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MARINE ALGAE *ENTEROMORPHA INTESTINALIS* ACTS AS A POTENTIAL GROWTH PROMOTER IN PRAWN FEED

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ABSTRACT

A newly prepared powder from marine algae *Enteromorpha intestinalis* (*Aqua*-Naturale) has been found as a potent growth promoter in freshwater prawn. Juveniles of *Macrobrachium rosenbergii* were fed with *Aqua*-Naturale at 5% level through fishmeal based supplementary diet for a period of 240 days. The study was an attempt to observe the responses of *Aqua*-Naturale diet towards muscle growth pattern of the culture species as well as the probable economic returns obtained from the experiment in order to commercialize the particular product in local aquafeed market. The desired level of *Aqua*-Naturale administration produced animals with significantly ($p<0.05$) higher body weight associated with expanded muscle fibres resulting in an increased muscle mass as revealed through histological analysis. An average weight gain of 32.48% and increased fibre surface area of 9.77% was obtained with inclusion of *Aqua*-Naturale in the diet which may be a direct consequence of hypertrophic muscle growth pattern observed in the species. An economic analysis also speaks in favour of *Aqua*-Naturale that generated significantly higher ($p<0.05$) income and BCR value through prawn sale. The present findings thus indicate that *Aqua*-Naturale may be used as a cost-effective and natural growth promoter in prawn feeds for higher profitability, better growth and production.

KEY WORDS: *E. intestinalis*, *M. rosenbergii*, Body weight, Muscle fibre, Hypertrophy, Economic analysis.

INTRODUCTION

Global population is expanding, assumed to reach a density of 9 billion by 2015. With that many people on planet the need for food is expected to double, specially high demand for fish. Aquaculture industry is likely to experience a double hike because fish is the only source

of cost effective protein for human consumption. In this context, high commercial productivity is required which is related to fast growth with maximum body weight, normally done by inclusion of synthetic growth promoters like steroid hormones, antibiotics, vitamins and several other chemicals into the formulated diets [1-5]. Food safety, environmental impacts and human health concerns have led to the search for alternative growth promoter of natural origin perhaps to replace the synthetic ones because of their detrimental effect through bioaccumulation within the body tissues of the culture species that eventually gets transferred to next consumer level by 'biomagnification' process. Advancement in scientific research has helped to develop ecofriendly and natural growth promoting supplements of floral origin in fish with no harmful impact on their health [6]. In continuation, the aquaculture researchers of University of Calcutta has come up with a new powder product derived exclusively from *E. intestinalis* (*Aqua*-Naturale), a dominant green seaweed found in the deltaic region of Sundarban mangroves with respect to its biochemical properties and performance as feed ingredient [7, 8]. Furthermore, it caused significant increase in biomass and muscle fibre surface area of the culture species thereby leading to strong economic growth while eliminating synthetic stimulants from feed. Considering the immense biological significance and practical applications of seaweed in aquaculture, the present study was carried out to investigate the growth boosting effect of *Aqua*-Naturale in palaemonid prawn *M. rosenbergii*, an economically important and major crustacean species in Indian aquaculture scenario.

MATERIALS AND METHODS

Collection and processing of marine algae

Live and healthy *E. intestinalis* was collected from the intertidal mudflat in the mangrove belt of Indian Sundarbans (21^{o}52'35.7" N latitude and 88^{o}11' 55.0" E longitude) during low tide condition. The collected material was washed in ambient water and then with freshwater to remove epiphytes and other extraneous matter, minimally processed in the form of powder to retain all the bioactive molecules.

Diet preparation and growth trial

The seaweed powder was mixed with fishmeal at a level of 5% within the formulated diet to meet the nutritional requirements of prawn. Simultaneously a control diet was also formulated with 0% inclusion of seaweed powder. A 240 day growth trial was conducted, prawn juveniles of mean initial weight of 2.5g were obtained from a local hatchery and

acclimated to the conditions prior to initiation of the experiment. Stocking at a density of 2 individuals/m^2 was done in the experimental ponds. Dietary treatments were randomly assigned to the ponds and feeding on the respective diets was initiated.

