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Performances of Green Seaweed *Enteromorpha intestinalis*, Salt-marsh grass *Porteresia coarctata* and Mangrove litter as Prawn feed Ingredients

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Abstract

Three experimental feeds were formulated by reducing fishmeal at a level of 5%, incorporated with selected ingredients like green seaweed, Enteromorpha intestinalis (referred as ENT feed); salt- marsh grass, Porteresia coarctata (referred as POT feed) and mangrove litter (referred as ML feed). Juvenile prawn, Macrobrachium rosenbergii were exposed to the feeds of floral origin during the experimental trial and performances were evaluated in terms of growth characteristics and muscle protein content. Incorporation of E. intestinalis in the formulated feed resulted in significantly higher ($p<0.01$) average body weight (80.48 gm), condition factor (1.30), specific growth rate (1.45% day^{-1}), feed conversion ratio (1.76) and survival (68.19%) as confirmed through ANOVA. Length-weight relationship varied significantly ($p<0.01$) thus exhibiting isometric growth ($b= 3.008$) in prawns treated with ENT feed as compared to others where allometric growth pattern was observed. Muscle protein content also reflected similar pattern with significantly greater ($p< 0.01$) in the group of prawns treated with ENT feed. The present study thus confirms the suitability of the particular seaweed species for its incorporation in the formulated prawn feed.

Keywords: Floral ingredients, *macrobrachium rosenbergii,* growth characteristics, muscle protein content.

Introduction

Feeds supplemented with ingredients of floral origin is currently gaining momentum in the area of organic feed formulation research perhaps to replace ingredients of animal origin[1,2] in order to increase disease resistance[3] and egg production[4]. However in aquatic animal nutrition, fishmeal happens to be the primary source of dietary protein and lipid because of its biological value. Interrupted availability and high price is gradually making this component very limited for incorporation in fish feeds by the manufacturers. Researchers are on the track screening alternative protein sources of plant origin as partial or total fishmeal replacement with a major focus on different terrestrial plants as protein sources in feed. As far as marine plant resources are concerned, incorporation of seaweeds as feed supplement have been used for shrimps and prawns[5-10], sea bass[11], snake head[12] and rainbow trout[13]. Apart from seaweed inclusion, feeds incorporated with salt-marsh grass, lucerne grass and mangrove litter have been reported for *Penaeus monodon*, *Macrobrachium rosenbergii*, *Metapenaeus monoceros* and *Cirrhinus mrigala*[14-17]. Research findings reveal that feed incorporated with seaweed meal has resulted in improved performance, better feed efficiency, better pellet stability and improved animal product quality which may be attributed to the enormous genetic potential of these lower group of plants with a genome that is more than twice the size of yeast which enables them in serving many industries such as food, animal feed, cosmetics, pharmaceuticals and biofuels[18]. Literature review enlightened some interesting facts which may serve as a baseline information for the present work. Such as green seaweed is found to be a good source of dimethyl sulfonyl propionate (DMSP) which has been proven for its attractant property thereby providing improved growth performance and increased feed efficiency in shrimps[19, 20], salt-marsh grass traditionally used as animal food and fodder[21] is found to contain chemical compounds like fatty acids, hydrocarbons, steroids, sterol ester, triacylglycerol, triterpenes, waxes[22] and finally mangrove litter (or foliage) where the presence of constituents like protein, polyphenol, tannin, potassium, phosphate and nitrogen[23-26] are reported. Thus the present experimental work is an attempt to evaluate the performances of these floral ingredients incorporated in *M. rosenbergii* feed, in terms of growth characteristics and muscle protein concentration.

Methodology

Experimental floral ingredients and feeds: Live and healthy plant materials (*E. intestinalis, P. coarctata* and mangrove litter) were collected from the estuarine zone of Uttar gobindapur village, Kakdwip block of Sundarbans (21°52'35.7" N latitude and 88°11' 55.0" E longitude) during low tide condition. All the collected plant materials were washed in ambient water and then with freshwater to remove epiphytes and other extraneous matter, dried in hot air oven under 55°C to preserve the biochemical constituents and finally processed. The experimental feeds were formulated to meet the nutritional requirements of prawn[27] as given in table 1. The proximate composition of the floral ingredients as well as the formulated feeds were estimated by the following methods: Lowry for

Protein[28], Dubois for Carbohydrate[29], Soxhlet for Lipid[30] and AOAC[31] for ash and moisture contents.

