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Original Article

Effect of different levels of *Chlorella* meal on growth and survival of freshwater prawns *Macrobrachium rosenbergii* juvenile

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Abstract

The experiment in triplicate group of prawn (30 prawn per group) with an initial weight of 2.5-2.6 g, were fed until apparent satiation with 32% crude protein diet containing various *Chlorella* levels ranging from 0 to 10% (Diet I, Diet II, Diet III, and Diet IV) for 56 days. At the end of the feeding trial, prawn given Diet IV gave the optimum growth and percentage survival which also contributed to the best feed efficiency. There was no significant difference in FCR in all treatments ($p<0.05$). There were significant differences ($p<0.05$) in growth rate among treatments and prawns fed Diet IV had the highest weight gain (WG) and specific growth rate (SGR) (3.58 g and 1.54 %/day), respectively. The percentage survival of *M. rosenbergii* ranged from 63-86% and similar by, prawn fed Diet IV had the highest survival with a value of 86%.

Keywords: *Chlorella*, *Macrobrachium rosenbergii*, growth and survival

1. Introduction

Microalgae are commonly used as live feed for all growth stages of crustaceans, mollusks and some fish in aquaculture industries. They can easily and rapidly grow, stable in diverse environmental conditions, and rich in good nutrient compositions. Algae have been used in aquaculture mostly for nutritional purposes (Muller-Feuga, 2000) and these are *Spirulina*, *Chlorella sp.*, *Tetraselmis sp.* and *Chaetoceros sp.* Microalgae can be given directly as feed or mixed in formulated diets for early larval stages. Microalgae with good nutritional value such as high PUFA, EPA and DHA content, can provide a high quality nutritional package for different stages of aquatic animals. Combinations of suitable algal species can provide a well-balanced diet, which facilitates the larval development and they are source of essential vitamins such as A, B, C, E, folic acid and panthothenic acid (Nakagawa and Montgomery, 2007; Priyadarshani and Rath, 2012). Furthermore, they are also rich source of essential amino acid (protein), minerals, carbohydrates and essential fatty acid such as linolenic acid (Quoc and Pascaud, 1996).

Chlorella is a genus of single-cell green algae, belonging to the phylum Chlorophyta. It is spherical in shape, about 2 to 10 μm in diameter. *Chlorella* consists of approximately 60% protein that contains 19 out of 22 different amino acids, and eight from that are essential amino acids. It also contains vitamins, minerals, carbohydrates, fiber, chlorophyll, enzymes, antioxidants and many other phytonutrients. *Chlorella* provides vitamin A, Beta-carotene, vitamins B, C, E and K. Its minerals include calcium, iron, phosphorus, potassium, magnesium, zinc, manganese, sulfur, and several other trace minerals. *Chlorella* also rich source of lutein, a powerful antioxidant known to be highly beneficial for the eyes. Lutein is known to ward off vision problems such as cataracts, macular degeneration and retinal problems. Some species of *Chlorella* (503 mg/100 mg) contain 50 times more

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lutein than spinach (10.2 mg/100 mg raw).

The freshwater prawn, *M. rosenbergii* has been popular as a diet for Asia and the Pacific region since the 1960's and the farming of this species has spread throughout the world (New, 2002). The freshwater prawn farming is in high demand in national and international markets (Soundarapandian *et al*., 2009). According to FAO (2009), India ranked the second placed after China in year 2006 with the production of 3.2 million tons of *M. rosenbergii*. The objective of this study was to determine the growth and survival of *M. rosenbergii* using different dietary levels of *Chlorella*.

2. Materials and Methods

2.1 Collection of *Chlorella* sp

The *Chlorella* sp (powder) was purchased from Indigo Herbs Limited, United Kingdom (CP value of 45.44%).

2.2 Experimental conditions

Experimental tanks with 200 L capacity, filled with 150 L filtered freshwater were used for culturing 30 juveniles per tank with mean initial body weight range from 2.5-2.6 g. Polyvinyl chloride (PVC) tubes and a few stones were placed at the bottom of the tank as substrate or sheltering place to reduce cannibalism. They were acclimatized for one week before the feeding experiment started. Each treatment diet was replicated was three replicates. Detritus, uneaten feed and dead larvae were removed every morning by siphoning out from the tank. Fifty percent of the water was exchanged daily (Soundarapandian *et al*, 2009). The water temperature ranged 27-30°C, pH 7.8-8.1 and dissolved oxygen 5.0 mg/L were measured every day.

