



Full Length Research Paper

Assessment on growth promoting ability of *Chlorella vulgaris* on *Macrobrachium rosenbergii* postlarvae

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The present study was conducted to assess the growth promoting ability of *Chlorella vulgaris* on *Macrobrachium rosenbergii* postlarvae. The experimental diets were prepared by fishmeal replacement with *C. vulgaris* at different levels (25, 50, 75 and 100%). The prepared feeds were offered to the *M. rosenbergii* postlarvae during 90 days in triplicate. At the end of the feeding experiment, survival rate, weight gain, specific growth rate and feed conversion efficiency were significantly ($P < 0.05$) higher in 50% *C. vulgaris* inclusion diet fed PL group. Similarly, total protein, amino acid, carbohydrate and lipid contents were significantly ($P < 0.05$) higher in specimens fed 50% *C. vulgaris* supplemented diet. The feeding rate, absorption rate, conversion rate were significantly ($P < 0.05$) higher in 50% *C. vulgaris* supplemented diet fed PL group. At this level of replacement, the activity level of digestive protease, amylase and lipase were significantly ($P < 0.05$) higher in 50% *C. vulgaris* supplemented group. Among all the experimental groups, the 50% fishmeal replacement with *C. vulgaris* inclusion diet fed group, showed significant performance. The present results revealed that the partial replacement of fish meal with *C. vulgaris* is favorable for *M. rosenbergii* postlarval culture.

Key words: *C. vulgaris*, digestive enzymes, growth performance, *M. rosenbergii*.

INTRODUCTION

The culture of freshwater prawn, *Macrobrachium rosenbergii* (Scampi), has received a great deal of attention in India as a preferred crustacean. Under controlled culture in freshwater and low saline ponds in inland, as well as coastal areas, it grows fastest among all freshwater prawns. It shows a wide range of temperature and salinity tolerance, acceptance of a large range of formulated diets, culture compatibility with non-

predaceous species of fish, and it has a shorter larval period. The *M. rosenbergii* breeding, larval culture and export is one of the important industries in south Indian states of Kerala, Andhra Pradesh and Tamilnadu. In recent years, the aquaculture industry has succeeded in reducing the inclusion rates of fishmeal and fish oil in the aquafeeds. However, due to the increase in production of all farmed species there is still a growing demand for

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these ingredients (Naylor et al. 2009). Fishmeal is the principal source of protein in commercial aquafeeds. As a result of the steep increase in price of fishmeal and the decline in fishery resources that goes in to fishmeal production, there is an interest in developing alternatives to this finite component. Finding and testing alternate protein and lipid sources is important to the aquatic feed industry (Kiron et al. 2012).

As microalgae protein is of good quality, with amino acid profiles comparable to that of other reference feed proteins, it could be a plausible alternative to fishmeal protein (Becker 2007). In addition, microalgae, which are the source of all photosynthetic ally fixed carbon in the food web of aquatic animals (Kwak and Zedler 1997), may be an ideal replacement for fishmeal in aquatic feeds. Meal from the cyanobacterium *Spirulina*, a brackish- water genus that is neither a eukaryote nor marine, has been incorporated into experimental fish feeds with little success (El-Sayed 1994; Olvera-Novoa et al., 1998; Nandeesha et al., 2001; Palmegiano et al. 2005).

Protein and vitamin content is a major factor determining the nutritional value of microalgae. In addition, polyunsaturated fatty acids (PUFA) e.g. eicosapentaenoic acid (EPA), arachidonic acid and docosahexaenoic acid (DHA) content is of major importance (Catarina et al. 2010). The most frequently used genera are *Chlorella*, *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema* and *Thalassiosira*. Combination of different algal species provides better balanced nutrition and improves animal growth better than a diet composed of only one algal specie (Spolaore et al. 2006).

The main applications of microalgae for aquaculture are associated with nutrition or as food additive for coloring the flesh of salmonids and for inducing other biological activities. Microalgae are required for larval nutrition during a brief period, either for direct consumption in the case of molluscs and peneaid shrimp or indirectly as feed for the live prey fed to small fish larvae (Muller-Feuga, 2000). In order to be used in aquaculture, a microalgal strain has to meet various criteria, such as ease of culturing, lack of toxicity, high nutritional value with correct cell size and shape and a digestible cell wall to make nutrients available (Patil et al. 2007).