Sampling

Prawn samples were collected at every 30 days interval. Body weight were measured with the help of field electronic balance prior to sacrificing the animals. The feed conversion ratio (FCR) was calculated as; FCR= estimated individual consumption / average individual increase in weight. The survival was calculated as: (final number / initial number) × 100, for each experimental pond.

Muscle Histology

Muscle samples for histological analysis were collected from 60 to 210 days of culture (DOC). Tissue from second abdominal segment was dissected and about 1.0 gm of tissue was fixed in Bouin's solution. After further processing of the tissues in the laboratory, the sections were rehydrated and stained with haematoxylin and counter stained with eosin for microscopic studies. Cross-sectional images were taken at regular intervals with the help of NIKON- Phase Contrast Microscope fitted with an image analyzing system. The surface area expansion of the fibre were measured using a 'Planimeter'. The areas of ten randomly selected fibre were measured per slide and the average was estimated.

Economic analysis

An economic analysis was performed during harvest to estimate the total income and benefit-cost ratio (BCR) obtained by incorporating *Aqua*-Natural*e* in the diet. The estimation was done according to the method outlined by Biswas et al., (2012) [9].

Statistics

To explore the relationships between body weight and muscle fibre expansion, scatterplots and allometric equations were computed. All the collected data were subjected to Analysis of Variance (ANOVA) and the calculations were performed with Web Agri Stat Package (WASP) statistical software for Windows.

RESULTS

1. The average initial body weight was 2.5 g and final body weight was found to be 72.04 g (control) and 80.47g (*Aqua*-Natural*e*) respectively (Table 1). Significant variations

($p<0.05$) in body weight was obtained among the treatments as represented by the growth pattern (Fig 1**)**.

2. Specific cytomorphological changes i.e. muscle fibre expansion occured during the time of observation. Initial fibre surface area was 10.60 ± 0.48 mm^2 (control) and 8.0 ± 1.08 mm^2 (*Aqua*-Natural*e*) whereas final observations exhibited an increased surface area of 18.2 ± 0.35 mm^2 (control) and 25.6 ± 0.92 mm^2 (*Aqua*-Natural*e*) respectively which clearly indicates the impact of *Aqua*-Natural*e* on muscle growth pattern of the culture species (Table 1). Fig 2 shows the variations in fibre surface area among the treatments which differed significantly ($p<0.05$).
3. An economic analysis resulted in significantly ($p<0.05$) higher total income as well as BCR value in the group of prawns treated with *Aqua*-Natural*e* incorporated diet compared to control. The comparison of economic parameters are provided in Table 2.

Table 1. Comparison of success parameters obtained through supplementation of *Aqua*-Natural*e* at 5% level in prawn diet

Parameters	Control diet	*Aqua*-Natural*e* diet
Final body weight (g)	72.04^b	80.47^a
Muscle fibre surface area (mm^2)	18.2^b	25.6^a
Average weight gain (%)	28.97^b	32.48^a
Net increase in fibre surface area (%)	4.22^b	9.77^a
FCR	1.81^b	1.76^a
Survival (%)	65.73^b	68.19^a

*****Means with different superscripts in a row differ significantly ($p<0.05$); values are means of three replicates.

Table 2. Comparison of economic parameters among control diet and *Aqua*-Natural*e* diet in *M. rosenbergii* culture experiment. Calculation was for 1 ha pond and 240 days of experimental duration. Currency mentioned is Indian Rupee (INR).

