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Effect of fishmeal replacement with *Artemia* biomass as a protein source in practical diets for the giant freshwater prawn *Macrobrachium rosenbergii*

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Abstract

A 30-day feeding experiment was conducted in 160-L plastic tanks to evaluate the potential use of *Artemia* biomass as a protein source in practical diets for postlarval *Macrobrachium rosenbergii* (initial mean weight of 12.12–12.29 mg). Nine isoenergetic and isonitrogenous experimental diets (approximately 40% crude protein) were formulated by replacing levels of the fishmeal (FM) protein difference either with dried or frozen *Artemia* (0, 25, 50, 75 and 100%). The 0% *Artemia* treatment, in which Peruvian FM was the only main protein source, was considered to be the control diet. The results showed that prawn postlarvae (PLs) fed the FM control diet had a lower survival (46%) compared with all *Artemia* diets. Significant differences ($P < 0.05$) were, however, only found at 75% and 100% *Artemia* protein inclusion levels (survival of 68–77%). A gradual increase in growth performance (live weight gain, specific growth rate and total length) of the prawns was achieved on increasing dietary inclusion of *Artemia* protein. Additionally, the size distribution exhibited the same response as growth performance. However, prawns fed the frozen *Artemia* diets showed a better performance than the ones fed the dried *Artemia* diets. It can be suggested that *Artemia* biomass may totally replace FM in practical diets for PLs of the freshwater prawn *M. rosenbergii*.

Keywords: *Artemia* biomass, fishmeal, growth, survival, size distribution, *Macrobrachium rosenbergii*

Introduction

Presently, in Vietnam, the aquaculture sector has been developing in terms of the culture area, production, number of species and degree of management intensity (Edwards, Tuan & Allan 2004). For example, the total aquatic production increased almost 7% in 2006, while aquaculture production increased 14.6% (Huong & Quan 2007). According to the Ministry of Agriculture & Rural Development, Vietnam, the availability of fishmeal (FM) is low and the price of imported FM from 2007 to the first 6 months of 2008 has increased significantly (1.1–1.4 USD kg^{-1}). Most feed manufacturers are using expensive imported FM as a protein source for aquafeeds (http://www.fistenet.gov.vn/details.asp?News). Therefore, assessment of cheaper or more readily available alternative protein sources such as by-products from fisheries, processing or other sources that may reduce the use of FM in feeds is necessary.

Among the alternative animal protein sources, *Artemia* biomass (adult *Artemia*) may be considered to be a suitable ingredient for replacing FM in fish and crustacean diets because of its high nutritional value (Sorgeloos 1980; Léger, Bengtson, Simpson & Sorgeloos 1986; Bengtson, Léger & Sorgeloos 1991; Lim, Soh, Dhert & Sorgeloos 2001, Maldonado-Montiel & Rodríguez-Canché 2005). Frozen adult *Artemia* included in a mixed diet improved the reproductive performance of *Penaeus vannamei* (Naessens, Lavens, Gomez, Browdy, McGovern-Hopkins, Spencer, Kawahigashi & Sorgeloos 1997; Wouters, Lavens,

Nieto & Sorgeloos 2001). In postlarval stages of penaeid shrimp and clawed lobster, *Artemia* has been shown to provide excellent nutrition (Wickins & Lee 2002; Tlusty, Fiore & Goldstein 2005). Live adult *Artemia* is also an ideal food for ornamental fish (Lim *et al.* 2001). According to Naegel and Rodriguez-Astudillo (2004), dried *Artemia* is a well-suited feed for postlarval *Litopenaeus vannamei*.

Vietnam offers a potential source of *Artemia* biomass as a by-product derived from the commercial cyst-oriented *Artemia* pond production systems, which yield an average *Artemia* biomass production of 0.2–0.3 tonnes ww ha^{-1} (Brands, Quynh, Bosteels & Baert 1995; Anh, Quynh, Hoa & Baert 1997). However, so far, only small amounts of this by-product have been utilized for production purposes. Therefore, the use of *Artemia* biomass as a protein source in feeds may contribute to the profitability of *Artemia* farmers and have a positive impact on the socio-economic aspects in the coastal area. Thus, the potential of this product should be evaluated further.

There has been a very rapid global expansion of freshwater prawn farming since 1995. The total global production of *Macrobrachium* is estimated at 7 50 000–10 00 000 tonnes year^{-1} by the end of this decade; most is produced in Asia, in which China is the leader, followed by Vietnam and India (New 2005). Currently, the giant freshwater prawn (*Macrobrachium rosenbergii*), which is indigenous to the Mekong Delta, Vietnam, is becoming an increasingly important target species for aquaculture. According to Sinh (2008), in 2006, the total culture area of *M. rosenbergii* in the Mekong Delta was 9077 ha and production increased to 9514 tonnes, with 111 hatcheries growing postlarvae (PLs) (production: 107 million PL) and about 300 millions of PL imported. *Macrobrachium* responds very well to extensive and semi-intensive culture systems, and its culture, especially in rice fields, is considered to have the potential to raise incomes among impoverished farmers and contribute towards enhancing rural development in Vietnam (Hai, Phuong, Hien, Bui, Son & Wilder 2003; Phuong, Hai, Hien, Bui, Huong, Son, Morooka, Fukuda & Wilder 2006). Because of the recent serious disease outbreaks in tiger shrimp (*Penaeus monodon*), prawn culture has been considered to be an alternative to shrimp in low-saline water areas and coastal saline soils as they can grow comfortably in waters having a salinity up to 10 g L^{-1} (New 2002; Cheng, Liu, Cheng & Chen 2003). An increase in prawn culture has led to a growing demand for PLs from hatcheries. Prawn farmers prefer to stock PL older than 10 days to ensure a high survival rate in their grow-out ponds. However, feed is the single largest cost item for *M. rosenbergii* culture, as it constitutes 40–60% of the operational costs (Phuong, Son, Bui, Tuan & Wilder 2003; Mitra, Mukhopadhyay & Chattopadhyay 2005). Moreover, the formulation of well-balanced diets and their adequate feeding are of utmost importance for successful aquaculture (Watanabe 2002). The nutritive value of formulated feeds depends on the ingredient composition and considerably affects the prawn performance, especially in the indoor-nursery phase, where the prawns rely solely on supplemented feed. Hence, development of formulated feeds to attain higher survival, better growth and more efficient feed conversion ratios is needed. The main goal of the study reported here was to evaluate the use of *Artemia* biomass as a high-quality protein source to improve postlarval diets for *M. rosenbergii*.