Chlorella vulgaris is unicellular green algae found in both fresh and marine water and it is widely used as food supplements (Kay 1991). Significant attention has recently been drawn to the use of microalgae for developing functional food, as microalgae produce a great variety of nutrients that are essential for human health. The nutritional value of *C. vulgaris* was initially determined in 1950s-1960s (Lubitz 1963) and first studied as a possible food source in Japan, United States and Germany after World War II (Miyachi, 1995; Ashraf et al., 2011). The previous researches suggested that *C. vulgaris* contain supreme level of crude protein,

carbohydrate, lipid, essential amino acids and minerals (Dawah et al., 2002; Tokusoglu and Unal, 2003; Janczyk et al., 2005; Vaikosen et al., 2007). In this study, the effect of fishmeal dietary replacement with *C. vulgaris* on growth performance, energy utilization, carcass composition and digestive enzyme activity in freshwater prawn *M. rosenbergii* postlarvae (PL) was evaluated.

MATERIALS AND METHODS

Culture of *Chlorella vulgaris*

Collection of pure mother culture of *C. vulgaris*: *C. vulgaris* mother culture were collected from Vivekananda Institute of Algal Technology (VIAT), R.K.M. Vivekananda College, Chennai, Tamil Nadu, India.

Preparation of inoculums: The microalgae, *C. vulgaris*, was inoculated in Bold Basal medium (100 ml mother culture + 900 ml basal medium – as used by Bischoff and Bold (1963) and Schuster et al. (1990), and the cultures were incubated for 15 days at 24 ± 1°C in a thermo-statically controlled room and illuminated with cool inflorescence lamps (Phillips 40 W, cool daylight 6500 K) at an intensity of 2000 lux in a 12: 12 h light dark regime.

Culture in glass tanks: Culture containers were well cleaned with bleach, rinsed and sun dried for 8 h. Then plastic troughs were filled with tap water up to 25 L and mixed well with the pure nutrient media (N-8 medium) (Vonshak1986). 1 L of mother culture of *C. vulgaris* was inoculated in the glass tanks. The tanks were vigorously aerated to provide required quantity of oxygen and to keep cells and media in suspension. The required concentration of algae was developed after 30 days of inoculation. The tanks were kept open under 100% outdoor light exposures. A constant temperature of 25-30°C was maintained throughout the growth period.

Counting of algal cells and filtering method: Sampling was done once in five days basis using 10 ml capacity vials. *Chlorella* cells in each vial were preserved by adding 2-3 drops of formalin. 1 ml of sample was carefully filled in Neubauer Hemocytometer groove (Bauer, 1990) and covered with glass slide. The cells were enumerated under compound microscope. Hand tally counter was used for reliable counting. Algal cells were calculated by the following mathematical expression:

$$\text{Cells (ml}^{-1}\text{)} = \text{Total number of cells counted}/10 \times 4 \times 10^{-6}$$

Filtering method: Printing polyester/ Nylon fabric cloth with a mesh between 30-60 microns was used as filter. After use, the filter was carefully washed, as quickly as possible, and then dried away from direct sunlight.

Experimental feeds

The processed feed grade fishmeal was purchased from Rosen fisheries (Marathakkara, Thrissur, Kerala, India). The other basal feed ingredients such as, soybean meal, groundnut oil cake, wheat bran, binders (Egg and tapioca flour), sunflower oil and vitamin capsules were purchased from local markets at Coimbatore (Tamilnadu, India). The replacement materials *C. vulgaris* was cultured in a laboratory as directed by Vonshak (1986) and Schuster et al. (1990).

The basal ingredients such as processed fishmeal and sundried

Table 1. Ingredients and proximate composition of experimental diets.

Ingredients (g kg ⁻¹)	Control	CL-25	CL-50	CL-75	CL-100
Fishmeal	250	187.5	125	62.5	0
Groundnut oil cake	250	250	250	250	250
Soybean meal	250	250	250	250	250
Wheat bran	100	100	100	100	100
Egg albumin	70	70	70	70	70
Tapioca flour	50	50	50	50	50
Sunflower oil	20	20	20	20	20
Vitamin mix*	10	10	10	10	10
<i>C. vulgaris</i>	0	62.5	125	187.5	250
Proximate composition of <i>C. vulgaris</i> incorporated feed (g kg⁻¹)					
Crude protein	420.20	414.70	409.30	403.80	398.40
Carbohydrate	204.80	206.80	208.80	210.50	212.70
Lipid (%)	147.00	136.30	134.10	132.40	131.30
Ash (%)	111.86	123.30	130.00	137.30	142.00
Moisture (%)	99.30	96.00	93.30	90.46	87.00
Gross energy (kcal kg ⁻¹)	3187.71	2930.13	2713.09	2651.96	2379.96