Operational cost (OC)	Quantity ha^{-1}	Rate (INR)	Control diet	*Aqua*-Natural*e* diet
Prawn juveniles	20000	5 fry^{-1}	100000	100000
Organic manure	500 Kg	22 kg^{-1}	11000	11000
Urea	300 kg	9 kg^{-1}	2700	2700
Single Super	300 kg	10 kg^{-1}	3000	3000

Phosphate (SSP)				
Formulated feeds	1714 kg (control)	59 kg^{-1}	101126	-
	1931 kg (*Aqua*-Naturale)	58 kg^{-1}	-	111998
Lime	2500 kg	6 kg^{-1}	15000	15000
Man power	60 man day	200 man-day^{-1}	12000	12000
Sub total			244826	255698
Interest on OC	8 months	12% annual	19586	20455
Total OC			**264412**	**276153**
Economic returns				
Prawn sale	947 kg (Control)	600 kg^{-1}	**568200**[b]	-
	1098 kg (*Aqua*-Naturale)	650 kg^{-1}	-	**713700**[a]
Net return			303788	437547
BCR			**2.148**[b]	**2.584**[a]

*Means with different superscripts in a row differ significantly ($p<0.05$); values are means of three replicates.

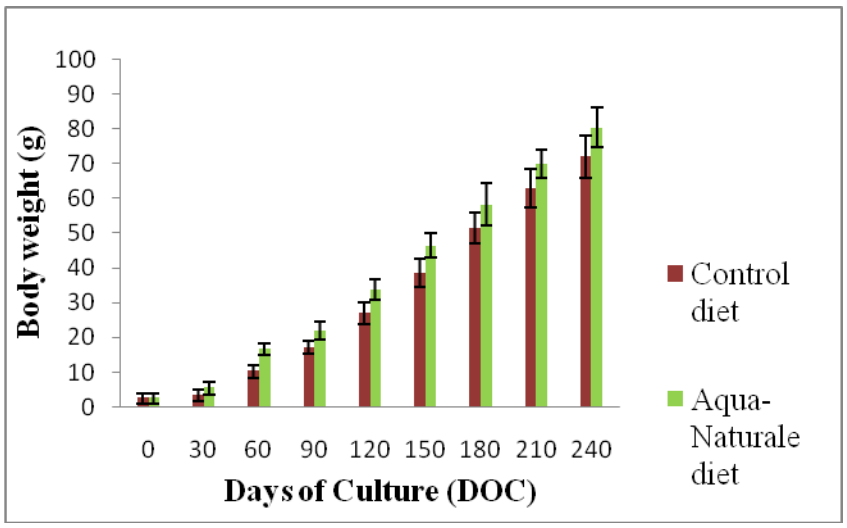

Fig.1. Body growth pattern of *M. rosenbergii* fed with formulated diets.

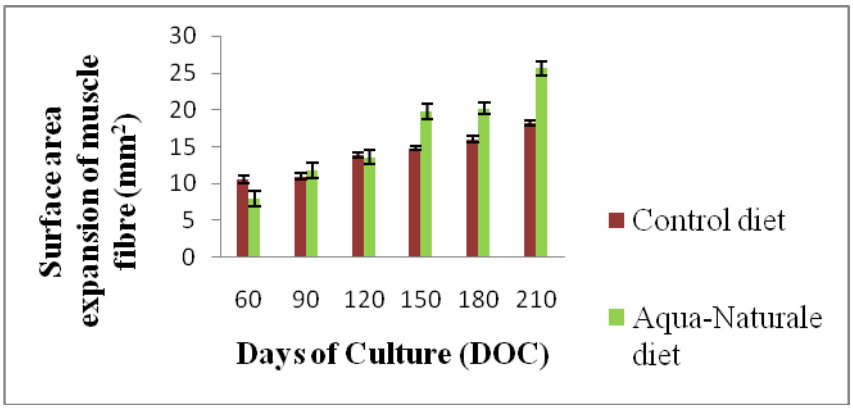

Fig.2. Variations in surface area expansion of muscle fibre in *M. rosenbergii* fed with formulated diets.