Feeding trial: A feeding trial was run at Uttar gobindapur village, Kakdwip block of Sundarbans (21°52'35.7" N latitude and 88°11' 55.0" E longitude) in grow-out ponds for 240 days of experimental duration as shown in figure 1. The experimental facility consisted of triplicate ponds for each dietary treatment. Each pond was well connected to adjacent estuary so that possible hydrological variations affect all the ponds simultaneously. The hydrological condition ranged within the optimum requirements recommended for freshwater prawn culture as given in table 2. Prawn juveniles were procured from a local hatchery and acclimated to the pond conditions prior to feeding trial. Juveniles were stocked at a density of 2 No/m^2 with average initial weight of 2.5 ± 0.14 gm and fed twice daily at 0630 and 1800 hrs. The uneaten feed was checked at regular intervals.

Growth characteristics of prawn: Individual weights were taken at every 30 days interval throughout the trial. The following response variables were determined from the experimental ponds:

Fulton's condition equation was used to find out the condition factor[32] as follows:

$$K = \frac{\overline{w}}{(\overline{TL})^3} \times 10^3$$

Where *K* is the condition factor, $\overline{w}$ is the average weight (g) and $\overline{TL}$ is the average total length (cm)

Specific growth rate (SGR) was calculated using the conventional equation:

$$SGR = \frac{\ln w_f - \ln w_i}{t} \times 100$$

Where W_f and W_i are the average final and initial weight in time *t*.

Final average body weight (ABW), feed conversion ratio (FCR) and survival were estimated after harvest by drag netting and dewatering the pond finally as:

FCR= total feed intake/ total biomass gain

Survival= (number of fish harvested/number of fish stocked) ×100

Length-weight relationship (*LWR*) was calculated by using the conventional formula[33] as given below:

$$W = a.TL^b$$

Where *W* is fish body weight (g), *TL* is total length (cm), *a* is the proportionality constant and *b* is the isometric exponent. The parameters *a* and *b* were estimated by non-linear regression analysis.

Table- 1
Formula and proximate composition of the experimental feeds

Feed Ingredients	ML feed	ENT feed	POT feed
Fish meal	30	30	30
Soybean oil cake	11	11	11
Mustard oil cake	11	11	11
Rice polish	23	23	23
Wheat flour	16	16	16
Oyster shell dust	2	2	2
Shark oil	2	2	2
Mangrove litter	5	-	-
E. intestinalis	-	5	-
P. coarctata	-	-	5
Proximate composition (% dry matter)			
Protein	34.73 ± 1.60	34.64 ± 1.20	34.09 ± 1.30
Carbohydrate	35.04 ± 0.27	33.19 ± 0.76	36.28 ± 0.90
Lipid	5.3 ± 0.46	6.12 ± 0.49	6.6 ± 0.32
Ash	10.4 ± 0.31	10.6 ± 0.12	12.4 ± 0.23
Moisture	10.2 ± 0.36	9.82 ± 0.15	10.7 ± 0.69

*Results are mean ± SD

Table- 2
Hydrological parameters of the experimental ponds treated with feeds of floral origin

Parameters	Ponds treated with ML feed	Ponds treated with ENT feed	Ponds treated with POT feed
Surface water temperature (^oC)	30.8 ± 1.57	30.81 ± 1.50	30.88 ± 1.45
Surface water salinity (psu)	1.52 ± 1.33	1.86 ± 1.09	2.15 ± 1.39
pH	7.47 ± 0.24	7.76 ± 0.11	7.70 ± 0.15
Dissolved oxygen (mg l^{-1})	5.38 ± 0.34	5.37 ± 0.44	5.50 ± 0.38
Nitrate concentration (µg-at l^{-1})	9.56 ± 2.03	10.85 ± 1.60	9.14 ± 1.53
Phosphate concentration (µg-at l^{-1})	0.98 ± 0.25	1.15 ± 0.17	0.98 ± 0.19

