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Partial Replacement of Fishmeal with Marine Algae *Turbinaria ornata* and *Gracilaria corticata* for Sustainable Culture of the Freshwater Prawn *Macrobrachium rosenbergii*

Gopalan Rajkumar[1], Periyakali Saravana Bhavan[1]*, Veeran Srinivasan[2], Rajendran Udayasuriyan[1], Madhayan Karthik [1], Thangavelu Satgurunathan[1]

[1] Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu, India

[2] Department of Animal Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Abstract: *This study was conducted to assess whether edible seaweeds, Turbinaria ornata and Gracilaria corticata can be partially replaced the fishmeal to promote the growth of the freshwater prawn, Macrobrachium rosenbergii post larvae (PL).Control was prepared with fishmeal, groundnut oilcake and soy bean meal as protein sources, wheat bran as carbohydrate source, sun flavor oil as lipid source, and topica flour and egg albumin as binding agents. Isonitric experimental diets were prepared by 25% and 50% replacements of the fish meal with T. ornata and G. corticata separately. These feeds were fed to M. rosenbergii PL for 90 days. Among these 25% fishmeal replaced diets with T. ornata and G. corticata produced significantly better survival and growth when compared with control. Among the fishmeal replacement seaweeds, G. carticata was performed better than that of T. ornata. The 50% replacements of the fishmeal showed poor performance when compared with control. In the 25% fishmeal replaced categories, the muscle total protein, amino acid, carbohydrate, lipid and ash, profiles of proteins, amino acids and fatty acids, and activities of digestive enzymes, such as protease, amylase and lipase were elevated due to the influence of T. ornata and G. corticata. Thus, 25% replacement of the fishmeal with these algae is recommended for sustainable production of M. rosenbergii.*

Keywords: *Turbinaria ornata, Gracilaria corticata, Macrobrachium rosenbergii, Survival, Growth, Protein, Amino acid, Fatty acid, Digestive enzymes.*

1. INTRODUCTION

The giant freshwater prawn, *Macrobrachium rosenbergii*, 'Scampi' is commercially cultured in India, China, Taiwan, Bangladesh, Vietnam, Thailand and in South America (New and Nair, 2012). It is one of the most important cultured crustaceans being farmed to promote rural livelihood and contributes to global food security. It represents a good source of protein, essential amino acids and polyunsaturated fatty acids, and very low in fat. Thus, it is being used as a delicious healthy choice of food for human consumption. However, its culture is relied upon availability of quality seed and feed. Live feeds can be given only to larval and early post-larval staged prawns. In the late post-larval, juvenile and grow-out stage, aqua-farmers need to procure artificial feeds, which constitute a major operational cost. Since the fishmeal is a depleting high-cost principal natural resource, the small farmers find it difficult to afford. According to Hardy (2001) the world fish meal production has been nearly constant, averaging about 62,00,000 mt over the past 15 years, and since demand for fish meal is growing, fish meal prices are expected to continue to increase. The formulation of feeds using locally available low cost agricultural, animal husbandry and industrial by-products have crucial role in aquaculture industry (Mitra *et al*., 2005; Langer, 2011). Literature shown that the fishmeal can effectively be replaced by alternative protein sources such as soybean protein and poultry by-products (Kaushik *et al*., 1995; Goda *et al*., 2007; Hernandez *et al*., 2010). In recent years, marine resources have attracted attention in the search for bioactive compounds to develop new drugs and healthy foods. It could not be completely replaced with any other material. However, only partial replacement can be possible with some plant/animal resources and their byproducts. Therefore, searching for alternate finite compound source for formulating a well-balanced diet and their adequate feeding are the most important for successful aquaculture of *M. rosenbergii*.

Seaweeds have been considered as potential sources for antibiotics, cancer therapeutics, hypocholesterolemic and antihelminthic substances (Salvador *et al*., 2007; Manilal *et al*., 2009; Manivannan *et al*., 2011). Marine macro algae are very important and commercially valuable resources for food, fodder, soil conditioners and pharmaceuticals (Yang *et al*., 2006). The brown algae are most commonly utilized for additive preparations or as a feed in animal nutrition (Indegaard and Minsaas, 1991). The proximate composition of brown algae have been reported to vary from species to species, they are ranging from 9 – 16% of protein, 14 – 25% of carbohydrate, 1.5 – 4% of lipids (Murugaiyan *et al*., 2012; Parthiban *et al*., 2013). Thus, the present study was dealt with partial replacement of fishmeal by edible marine macro algae, *Turbinaria ornata* (brown) and *Gracilaria corticata* (red) which are available aplenty in coastal regions of Indian subcontinent. First their proximate biochemical compositions including fatty acid profile were assessed. And then their potential have been evaluated by studying effects on the survival, growth, nutritional indices, concentrations of basic biochemical constituents (total protein, carbohydrate, lipid and amino acid), profiles of protein and amino acids and activities of digestive enzymes (protease, amylase and lipase) on the late aged post-larvae of *M. rosenbergii*.

