



Original Article

Development of Formulated Feed for Improving Growth and Pigmentation of Shrimp (*Penaeus Monodon*) Juveniles

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ABSTRACT

Traditional method of shrimp (*Penaeus monodon*) culture is a common practice in Indian Sundarbans which is done without any sound scientific back-up especially proper feed and water quality management. The shrimp farmers use traditional feed of animal origin that often results in fouling of water quality and disease outbreak in cultured species. The present study focuses on the effect of total replacement of animal by products in shrimp feed with dust of saltmarsh grass *Porteresia coarctata* (as principal floral ingredient) on weight gain, condition index (C.I.), feed conversion ratio (FCR), survival and body pigmentation (astaxanthin level). Higher C.I. values, survival rate and gain in shrimp weight were observed in experimental pond (E) compared to control pond (C). Low FCR values were observed in the experimental pond than the control pond. Astaxanthin values in shrimps of the experimental pond were also higher than the control pond which points towards *P. coarctata* as the source of carotenoid in the shrimp tissue.

Keywords: Astaxanthin, *Porteresia coarctata*, *Penaeus monodon*, Indian Sundarban.

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INTRODUCTION

The culture of black tiger shrimp *Penaeus monodon*, Fabricius 1798 (Crustacea: Decapoda) is practiced in the brackish water system of the Indian subcontinent with the aim to increase the fish production basically for human consumption. Feed is a significant factor in increasing the productivity and profitability in the aquaculture sector (Jamu and Ayinla, 2003). From economic point of view, feed cost and feed management accounts for at least 60% of the production cost and appears to be one of the major constraints against the greater expansion of aquaculture (Kaushik, 1990). Today, fish meal replacement in the diets by another protein sources for fish and shrimp is becoming necessary and the main trend for sustainable aquaculture. In which, SM (Soybean meal) and PBM (poultry by-product meal) are basically considered as the appropriate alternatives of FM (Fish meal) in the diets for many cultured aquatic species (Hasan *et al.*, 1997; Gatlin and Hardy, 2002; Emre *et al.*, 2002;

James and Sampath, 2003; Weeks and Garling, 2010; Milamena, 2004; Stankovic *et al.*, 2011).

Considering the marine source of astaxanthin, the present paper is aimed at formulation of shrimp feed by using astaxanthin derived from coastal floral sources (*Avicennia marina*, *Avicennia alba*, *Avicennia officinalis*, *Sonneratia apetala*, *Porteracia coarctata*, *Enteromorpha intestinalis*, *Ulva lactuca*, *Catenella repens* and *Sueda sp.*) This is the first hand approach to study the effect of floral based formulated feed on water quality, shrimp quality and production, survival rate and FCR in a shrimp culture unit of Indian Sundarbans.

MATERIALS AND METHODS

Screening of mangroves for astaxanthin

The entire network of the present programme encompassed the sampling of the leaves of ten dominant mangrove species during the low tide period from the Jharkhali Island during May, 2012 to April, 2013. Leaves of the selected species were collected from two different portions (submerged lower zone and exposed upper zone) of the same plant. The lower region of the tree gets inundated during the high tide condition and upper region of the same plant remains unexposed to tidal water. In addition to true mangrove species, the astaxanthin level of few associates like *Porteracia coarctata*, *Enteromorpha intestinalis*, *Ulva lactuca*, *Catenella repens* and *Sueda sp.* were also monitored. Salinity, pH, temperature, dissolved oxygen and nutrient load of the ambient water were analysed simultaneously to pinpoint the hydrological parameters to which the plant species are exposed in natural condition. The collected leaves were thoroughly washed with ambient water followed with deionized water and oven dried at 110°C overnight. The extraction of astaxanthin was done in organic solvent as per the standard method and analysed spectrophotometrically. The mean results of all the analyses of 12 months; May, 2012 to April, 2013) are shown in Table 1.

Table 1: Mean astaxanthin content in mangrove and associated species collected from Jharkhali island of Indian Sundarbans during May, 2012 to April, 2013