*Results are mean ± SD of three replicates

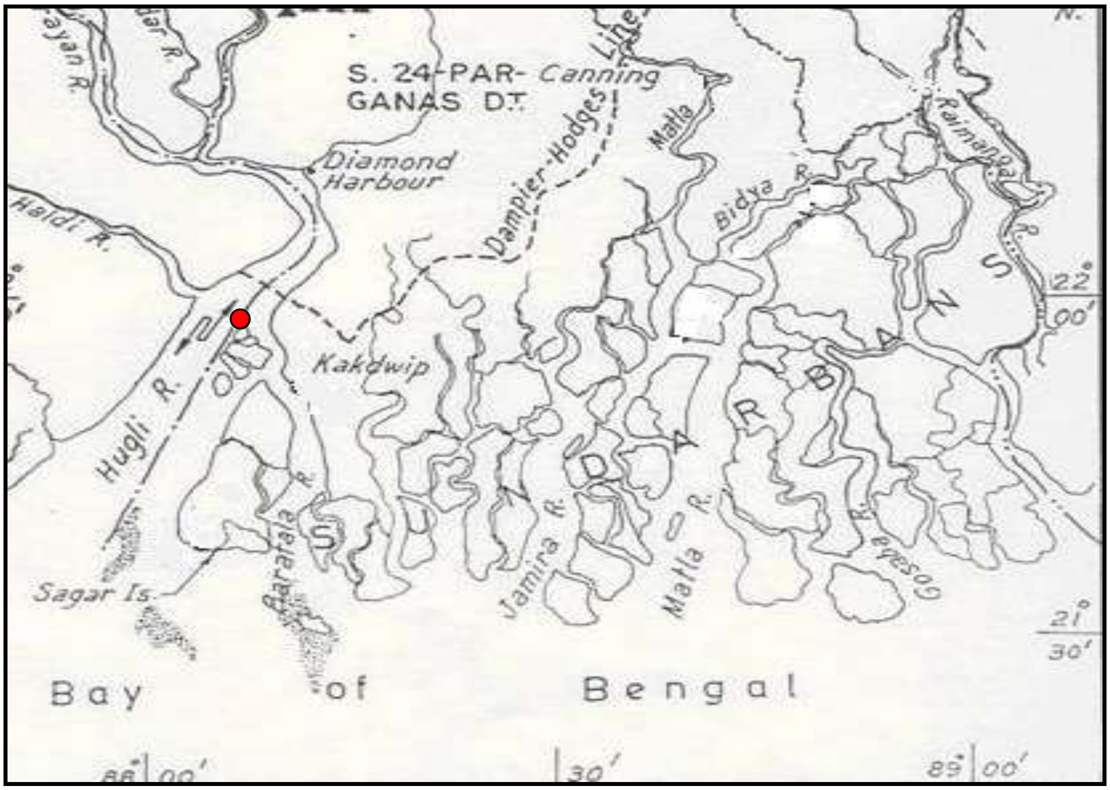


Figure- 1
Map showing the *M. rosenbergii* culture site at Uttar gobindapur village, Kakdwip block of Sundarbans

Muscle protein concentration of prawn: Protein content was analyzed as per the standard Lowry's method. Protein present in the sample reacts with folin-ciocalteau reagent to give a coloured complex which is produced by the reduction of phospho-molybdate by tyrosine and tryptophan liberated from the protein by the action of alkaline copper. The intensity of the colour depends on the amount of protein present in the sample and is measured spectrophotometrically against standards and blank.

Statistical analysis: The collected data were finally subjected to one-way analysis of variance (ANOVA). All statistical calculations were performed with SPSS 9.0 for windows.

Results and Discussion

Proximate composition of the selected floral ingredients: The proximate compositions of the floral ingredients like *E. intestinalis*, *P. coarctata* and mangrove litter as observed from the study region are provided in table 3. The protein content (% DW) in the selected samples varied as per the order, *E. intestinalis* > mangrove litter > *P. coarctata*. In case of carbohydrate content (% DW), the order is *P. coarctata* > mangrove litter > *E. intestinalis*. The lipid content (% DW) varied as per the sequence, mangrove litter > *E. intestinalis* > *P. coarctata*. The order of ash content (% DW) is *E. intestinalis* > *P. coarctata* > mangrove litter. The sequence of moisture content (% DW) is *P. coarctata* > mangrove litter > *E.*

intestinalis. Earlier studies conducted shows that crude protein, crude lipid, total carbohydrate, ash and moisture contents in *E. intestinalis* ranged from 4-16%, 21-38%, 2-7%, 16-39% and 21-45% respectively[34-40], while for *P. coarctata* these values varied from 5-12%, 35-50%, 0.9-2%, 13-23% and 42-68%[41-44] and for mangrove litter they ranged from 6-14%, 5-45%, 7-16%, 2-18% and 59-75% respectively[45-49].