2. MATERIALS AND METHODS

2.1. Collection and identification of marine algae

The marine algae (*T. ornata* and *G. corticata*) were collected from the intertidal region of Mandapam coast (Lat. 9° 17'N; Lon. 79° 19'E) of Gulf of Mannar, south-east coast of Tamil Nadu, India. The macro alga species was identified based on its morphology by using identification manual of "Economically Important Seaweeds" (Kaliaperumal *et al*., 1995) published by Central Marine Fisheries Research Institute (ICAR), Kochi, India. Finally, the species (BSI/SRC/23/2015/Tech./975; BSI/SRC/23/2015/Tech./976) were authenticated by Botanical Survey of India (BSI), Coimbatore, India.

2.2. Proximate composition and fatty acid profile of marine algae

The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles and necrotic parts, and brought to the laboratory in plastic bags. The samples were then thoroughly washed with freshwater, blotted, spread out and dried at room temperature for 2 weeks. Shade dried samples were ground to fine powder and stored in sterilized containers for further usage. The powdered algae were subjected to proximate composition analysis by adopting the methodology of Castell and Tiews (1980) as given in AOAC (1995) and the results are presented in table 1, which contain, 14.40 –19.30% of crude protein, 17.49 – 25.14% of crude fibre and 1.67 – 1.80% of etheric extract, 25.11- 29.90% of total ash, 9.40-10.40% of moisture, 26.26-28.34% of nitrogen free extract, 1.23-2.12% of sand and silica, 0.25-0.46% of calcium, 1.54-1.75% of phosphorus, 1.11-1.34% of salt and 3546-3781 kcal/kg of gross energy. The powdered algal samples were also subjected to analysis of fatty acid profile by adopting Gas Chromatographic method of Nichols et al. (1993) and the results are given in table 2, which contain fatty acids profile of 50.27-53.56 % of SFA, 25.11 – 29.90% MUFA (olecic acid), and PUFA % of 10.55 – 11.34 and 1.65 – 1.98 for linoleic and linolenic acids respectively.

Table 1: *Proximate biochemical composition (%) of marine algae*

Composition	***T. ornata***	***G. corticata***
Crude protein	14.40±1.20	19.30±1.20
Crude fibre	17.49±1.87	25.14±1.87
Etheric extract	1.67±0.34	1.80±0.34
Total ash	25.11±2.30	29.90±2.30
Moisture	10.40±0.57	9.26±0.57
Nitrogen free extract	26.14±6.28	28.34±6.28
Sand and silica	1.23±0.23	2.12±0.23
Calcium	0.25±0.03	0.46±0.03
Phosphorus	1.54±0.31	1.75±0.31
Salt	1.34±0.24	1.11±0.24
Gross energy (kcal/kg)	3546±88.00	3781±88.00

Each value is mean ± standard deviation of three individual observations.

Table 2: *Profiles of fatty acids (%) of marine algae*

Fatty acids		***T. ornata***	***G. corticata***
SFA	Myristic acid (C14:0)	6.54±1.10	6.79±1.23
	Palmitic acid (C16:0)	34.98±2.87	36.62±2.93
	Stearic acid (C18:0)	4.18±0.93	4.77±1.05
	Arachidic acid (C20:0)	0.76±0.18	1.15±0.26
	Behenic acid (22:0)	3.87±0.63	4.23±0.84
MUFA	Olecic acid (C18:1)	25.11±3.57	26.32±3.87
PUFA	Linoleic acid (C18:2n-6)	10.55±1.71	11.34±1.89
	Linolenic acid (C18:3n-3)	1.65±0.38	1.98±0.49
∑FA		87.64±11.37	93.20±12.56
∑SFA		50.33±5.71	53.56±6.31
∑MUFA		25.11±3.57	26.32±3.87
∑PUFA		12.20±2.09	13.32±2.38
n-3		1.65±0.38	1.98±0.49
n-6		10.55±1.71	11.34±1.89

Each value is mean ± standard deviation of three individual observations.

FA, fatty acids; SFA, saturated fatty acids; MUFA, mono unsaturated fatty acids; PUFA, poly unsaturated fatty acids.

2.3. Experimental animal

The post larvae (PL-30) of the freshwater prawn, *M. rosenbergii* were procured from ADAK Hatchery, Odayam, Varkala, Thiruvananthapuram, Kerala, India. They were transported to the laboratory in polythene bags filled with oxygenated water. The prawns were acclimatized to ambient laboratory conditions for 2 weeks in large cement tank (1000 L) with ground water (temperature, 28±2.0; pH, 7.0±0.20; total dissolved solids, 0.96±0.07 g L^{-1}; dissolved oxygen, 7.10±0.30 mg L^{-1}; BOD, 32.0±3.00 mg L^{-1}; COD, 136.0±11.00 mg L^{-1}; ammonia, 0.030±0.007 mg L^{-1}), APHA (2005). During acclimatization the prawns were fed with boiled egg albumin and artificially formulate feed (our laboratory prepared feed). More than 50% of tank water was routinely changed every day in order to maintain a healthy environment and aeration was also provided. The unfed feed, faeces, moult and dead prawns if any were removed by siphoning without disturbing the prawns.