Materials and methods

Experimental diets

Peruvian FM was supplied by CATACO Company, Can Tho city; dried and frozen *Artemia* biomass were obtained from the experimental *Artemia* biomass ponds in the experimental station of Can Tho University in Bac Lieu province, Vietnam. Dried *Artemia* meal was obtained by sun drying thin layers of *Artemia* biomass. Other ingredients such as soybean meal, soybean oil, squid oil, gelatine, wheat flour . . . were purchased from commercial suppliers. The dietary ingredients were analysed for chemical composition (Table 1) before the formulation of the diets.

Nine experimental diets were formulated by replacing 0%, 25%, 50%, 75% and 100% of the FM protein in a standard diet with either dried or frozen *Artemia* biomass (Table 2). In the 0% *Artemia* treatment,

Table 1 Proximate composition (% of dry matter) of the ingredients used in the experimental diets

Ingredients	Peruvian fishmeal	Dried *Artemia* meal	Frozen *Artemia*	Soybean meal	Wheat flour
Dry matter	91.38	86.57	12.44	89.19	88.78
Crude protein	59.20	50.76	50.89	47.60	11.62
Crude lipid	5.62	9.89	9.93	3.17	1.92
Ash	33.29	24.97	23.98	7.38	0.71
Crude fibre	0.62	2.50	2.48	6.99	1.25

Table 2 Composition (g 100 g^{-1} dry matter) of the nine experimental diets

Ingredients	0% A	25% DA	25% FA	50% DA	50% FA	75% DA	75% FA	100% DA	100% FA
Fishmeal	49.35	37.00	37.03	24.66	24.69	12.33	12.35	0.00	0.00
Dried *Artemia*	0.00	14.39	0.00	28.77	0.00	43.14	0.00	57.55	0.00
Frozen *Artemia*	0.00	0.00	14.36	0.00	28.73	0.00	43.11	0.00	57.40
Soybean meal	12.34	12.58	12.85	13.36	13.35	13.87	13.86	14.34	14.35
Wheat flour	25.08	23.03	22.89	20.98	20.71	18.94	18.52	16.90	16.86
Soybean oil	1.68	1.33	1.33	0.97	0.97	0.62	0.62	0.27	0.26
Squid oil	1.67	1.32	1.32	0.97	0.97	0.62	0.61	0.26	0.26
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin Premix	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Gelatin	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Cellulose	2.38	2.58	2.73	2.78	3.08	2.98	3.43	3.18	3.37

A, *Artemia*; DA, dried *Artemia*; FA, frozen *Artemia*.

(1) Vitamin produced by Rhone-Poulenc, namely Vemevit. In 1 kg of mixture, there are vitamin A: 2 000 000 UI; vitamin D_3: 400 000 UI; vitamin E: 12 000 mg; vitamin K: 480 mg; vitamin B_1: 800 mg; vitamin B_2: 800 g; vitamin B_6: 500 mg; nicotinic acid: 5000 mg; calcium: 2000 mg; vitamin B_{12}: 2000 mg; folic acid: 160 mg; Microvitamin H: 2000:1000 mg; vitaminC: 100 000 mg; Fe^{2+}: 1000 ppm; Zn^{2+}: 3000 ppm; Mn^{2+}: 2000 ppm; Cu^{2+}: 100 ppm; iodine: 20 ppm; Co^{2+}: 10 ppm and some others.

(2) Gross energy was calculated based on protein = 5.56; lipid = 9.54 and NFE = 4.20.

NFE, nitrogen-free extract.

Peruvian FM was the main protein source of mixed ingredients. All diets were formulated to be approximately isolipidic, isoenergetic and isonitrogenous (40% dietary protein). The 'SOLVER' program in Microsoft Excel was used to establish the formulated feeds. In this program, the proximate composition of the ingredients and those of the diets are preset, in which the proportion of FM protein substituted by *Artemia* protein must be precise. Based on the composition of FM and *Artemia* meal, it is therefore possible that other ingredients (e.g. amount of wheat flour, soybean oil and squid oil) vary as well in order to keep the gross composition of the resulting diets as similar as possible. The diets were made into sinking pellets (700 μm) using a pellet machine, oven-dried at 60 °C and stored at 4 °C.

Chemical analysis

Proximate analysis (moisture, crude protein, total lipid, fibre and ash) of the ingredients and experimental diets was carried out according to the standard methods of Association of Official Analytical Chemists (1995). Nitrogen-free extract was estimated on a dry weight basis by subtracting the percentages of crude protein, lipids, crude fibre and ash from 100%.

The amino acid contents of the test diets were analysed by liquid chromatographic analysis (HP, Hewlett-Packard AOS – 1090 Merck, Darmstadt, Germany) using the conductivity of pre-column OPA (*O*-phtal aldehyde) and FMOC (9-fluorenylmethyl chloroformate).

The fatty acid composition of the experimental diets was determined by gas chromatography. Fatty acid methyl esters (FAMEs) were prepared via a procedure modified from Lepage and Roy (1984).