Table-3
Proximal composition of the experimental floral ingredients (% dry matter)

Proximate composition	Mangrove litter	*E. intestinalis*	*P. coarctata*
Protein	15.47 ± 1.67	17.23 ± 1.74	10.23 ± 1.51
Carbohydrate	33.12 ± 1.27	32.46 ± 1.16	46.7 ± 1.36
Lipid	9.02 ± 0.62	4.67 ± 1.10	1.26 ± 0.35
Ash	9.36 ± 1.03	25.43 ± 1.28	17.53 ± 1.02
Moisture	54.83 ± 1.36	30.77 ± 0.46	55.3 ± 0.57

*Results are mean ± SD

Proximate composition of the formulated feeds: The feed composition is presented in table 1. The protein content ranged between 34.09 ± 1.30% (POT feed) to 34.73 ± 1.60% (ML feed), lipid content ranged between 5.30 ± 0.46% (ML feed) to 6.60 ± 0.32% (POT feed), carbohydrate content ranged between 33.19 ± 0.76% (ENT feed) to 36.28±0.90% (POT feed), ash content ranged between 10.4 ± 0.31% (ML feed) to 12.40±0.23% (POT feed) and moisture content ranged between 9.82 ± 0.15% (ENT feed) to 10.70 ± 0.69% (POT feed) respectively. The results obtained in the present study are in agreement with the values of several research findings[50-53].