2.4. Preparation of experimental diets

The following branded feed basal ingredients (BI) were taken to formulate the experimental feed. The fish meal, groundnut oilcake and soybean meal as protein sources, wheat bran as carbohydrate source, sunflower oil as lipid source, and tapioca flour and egg albumin were used as binding agents. The fish meal, groundnut oilcake, soybean meal, wheat bran and tapioca flour were thoroughly mixed, a dough was prepared with sterilized water, then it was steam cooked and cooled at room temperature. Then the Sunflower oil and egg albumin were added to the dough and mixed well. *T. ornata* and *G. corticata* powders were incorporated separately with the dough of BI at two different concentrations, 25% and 50% by replacing the right quantity of fishmeal, and in order to prepare iso-nitric diets, the protein level was maintained by adjusting the groundnut oilcake and soybean meal (Table 3). Sterilized water was adequately added for maintaining the dough in moist and paste form. Then it was pelletized in a manual pelletizer (Kolkata, India) fixed with 3 mm diameter mesh. The pellets were dried in a thermostatic oven (M/s Modern Industrial, Mumbai, India) at 40ºC until they reached constant weight and stored in airtight jars at room temperature. The pelletized feeds were subjected to proximate composition analyses (AOAC, 1995) and the results are also presented in table 3. The total organic matters present in the basal diet (control) and experimental diets contained 42.26-42.55% of crude protein, 4.20-4.39% of crude fat, 1.16-1.24% of crude fibre, 7.50-7.63% of total ash, 8.89-8.98 % of moisture, 35.30-35.89% of total carbohydrate and 4224-4347 (kcal/kg) gross energy. It is important to mention here that freshwater prawn requires 30-40% crude protein, 25-35% carbohydrate and 3-7% lipid (Swamy, 1995; Mitra, 2005). Therefore, the formulated feeds satisfied the prescribed proximate composition.

Table 3: *Ingredients used to formulate iso-nitric diets, and proximate composition (g) of marine algae*

Basal ingredients (BI)	**Control**	**Partially fishmeal replaced diets**			
		T. ornata		***G. corticata***	
		25%	50%	25%	50%
Fish meal	25.00	18.75	12.50	18.75	12.50
Groundnut oil cake	25.00	29.00	34.00	29.00	34.00
Soybean meal	25.00	29.00	34.00	29.00	34.00
Wheat bran	10.00	11.00	12.00	11.00	12.00
Egg albumin	07.00	07.70	08.40	07.70	08.40
Tapioca flour	05.00	05.50	06.00	05.50	06.00
Sunflower oil	02.00	02.20	02.40	02.20	02.40
Vitamin mix*	01.00	01.10	01.20	01.10	01.20
T. ornata / G. corticata		06.25	12.50	06.25	12.50
Total	100.00	110.50	123.00	110.50	123.00
Proximate composition of diets					
Crude protein (%)	42.55	42.34	42.26	42.38	42.31
Crude fat (%)	4.39	4.26	4.20	4.30	4.25
Crude fibre (%)	1.24	1.20	1.16	1.21	1.18
Ash (%)	7.63	7.56	7.51	7.59	7.50
Moisture (%)	8.89	8.94	8.98	8.92	8.96
Total nitrogen free extract	35.30	35.70	35.89	35.60	35.80
Gross energy (kcal/kg)	4281	4312	4224	4347	4248

**- Each capsule contains, Total mg = 438.5 mg; Thiamine Mononitrate 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, 100 mcg; Ascorbic acid IP, 150 mg manufactured by Pfizer.*

BI, basal ingredients.

2.5. Feeding trials

M. rosenbergii PL (PL-45; 2.02±0.02cm length; 0.12±0.01g weight) was starved for 24 h before commencing the feeding trial. Five groups, each with 30 PL were maintained in 30 L plastic tanks under a triplicate experimental set-up. One group served as control and fed with feed formulated by without incorporation of *T. ornata* and *G. corticata*, and the other four groups were fed with experimental feeds prepared by incorporation of *T. ornata* (at 25% and 50%) and *G. corticata* (at 25% and 50%) respectively by replacing the right quantity of the fishmeal. The feed was allocated to the prawns for two times a day (7:00 am and 7:00 pm) at 10% of body weight. The experiment was extended for a period of 90 days, by this time it reached juvenile stage. The unfed feed, feces and moult (if any) were collected on daily basis by siphoning method causing minimum disturbance to the prawns during renewal of water. For morphometric and nutritional analysis 10 prawns from each group were randomly measured and the mean was considered as a single value (mean of 10 individual measurements = one observation), and three such measurements were made to fulfill the triplicate analysis.

