$20.82^{b} \pm 0.31$
II	$20.9^{b} \pm 0.24$	$23.58^{a} \pm 0.24$	$24.51^{a} \pm 0.30$	$25.08^{a} \pm 0.51$	$21.42^{b} \pm 0.42$

The mean values in same row with different superscripts are significantly different from each other ($p < 0.05$).

Discussion

Size of post-larvae at stocking play an important role in getting the maximum productivity as well as the maximum profit in the aquaculture of *M. rosenbergii* (Reddy 1997). Therefore, the growth of post-larvae has received high attention of growers and researchers. Variety of feeds have been formulated and tested to achieve the highest growth and maximum survival of post-larvae. Tested feeds include fish meal, *Artemia*, *Moina*, *Tubifex*, chironomid larvae, crab meat, poultry eggs, beans, various grains, clam meat, mussel meat and shrimp (Reddy 1997, Indulkar and Belsare 2004). In addition, feeding experiments with probiotics, nauplii larvae, liquid shrimp diet, spray dried algal cells and feeds formulated using various ingredients have also been carried out (Asoka and Hettiarachchi 2004; Venkat et al. 2004; Parakrama et al. 2009; Shailender et al. 2013). This is the first record of research carried out in Sri Lanka on the growth and survival of post-larvae of *M. rosenbergii* when fed with feeds formulated with mussel meat, trash fish and cattle intestines as protein sources.

Significantly higher values for specific growth rates observed when fed with formulated experimental feeds than when fed with poultry feed may be due to higher protein content in the former. The protein content in the commercially available poultry feed starter-I (20-21%), which is used to feed the post-larvae of *M. rosenbergii* in semi-intensive pond culture in Sri Lanka at present is less than the minimum required level of 23% (Corbin et al. 1983) whereas in the formulated feed used in the present study, these values were higher than the required minimum. However, these values were slightly lower than that of the feed used by Parakrama et al. (2009). Higher values observed for specific growth rate when fed with formulated experimental feeds indicate that larger size can be attained with lesser time than when fed with poultry feed starter-I. Specific growth rates observed in the present study when fed with formulated experimental feeds are higher than those recorded in the feeding experiments with *Lactobacillus*-based probiotics (Venkat et al. 2004) and diets enriched with vitamins E and D, cod liver oil and astaxanthin (Parakrama et al. 2009). However, the specific growth rates recorded in the present study are lower than those recorded when fed with *Artemia, Moina*, *Tubifex*, clam meat and egg

custard (Indulkar and Belsare 2004). This may be due to the higher nutritive value, especially the higher content of crude protein of these food items (44-66%) than that of the experimental feeds used in the present study (33-35%).

Survival rates observed in the present study when fed with formulated experimental feeds (46-50%) were higher than those recorded when fed with *Artemia* (44%) or *Moina* (33%) alone but were lower than those recorded when fed with a diet consisting of 50:50 combination of *Artemia* and *Moina* (56%) (Alam et al. 1993). Parakrama et al. (2009) also recorded higher survival rates (57-77%) in their feeding experiments with vitamin enriched formulated feed. Indulkar and Belsare (2004) have also recorded high survival rates (70-92%) in their feeding experiments with *Artemia, Moina*, *Tubifex*, clams and egg custard. In feeding experiments with *Lactobacillus*-based probiotics, Venkat et al. (2004) recorded 100% survival of post-larvae. However, survival rates recorded in the present study, when fed with formulated experimental feeds were higher than those recorded by Islam and Khan (1990), Adisukrenso et al. (1982) and Islam et al. (2000) which were 11.9%, 10.2% and 30% respectively. Significantly higher survival rates recorded when formulated experimental feeds were used than when poultry feed starter-I was used (18-21%) may probably be due to the higher nutritive value of the former as evident by the higher crude protein levels, which may have resulted in healthier and more robust post-larvae.

Significantly higher final weights of the post-larvae recorded when fed with formulated experimental feeds than when fed with poultry feed starter-I may also be due to the high nutritive value of the experimental feed as indicated above.

The higher weight gain observed during the culture cycle II than during the culture cycle I may be due to higher initial size of the post-larvae used. Other parameters such as the survival rates, specific growth rates and food conversion ratios are similar in both culture cycles indicating that these parameters are not affected by the initial size of the post-larvae.

Significantly higher food conversion ratios observed when fed with poultry feed starter-I (4.9-5.0) than when fed with formulated experimental feeds (2.5-3.2) indicate that higher amount of the former is required for a unit increase in weight of post-larvae than the latter. This may also be due to the poor nutritive quality of the commercial feed as indicated by low level of crude protein. Food conversion ratios similar to those recorded in the present study have been recorded by Venkat et al. (2004) in their feeding experiments with *Lactobacillus*-based probiotics (2.19-2-75). The food conversion ratios recorded by Parakrama et al. (2009) in their feeding experiments with vitamins enriched formulated feeds (0.87-1.88) lower than those recorded in the present study probably due to their high nutritive value. Enriched vitamins would have enhanced the assimilation of food.

Dissolved oxygen content, temperature, pH, dissolved ammonia and nitrite contents recorded in the present study were within the ranges stipulated by New (2002) for freshwater prawn nurseries and grow-out facilities. However, the alkalinity and hardness values recorded were higher than those recommended by New (2002). Nevertheless, the survival rates, specific growth rates and food conversion ratios were in the acceptable range as recorded by many researchers.

Results of the present study indicates that the feeds formulated with mussel meat, trash fish or cattle intestines as the protein source yield better survival rates, higher specific growth rates and lower food conversion ratios of post-larvae of *M. rosenbergii* than the poultry feed starter-I. Since these protein sources are presently discarded, they could be purchased at a very low price, thus enabling the production of these feeds at a low cost which will ultimately contribute to reduce the cost of production of *M. rosenbergii* in semi-intensive aquaculture in ponds and tanks.

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