Chemical composition of *C. vulgaris*: Crude protein- 55.70%; Carbohydrate- 15.28%; Lipid- 10.65%; Ash- 9.00%; Moisture- 6.30%. *Becosules capsules (Each capsule contains); Thiamine mononitrat (IP): 10 mg; Riboflavin (IP): 10 mg; Pyridoxine hydrochloride (IP): 3 mg; Vitamin B₁₂ (as tablets 1:100) (IP): 15 mcg; Niacinamide (IP): 100 mg; Calcium pantothenate (IP): 50 mg; Folic acid (IP): 1.5 mg; Biotin USP (IP): 100 mcg; Ascorbic acid (IP): 150 mg.

soybean meal, groundnut oil cake and wheat bran were ground separately and filtered with a 60-mesh sieve. The sieved feed ingredients were blended with manual blender and the blends were used for preparation of experimental diets, one control diet without microalgae and four diets with *C. vulgaris* at the concentration of 25% (CL-25), 50% (CL-50), 75% (CL-75) and 100% (CL-100) of fishmeal replacement. The blends were steam cooked for 15 min at 95-100°C and allowed to cool at room temperature. The steam cooked blends were mixed with replacement material (*C. vulgaris* at the respective concentrations), vitamin tablets, sunflower oil and binder (egg albumen and tapioca flour). The blends again blended with manual blender and added boiled water to prepare a dough form. The dough was pelletized with a manual pelletizer fixed with 3 mm die and the pellets were collected in aluminum trays. Then the feeds were dried in thermostatic oven until the moisture content is less than 10%. The dried pellets were physically examined for visual appearance, uniformity, colour and fragrance. The selected ingredients and feed proximate composition are shown in Table 1.

Animals

The postlarvae PL of *M. rosenbergii* (PL 15) were purchased from Government prawn hatchery (Azhikkode, Thrissur, Kerala, India). They were safely brought to the laboratory in well-oxygenated plastic bags. They were stocked in a large cement tank (6'x4'x3') and acclimatized for 2 weeks under laboratory conditions. During the period of acclimatization they were fed with boiled egg albumin (egg custard), *Artemia* nauplii and crumble feed alternatively twice a day. The unused feed and fecal matters were removed daily by the syphoning method in the acclimatized tank, three fourth of the water was renewed daily and adequately aerated.

Feeding experiment

M. rosenbergii (PL-30) with the length and weight range of 15.60 ±

2.90 mm and 2.20 ± 0.39 g were taken for the feeding experiment. Feeding trial was carried out in experimental aquarium (5 x 3 = 15) containing 40 L of water, the experimental prawn PLs were introduced in the aquarium (30 PLx15 aquarium = 450 PL), and fed experimental diets for 90 days. The feeding was adjusted to two times a day (6:00 am and 6:00 pm) at the ration of 10% of the body weight of PL. The feeding experiment was prolonged for 90 days; mild aeration was given continuously in order to maintain the oxygen level. In each experimental group, 10 prawns sacrificed for growth parameter and energy utilization parameters (10 PLx3 = 30 PL/group x 5 = 150 PL); 5 PL was sacrificed for proximate composition analysis (5 PLx3 = 15 PL/group x 5 = 75 PL); 5 PL was sacrificed for digestive enzyme analysis (5 PLx3 = 15 PL/group x 5 = 75 PL). Therefore, totally 300 PLs (150+75+75 = 300 PL) were sacrificed for these parameters.

Analyses of the proximate composition of the experimental diets

Analysis of crude protein, moisture, lipid and ash in the formulated feed was performed according to AOAC (1995) procedures. The crude protein was analyzed in the Kjeldahl apparatus after acid digestion (Pelican equipment; KELPLUS- KES 04 LR with automatic digestion system); total lipid was extracted with petroleum ether by the Soxhlet method after acid hydrolysis; ash was determined using Muffle furnace and moisture was determined by thermostatic oven drying method. The formulated feed gross energy was determined using the Oxygen Bomb Calorimeter (230 VAC; SI. No. 26036; Advance Research Instrument Company, New Delhi, India).