2.6. Evaluations of growth and nutritional indices

After the 90 days feeding trial, the growth parameters, such as survival rate (SR), length gain (LG), weight gain (WG), specific growth rate (SGR) and food conversion rate (FCR) were determined (Tekinay and Davies, 2001).

$$\text{Survival rate, SR (\%)} = \frac{\text{Total No. of live prawns}}{\text{Total No. of prawns introduced initially}} \text{X } 100$$

$$\text{Length gain, LG (cm)} = \text{Final length (cm)} - \text{Initial length (cm)}$$

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

$$\text{Specific growth rate, SGR (\%)} = \frac{\log w2 - \log w1}{t} \text{X } 100$$

where,

w1 & $w2$ $represents\ initial\ and\ final\ weight\ (g)\ respectively$

't' is the total number of experimental days

$$\text{Food conversion rate, FCR (g)} = \frac{\text{Total quantity of feed intake (g)}}{\text{Weight gain of the prawn (g)}}$$

2.7. Estimation of basic biochemical constituents

The concentrations of basic biochemical constituents, such as total protein, carbohydrate, lipid, amino acid, moisture and ash in the muscle of prawns were determined. Concentration of total protein was estimated by the method of Lowry et al. (1951) using ethanolic precipitated sample. Concentration of total carbohydrate was estimated by the method of Roe (1955) using TCA extracted sample. Concentration of total lipid was extracted by following the method of Folch et al. (1957) and estimated by the method of Barnes and Blackstock (1973). Amino acids were extracted using sodium tungstate and H_2SO_4. The content of total amino acid was assayed by the method of Moore and Stein (1948). Ash and moisture were analyzed by the method of APHA (2005). All analysis was carried out in triplicates. For these parameters, tissues from five prawns were pooled together from each group to constitute a single observation and three such observations were made to fulfill the triplicate analysis.

2.8. Analyses of profiles of proteins

SDS-PAGE analysis was done in the muscle samples of prawns fed with control and 25% of fishmeal replaced with *T. ornata*, and 25% with *G. corticata* (the best concentration in each category) feeds. The muscle tissue sample was first defrosted in phosphate buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 2 mM KH_2PO_4, pH-7.4), homogenized under ice cold condition and centrifuged at 1500 rpm under 4 ºC for 5 min. The soluble protein content in supernatant was determined (Lowery *et al*., 1951). SDS-PAGE was performed on vertical slab gel with 4% stacking and 10 % separating gels (Laemmli, 1970) along with protein markers of Medox-Biotech Pvt. Ltd., India (β-galactosidase -116 kDa, bovine serum albumin - 66 kDa, ovalbumin - 45 kDa, carbonic anhydrase - 29 kDa, soybean trypsin inhibitor - 20 kDa and lysozyme - 14 kDa). The polypeptides banding patterns between control and test prawns were compared by using the information on apparent molecular masses of bands and their intensities.

2.9. Analyses of profiles of amino acids

The profiles of amino acids were analyzed in the muscle samples of prawns fed with control and 25% of fishmeal replaced with *T. ornata*, and 25% with *G. corticata* (the best concentration in each category) feeds by using the High Performance Thin Layer Chromatographic (HPTLC) method (Hess and Sherma, 2004). The prawns were dried (80 ºC for 3 h), digested with 6M aqueous HCL and dried under vacuum. Samples (5 µl of distilled H_2O dissolved) were loaded on TLC plate pre-coated with Silica gel-60F254 (8 mm thick; 20 cm×15 cm), processed by using CAMAG-LINOMAT 5 instrument and developed under Butane-Ammonia-Pyridine-Water (3.9:1:3.4:2.6) as mobile phase. The gel was sprayed with ninhydrin reagent prepared in propan-2-ol and dried. The developed gel was documented under CAMAG-11REPROSTAR 3 at 254 nm and 366 nm UV lights. Finally, the gel was scanned at 500 nm using CAMAG-TLC SCANNER 3. TLC for four groups of standard amino acids: lysine, asparagine, glutamine, glutamic acid and metheonine (group-I); proline, serine, cystine, tyrosine and tryptophan (group-II); histidine, arginine, aspartic acid, threonine and leucine (group-III); and glycine, alanine, valine, isoleucine and phenyl alanine (group-IV) were also performed simultaneously. The peak area of the sample was compared with standard amino acids and quantified.

2.10. Analyses of profiles of fatty acids

The profiles of fatty acids were analyzed in the muscle samples of prawns fed with control and 25% of fishmeal replaced with *T. ornata*, and 25% with *G. corticata* (the best concentration in each category) feeds by using the Gas Chromatographic (GC) method (Nichols *et al*., 1993). Fatty acid samples were obtained from lipid by saponification using NaOH dissolved in methanol-H_2O mixture (hydrolysis with alkali). They were methylated into fatty acid methyl ester using methanol-HCl mixture. The fatty acid methyl ester was separated using hexane-anhydrous diethyl ether mixture. For the organic phase aqueous NaOH was used as base wash and the upper organic layer was separated. 2

µL of sample was injected and analyzed using Chemito 8610 Gas Chromatography, with BPX70 capillary column and flame ionization detector. Nitrogen was used as carrier gas. Standard fatty acids were analyzed simultaneously. Based on the retention time of the standard fatty acids, each fatty acid in the unknown sample was identified. The peak areas of standard and unknown were compared and quantified.