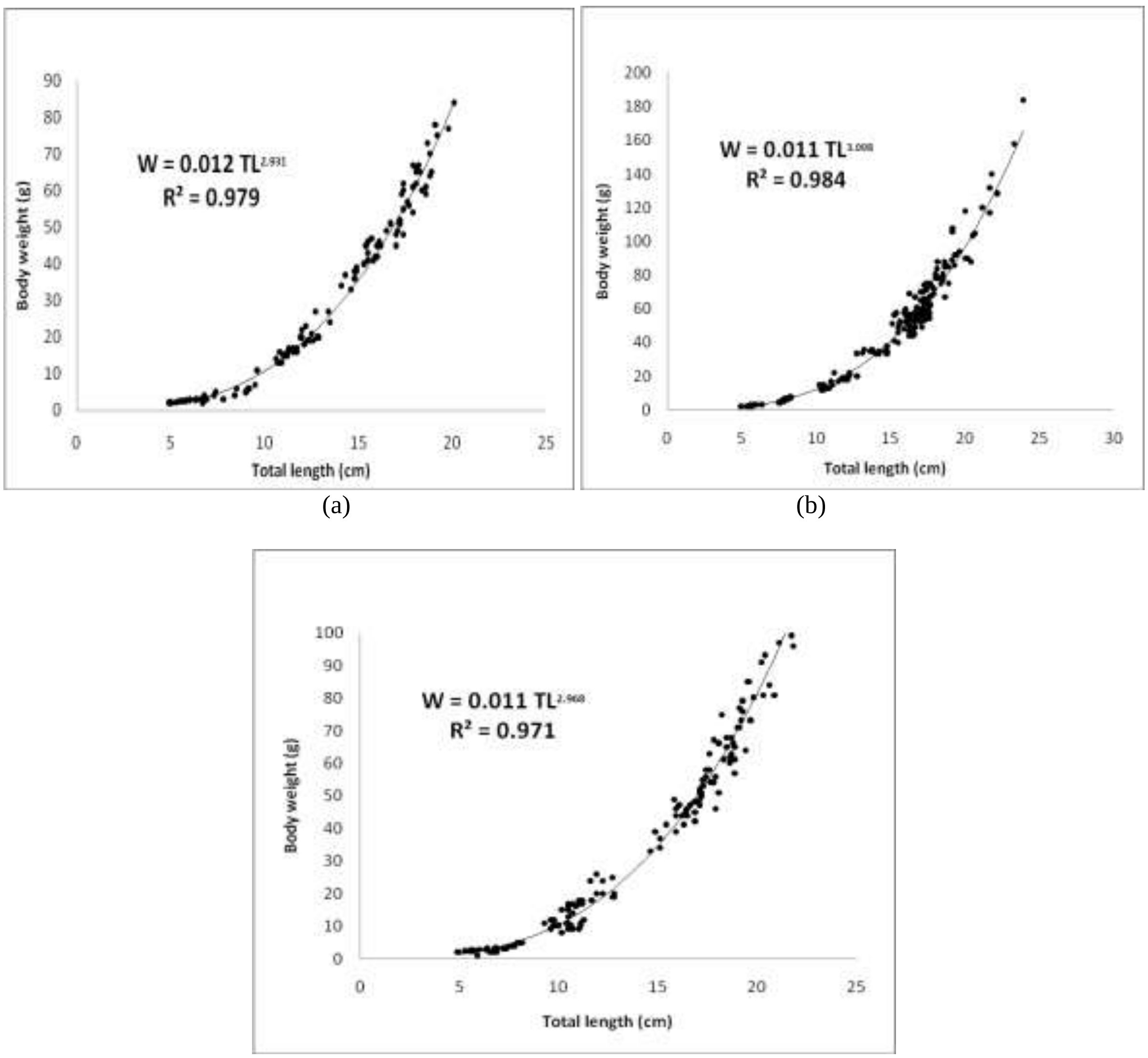
Growth characteristics of prawn: Prawns treated with ENT feed represented higher average body weight (80.48 gm), better condition factor (1.3±0.07), low FCR (1.76) and high SGR (1.45±0.13% day^{-1}), the differences being statistically significant at 1% level as provided in table 4. At harvest, survival was 56.28%, 68.19% and 61.55% as recorded from respective treatments which also varied significantly ($p<0.01$). Feeds of floral origin and its application in *M. rosenbergii* farming is the pioneer work from the present study region. A similar approach was undertaken by researchers through inclusion of floral ingredients in different regions of Indian subcontinent, but they were mostly related to replacement of fishmeal with terrestrial plant proteins. In this context, better weight gain, SGR and survival was observed in *M. rosenbergii* treated with feeds containing 7% *Murraya koenigi* extract[54]. Similar findings was obtained with significantly better growth performance and body pigmentation of *P. monodon* fed with formulated feed containing 5% of red seaweed, *Catenella repens* extract from Sundarbans. Inclusion of salt-marsh grass, *P. coarctata* in feeds of *P. monodon* at 100 g kg^{-1} is also reported by workers[55]. Even better growth was obtained in *M. rosenbergii* through inclusion of *E. intestinalis* and *P. coarctata* in the feed. The inclusion of decomposed mangrove leaves or foliage in formulated feeds of *Metapenaeus monoceros* have been reported previously with better growth and conversion efficiency. However the present work reflected comparatively inferior growth performance in prawns applied with mangrove litter incorporated feed which may be attributed to the presence of certain organic or inorganic constituents in litter (such as polyphenols, tannins etc.) that might have contaminated the feed to certain extent resulting in reduced digestibility and inferior growth performance[56-58]. Supplementation of feeds using *Enteromorpha* sp. is also reported for *Penaeus monodon*, *P. indicus*, *P. stylirostris*, *Litopenaeus vannamei* and *M. rosenbergii* with better growth performance and FCR[59-62]. Superior growth performance of cultured species may be attributed to the formulated feed incorporated with *E. intestinalis*, although inclusion rate was low. Another probable reason could be the presence of compound dimethyl sulfonyl propionate (DMSP) in this particular order of seaweed (Ulvales) which has been proven as an attractant, thereby accelerating faster feed consumption and subsequent digestion by the culture species. The growth characteristics of prawn thus exhibited in the present study may be a direct consequence of inclusion of *E. intestinalis* in the feed which is under order Ulvales.

Table- 4
Variations in growth characteristics of prawn related to incorporation of floral ingredients in feed

Parameters	Prawns treated with ML feed	Prawns treated with ENT feed	Prawns treated with POT feed
Condition factor (K)	1.14 ± 0.11^{c}	1.30 ± 0.14^{a}	1.19 ± 0.14^{b}
Survival (%)	56.28^{c}	68.19^{a}	61.55^{b}
SGR (% day^{-1})	1.18 ± 0.99^{c}	1.28 ± 1.16^{a}	1.21 ± 1.08^{b}
FCR	2.0^{a}	1.76^{c}	1.88^{b}
ABW (gm)	61.76^{c}	80.47^{a}	66.16^{b}
LWR (b)	2.890^{c}	3.008^{a}	2.931^{b}

*Means with different superscripts in a row differ significantly ($p<0.01$); values are means of three replicates.