Determination of growth parameters

After the 90 days feeding trial, the growth parameters such as

survival rate, weight gain, specific growth rate, feed conversion rate, condition factor and feed conversion efficiency were individually determined by the following equations (Tekinay and Davies 2001):

$$\text{Survival Rate (SR)} = \frac{\text{No. of live prawns}}{\text{No. of prawns introduced}} \times 100$$

$$\text{Weight Gain (WG)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Specific Growth Rate (SGR)} = \frac{\log \text{ of Final weight (g)} - \log \text{ of Initial weight (g)}}{\text{No. of days}} \times 100$$

$$\text{Condition Factor (CF)} = \frac{\text{Final weight (g)}}{\text{Final length}^3 \text{ (cm)}} \times 100$$

$$\text{Food Conversion Rate (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

$$\text{Feed conversion efficiency (FCE)} = \frac{\text{Biomass (g)}}{\text{Total feed intake (g)}}$$

Energy utilization

The parameters of energy utilization, such as feeding rate, mean absorption, mean conversion and metabolic rate were calculated. The energy content of whole prawn, feeds, faeces and exuvia were calculated by the Oxygen Bomb Calorimetrically derived heat energy value. The scheme of energy budget followed in the present study is that of IBP formula (Petrušewicz and Macfadyen 1970) represented as $C = P + R + U + F$, where C is the energy consumed, P the growth, R the energy lost as heat due to metabolism, F the faeces and U the nitrogenous waste. The daily excretion of ammonia by the prawn was estimated after feeding as per the phenol hypochloride method of Solorzano (1969). The energy loss due to ammonia excretion was calculated using the ammonia calorific quotient, 1 mg NH₃: 5.9 cal. (Elliot 1976).

$$\text{Feeding Rate} = \frac{\text{Mean Food Consumption (k.cal/day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{Mean Absorption} = \frac{\text{Mean Food Consumption (k.cal/day)}}{\text{Mean Food Excreted as Faeces (k.cal/day)}}$$

$$\text{Absorption Rate} = \frac{\text{Mean Absorption (k.cal/day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{Mean Conversion} = \text{Mean weight gain (k.cal/day)} + \text{Mean exuvial weight (k.c)}$$

$$\text{Conversion rate} = \frac{\text{Mean Conversion (k.cal/day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{NH}_3 \text{ Excretion Rate} = \frac{\text{Mean NH}_3 \text{ Excretion (k.cal/day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{Metabolic Rate} = \text{AR (k.cal/g/day)} - \text{CR (k.cal/g/day)} + \text{NH}_3 \text{ER (k.cal/g)}$$

(Where AR= Absorption rate; CR= Conversion rate; ER= Excretion rate)

Digestive enzymes

At the end of the experimental period, the experimental prawns weighed with no significant differences between groups. Prawn feeding was discontinued 24 h before sampling and five animals

per tank (fifteen per treatment) were randomly sampled and killed with a sharp blow in the head. The digestive tract was excised and it was checked that there was no food in any portion. The isolated digestive sections were immediately frozen in liquid nitrogen, and stored at -80°C. Each part was homogenized in nine volumes of ice-cold 100 mM Tris-HCl buffer containing 0.1 mM EDTA and 0.1% (v/v) Triton X-100, pH 7.8. All procedures were performed on ice. Homogenates were centrifuged at 30,000 g for 30 m at 4°C and the resultant supernatants were kept in aliquots and stored at -80°C for further digestive enzyme assays.

Total protease activity was determined by the casein-hydrolysis method described by Furne et al. 2005. The assay buffer consisted of 0.1 M glycine-NaOH (pH 10.0). The reaction mixture consisted of casein at 1% (w/v) (0.25 mL), buffer (0.25 mL) and supernatant from the homogenates (0.1 mL). This reaction mixture was incubated for 1 h at 37°C. The reaction was stopped by adding 0.6 mL 8% (w/v) trichloroacetic acid solution and kept for 1 h at 2°C then centrifuged at 1800 g for 10 m and the supernatant absorbance was measured at 280 nm against blanks. For the blank preparation, the supernatant from the homogenates was added at the end of the incubation period, just before adding trichloroacetic acid. Tyrosine solution was used as standard. One unit of enzyme activity was defined as the amount of enzyme needed to catalyze the formation of 1.0 μmol of tyrosine per min.

Amylase activity was determined by the starch-hydrolysis method of Bernfeld (1955). The reaction mixture consisted of 2% (w/v) starch solution (0.125 mL), 0.1 M citrate-phosphate buffer at pH 7.5 (0.125 mL) and supernatant from the homogenates (0.5 mL). This was incubated for 1 h at 37°C. The absorbance was measured at 600nm against a blank. For the blank, the supernatant from the homogenates was added just after the incubation period. Maltose solution was used as standard. One unit of amylase activity was defined as the amount of enzyme that produced 1.0 μmol of maltose per min.