2.11. Activities of digestive enzymes

The whole flesh except eye stalk and exoskeleton was homogenized in ice cold distilled water and centrifuged at 10,000 rpm under 4ºC for 20 minutes. The supernatant was used as crud enzyme source. The activity of protease was estimated by the method of Furne et al. (2005). One unit of enzyme activity represents the amount of enzyme required to liberate one µg of tyrosine min-1 under assay conditions. The activity of amylase was assayed by following the method of Bernfeld (1955) in which the increase in reducing power of buffered starch solutions was measured. One unit of amylase activity was calculated as quantity (mg) of maltose liberated/ g of protein/ h (mg/g/h). The activity of lipase was assayed by the method of Furne et al. (2005). One unit of lipase activity was defined as the amount of free fatty acid released from triacyl glycerol per unit time was estimated by the amount of NaOH required to maintain pH constant and represented as mille equivalents of alkali consumed.

2.12. Statistical analysis

Data between control versus experiments and between experiments were subjected to statistical analysis through one-way ANOVA and subsequent post hoc multiple comparison with DMRT by adopting the SPSS v16. All the details of statistical analyses were given in respective tables. The P values less than 0.05 were considered statistically (95%) significant.

3. RESULTS

3.1. Growth, nutritional indices and basic biochemical constituents

The morphometric parameters (length and weight gains), nutritional indices (survival rate, specific growth rate and protein efficiency ratio) and concentrations of basic biochemical constituents (total protein, carbohydrate, lipid, amino acid and ash) were found to be significantly increased ($P < 0.05$) in 25% fishmeal replaced feeds fed prawns when compared with control, whereas the 50% fishmeal replacements showed significantly decreased growth, nutritional indices and contents of basic biochemical constituents when compared with control (Tables 4 and 5). The FCR was appeared reverse, that was lowest in 25% fishmeal replaced feeds fed prawns and highest in 50% fishmeal replaced feeds fed prawns when compared with control (Table 4). The lowest FCR recorded represents the best quality of feed. Among these two algae, *G. corticata* has produced better growth, nutritional indices and contents of basic biochemical constituents than that of *T. ornata* (Tables 4 and 5).

Table 4: *Morphometric and nutritional indices of M. rosenbergii fed with diets prepared by partial replacement of fishmeal with T. ornata and G. corticata*

Parameter		**Fishmeal replaced diets**			
		T. ornata		***G. corticata***	
	Control	25%	50%	25%	50%
SR (%)	84.25 ± 2.63^{c}	86.33 ± 2.51^{b}	77.00 ± 1.00^{e}	89.00 ± 1.07^{a}	79.11 ± 1.00^{d}
LG (cm)	2.48 ± 0.93^{bc}	2.50 ± 0.90^{b}	1.76 ± 0.13^{e}	2.74 ± 0.17^{a}	1.85 ± 0.17^{d}
WG (g)	0.32 ± 0.02^{c}	0.37 ± 0.02^{b}	0.27 ± 0.01^{de}	0.40 ± 0.04^{a}	0.29 ± 0.02^{d}
SGR (%)	0.63 ± 0.06^{c}	0.68 ± 0.05^{b}	0.56 ± 0.04^{de}	0.74 ± 0.05^{a}	0.57 ± 0.04^{d}
FCR (%)	1.18 ± 0.02^{c}	1.14 ± 0.01^{cd}	1.35 ± 0.02^{a}	1.12 ± 0.03^{d}	1.36 ± 0.03^{ab}
PER (%)	1.92 ± 0.03^{c}	1.98 ± 0.02^{b}	1.73 ± 0.01^{e}	2.04 ± 0.02^{a}	1.76 ± 0.02^{d}

Each value is mean ± standard deviation of three individual observations.

Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at P<0.05 (one-way ANOVA and subsequent post hoc multiple comparison with DMRT.

BI, basal ingredients; SR, survival rate; LG, length gain; WG, weight gain; SGR, specific growth rate; FCR, food conversion ratio; PER, protein efficiency ratio.