Experimental design

A feeding trial was conducted for 30 days in the shrimp hatchery of the College of Aquaculture and Fisheries, Can Tho University, Vietnam. The test was set up as a completely randomized design with three replicates per treatment.

The plastic 160 L tanks were filled with 120 L of de-chlorinated tapwater. Each tank was provided with continuous aeration and black plastic nets were distributed throughout the water column as a substrate for PL prawns in order to reduce the opportunity for cannibalism. Postlarvae from one single batch were purchased from a commercial hatchery in Can Tho city and reared in a 1 m^3 tank for 5 days before the start of the trial.

At the start of the experiment, all PL were deprived of food for 1 day. 120 PL (mean initial weight of 12.16–12.29 mg) were transferred into each tank. 50% of the tank volume was exchanged every 3 days at 14:00 hours. Daily water temperature and pH were measured at 7:00 and 14:00 hours using a thermo-pH meter (YSI 60 Model pH meter, HANNA instruments, Mauritius). Total ammonia nitrogen (NH_3/NH_4^+),

NO_2-N and NO_3-N were monitored weekly using a spectrophotometer according to American Public Health Association (1998).

Prawns were fed four times a day at about 8:00, 12:00, 16:00 and 20:00 hours at 15% of their body weight. Uneaten feed and faecal matter were siphoned out before the first feeding in the morning. Thirty prawns in each tank were randomly sampled at the start of the experiment and at 10-day intervals during the experimental period. The amount of diet given was adjusted according to these weight measurements. Prawns were weighed in groups of 30 on a digital Mettler scale (Denver Instrument, Denver, Colorado) and the mean weights were determined. Prawns were then returned to their original tanks. At the end of the experiment, the survival, mean individual weight and total length of the prawn were determined.

Performance parameters

Performance was evaluated by live weight gain, specific growth rate (SGR), total length and survival, which were calculated as follows:

Live weight gain (mg) = final weight − initial weight

SGR $(\%\,\text{day}^{-1}) = 100 \times [(\ln \text{ final weight} - \ln \text{ initial weight})/\text{days of experiment}]$

Survival (%) = 100 × (final prawn number/initial prawn number)

The total length (mm) of prawns was measured from the tip of the rostrum to the telson using a calibrated ruler.

Statistical analysis

Data for all measured parameters were analysed using SPSS for Windows, Version 11.0. Variations from dietary treatment were compared by one-way ANOVA. The Tukey HSD *post hoc* analysis was used to detect differences between means. Significant differences were considered at $P < 0.05$. All percentage values were normalized through a square root arcsine transformation before statistical treatment.

Results

Water quality

Daily water temperatures ranged from 27.5 to 30.5 °C, and pH fluctuated from 7.2 to 7.8; variations in the levels of total ammonia nitrogen, NO_2-N and NO_3-N were 0.02–0.61, 0.02–0.32 and 0.08–0.19 mg L^{-1}, respectively, throughout the experimental period. Generally, the water quality was not much different between treatments and remained within the suitable range for the normal growth of *M. rosenbergii* PL (D'Abramo, Ohs, Fondren, Steeby & Posadas 2003; Niu, Lee, Goshima & Nakao 2003).

Experimental diets

Proximate analysis showed that all diets used in this study were nearly isonitrogenous, isolipidic and isocaloric (Table 3). However, a slightly lower lipid content in the FM-based control diet compared with the 100% *Artemia*-based diets was noted.

The analysed amino acid concentrations in the experimental diets (Table 4) indicated that the diets containing *Artemia* protein had, for all amino acids, equal or slightly higher levels than the control diet. In general, these differences among the diets were negligible.

The fatty acid profiles of the experimental diets are shown in Table 5. The concentrations of linoleic (18:2n-6), linolenic (18:3n-3), eicosapentaenoic acid, (EPA) (20:5n-3) and arachidonic acid (ARA) (20:4n-6), total monounsaturated fatty acids, polyunsaturated fatty acids (PUFA), n-3 and n-6 fatty acids in the

Table 3 Chemical compositions (% of dry matter) of the nine experimental diets

Ingredients	0% A	25% DA	25% FA	50% DA	50% FA	75% DA	75% FA	100% DA	100% FA
Dry matter	91.91	92.76	90.01	92.07	91.25	90.72	92.61	89.62	92.75
Crude protein	39.95	39.87	39.64	39.69	39.79	39.78	39.81	39.91	39.94
Crude lipid	7.47	7.93	7.95	8.03	7.96	7.99	8.10	8.78	8.15
NFE	29.19	28.40	27.63	26.43	26.96	27.12	27.52	26.74	27.96
Ash	20.57	20.69	21.49	21.77	22.04	21.09	21.74	20.73	21.42
Crude fibre	2.82	3.11	3.29	4.08	3.25	4.02	2.83	3.84	2.53
Energy (kcal g^{-1})	4.19	4.20	4.15	4.11	4.13	4.14	4.17	4.21	4.20

NFE, nitrogen-free extract.