Lipase activity was determined following the method of Furne et al. (2005) by degrading triacylglycerol to free fatty acids. A solution of 1% polyvinyl alcohol (PVA) and 5 mL of 0.1 N HCl in 1 L of distilled water was heated to 75-85°C, cooled, filtered and adjusted to pH 8.0 with 0.1 N NaOH. Virgin olive oil was added to an aliquot of the previous solution obtaining a substrate concentration of 0.1 M. This mixture was emulsified for 5 m. In addition, McIlvaine buffer was prepared from 0.1 M citric acid and 0.2 M disodium phosphate. A reaction mixture containing PVA solution-emulsified substrate (1 mL), McIlvaine buffer at pH 8.0 (0.5 mL), and supernatant from the homogenates (0.5 mL) was incubated for 4 h at 37°C. Afterwards, 3 ml of 1:1 ethanol-acetone solution was added to stop the reaction and break the emulsion. Phenolphthalein in ethanol 1% (w/v) was added to the reaction mixture and titrated with 0.01 M NaOH. For the blanks, the same procedure was followed but boiled enzyme was used. One unit of lipase activity was defined as the hydrolysis of 1.0 micro equivalent of fatty acids from triacylglycerols in 1 h at pH 8 and 37°C. Specific activities were expressed in unit per mg of soluble protein (U/mg protein).

Corporal chemical composition of postlarvae

At the end of the experimental period, the experimental prawns were weighed and sacrificed for corporal chemical composition analyses. Five animals per tank (fifteen per treatment) were randomly sampled and killed with a sharp blow in the head. The animals head, alimentary track and exoskeleton were removed. The isolated muscle portions were labeled and kept in -20°C for further analyses.

Concentration of total protein in enzymatic extracts and postlarvae carcasses was estimated by the method of Lowry et al. (1951) using ethanolic precipitated sample. The blue colour was a result of biuret reaction of protein with copper ions in alkali solution

and reduction of the phosphomolybdic-phosphotungstic of Folin reagent by the tyrosine and tryptophan present in the treated protein. This colour intensity was measured at 650 nm against a blank devoid of protein sample. Bovine serum albumen (BSA) was used as a standard. The content of amino acid was estimated by the method of Moore and Stein (1948). The total amino acid was extracted with sodium tungstate and H₂SO₄. When amino acids are heated with ninhydrine, they undergo deamination. The reaction of amino acid-hydratin complex with ninhydrin produced purple colour, which was measured at 540 nm against a blank. Leucine was used as standard.

The carbohydrate was estimated by the method of Roe (1955) using TCA extracted sample. Carbohydrates were hydrolysed into simple sugars by diluted HCl in hot acidic medium. Glucose is dehydrated into hydroxyl-methyl furfural. This compound reacts with anthrone and produced green colored product, which was measured at 630 nm against a blank. Glucose was used as standard.

Total lipid was extracted with chloroform-methanol mixture following the method of Barnes and Black-Stock (1973), and estimated by the method of Folch et al. (1957). Lipid reacts with vanillin in a medium of H₂SO₄ and phosphoric acid to form a pink coloured chromogen, which is proportional to the lipid content of the sample, which was measured at 540 nm against a blank. Olive oil was used as standard.

Statistical analysis

The results were expressed as Mean \pm SD. Statistical analysis was carried out by Analysis of Variance (one way ANOVA) followed by DMRT were considered as indicative of significance level of $P < 0.05$, as compared to the control group. All calculations were performed using: SPSS, version 16.0 for Windows (PSS Inc., 444 N. Michigan Ave., Suite 3000, Chicago, IL 60611).

RESULTS AND DISCUSSION

Growth, nutritional indices and energy utilization

Growth performance, nutritional and energy utilization of *M. rosenbergii* PL fed experimental diets are presented in Table 2. The initial average body length and weight of PL was 15.6 ± 2.9 mm and 2.2 ± 0.39 g respectively, in all the groups. At end of the feeding experiment, the final length and weight were significantly ($P < 0.05$) improved in CL-50 group, followed by the CL-75 and CL-25 groups when compared with control treatment. Similarly, the growth parameters and nutritional indices (SR, WG, SGR and FCE) were found to be maximum in specimens fed on 50% *C. vulgaris*. The same experiment group (CL-50) showed a significance increase in feeding, absorption and conversion rates, ammonia excretion and metabolic rates. The groups fed on CL-100 showed no significant difference when compared with the control group.

Ferouz et al. (2012) reported in his review study that alternative protein sources such as cereals, pulses, oil seeds and some of the animal proteins were present in aquaculture species. Still, the alternative protein study needed improvement in proximate composition and anti-nutritional factors analyses. Previous studies have shown that fishmeal can be effectively replaced by alternative

protein sources such as soybean protein for *Oncorhynchus mykiss* (Kaushik et al., 1995); plant protein based diets for tilapia (Goda et al. 2007) and poultry by-products for *Oreochromis niloticus* (Hernandez et al. 2010).