Mangrove species	Astaxanthin content (mg/kg)		Percentage increase
	Submerged	Exposed	
<i>Avicennia officinalis</i>	396.56±8.9	297.75±7.8	33.19 %
<i>Avicennia alba</i>	441.33±9.6	328.02±8.6	34.54%
<i>Avicennia marina</i>	435.59±7.9	319.10±7.9	36.51%
<i>Sonneratia apetala</i>	162.80±3.2	103.49±3.7	57.31%
<i>Aegiceros corniculatum</i>	120.86±2.1	98.15±1.2	23.14%
<i>Aegialitis rotundifolia</i>	105.92±1.8	82.41±0.99	28.53%
<i>Ceriops decandra</i>	91.09±0.99	67.44±0.87	35.07%
<i>Heritiera fomes</i>	761.00±10.6	398.54±6.7	90.95%
<i>Rhizophora apiculata</i>	84.90±0.98	56.22±0.77	51.01%
<i>Bruguiera gymnorhiza</i>	461.38±3.4	397.11±5.8	16.18%
* <i>Porteracia coarctata</i>		607.56±4.6	
* <i>Enteromorpha intestinalis</i>		120.78±0.99	
* <i>Ulva lactuca</i>		56.43±0.67	
* <i>Catenella repens</i>		129.05±1.4	
* <i>Sueda sp.</i>		87.55±0.69	

Values are the mean ± SD

Remark: Mangrove associates (denoted with * sign) were not monitored during 2 tidal phases. They were collected during low tide condition

Shrimp Nutrition

Experimental Design and Layout

The study area for culturing shrimp (*P. monodon*) was selected in the central part of Indian Sundarbans in Jharkhali located in South 24 Parganas district of the state of West Bengal (22°16'40.6" N latitude & 88°38'18.4" E longitude) during January to April, 2014. The culture site is located in Sundarban Biosphere Reserve (SBR) on the bank of River Matla adjacent to Herobhanga Island (River Salinity – 22.50 ±0.68 psu). Two ponds were selected in the study

site out of which one was treated as control (C) which is 390m² and the other was treated as experimental (E) which is 780m². On the basis of astaxanthin concentration, *Porteresia coarctata* was selected for feed preparation because of its high astaxanthin content. The cultured species (*P. monodon*) in the experimental pond was provided with the *Porteresia coarctata* based formulated feed and the control pond were provided with traditional feed. Good quality shrimp seeds obtained from a local shrimp farm were stocked after proper acclimatization with ambient environmental conditions. The stocking density was 5 PL₂₀/m² in both the control and experimental ponds. The shrimps were fed initially at 15% of the biomass in each pond and the ration was then adjusted to actual consumption every day, thus reducing uneaten feed to a minimum.

Water quality parameters were monitored continuously 90 days culture period (15th January to 15th April, 2014) which were in acceptable ranges. Dissolved oxygen (D.O.), pH, transparency and nutrients were analyzed following the standard spectrophotometric method (Strickland and Parsons, 1968, 1972). Phytopigment concentration (Chl *a*) was analysed as per the method of Jeffrey and Humphrey, 1975. Organic carbon content of pond bottom soil was estimated by the standard titration method (Walkey and Black, 1934).

Diet Preparation

The experimental pond was provided with *Porteresia coarctata* dust to replace totally the fish and shrimp meal component (Table 2) of the traditional fish feed. Commercial diet from local market was given to the control pond. The ingredients used in the feed were accurately weighed and the feeds were prepared following the method by Jayaram and Shetty (1981).

Table 2: Ingredients (gm) and proximate composition (% DW) of diets of experimental pond and control pond

Component	Control (gm)	Experimental (gm)
Rice bran	250	250
Wheat bran	250	250
Fish meal	150	Salt marsh grass dust = 300
Shrimp meal	150	
Sun flower oil cake	50	50
Mustard oil cake	50	50
Vit. + Min. Mixture	50	50
Binder	30	30
Shark oil	20	20
Proximate Analysis (%)		
Crude protein	34.37±1.2	36.78±1.5
Soluble carbohydrate	32.67±2.6	38.10±0.67
Crude Fat	7.1±0.80	6.5±0.22
Ash	9.1±0.12	13.1±0.34
Moisture	8.9±0.10	10.7±0.98

Zoo-Technical Parameters and Statistical Analysis

Individual weights and lengths of shrimps were taken at fortnightly interval for 90 days culture period and the relevant response variables were determined for each control and experimental ponds. Condition Index (C.I.) was analysed at fortnightly interval during the culture period as per the expression; $C.I. = W/L^3 \times 100$, where W = weight of the cultured species (in gm) and L = length of the cultured species (in cm). Percentage weight gain was calculated as the difference in weight from the average final weight with respect to the initial weight; $\text{weight gain} = [(\text{average individual final weight} - \text{average individual initial weight}) / \text{average individual initial weight}] \times 100$. Feed consumption reported was the total of the consumption estimated for 90 days period. The survival rate was determined as percentage of the difference of stocking number and number recovered at the end of the experimental

trial. Feed Conversion Ratio (FCR) was analysed after the harvesting of shrimps as per the expression: $FCR = f/b$, where, f = Change in feed biomass and b = Change in body biomass of the cultured species. Astaxanthin in the shrimp tissue was estimated as per the standard spectrophotometric method (Schuep and Schierle, 1995) and body pigmentation was assessed for each treatment on shrimp cooked for 5 min in boiling water and comparing the orange colouration with Roche SalmoFan™ colour score.