Table 4 Amino acid composition of the experimental diets (% of dry matter)

Amino acid	0% A	25% DA	25% FA	50% DA	50% FA	75% DA	75% FA	100% DA	100% FA
Essential amino acid									
Arginine	1.83	1.67	1.71	1.89	1.96	2.07	2.10	2.11	2.18
Histidine	0.75	0.70	0.72	0.74	0.74	0.82	0.85	0.85	0.84
Isoleucine	0.95	1.03	1.08	1.02	1.08	1.20	1.14	1.21	1.26
Leucine	1.69	1.64	1.85	1.74	1.93	1.93	1.96	2.01	2.08
Lysine	1.43	1.39	1.53	1.42	1.58	1.37	1.68	1.46	1.65
Methionine	0.66	0.59	0.61	0.62	0.65	0.65	0.69	0.67	0.70
Phenylalanine	0.96	1.06	1.12	1.05	1.12	1.17	1.16	1.22	1.16
Threonine	0.98	1.04	1.11	1.11	1.24	1.26	1.26	1.28	1.27
Valine	1.15	1.22	1.21	1.12	1.13	1.34	1.25	1.32	1.33
Non-essential amino acid									
Alanine	1.64	1.74	1.83	1.78	1.80	1.83	1.93	1.88	1.95
Aspartic	2.07	1.99	2.09	2.21	2.20	2.08	2.51	2.46	2.53
Glutamic	4.37	3.81	4.23	4.01	4.30	4.18	4.53	4.34	4.46
Glycine	1.28	1.69	1.73	1.45	1.69	1.79	1.67	1.73	1.72
Proline	1.35	1.45	1.40	1.59	1.60	1.71	1.87	1.74	1.95
Serine	1.26	1.35	1.38	1.38	1.42	1.46	1.52	1.67	1.67
Tyrosine	0.92	0.94	1.06	1.02	0.91	0.98	1.02	1.18	1.19

Artemia diets were significantly ($P < 0.05$) higher than in the FM control diet. The ratio of n-6/n-3 was similar among the treatments. In contrast, the total saturated fatty acid in the FM control diet was higher as compared with the *Artemia* diets. Especially, docosahexaenoic acid (DHA) (22:6n-3) was also six to eight times higher in the control diet than in the frozen and dried *Artemia* diets. Overall, the levels of unsaturated fatty acids increased with inclusion of *Artemia* protein in the diets. In addition, amino acid and fatty acid levels in the dried *Artemia* diets were slightly lower in comparison with the frozen *Artemia* diets.

Prawn performance

The effect of the dietary FM replacement level on the survival and growth parameters of the freshwater prawn *M. rosenbergii* is presented in Table 6. Prawn fed the FM control diet had the lowest survival (46%). However, a significant difference ($P < 0.05$) was only found at the 75% and 100% replacement levels (68–77%). The polynomial regression (Fig. 1) and R^2 value ($R^2 = 0.9507$) indicated that there was a positive correlation between the survival of the prawn and the dietary *Artemia* protein inclusion level. After 30 days of feeding, the growth performance of the prawns was noticeably affected by the treatments (Table 6). Overall prawn PL in all experimental groups grew at rates that were consistent with the values routinely obtained in our research facility.

A gradual increase in the growth performance (live weight gain, SGR and total length) of the prawn occurred with increasing dietary inclusion of *Artemia* protein. Differences with the control, however, only became significant from the 50% replacement level upwards. As for survival, there was a similar significant positive correlation between the SGR of the prawns and the dietary *Artemia* level. R^2 values were 0.9580 and 0.9952 for frozen and dried *Artemia* respectively (Fig. 2). At the same level of *Artemia* inclusion in the diet, no significant difference was observed between the dried and the frozen *Artemia* form. However, prawn fed frozen *Artemia* diets overall showed a better performance than the ones fed dried *Artemia* diets.

The size distribution based on weight categories of prawn PL revealed the same trend as for growth performance (Fig. 3). The proportion of prawns in the 0.21–0.25 g category was higher in the treatments fed the diets with 50%, 75% and 100% *Artemia* protein inclusion (15.6–31.1%) than animals fed FM control and 25% *Artemia* diet groups (2.2–7.8%). In addition, the proportion of the large size class (> 0.25 g) in the groups fed diets with 50% *Artemia* inclusion and upwards ranged from 11.1% to 27.8%, while this size was not found in the FM control and 25% *Artemia* replacement diet groups. On the contrary, the proportion of the small size class (< 0.1 g) was the highest (66.7%) in the FM control group and this size class was not present in the treatment with a complete FM substitution.

Table 5 Fatty acid profile (mg g^{-1} dry weight) of the experimental diets containing different levels of dried or frozen *Artemia* protein