Table 5: *Basic biochemical constituents of M. rosenbergii fed with diets prepared by partial replacement of fishmeal with T. ornata and G. corticata*

Parameters (Total form)	Control	Partially fishmeal replaced diets			
		T. ornata		*G. corticata*	
		25 (%)	50 (%)	25%	50%
Protein (mg/g wet wt.)	141.65±2.46^{c}	147.43±2.00^{b}	94.76±2.35^{de}	151.20±4.78^{a}	96.15±2.92^{d}
Carbohydrate (mg/g wet wt.)	37.15±1.31^{c}	40.98±1.23^{b}	19.67±1.88^{de}	44.23±2.80^{a}	20.81±1.65^{d}
Lipid (mg/g wet wt.)	36.75±0.85^{b}	39.68±0.99^{ab}	15.87±1.38^{e}	40.94±1.32^{a}	18.20±1.43^{d}
Amino acid (mg/g wet wt.)	92.20±2.11^{b}	97.58±3.85^{ab}	81.22±2.57^{e}	99.61±2.43^{a}	83.68±2.31^{d}
Ash (%)	16.91±0.39^{bc}	18.32±0.40^{b}	12.00±1.00^{d}	21.09±0.78^{a}	14.26±1.43^{c}
Moisture (%)	65.28±1.43^{c}	63.21±1.15^{d}	76.20±1.46^{a}	60.24±2.20^{e}	74.20±1.46^{b}

Each value is mean ± standard deviation of three individual observations.

Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at P < 0.05 (one-way ANOVA and subsequent post hoc multiple comparison with DMRT.

3.2. Profiles of proteins

Polypeptide bands of molecular weight between 116-14 kDa were resolved in the muscle of prawns (Fig. 1). The Coomassie blue stained protein bands at some regions in 25% fishmeal replaced feeds fed prawns (71, 45, 29 and 20kDa of *T. ornata* incorporated feed, and 71, 66, 50, 45, 29, 26, 20, 15 and 14kDa of *G. corticata* incorporated feed) were found to be stained more intense than that of control. The resolved SDS-PAGE pattern revealed that among these two algae *G. corticata* showed better profile than that of *T. ornata*.

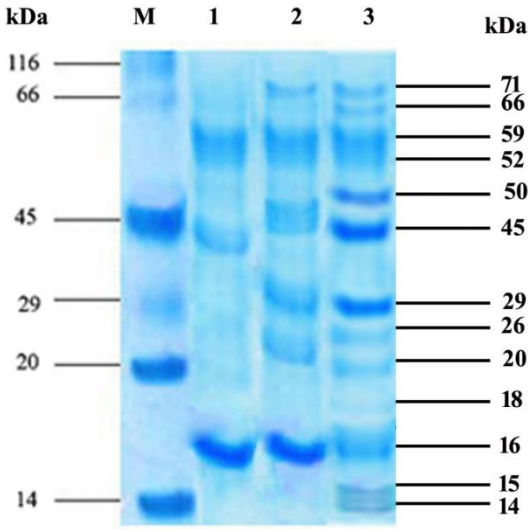

Fig. 1. *SDS-PAGE pattern of muscle protein of M. rosenbergii PL fed with diets prepared by partial replacement of fishmeal with T. ornata and G. corticata.*

Lane 1: Marker; Lane: 2; Control; Lane 3: 25% of fishmeal replaced diet with *T. ornata*. Lane 4: 25% of fishmeal replaced diet with *G. corticata*. kDa, kilo Dalton; 116, β-galactosidase; 66, Bovine serum albumin; 45, ovalbumin; 29, carbonic anhydrase; 20, soybean trypsin inhibitor; 14, lysozyme.

3.3. Profiles of amino acids

There were eighteen amino acids detected in the muscle of prawns, of which eleven were essential amino acids (EAA), lysine, histidine, arginine, glycine, asparagine, threonine, valine, methionine, isoleucine, leucine and phenyl alanine, and seven were non-essential amino acids (NEAA), proline, glutamine, cystine, aspartic acid, alanine, glutamic acid and tyrosine (Table 6). Generally the content of all the EAA and NEAA were found to be significantly higher ($P < 0.05$) in 25% fishmeal replaced feeds fed prawns when compared with control (Table 6). Among these two marine algae *G. corticata* produced better results.

Table 6: *Profiles of amino acids (g/ 100 g dry wt.) in the muscle of M. rosenbergii fed with diets prepared by partial replacement of fishmeal with T. ornata and G. corticata*