2.3 Diets formulation and preparation

Four experimental diets were prepared containing four different *Chlorella* level, designated as Diet I (0 %), Diet II (2.5%), Diet III (5%) and Diet IV (10 %). The selected feedstuff and *Chlorella* sp. were mixed to achieve 32% of dietary protein. The composition and chemical analysis of the experimental diets are presented in Table 1. Chemical analysis on feedstuffs, homogenized prawn and experimented diets were carried out according to the method of AOAC (2000). *M. rosenbergii* juveniles were fed manually (hand-fed) to satiation twice a day (Asaduzzaman *et al*., 2009), seven days a week, for 56 days.

2.4 Sample collection

Juvenile prawns in each tank were sampled every seven days for total weight and percentage survival. At the end of the experiment, the juveniles in all tanks were counted and weighed. Their weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and percentage survival were calculated as follows (Ridha and Cruz, 2001; Saad *et al*., 2009):

Weight Gain (WG) = Final wt. (g) – Initial wt. (g)

Specific Growth Rate (SGR) =

$$\frac{(\text{Ln Final wt.(g)} - \text{Ln Initial wt.(g)})}{t} \times 100$$

$$\text{Survival (\%)} = \frac{\text{Final No. of Prawn}}{\text{Initial No. of Prawn}} \times 100$$

Feed Conversion Ratio (FCR) =

$$\frac{\text{Total weight of dry feed given}}{\text{Total wet weight gain}} \times 100$$

Table 1. Composition and chemical analysis of the experiment diets (*Chlorella*) for juvenile *M. rosenbergii*.

Ingredients (%)	Diet I	Diet II	Diet III	Diet IV
Fish meal	27.68	26.00	23.31	21.05
Cornmeal	2.19	2.47	2.76	3.04
Rice bran	5.60	5.63	5.66	5.46
Soybean meal	20.53	19.40	18.27	16.00
Filler	0	0	0	0.45
Vegetable oil[1]	1	1	1	1
Vitamin C	1	1	1	1
Vitamin premix[2]	1	1	1	1
Mineral Premix[3]	1	1	1	1
Chlorella powder	0	2.5	5	10
Copra meal	20	20	20	20
Tapioca	20	20	20	20
Total	100	100	100	100
Proximate analysis (%)				
Crude Protein	31.95	32.00	31.89	32.21
Crude Fat	4.24	4.04	4.02	4.11
Crude Fiber	24.07	24.04	24.01	23.93
Moisture	8.28	8.04	7.79	7.95
Ash	10.67	10.39	10.12	9.56
Energy	3970	3993	4017	4076

*[1] Mozala Sunflower oil. *[2] Vitamin premix (g/kg premix): ascorbic acid, 45; myo-inositol, 5; choline chloride, 75; niacin, 4.5; riboflavin, 1; pyridoxine, 1; thiaminmononitrate, 0.9; Ca-pantothenate, 3; retinyl acetate, 0.6; cholecalciferol, 0.08; vitamin K menadione, 1.7; α-tocopheryl acetate (500 IU/g), 8; biotin, 0.02; folic acid, 0.1; vitamin B12, 0.001; cellulose, 845.1. *[3]Mineral premix: (g/kg pemix): KCL, 90; KI, 0.04; $CaHPO_4.2H_2O$, 500; NaCl, 40; $CuSO_4.5H_2O$, 3; $ZnSO_4.7H_2O$, 4; CoO_4, 0.02; $FeSO_4.7H_20$, 20; $MnSO_4.H_2O$, 3: $CaCo_3$, 215; MgOH, 124: Na_2SeO_3, 0.03; NaF, 1.

2.5 Statistical analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA) to find any significant differences among the experimental groups and Duncan multiple range test was used to compare the differences between means ($p<0.05$).

3. Results and Discussion

The effects of dietary *Chlorella* sp supplement on *M. rosenbergii* were shown in Table 2. Final weight (FW), weight gain (WG), specific growth rate (SGR %/day), and feed conversion ratio (FCR) of juvenile were affected significantly ($p<0.05$) by different levels of *Chlorella* sp in the diet. Prawn fed Diet IV containing 10% of *Chlorella* sp gave the highest significant value of final weight (6.08 g), weight gain (3.58 g or 58.88%) and specific growth rate (1.54 %/day). However, the least value of FW, WG and SGR were recorded with prawn fed Diet 1 (Control) with values of 4.62 g, 2.12 g or 45.88% and 1.10 %/day, respectively. There was an increasing trend in WG and SGR with an increase of the dietary level of *Chlorella* sp. from 0 to 10%.