Experimental diets									
Fatty acids	**0% A**	**25% DA**	**25% FA**	**50% DA**	**50% FA**	**75% DA**	**75% FA**	**100% DA**	**100% FA**
18:2n-6 (linoleic)	$13.17^{a} \pm 0.04$	$14.55^{b} \pm 0.11$	$14.88^{bc} \pm 0.01$	$15.56^{cd} \pm 0.28$	$15.51^{cd} \pm 0.04$	$16.03^{de} \pm 0.47$	$16.32^{def} \pm 0.31$	$16.69^{ef} \pm 0.11$	$16.93^{f} \pm 0.08$
18:3n-3 (linolenic)	$1.30^{a} \pm 0.02$	$1.49^{b} \pm 0.01$	$1.56^{bc} \pm 0.02$	$1.59^{c} \pm 0.01$	$1.62^{c} \pm 0.01$	$1.73^{d} \pm 0.04$	$1.76^{d} \pm 0.03$	$1.87^{e} \pm 0.02$	$1.94^{e} \pm 0.01$
20:4n-6 (ARA)	$0.56^{a} \pm 0.01$	$1.29^{b} \pm 0.01$	$1.26^{b} \pm 0.04$	$1.92^{c} \pm 0.01$	$1.94^{c} \pm 0.01$	$2.45^{d} \pm 0.02$	$2.67^{d} \pm 0.02$	$3.22^{e} \pm 0.04$	$4.43^{f} \pm 0.01$
20:5n-3 (EPA)	$5.99^{a} \pm 0.16$	$7.67^{b} \pm 0.03$	$8.24^{c} \pm 0.02$	$9.27^{d} \pm 0.02$	$9.46^{d} \pm 0.04$	$10.56^{e} \pm 0.04$	$10.91^{f} \pm 0.04$	$11.61^{g} \pm 0.04$	$12.14^{h} \pm 0.04$
22:6n-3 (DHA)	$2.74^{a} \pm 0.08$	$2.23^{b} \pm 0.27$	$2.14^{b} \pm 0.04$	$1.53^{c} \pm 0.01$	$1.58^{c} \pm 0.02$	$0.93^{d} \pm 0.01$	$1.00^{d} \pm 0.02$	$0.34^{e} \pm 0.03$	$0.43^{e} \pm 0.01$
Total SFA	$26.54^{a} \pm 0.21$	$25.73^{ab} \pm 0.48$	$25.46^{ab} \pm 0.57$	$26.01^{ab} \pm 0.30$	$24.36^{bc} \pm 0.37$	$23.48^{bc} \pm 0.36$	$23.24^{cd} \pm 0.56$	$22.79^{cd} \pm 0.36$	$22.24^{d} \pm 0.62$
Total MUFA	$19.20^{a} \pm 0.10$	$21.10^{b} \pm 0.02$	$21.68^{b} \pm 0.01$	$22.54^{c} \pm 0.01$	$24.15^{d} \pm 0.11$	$23.86^{d} \pm 0.47$	$25.34^{e} \pm 0.14$	$25.84^{e} \pm 0.28$	$27.47^{f} \pm 0.17$
Total PUFA	$12.01^{a} \pm 0.30$	$14.43^{b} \pm 0.13$	$15.37^{b} \pm 0.11$	$16.91^{c} \pm 0.06$	$17.76^{c} \pm 0.06$	$19.08^{d} \pm 0.07$	$20.28^{e} \pm 0.10$	$20.99^{e} \pm 0.71$	$23.74^{f} \pm 0.14$
Total n-3	$11.19^{a} \pm 0.27$	$12.78^{b} \pm 0.16$	$13.67^{bc} \pm 0.06$	$14.23^{cd} \pm 0.03$	$14.96^{de} \pm 0.08$	$15.58^{ef} \pm 0.09$	$16.45^{f} \pm 0.04$	$16.52^{f} \pm 0.71$	$17.93^{g} \pm 0.08$
Total n-6	$13.99^{a} \pm 0.01$	$16.21^{b} \pm 0.08$	$16.58^{b} \pm 0.07$	$18.24^{c} \pm 0.25$	$18.31^{c} \pm 0.06$	$19.54^{d} \pm 0.45$	$20.15^{d} \pm 0.25$	$21.16^{e} \pm 0.11$	$22.74^{f} \pm 0.01$
n-6/n-3 ratio	$1.25^{a} \pm 0.03$	$1.27^{a} \pm 0.01$	$1.21^{a} \pm 0.00$	$1.28^{a} \pm 0.02$	$1.22^{a} \pm 0.01$	$1.25^{a} \pm 0.02$	$1.22^{a} \pm 0.02$	$1.28^{a} \pm 0.06$	$1.27^{a} \pm 0.01$

Data are mean values ($\pm$ SD) of two determinations. Means in the same row with different superscripts are significantly different ($P < 0.05$). ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Discussion

The result of the present study indicated that the growth performance of a prawn *M. rosenbergii* PL fed an FM control diet was inferior to prawn fed *Artemia* diets. Hari and Kurup (2003) obtained the highest SGR (3.72% day^{-1}) of *M. rosenbergii* (0.264 g initial weight) fed with diets containing 30% protein and varying amounts of trash FM and groundnut oilcake. Du and Niu (2003), when evaluating dietary substitution of soya bean meal for FM on growth of prawn (initial weight of 0.32 g), achieved the best SGR of 2.5% day^{-1} in the FM control diet. A study by Gitte and Indulkar (2005) evaluated the use of five different marine FM diets (40% protein) for *M. rosenbergii* PL (initial weight of 10.8 mg) and found that the SGR of prawn ranged from 4.82% to 5.86% day^{-1}.

Furthermore, Marques, Lombardi and Boock (2000) studied the stocking densities for a nursery-phase culture of *M. rosenbergii* PL in cages at densities between 2 and 10 PL L^{-1}. The initial weight of the prawns was 0.011 g. Prawns were fed with a 35% protein commercial pellet. After 20 days of culture, survival and final mean weight ranged from 63% to 95% and 0.042 to 0.061 g (SGR: 6.70–8.65% day^{-1}) respectively. Hossain and Islam (2007) found that PLs of *M. rosenbergii* (initial weight of 26 mg) raised in a recirculatory system for 60 days and fed a commercial shrimp nursery diet (30% protein) achieved survival of 76%, a final weight of 502.33 mg and an SGR of 3.28% day^{-1}. A study by Phuong, Khanh and Wilder (2003) investigated the nursing of *M. rosenbergii* PL in 400–1200 m^2 earthen ponds with stocking densities of 50 and 100 PL m^{-2}. The initial weight was 6.8 mg. A commercial feed (Grobest, Dong Nai, Vietnam) containing 33% crude protein was used. After 45 days, the survival of the prawns varied around 76–77% and the final weight was 0.33–0.68 g (SGR: 8.63–10.23% day^{-1}).

From these results, it can be suggested that the SGR of *M. rosenbergii* PL fed the FM control diet in the current study (6.46% day^{-1}) was comparable to or better than the prawn fed an FM-based diet in other studies. Moreover, prawn fed the *Artemia* diets demonstrated a similar range of survival (59–77%) and an SGR (7.04–9.50 day^{-1}) that was equal to or superior than prawn fed commercial feeds in the studies reported above.