In order for protein sources to be considered effective replacements for FM, they must be economically competitive, capable of being produced in large quantities (Hardy et al. 2002), contain balanced amino acid profiles and proper crude protein levels, and not compromise the growth or health of the fish. It is also helpful if they are easily handled and stored and do not lead to

environmental contamination from release of phosphorous and nitrogen. Furthermore, such protein sources must be commodity traded (Lunger et al. 2006). Also, the present study represented that the alternative protein source is well utilized by the prawn PL, because the experimental diets fed groups showed significant improvement in length, weight gain, survival, specific growth rate and feed conversion efficiency. In this feeds the 50% fishmeal replaced by *C. vulgaris* feed fed group showed better performance in growth parameters. These results are in agreement with those obtained by Dawah et al. (2002) who found that the addition of algae in fish diets improved growth performance of Nile tilapia (*O. niloticus*).

Also, Zeinhom (2004) observed that inclusion of algae in fish diets significantly improve the live body weight and SGR. The fresh biomass of *Chlorella* sp., *Tetraselmis* sp., *Isochrysis* sp., *Synechococcus* sp. and *Phormidium* sp. were used as feed for shrimp *Penaeus monodon* and it enhanced the growth, weight gain and survival rate. Similarly, microalgae (*Chlorella* sp., *Tetraselmis* sp., *Isochrysis* sp.) and cyanobacteria (*Synechococcus* sp., and *Phormidium* sp.) live feed fed shrimp had improve the growth, survival and body carcass composition (Sivakumar et al. 2011); live *C. vulgaris* fed *Moina micrura* had significant growth and survival (Habib et al., 2003) and partial replacement of fish meal with dried microalgae *Chlorella* sp and *Scenedesmus* sp. feed fed *O. niloticus* gain significant improvement in survival, growth and body proximate composition (Tartiel et al. 2008). Nandeeshha et al. (1998) reported that body weight gain of Nile tilapia (*O. niloticus*) increased linearly with increasing the level of algae in fish diet at levels less than 20%.

In the present study, the feeding rate of *C. vulgaris* supplemented groups was comparatively higher than in the control group. The CL-50 showed higher feeding rate, but the level was slightly decreasing in CL-75 and CL-100 groups. Part of the food ingested is assimilated in the gut and the remaining fraction is eliminated as faeces. It is indicated that the *C. vulgaris* protein was easily absorbed and assimilated by prawn PL, so the fecal output is lower. In this experiment, the groups fed on *C. vulgaris* supplemented diets showed better feed conversion rate and growth performance. The present study revealed that the experimental feed was well utilized by the PL, and the

Table 2. Growth, nutritional indices and energy utilization parameters of *M. rosenbergii* PL fed with experimental diets.

Tests	Parameters	Control	CL-25	CL-50	CL-75	CL-100	F value	
Morphometric data	Length (mm)	Initial	15.60 ± 2.90	15.60 ± 2.90	15.60 ± 2.90	15.60 ± 2.90	15.60 ± 2.90	-
		Final	44.20 ± 3.00 ^c	50.20 ± 1.60 ^b	58.00 ± 2.90 ^a	51.60 ± 1.50 ^b	43.00 ± 3.10 ^c	39.720
	Weight (g)	Initial	2.20 ± 0.39	2.20 ± 0.39	2.20 ± 0.39	2.20 ± 0.39	2.20 ± 0.39	-
		Final	16.50 ± 1.00 ^c	21.60 ± 1.60 ^b	28.80 ± 1.50 ^a	23.70 ± 2.40 ^b	16.10 ± 1.00 ^c	61.091
	Nutritional indices	Survival rate (%) SR	76.66 ± 3.5 ^c	86.66 ± 2.5 ^b	93.33 ± 2.50 ^a	80.00 ± 3.00 ^c	80.00 ± 4.00 ^c	13.399
		Weight gain (g) WG	16.10 ± 1.00 ^c	19.40 ± 1.20 ^b	27.30 ± 1.70 ^a	21.50 ± 1.50 ^b	13.90 ± 0.70 ^c	48.125
Specific growth rate (g day ⁻¹) SGR		0.997 ± 0.05 ^d	1.102 ± 0.07 ^c	1.252 ± 0.04 ^a	1.146 ± 0.06 ^b	0.960 ± 0.08 ^e	146.54	
Condition factor		1.91 ± 0.21 ^a	1.70 ± 0.2 ^{ab}	1.51 ± 0.23 ^b	1.72 ± 0.13 ^{ab}	2.02 ± 0.14 ^a	3.339	
Feed conversion rate (%) FCR		1.65 ± 0.19 ^a	1.50 ± 0.12 ^{ab}	1.21 ± 0.16 ^c	1.25 ± 0.14 ^{bc}	1.73 ± 0.10 ^a	7.694	
Feed conversion efficiency (%) FCE		1.98 ± 0.23 ^b	1.99 ± 0.18 ^b	2.54 ± 0.22 ^a	2.41 ± 0.19 ^a	1.66 ± 0.21 ^b	8.915	
Feeding rate		1.65 ± 0.12 ^c	2.20 ± 0.90 ^a	2.31 ± 0.04 ^a	2.16 ± 0.09 ^a	1.78 ± 0.07 ^b	38.91	
Energy utilization (kJ day ⁻¹)	Absorption rate	1.36 ± 0.07 ^c	1.67 ± 0.06 ^b	1.95 ± 0.09 ^a	1.54 ± 0.10 ^c	1.39 ± 0.50 ^d	42.02	
	Conversion rate	0.92 ± 0.09 ^c	1.20 ± 0.11 ^b	1.45 ± 0.13 ^a	1.07 ± 0.09 ^{bc}	0.90 ± 0.10 ^{cd}	16.89	
	NH ₃ Excretion	0.07 ± 0.01 ^b	0.11 ± 0.01 ^{ab}	0.12 ± 0.01 ^a	0.10 ± 0.01 ^b	0.08 ± 0.01 ^c	16.80	
	Metabolic rate	0.51 ± 0.03 ^c	0.58 ± 0.02 ^a	0.61 ± 0.06 ^a	0.56 ± 0.02 ^a	0.53 ± 0.02 ^a	0.061	