No significant differences in FCR were observed with varying *Chlorella* sp levels from 0 to 10% in the diets (Table 2). The result also showed the fluctuation values in the FCR among treatments. However, the best FCR was observed in prawn fed Diet III. However prawn fed Diet IV had a high numerical value (2.86) than the other treatments.

Percentage survival at the end of the experiment showed that there were significant differences among the treatments. It ranged between 63 to 86%. The best percentage survival was recorded in prawn fed Diet IV with the 86%. However it was not significantly different with prawn fed Diet III. Prawn fed Diet III had 80% of survival compared to prawns in other treatment which had less amount of dietary *Chlorella* sp. such as Diet I and Diet II. Prawn fed Diet I with 0% *Chlorella* sp. (control) recorded the lowest significantly value (63%) for survival followed by prawn fed Diet III and Diet II with values of 80 and 83%, respectively.

Chlorella have high content of protein or unsaturated fatty acids (Cho *et al*., 2007) and normally used as feed for zooplankton such as rotifer (Mostary *et al*., 2010). According to Jean and Sung (2011), *Chlorella* and *Nanncochloropsis* sp. were used in mass culture of rotifer. Rotifer fed on *C. vulgaris* had the highest content of total amino acids compared with those fed with *Nannochloropsis* sp and *Nannochloris* spp.

Result from the study of the feeding experiment, indicate that growth performances of juvenile freshwater prawn were significantly affected by different levels of *Chlorella* sp. Prawn had a better growth with an increased in dietary level of *Chlorella* sp. up to 10%. However, Habashy (2009) concluded that juvenile freshwater prawns required a dietary protein level of around 25 to 35%. In addition, Mustafa and Nakagawa (1995), reported that algae also contributed to an increased in food utilization.

In the other study using different algae by Hemtanon. (2005), found that the growth rate of *Penaeus monodon* larvae fed diets containing 5% of *S. platensis* was significantly higher than larvae fed the control diet. Also Nakagawa and Gòmez-Díaz (1995) showed that growth performance and feed utilization of *M. rosenbergii* improved by feeding *spirulina* irrespective of the dietary level. According to Davassi (2011), the best percentage survival in his study was 40% after culturing *M. rosenbergii* for six months.

The higher survival for prawns fed Diet IV, along with better growth, indicates this diet was of a higher nutritional value. Considering *Chlorella* is high in vitamin and minerals this may have contributed to this finding. Besides, *Chlorella* also contained vitamin A, B, C, E, Beta-carotene and minerals such as calcium, iron, phosphorus, potassium, magnesium, zinc, manganese, sulfur, and several other trace minerals.

4. Conclusions

From this experiment, it is suggested that a dietary level of 10% *Chlorella* is a good supplement material in feed of *M. rosenbergii* for the juvenile stage since this significantly increased their growth and survival.

Table 2. Effects of dietary *Chlorella* supplement on the final weight, weight gain, specific growth rate, feed conversion ratio and survival rate of giant freshwater prawn during 56 days rearing.

Parameter treatment	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain (%)	Specific growth rate (%/day)	FCR	Percentage Survival (%)
Diet I	2.50 ± 0.00^{a}	4.62 ± 0.23^{a}	2.12 ± 0.77^{a}	45.88 ± 0.77^{a}	1.10 ± 0.09^{a}	2.97 ± 0.17^{a}	63 ± 0.58^{a}
Diet II	2.52 ± 0.03^{a}	$4.96\pm0.20^{a,b}$	$2.44\pm0.83^{a,b}$	$49.19\pm0.83^{a,b}$	$1.21\pm0.09^{a,b}$	3.03 ± 0.17^{a}	83 ± 1.53^{b}
Diet III	2.50 ± 0.01^{a}	$5.53\pm0.13^{a,b}$	$3.03\pm0.96^{a,b}$	$54.79\pm0.96^{a,b}$	$1.42\pm0.04^{b,c}$	2.86 ± 0.05^{a}	80 ± 1.00^{c}
Diet IV	2.55 ± 0.05^{a}	6.08 ± 0.76^{b}	3.58 ± 1.20^{b}	58.88 ± 1.20^{b}	1.54 ± 0.24^{c}	2.91 ± 0.07^{a}	86 ± 1.15^{d}

Values are mean ± SD of triplicate groups. Mean with different superscripts a, b, c in column are significantly different ($p<0.05$).

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