Amino acids	Control	*T. ornata* (25%)	*G. corticata* (25%)
Lysine[E]	3.26±0.16[c]	3.96±0.02[b]	4.34±0.03[a]
Histidine[E]	2.36±0.19[c]	2.96±0.02 [b]	3.12±0.04[a]
Arginine[E]	3.19±0.12[c]	4.33±0.03[b]	4.67±0.04[a]
Glycine[E]	10.86±0.59[c]	12.13±0.02[b]	13.3±0.03[a]
Asparagine[E]	7.11±0.03[c]	9.51±0.03[b]	9.81±0.03[a]
Threonine[E]	4.34±0.05[c]	4.71±0.02[b]	5.02±0.04[a]
Valine[E]	3.38±0.08[c]	3.73±0.03[b]	3.89±0.03[a]
Methionine[E]	2.89±0.15[c]	2.94±0.04[b]	3.31±0.04[a]
Isoleucine[E]	2.25±0.20[c]	2.40±0.04[b]	2.83±0.03[a]
Leucine[E]	4.10±0.24[c]	4.75±0.01[b]	4.95±0.02[a]
Phenyl alanine[E]	3.47±0.07[c]	4.16±0.05[b]	4.59±0.05[a]
Proline[NE]	6.54±0.55[c]	7.32±0.02[b]	8.05±0.03 [a]
Glutamine[NE]	1.26±0.07[c]	1.90±0.03[b]	2.46±0.04[a]
Cystine[NE]	1.61±0.29[c]	2.16±0.04[b]	2.55±0.04[a]
Aspartic acid[NE]	4.14±0.05[c]	4.87±0.06[b]	5.12±0.05[a]
Alanine[NE]	1.86±0.08[c]	2.75±0.10[b]	2.93±0.07[a]
Glutamic acid[NE]	1.96±0.11[c]	2.41±0.02[b]	2.78±0.12[a]
Tyrosine[NE]	3.91±0.08[c]	4.21±0.06[b]	5.16±0.09[a]
∑AA	68.80±2.69[c]	81.24±0.70[b]	88.88±0.81[a]
∑EAA	47.49±1.92[c]	55.61±0.34[b]	59.83±0.38[a]
∑NEAA	21.30±0.71[c]	25.62±0.35[b]	29.05±0.46[a]

Each value is mean ± standard deviation of three individual observations.

Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at P < 0.05 (one-way ANOVA and subsequent post hoc multiple comparison with DMRT.

AA, amino acid; [E], essential amino acids; [NE], non essential amino acids

3.4. Profiles of fatty acids

There were ten fatty acids detected in the muscle of prawns (Table 7), of which five saturated (lauric acid, myristic acid, palmitic acid, stearic acid and arachidic acid), two mono saturated (palmitoleic acid and oleic acid) and three poly unsaturated fatty acids [linoleic acid (n-6), EPA (n-3) and DHA (n-3)]. Generally the total content of all these three groups of fatty acids were found to be significantly higher ($P < 0.05$) in 25% fishmeal replaced feeds fed prawns when compared with control (Table 7). The ∑MUFA (palmitoleic acid and olecic acid) and ∑PUFA (Linoleic acid, EPA and DHA) elevation was higher when compared with ∑SFA in 25% fishmeal replaced feeds fed prawns when compared with control (Table 7). Among these two marine algae *G. corticata* produced better results.

Table 7: *Profiles of fatty acids (%) in the muscle of M. rosenbergii fed with diets prepared by partial replacement of fishmeal with T. ornata and G. corticata*

Fatty acids		Control	*T. ornata* (25%)	*G. corticata* (25%)
SFA	Lauric acid (C12:0)	0.55±0.03[c]	1.20±0.06[b]	1.67±0.03[a]
	Myristic acid (C14:0)	0.40±0.02[c]	1.18±0.02[b]	1.47±0.02[a]
	Palmitic acid (C16:0)	13.13±0.10[c]	15.30±0.16[b]	16.94±0.95[a]
	Stearic acid (C18:0)	8.25±0.11[c]	9.66±0.12[b]	9.72±0.53[a]
	Arachidic acid (C20:0)	0.72±0.05[c]	1.25±0.05[b]	2.09±0.57[a]
MUFA	Palmitoleic acid (C16:1)	9.18±0.05[c]	11.52±0.10[b]	11.73±0.26[a]
	Olecic acid (C18:1)	7.54±0.03[c]	10.24±0.25[b]	11.71±0.08[a]
PUFA	Linoleic acid (C18:2 n-6)	8.64±0.09[c]	11.08±0.22[b]	12.92±0.05[a]
	EPA (C20:5 n-3)	1.15±0.07[c]	2.19±0.15[b]	3.20±0.09[a]
	DHA (C22:6 n-3)	1.25±0.05[c]	3.25±0.05[b]	4.44±0.03[a]
∑FA		50.84±0.03[c]	66.90±0.07[b]	75.92±0.31[a]
∑SFA		23.06±0.04[c]	28.60±0.05[b]	31.90±0.39[a]
∑MUFA		16.73±0.01[c]	21.76±0.03[b]	23.44±0.12[a]
∑PUFA		11.04±0.02[c]	16.52±0.08[b]	20.57±0.02[a]
n-3		2.40±0.01[c]	5.44±0.07[b]	7.65±0.03[a]
n-6		8.64±0.09[c]	11.08±0.22[b]	12.92±0.09[a]

Each value is mean ± standard deviation of three individual observations.

Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at P < 0.05 (one-way ANOVA and subsequent post hoc multiple comparison with DMRT.

FA, fatty acids; SFA, saturated fatty acids; MUFA, mono unsaturated fatty acids; PUFA, poly unsaturated fatty acids.

3.5. Activities of digestive enzymes

Activities of digestive enzymes, protease, amylase and lipase were significantly (P<0.05) elevated in 25% fishmeal replaced feeds fed prawns when compared with control. Among these two marine algae *G. corticata* produced more elevation in activities of these enzymes (Table 8).