Each value is a mean ± SD of three replicate analysis, within each row means with different superscripts letters are statistically significant $P < 0.05$ (one way ANOVA and subsequently *post hoc* multiple comparison with DMRT).

feeds contained nutrients which are easily absorbed and converted to energy, because it is ultimately producing the significant growth and body carcass composition in experimental diet fed group. A balanced energy budget is a tool for bioenergetics modeling in aquaculture and fisheries management. In contrast, for crustacean species, notably penaeid shrimp information is limited (Bureau et al., 2000). Similarly, the balanced energy budget study was reported in various studies of *M. rosenbergii* PL fed with cereals, pulses based feed (Bhavan et al. 2010); probiotic incorporated feed (Seenivasan et al., 2012; 2013) and medicinal herbs incorporated feed (Shanthi et al., 2012; Radhakrishnan et al., 2014a, b).

Corporal chemical composition

Total protein, amino acid, carbohydrate and lipid content of *M. rosenbergii* PL was affected by partial replacement of fishmeal *C. vulgaris*. The chemical contents were significantly ($P < 0.05$) increased in specimens fed on 50% of inclusion level, followed by groups fed on CL-25 and CL-75% when compared with the control group. The 100% *C. vulgaris* inclusion feed fed group showed no significant difference when compared with the control (Table 3). The present results indicate the *C. vulgaris* protein was well utilized by the PL of *M. rosenbergii*. Similarly, Bakhtiyar et al. (2011) reported that *Chlorella* bioenriched zooplanktons

fed *Labeo rohita*, gained significant improvement of survival and body carcass composition. Also, Sivakumar et al. (2011) reported that live three micro algal strains (*Chlorella* sp., *Tetraselmis* sp., and *Isochrysis* sp) two cyanobacterial (*Synechococcus* sp. and *Phormidium* sp) strains fed *P. monodon* had significant improvement in growth, survival and body composition. Gouveia et al. (1998) suggested that the dry *C. vulgaris* biomass is an effective and digestible source of carotenoid pigments.

Digestive enzymes activity

The protease, amylase and lipase activity levels in

Table 3. Body chemical composition and activities of digestive enzymes of *M. rosenbergii* PL fed with experimental diet.