All data were expressed in terms of mean and standard deviations / range (\pm SD/ range). Analysis of variance (ANOVA) was computed between all the selected parameters (physico-chemical and zoo technical) considering both control and experimental ponds to evaluate the differences caused by inclusion of *Porteresia coarctata* dust in the feed.

RESULTS AND DISCUSSION

The astaxanthin level in the selected mangrove species (collected from Jharkhali region) exhibited significant variations. It is of the order *Heritiera fomes* > *Bruguiera gymnorrhiza* > *Avicennia alba* > *Avicennia marina* > *Avicennia officinalis* > *Sonneratia apetala* > *Aegiceros corniculatum* > *Aegialitis rotundifolia* > *Ceriops decandra* > *Rhizophora apiculata* (Table 1). The relatively greater astaxanthin content in the submerged leaves of mangroves confirms the synthesis of astaxanthin content under stressful condition. Our results agree with several reports of decrease content of carotenoids by salinity as reported in a number of glycophytes (Gadallah., 1999; Agastian *et al.*, 2000, Mitra and Banerjee, 2010). However, more studies are needed to confirm the influence of tidal influx and subsequent salinity fluctuation on astaxanthin level in the mangrove floral parts. The present data may serve as baseline information on the regulatory role of tidal submergence on astaxanthin level in the estuarine and coastal vegetation as mentioned by Mitra *et al.*, 2006. The enhancement of astaxanthin production under stressed condition of organisms is a matter of interest and several researches are still being undertaken to pinpoint the reaction pathway of astaxanthin production by inducing stress of varied nature (Mitra *et al.*, 2006). Many types of yeast have been described with an increase ability to produce carotenoids when they grow under unfavourable environment (Certik *et al.*, 2005). Several workers have reported both in the dark and light the enhancement of the accumulation of astaxanthin in cysts of *Haematococcus pluvialis* under salt stress conditions (Harker *et al.*, 1996; Lee, 1999; Boussiba, 2000; Park and Lee, 2000, 2001; Steinbrenner and Linden, 2003 and Chio *et al.*, 2003). The present study points to higher astaxanthin level in those leaves of the mangroves that are inundated for 10 to 12 hours by tidal waters of Jharkhali station having typical estuarine water characteristics (salinity = 10 - 25.85 psu, pH = 7.98 - 8.28, temperature = 29.8 - 31.5°C, dissolved oxygen = 5.93 - 6.10mg/l, NO₃ = 15.09 - 21.04 µgat/l, PO₄ = 1.12 -1.39 µgat/l and SiO₃ = 64.44 - 83.16 µgat/l). The enhancement of astaxanthin level in the inundated Sundari leaves (*Heritiera fomes*) clearly reflects the highest degree of stress posed by water salinity on this species. *Heritiera fomes*, being fresh water loving mangrove species cannot tolerate high salinity (Mitra and Pal, 2002) and thus acceleration of astaxanthin production may probably be a part of its adaptation to cope with the stenohaline condition of coastal and estuarine environment that becomes acute during high tide. The astaxanthin level of mangrove flora is thus a function of its physiological system, which is extremely species specific. Highest astaxanthin was recorded in mangrove associate *Porteresia coarctata* (commonly known as salt marsh grass) in comparison to other species and therefore considered for feed preparation.

Shrimp Nutrition and Growth

Shrimps fed with *Porteresia* diet exhibited higher final weights and better weight gain (Table 3) at the end of the experiment (27.2 gm final weight) in comparison to control pond (20.5 gm final weight). Condition Index values of shrimp were also higher in experimental ponds (3.87 ± 0.67) than control pond (2.98 ± 0.55) (Table 3). The FCR value for control pond was 1.67

and for experimental pond was 1.27. The survival rate was found to be 58.4% in the control pond and 69.2% in experimental pond. The present study speaks in favour of healthy pond environment, better growth, higher survival rate and low FCR values through use of *Porteresia* based feed.