Growth of prawns depends on sex, stage and environmental factors such as nature of food, water temperature and salinity[63]. *LWR* is an important indicator to measure the growth variations in a particular organism[64] as well as for stock assessment[65]. Easier techniques of calculating average weight at given length group is usually done by the method of *LWR*[66,67]. The health conditions of a fish population[68, 69] is also assessed by this procedure. Variation in *LWR* obtained between the culture species may be attributed to the different feed composition, population density, environmental conditions and genetic make up of the species which has been well documented in earlier studies[70-74]. A curvilinear growth pattern was observed irrespective of treatments with a gradual increase over the feeding trial as shown in figures 2a, 2b and 2c. The isometric exponent (*b*) values showed significant variation ($p<0.01$) with the highest being obtained from the culture species fed with *E. intestinalis* incorporated feed as given in table 4. This value exactly coincides with the ideal condition of isometric growth pattern, when *b= 3.0*[75]. Difference in ingredient selection of feed clearly affected *LWR* of individual organisms, which coincides with the earlier observations that *b* value close or equal to 3.0 reflects better feed acceptance/ or environmental condition in a culture system. Hence the greater preference for ENT feed by the culture species would have affected this parameter.



(a) (b)

(c)

Figure- 2
Length-Weight Relationship curves of *M. rosenbergii* fed with different experimental feeds
(a= ML feed, b= ENT feed, c= POT feed)

Muscle protein concentration of prawn: Protein is one of the major component which indicates shrimp/prawn meat edibility in terms of its amino acid profile. The present study highlights the highest protein content in the cultured species treated with *E. intestinalis* incorporated feed followed by the groups treated with *P. coarctata* and mangrove litter incorporated feeds as given in table 5. ANOVA results confirm significant variation ($p<0.01$) in muscle protein content of prawns which may be attributed to the influence of algal protein in the formulated feed that are consumed by the culture species and due to the nature of enzymes present in the digestive tract which are indicative that *M. rosenbergii* can digest protein of both plant and animal origin[76-81]. Higher protein concentration is directly linked with the translation mechanism occurring within the cells, which is the prime source of protein synthesis[82]. The values obtained were similar to the results as reported earlier where isonitrogenous feeds were prepared from fishmeal, acid silage and fermented fish silage in *M. rosenbergii* culture[83]. Similar observations were reported in the case of protein value while experimenting with some alternative practical feeds for *M. rosenbergii*[84]. Higher protein values were recorded in cultured prawn (*M. rosenbergii*)[85], whereas, similar result was found in *M. idella idella*[86] where the protein ranged from 58.60 to 64.28% in males and 58.36 to 61.86% in females. The protein content varied between 58.17% to 64.15% in *M. malcolmsonii*[87]. The protein content was maximum in small sized males of *M. scabriculum* 59.15%[88] and *M. idae* 61.44%[89]. Juveniles of *M. rosenbergii* when exposed to formulated feeds containing different levels of cuttlefish liver lipid was also found to have muscle protein content between 64 - 66%[90] which are in agreement to those reported in the present study where the feeds are incorporated with floral ingredients. Feed supplemented with 50% methanolic extract of *Ricinus communis* as feed additive when applied to groups of *P. monodon* exhibited muscle protein content of around 62%[91]. Thus the results are indicative that the feed protein is efficiently utilized for the increase in biomass by boosting up the growth of prawns.

Table- 5
Effect of experimental feeds on muscle protein concentration of *M. rosenbergii*

	Prawns treated with ML feed	Prawns treated with ENT feed	Prawns treated with POT feed
Protein (% dry weight)	64.17 ± 1.76^c	68.78 ± 5.05^a	65.92 ± 2.75^b

*Means with different superscripts in a row differ significantly ($p<0.01$); values are means of three replicates.

Conclusion

In the present study, *E. intestinalis* incorporated feed exhibited the best results in terms of growth characteristics and muscle protein content. The availability of this particular seaweed from the mangrove dominated Indian Sundarbans creates a viable opportunity for utilizing them in fish feed, which could be a step towards organic feed formulation in future. The species can also be cultured through "rope culture technology", which is cost-effective. The present programme can thus open the avenue of alternative livelihood for the island dwellers of Indian Sundarbans.

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