Polynomial regression analysis indicated that when the dietary *Artemia* protein inclusion level increased from 0% to 100%, the survival and growth rate of prawn PL increased significantly ($P < 0.05$)

Table 6 Survival, live weight gain, specific growth rate and total length of PL *Macrobrachium rosenbergii* after a 30-day feeding trial with different *Artemia* protein inclusion levels

Treatment	Survival (%)	Initial weight (mg)	Final weight (mg)	Live weight gain (mg)	SGR (% day^{-1})	Total length (mm)
0% A	45.83 ± 6.29^{a}	12.16 ± 0.15	84.58 ± 23.93	72.41 ± 5.70^{a}	6.46 ± 0.25^{a}	21.17 ± 3.34^{a}
25% DA	59.17 ± 5.20^{ab}	12.34 ± 0.20	102.32 ± 31.05	89.98 ± 8.50^{ab}	7.04 ± 0.33^{ab}	23.83 ± 3.42^{b}
25% FA	59.72 ± 3.37^{ab}	12.22 ± 0.16	111.43 ± 29.44	99.21 ± 4.63^{ab}	7.37 ± 0.71^{b}	24.51 ± 3.41^{b}
50% DA	61.67 ± 3.33^{ab}	12.25 ± 0.15	117.04 ± 26.91	104.79 ± 4.20^{b}	7.52 ± 0.09^{b}	25.38 ± 3.64^{bc}
50% FA	62.50 ± 3.82^{abc}	12.29 ± 0.38	157.26 ± 31.13	144.97 ± 9.96^{c}	8.49 ± 0.29^{c}	26.47 ± 3.58^{cd}
75% DA	67.78 ± 5.42^{bc}	12.21 ± 0.23	147.39 ± 35.49	135.18 ± 6.98^{c}	8.30 ± 0.20^{c}	26.67 ± 3.60^{cd}
75% FA	68.33 ± 5.20^{bc}	12.30 ± 0.52	163.37 ± 34.40	151.07 ± 8.92^{c}	8.62 ± 0.20^{c}	28.06 ± 3.70^{d}
100% DA	75.00 ± 3.82^{bc}	12.24 ± 0.30	174.29 ± 31.58	162.06 ± 17.62^{c}	8.84 ± 0.41^{cd}	28.08 ± 3.51^{d}
100% FA	77.22 ± 7.47^{c}	12.23 ± 0.47	211.33 ± 36.08	199.10 ± 12.97^{d}	9.50 ± 0.27^{d}	30.67 ± 4.09^{e}

Means with different superscripts in the same column are significantly different ($P<0.05$); SGR, specific growth rate; PL, postlarvae.

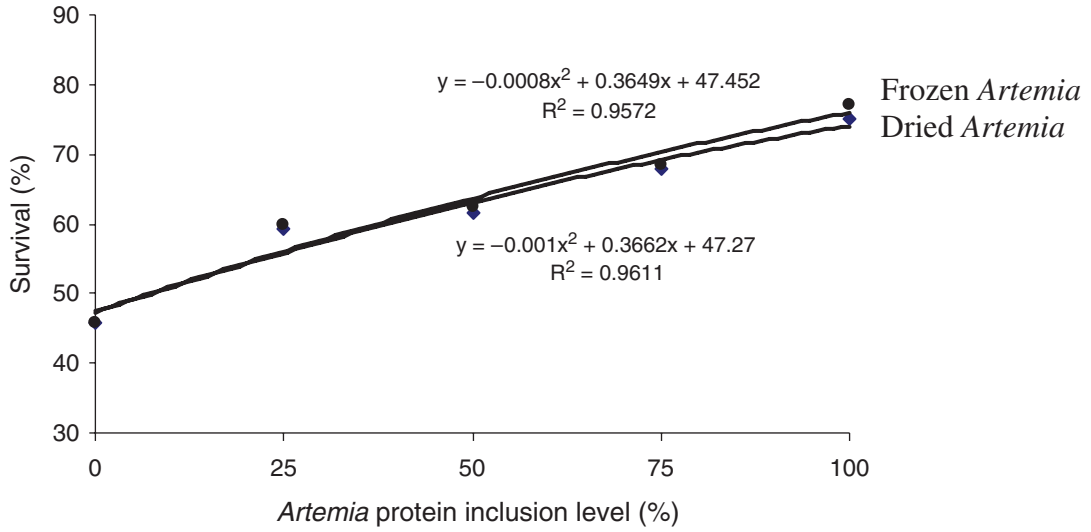

Figure 1 Relationship between survival and dietary *Artemia* protein inclusion level in *Macrobrachium rosenbergii* at the end of the 30-day feeding trial.

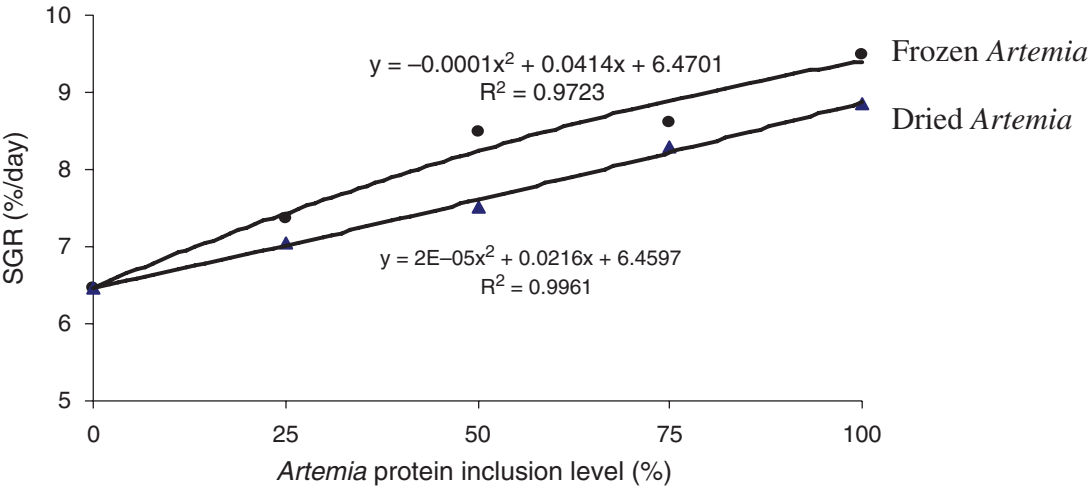

Figure 2 Relationship between specific growth rate and dietary *Artemia* protein inclusion level in *Macrobrachium rosenbergii* at the end of the 30-day feeding trial.