DISCUSSION

Natural plant products have been reported to promote various activities like antistress, growth promotion, appetite stimulation, tonic and immunostimulation, and to have aphrodisiac and antimicrobial properties in aquaculture operations due to the presence of active components such as alkaloids, flavanoids, pigments, phenolics, terpenoids, steroids and essential oils [10-14]. In this context, commercial herbal products having medicinal value had been evaluated in the diet of marine shrimps *Penaeus indicus* and *P. monodon* for their efficacy as growth promoter which resulted in increased growth, survival and molting efficiencies [15, 16]. Different categories of growth promoting agents have also been tested in the diet of freshwater prawn *M. rosenbergii* with proven results of significantly higher weight gain, tissue protein level, survival, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER). Growth promoting agents like brewer's yeast *Saccharomyces cerevisiae* [17], probiotics based feed [18-20], medicinal herbs *Alteranthera sessilis, Eclipta alba, Cissus quadrangularis* [21]; *Piper longum, P. nigram, Zingiber officinale* [22], *Myristica fragrans, Glycyrrhiza glabra, Quercus infectoria* [23], garlic, ginger, turmeric, fenugreek [24], *Ocimum sanctum* and *Withania somnifera* [25] had been the potential candidates of research. Marine algae (or seaweeds), comprising a category of lower group of plants are known to be the storehouse of novel bioactive compounds. Beneficial impacts of their extracts on humans, animals and plants were well recognized in the past but current advances in scientific research has led to the development of new age biotechnological products. They contain many different forms of polysaccharides, which differ in their structural patterns related to respective taxonomic classification and cell structure [26, 27]. Prebiotic role of algal polysaccharides are reported to be one of the significant factors that exert growth promoting and health improving effects [28]. But apart from polysaccharides, significant positive effects on growth and immune system are also reported due to the presence of active compounds in seaweeds as evidenced in case of aquatic animals [26]. Works have been reported on *M. rosenbergii* from the present study region where diets supplemented with green seaweed *E. intestinalis* had significant influence on growth and biochemical characteristics [7, 8]. The present study exhibits considerable increase in biomass and muscle fibre surface area of prawns fed with *Aqua*-Naturale diet as compared to control. Considering if growth of *M. rosenbergii* are dictated primarily by the efficiency at which feed is converted into muscle mass, it is likely that diet plays a specific regulatory role in controlling the expression levels of particular genes responsible for muscle growth and development in crustaceans which could provide a good molecular marker of

individual growth potential as documented earlier [29]. Observations were reported in case of Atlantic pink shrimp *Farfantepenaeus paulensis* where feed had a positive impact in regulating the expression levels of certain candidate genes related to muscle growth [30]. A strong correlation was found between body weight and expression levels of cyclophilin-like proteins in the tiger shrimp *P. monodon* [31] which may also serve as a baseline information for the present experimental work. However further studies are needed to confirm these observations. Cytomorphological analysis reveal gradual expansion of muscle fibre with time (Fig 2). The average weight gain was 32.48% with *Aqua*-Naturale diet and 28.97% with control diet whereas increment in fibre surface area was 9.77% and 4.22% respectively. ANOVA results confirm significant variations in all the selected parameters between the treatments. It is noteworthy that prawn body weight is accounted solely due to muscle fibre expansion (hypertrophy) (Fig 3 and 4). The degree of dependency is however a function of ambient ecology, since hydrological condition also plays a significant role in affecting growth rate and muscle fibre recruitment in fish [32]. Thus, a uniform allometric equation representing inter-relationship between body weight and muscle fibre expansion cannot be established because results obtained at one culture system may not be equally applicable to another system.