Table 8: *Activity of digestive enzymes of M. rosenbergii fed with diets prepared by partial replacement of fishmeal with T. ornata and G. corticata*

Parameters	**Control**	**Partially fishmeal replacement diets**	
		***T. ornata* (25%)**	***G. corticata* (25%)**
Protease (U/mg protein)	1.53 ± 0.49^{c}	1.61 ± 0.55^{b}	1.73 ± 0.52^{a}
Amylase (U/mg protein)	0.90 ± 0.23^{bc}	0.93 ± 0.21^{b}	1.13 ± 0.30^{a}
Lipase ($\times10^{2}$ U/mg protein)	0.35 ± 0.05^{c}	0.43 ± 0.07^{b}	0.52 ± 0.04^{a}

Each value is mean ± standard deviation of three individual observations.

Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at P < 0.05 (one-way ANOVA and subsequent post hoc multiple comparison with DMRT.

4. DISCUSSION

In the present study, the result recorded clearly indicated the fact that 25% fishmeal replaced feeds with *T. ornata* and *G. corticata* produced better survival, growth, protein, amino acids (EAA and NEAA), fatty acids (SFA, MUFA and PUFA), and digestive enzymes (protease, amylase and lipase) activities when compared with control by accumulation of nutrients due to active principles of these algae. Body biochemical composition is a good indicator for physiological condition and easy to assess the cultivable organisms. Protein is one of the major components of prawn feeds as larvae and juveniles have greater protein prerequisite than adults for growth and metabolism. Dietary protein supplies amino acids required to build body tissues essential for growth and production of hormones, antibodies, enzymes etc., (Gimenez *et al*., 2009). Generally, crustacean muscles contain high concentration of free amino acids, such as arginine, glycine, proline, glutamine, alanine, lysine, tryptophan, valine and histidine, which are involved in energy metabolism and protein synthesis (Bhavan *et al*., 2010). The free amino acids are plays an important role in osmoregulation, neurotransmitter, protein synthesis etc., (Fang *et al*., 1992; Mullen and Martin, 1992; Wilson, 2002). One of the major requirements of prawn culture is the transformation of dietary protein into tissue protein, which is essential for normal function, growth and maintenance. Dietary lipids are vital in providing essential fatty acids as they are yield energy, maintain the structural integrity of biological membranes, functions as precursors for important steroids, phospholipids, act as carriers of fat soluble vitamin A, D, E and K, and essential for growth, moulting and reproduction including egg hatchability and larval survival (Corbin *et al*., 1983; Kanazawa *et al*., 1985; Xu *et al*., 1994; Yepiz-Plascencia *et al*., 2000; Vasagam *et al*., 2005). Carbohydrates are the most economical and inexpensive source of energy. It together with proteins and lipids form dietary source of energy, and are important in synthesis of chitin, steroid, fatty acids and glycogen (Mukhopadhyay *et al*., 2003). Therefore, 25% fishmeal replaced with *T. ornata* and *G. corticata* have more influences on growth and nutritional profiles of *M. rosenbergii* due to the enhanced activities of digestive enzymes, as they have been reported to regulate the growth and moult cycle directly or indirectly (Lovett and Felder, 1990; Sun *et al*., 2011). Actually, crustaceans can able to digest a variety of complex food materials as they contain high concentration of protein, carbohydrate and lipid digesting enzymes, such as pepsin, trypsin, chymotrypsin, carboxypeptidases-A and B, leucine, aminopeptidase, amylase, collagenase, esterase and lipase (Gamboa-Delgado *et al*., 2003; Debnath *et al*., 2007).

The maximum survival, growth and nutritional indices have been reported in prawns fed with 50% partial replacement of the fishmeal with *Chlorella vulgaris* (Radhakrishnan *et al*., 2015). The growth-

promoting effect of *Chlorella* has also been reported in the fish Gibel carps, *Carassius auratus gibelio* (Xu *et al*., 2014). The partial replacement of fish meal by microalgae *Spirulina platensis*,, *Hypnea cervicornis* and *Cryptonemia crenulata* have also been reported in juvenile Pacific white shrimp, *Litopenaeus vannamei* with significant increase of growth (Hanel *et al*., 2007). The marine alga, *Enteromorpha* sp. supplementation has also been reported for better growth performance and FCR in *Penaeus monodon, Penaeus indicus, Papeneopsis stylirostris, L. vennamei* and *M. rosenbergii* (Bray *et al*., 1990). Sunitha and Rao (2003) have reported better weight gain in *Tilipia mossambica* when fed with blue green algae (*Chlorella, Anabaena, Oscillatoria, Nostoc*). Effects of *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* have been evaluated in the juveniles of the European seabass, *Dicentrarchus labrax* (Valente *et al.*, 2006).

5. CONCLUSION

Finally, this study recommends 25% of fishmeal can be replaced by *T. ornata* and *G. corticata* raw powders as these macro algae are low cost materials and available aplenty. They can be utilized for sustainable development of *M. rosenbergii* culture.

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