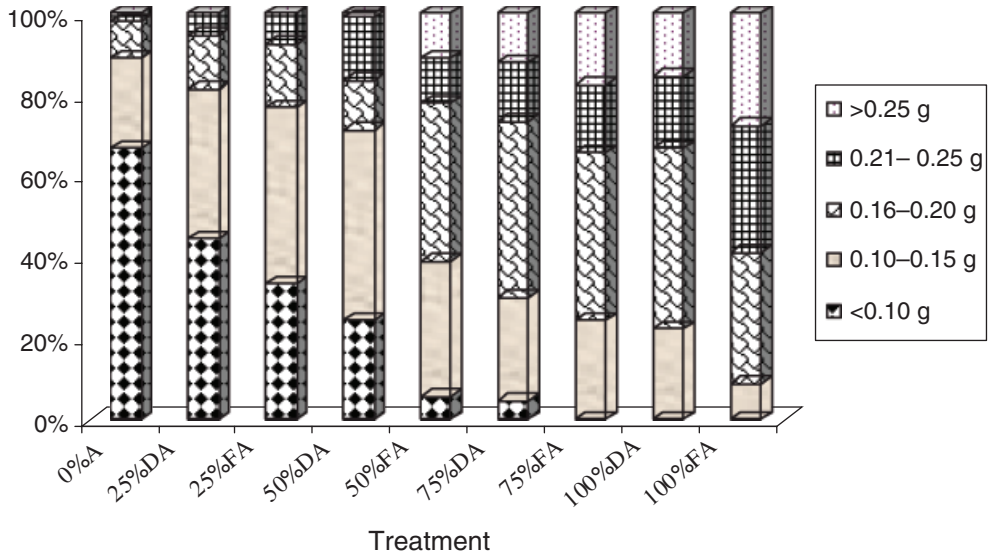

Figure 3 Size distribution of *Macrobrachium rosenbergii* after 30 days fed diets with different *Artemia* inclusion level based on weight categories.

and the best survival and growth performances occurred at 100% replacement of FM protein by *Artemia* protein. The high survival and good growth performance of prawn fed *Artemia* diets as compared with an FM control diet may be due to an increased feed intake caused by a better palatability of the *Artemia* diets. Cook, Rust, Massee, Majack and Peterson (2003) compared the transfer of erythromycin from bioencapsulated live and freeze-dried adult *Artemia* and pellets to fry of sockeye salmon, *Oncorhynchus nerka* (Walbaum), and found that both *Artemia* feeds are so palatable that they were more immediately and completely consumed by the fish relative to the medicated pellets.

From observation of the feeding behaviour in the present study, it was observed that prawn receiving diets containing 50% *Artemia* protein and more showed a faster response to the feed pellets than animals in the FM control diet. Therefore, although feed intake was not determined in this study, the *Artemia* diets are apparently more attractive to the prawns than the FM control diet. This may result in better feed satiation of the prawns and hence high survival (lower cannibalism) and good growth performance.

Previous studies demonstrated that the amino acid requirements of freshwater prawn are relatively constant during larval life and can be satisfied by suitable protein sources that resemble the larval amino acid profile (Roustaian, Kamarudin, Omar, Saad & Ahmad 2000). Moreover, *M. rosenbergii* require the same ten essential amino acids as other crustacean and fish species, but quantitative requirements have not been well established (D'Abramo 1998; Mitra *et al.* 2005). In the present study, all experimental diets had nearly the same protein content and similar amino acid levels. It is therefore unlikely that this explains the different response of the prawn PL to the diet.

Fatty acids play important roles in the nutrition of crustaceans. Their main functions are as a source of energy and for the maintenance of the functional integrity of biomembranes (Reigh & Stickney 1989). The highly unsaturated fatty acid (HUFA) content of artificial diets has been found to have a strong impact on the survival, growth, feed conversion, fecundity, egg hatchability, moulting and stress tolerance of *Penaeus* and *Macrobrachium* species. Particularly, 18: 2n-6 (linoleic acid), 18:3n-3 (linolenic acid), 20:5n-3 (EPA) and 22:6n-3 (DHA) fatty acids are essential to increase the growth and survival of larvae and juveniles of these genera (Querijero, Teshima, Koshio & Ishikawa 1997; D'Abramo 1998; Roustaian, Kamarudin, Omar, Saad & Ahmad 1999; Mitra *et al.* 2005). For *M. rosenbergii*, the dietary requirements for HUFA are relatively small: both n-3 and n-6 HUFA at dietary levels of 0.075% were found to increase remarkably the weight gain and feed efficiency of prawn (Mitra *et al.* 2005). A similar observation by Teshima, Koshio and Oshida (1992) found the highest weight gain in *M. rosenbergii* fed diets containing a mixture of 18:3n-3 and 18: 2n-6. On the contrary, *M. rosenbergii* did not show any significant increase in weight gain

with supplements of either 18:3n-3 or 18:2n-6 dietary fatty acids; however, their absence was associated with significant reductions in the midgut somatic index (D'Abramo & Sheen 1993). Moreover, González-Baró and Pollero (1998) reported that prawns are unable to synthesize either 20:4n-6 (ARA) or 20:5n-3 from shorter chain fatty acids and these fatty acids must be supplied by the diet.