Assays	Parameters	Control	CL-25	CL-50	CL-75	CL-100	F value	
Biochemical constituents dry basis (g kg ⁻¹)	Protein	563.00 ± 15.80 ^C	640.00 ± 21.00 ^b	698.00 ± 19.50 ^a	636.00 ± 12.60 ^b	552.20 ± 18.60 ^C	34.72	
	Amino acid	350.00 ± 11.70 ^d	400.60 ± 5.20 ^b	438.30 ± 5.80 ^a	370.20 ± 6.80 ^C	272.30 ± 11.10 ^e	155.917	
	Carbohydrate	224.10 ± 13.60 ^C	258.90 ± 15.70 ^{ab}	282.40 ± 17.80 ^a	238.70 ± 17.80 ^{bc}	213.50 ± 12.30 ^C	11.38	
	Lipid	174.20 ± 19.80 ^b	179.60 ± 4.10 ^b	212.50 ± 6.40 ^a	190.10 ± 13.40 ^b	140.90 ± 6.10 ^d	15.16	
	Ash (%)	11.90 ± 0.28 ^b	12.12 ± 0.26 ^b	13.15 ± 0.25 ^a	12.12 ± 0.23 ^b	12.03 ± 0.15 ^b	13.390	
	Moisture (%)	74.45 ± 1.10 ^{ab}	73.26 ± 1.15 ^a	72.41 ± 1.50 ^b	73.11 ± 1.65 ^{ab}	75.2 ± 1.20 ^a	2.103	
Digestive enzyme	Protease	Initial	0.39 ± 0.09	0.39 ± 0.09	0.39 ± 0.09	0.39 ± 0.09	0.39 ± 0.09	-
		Final	1.16 ± 0.08 ^{bc}	1.26 ± 0.07 ^{ab}	1.31 ± 0.02 ^a	1.21 ± 0.07 ^{abc}	1.10 ± 0.08 ^C	3.815
	Amylase	Initial	0.27 ± 0.12	0.27 ± 0.12	0.27 ± 0.12	0.27 ± 0.12	0.27 ± 0.12	-
		Final	0.81 ± 0.05 ^C	0.94 ± 0.04 ^b	1.04 ± 0.03 ^a	0.85 ± 0.06 ^{bc}	0.78 ± 0.08 ^C	12.389
	Lipase	Initial	0.28 ± 0.07	0.28 ± 0.07	0.28 ± 0.07	0.28 ± 0.07	0.28 ± 0.07	-
		Final	0.76 ± 0.05 ^b	0.75 ± 0.06 ^b	0.86 ± 0.03 ^a	0.80 ± 0.04 ^{ab}	0.61 ± 0.03 ^C	13.195

Values are represented for protease and amylase in Unit mg protein⁻¹; lipase represented in Unit ×10². Each value is a mean ± SD of three replicate analysis, within each row means with different superscripts letters are statistically significant. P < 0.05 (one way ANOVA and subsequently *post hoc* multiple comparison with DMRT).

enzymatic extracts were assayed in the initial and final day of the study (Table 3). The protease enzyme activity level was significantly higher in animals of CL-50 fed group, followed by CL- 25 and CL-75 groups, but the PL fed on 100% *C. vulgaris* diet showed no significance when compared to control group. The amylase and lipase activities showed the same tendency with that of protease activity. The statistical analysis revealed that the level of digestive enzymatic activities between control and experimental feed prawns and microalgae supplemented groups were statistically significant (*P* < 0.05).

Similarly, Nandeesh et al. (1998) reported that *Spirulina* inclusion diets fed *C. carpio* had significantly increased hepatopancreas protease, amylase and lipase activity when compared to control. Also, Umesh et al. (1994) reported that 50% of *S. platensis* dietary inclusion significantly improved

the protein digestibility in common carp *C. carpio*. Nandeesh et al. (1994) reported that 25, 50, 75 and 100% level of fishmeal replacement with *S. platensis* significantly increased the digestibility in catla, rohu and common carp mixed culture. Mustafa and Nagakawa (1995) suggested that the dietary inclusion of algae contribute to an increase in protein assimilation and feed utilization.

There is hardly any information on the use of microalgae as a dry feed component for shrimps, though there are ongoing efforts to replace fishmeal protein using terrestrial plant proteins. *L. vannamei* has been successfully grown on a predominantly plant protein diet containing solvent-extracted soybean meal, corn gluten meal and corn fermented soluble, which together accounted for nearly 98% of the total dietary protein of 36% (Amaya et al., 2007a). The same research group has verified the concept to

fishmeal-free shrimp feed in a pond trial (Amaya et al. 2007b). Furthermore, beneficial impact of algal inclusion on shrimp health has been reported recently. *L. vannamei* fed diets supplemented with marine algal meals rich in docosahexaenoic acid and arachidonic acid demonstrated significant improvement in immune responses (Nonwachai et al. 2010).

The present study exhibits, dried *C. vulgaris* can be used as a partial replacement of fishmeal protein in aquafeeds. It was revealed that, the growth of *M. rosenbergii* fed *C. vulgaris* meal up to 50% level increased significantly. At 75% and 100% inclusion level the growth and nutritional utilization was slightly reduced. Hence, the result of this study exposed that the partial replacement of fishmeal with *C. vulgaris* could be beneficial for nursery maintenance in *M. rosenbergii* postlarvae culture.

Conflict of Interest

The authors have not declared any conflict of interest.

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