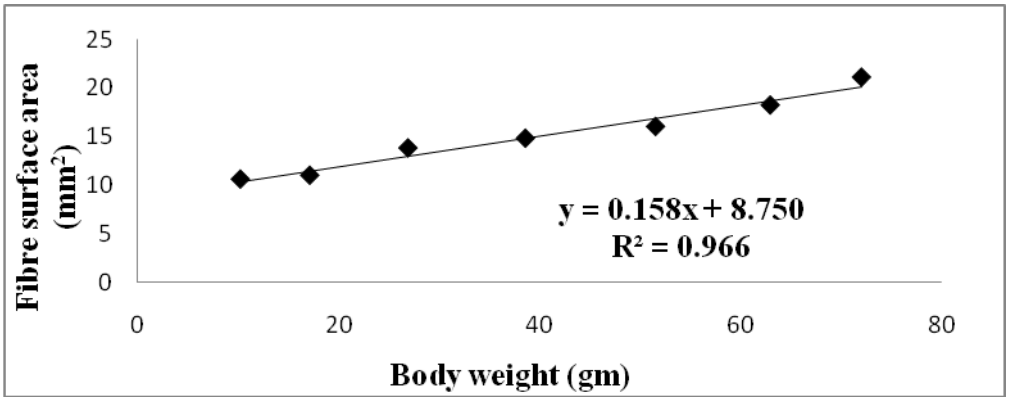

Fig 3. Allometric equation for *M. rosenbergii* fed with control diet.

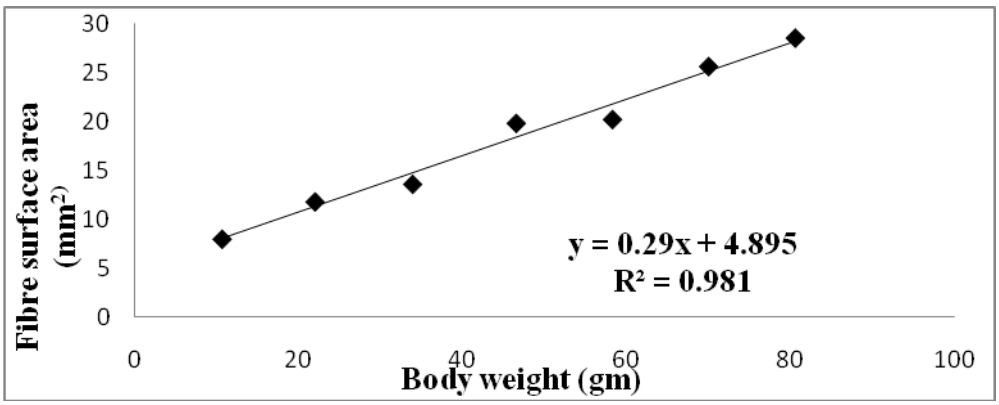

Fig 4. Allometric equation for *M. rosenbergii* fed with *Aqua*-Naturale diet.

Higher animal production and economic returns remains an integral part in any aquaculture endeavor, especially when performed with a newly developed feed supplement in order to judge whether the particular product has any probable chances of market acceptance. Usually benefit - cost ratio analysis (BCR) is a common method applied to estimate such difference. It takes into account the amount of monetary gain realized by performing an experiment versus the amount it costs to execute the experiment. The higher the BCR, the better the investment. A simple economic analysis was performed to estimate the total income and BCR derived from the *M. rosenbergii* culture experiment through supplementation of *Aqua*-Natural*e* in the diet (Table 2). Significant variations ($p<0.05$) in BCR and total income obtained among the treatments may be attributed to increased prawn production and low FCR, which are the primary success indicators in aquaculture [33]. Therefore inclusion of *Aqua*-Natural*e* in the diet resulted in higher economic return and profitability through prawn sale. The availability of *E. intestinalis* from the present geographical locale provides an opportunity for utilizing them in aquafeeds, which could be a step towards development of ecofriendly and sustainable pisciculture technology. The seaweed species can also be cultured through "rope culture technology", which is cost-effective.

CONCLUSION

The present study convey a clear message that supplementation of *E. intestinalis* derived powder (*Aqua*-Natural*e*) under the desired concentration level promotes growth in *M. rosenbergii* by enhancing biomass and muscle fibre expansion (in terms of surface area), which was evident through growth studies and histological analysis. However, this study needs further clarification with an aim to observe the effects of formulated diets of floral origin on the muscle genomic expression of the organism to arrive at more meaningful conclusion. Anyhow, the particular algae can be supplemented in prawn feed formulations for higher profitability and better economic returns.

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