From a nutritional viewpoint in terms of fatty acids, most levels of important fatty acids such as n-3 and n-6 PUFA in the diets containing 50% or more *Artemia* protein were significantly higher than those in the FM control diets, which may have contributed to the better survival and growth of PLs in this experiment.

The beneficial effect of *Artemia* protein inclusion level in practical diets on the growth performance of the prawn was consistent with the results of size distribution. Higher proportions of prawns in the 0.21–0.25 g and >0.25 g categories were found mainly in the diets with 50%, 75% and 100% *Artemia* protein inclusion. The proportion of small (<0.1 g) size classes was the highest (66.7%) in the FM-control group and this category was not present in the treatment with a complete FM substitution. Size variation in prawns is an important factor affecting the economic efficiency of nursing practice. Ra'anan and Cohen (1985) pointed out that *M. rosenbergii* express perceptible individual variation in growth rates and the wide variation in growth rate is believed to be induced primarily by intrinsic factors associated with social hierarchy rather than by environmental factors. Starting with a cohort of PLs with a normal size distribution, the population at the time of harvest is highly heterogeneous. However, a combination of batch- and size-graded PLs can reduce heterogeneous individual growth in prawns (Ranjeet & Kurup 2002). Size grading of juveniles from a nursery-grown population before stocking into production ponds is an effective method to increase the mean individual weight and total yield at harvest (D'Abramo *et al.* 2003; Tidwell, Coyle & Dasgupta 2004).

Generally, the levels of amino acids and fatty acids in the dried *Artemia* diet were slightly lower than in the frozen *Artemia* diet. This is probably due to the loss of nutrients during the direct sun drying process. Similar results have been reported in the literature for sun drying of various fruits and vegetables. According to Jayaraman and Gupta (1995), sun drying causes increased loss of ascorbic acid. This may be due to the long drying time (18–24 h) and the large surface area or open pore structure of the dried products, which facilitates oxidation of ascorbic acid. Considerable loss of ascorbic acid content in sun-dried aonla fruit, and sun-dried tomatoes when compared with fresh samples was found by Pragati, Dahiya and Dhawan (2003); Latapi and Barrett (2006) respectively. In addition, Negi and Roy (2000) found that sun drying resulted in high losses of β-carotene and ascorbic acid of leafy vegetables, and the β-carotene content of sun-dried apricots was significantly lower than in the original materials (Karabulut, Topcu, Duran, Turan & Ozturk 2007). In this study, besides the lower content of amino acids and fatty acids in a dried *Artemia* diet, other nutrients may also be less than that of the frozen *Artemia* diet. This could explain why, at the same replacement level, overall a poorer performance was observed for prawns fed the dried *Artemia* diets.

The results of this study are in agreement with those of Abelin, Tackaert and Sorgeloos (1989), who reported that PL30-45 of *P. monodon* and PL15 of *P. vannamei* fed a diet containing a freeze-dried *Artemia* meal had a significantly better growth as compared with the FM-based diet. Naegel and Rodriguez-Astudillo (2004) demonstrated that feeding *L. vannamei* shrimp PL with dried *Artemia* biomass resulted in a significantly higher survival and larger size compared with four commercial feeds and three crustacean meals. Moreover, juvenile American lobsters (*Homarus americanus*) fed enriched frozen adult *Artemia* showed the greatest weight gain ($>6\%\,\text{day}^{-1}$) compared with formulated diets (Tlusty *et al.* 2005). Naessens *et al.* (1997) found an enhanced reproductive performance when using frozen *Artemia* as a dietary supplement for *P. vannamei*. Similar to the above studies, our experiment again demonstrates the potential of *Artemia* biomass as a diet ingredient in aquaculture.

Overall, the formulated diets containing *Artemia* biomass ensured that the essential nutrient requirements for *M. rosenbergii* PL were met and resulted in excellent growth performance and a high survival. Among the experimental diets, total replacement of FM protein with frozen *Artemia* protein seems to be the best for prawn PL. On the other hand, more work is needed to test other target species and to optimize the use of *Artemia* biomass as a promising alternative feed that could bring about important socio-economic aspects. The interesting issue is that in coastal areas in Vietnam, about 0.2–0.3 tonnes ha^{-1} of live *Artemia* biomass, a by-product from commercial cyst-oriented *Artemia* production in solar saltpans, can be harvested when the culture season ends (Brands *et al.* 1995; Anh *et al.* 1997). According to

Hoa (2006), the production area in the 2006 culture season was approximately 350 ha, and thus more than 100 tonnes of *Artemia* biomass could be obtained from these regions. Until now, this product has been not efficiently utilized. Therefore, use of *Artemia* biomass as a protein source for feeding aquatic species or other animals may not only help the *Artemia* producers to improve the cost-effectiveness of their operations but may also contribute to reduce the use of FM in aquafeeds. Although a full economic comparison between the costs of FM and *Artemia* biomass in practical diets for the *M. rosenbergii* PL was beyond the scope of this study, some considerations can be made. In Vietnam, labour cost is cheap and sun drying or solar drying of *Artemia* biomass is a very inexpensive method. In the present study, *Artemia* biomass was collected from experimental ponds; hence, it is difficult to estimate its cost. It can be assumed *Artemia* biomass; a by-product was used, even when labour and processing costs are accounted for would still be cheaper or at most similar in price as imported FM (1.1–1.4 USD/kg). The results of this study moreover demonstrated that survival of prawn fed the *Artemia* diet was about 1.5 times higher than prawn fed the FM diet; the growth response was also better. Therefore, the nursery phase can be shorter and more profitable.

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