An important factor governing the consumer acceptance and market value of many cultivated fish and shrimp species is the pink or red colouration of their flesh or boiled exoskeleton (Brun and Vidal, 2006). In the wild, this colouration is achieved through the ingestion of carotenoid pigments particularly astaxanthin contained within invertebrate food organisms (Johnson *et al.*, 1977; Ibrahim *et al.*, 1984). The *Porteresia* based feed in the present study resulted in higher astaxanthin values in shrimps of experimental pond (15.32 ± 1.22 ppm; $n = 10$) as reflected through darker orange-red colouration of shrimp exoskeleton in comparison to control pond (8.66 ± 0.78 ppm). The Roche SalmoFan™ colour score showed the color value of 23 and 29 for shrimps from control pond and experimental ponds respectively (Table 3), which confirms the variation of astaxanthin level due to different feed ingredients.

Table 3: Growth parameters of *Penaeus monodon* fed *Porteresia* based feed

Parameters	Control pond	Experimental pond
Condition Index of shrimp	2.98 ± 0.55	3.87 ± 0.67
Wt. at harvest (g)	20.2	27.5
Astaxanthin in shrimp (ppm)	8.66 ± 0.78	15.32 ± 1.22
Roche SalmoFan™ colour score	23	29
Survival rate (%)	58.4	69.2
FCR	1.67	1.27

Water Quality Analysis

Hydrological parameters of the shrimp culture ponds are a reflection of the quality of feed provided and its response in morphometric parameters. (Maceina and Murphy, 1998) (Table 4).

Surface water temperature in both the culture ponds showed more or less parallel trend of variation throughout the study period. The uniformity in temperature profile is due to the location of both the ponds in the same site that experienced similar weather conditions. Water temperature plays a major role in shrimp enzyme kinetics which may have a regulatory influence on their growth (Mitra *et al.*, 2006). It also affects the process of molting during the post larval stage of shrimps (WWF-India, 2006).

The salinity of the Hugli-Matla estuarine complex is known to exhibit intensive variations (Saha *et al.*, 1995). The difference in salinity between ponds may be attributed to the different soil salinity (as substratum of the pond) that leaches soluble salt to the pond water. The relatively higher C.I. values in the experimental pond with less salinity proves the efficiency of formulated feed in combating the stress posed by salinity.

Shrimp culture directly affects the pH of the pond bottom through deposition of excess feed, shrimp excreta, dead shrimps, etc. This shifts the soil and overlying aquatic pH towards acidic condition. In the present study, such condition was not observed owing to the traditional practice of liming at a regular interval of time.

Dissolved oxygen (D.O.) is a vital parameter regulating the aquatic life. The shrimp health is a direct function of dissolved oxygen and its diurnal variation. Excessive organic load in the system results in lowering the D.O. value during night/dawn posing threat to the survival of aquatic life. In the present study the D.O. level in the control pond showed lower value owing to deposition of organic carbon at the bottom of the pond. The significant variation of D.O. between ponds may be attributed to different growth rate of the culture species and also the use of different types of feed. Traditional feed contains dry fish dust and trash shrimp dust which lowers the D.O. due to their utilization for oxidizing the residual matter (BOD value

increases under this situation). Floral based feed, on the other hand generates very limited residue due to which D.O. remains almost unaltered.

Transparency controls the phytoplankton standing stock in shrimp culture ponds due to their dependency on the solar radiation for photosynthesis. The experimental pond provided with formulated feed showed increased transparency due to its unique binding property. The ready acceptance of the *Porteresia* based feed by the cultured species in the experimental pond may be the basis of reduced suspended particulate matter in the aquatic phase of the experimental pond.

Nutrients (comprising of nitrate, phosphate and silicate) budget in the aquatic phase of the culture ponds are regulated through quantum of excretory products of the cultured species, left over feed and also by the churning of the pond bed (due to run-off from the adjacent land masses). High concentration of nitrate in the control pond may be due to leaching of the feed ingredients (particularly from animal component in traditional feed) in pond water and also the faecal matter that generates ammonia (Mitra and Choudhury, 1995). The phosphate concentration during the study period showed no significant variation between the ponds owing to ban imposed on washing utensils, clothes and other daily house-hold activities during the culture period. The silicate level of the ponds may be attributed to substratum or pond bottom composition. In both the control and experimental ponds no significant variation in silicate was observed.

Soil organic carbon was greater in control pond due to more generation of residual feed and excreta in the absence of any feed management. On contrary, the lower value of organic carbon in the experimental pond is an indication of better acceptability of *Porteresia* based feed by shrimp due to which wastage was minimum.

Table 4: Variation in the physico-chemical parameters of the culture ponds

Parameters	Control pond	Experimental pond
Surface water temperature (°C)	29.4 ± 0.22	29.4 ± 0.22
Surface water salinity (psu)	18.56 ± 0.20	15.08 ± 0.13
pH	7.88 ± 0.35	8.08 ± 0.03
Transparency (cm)	17.02 ± 2.02	24.3 ± 2.58
Dissolved oxygen (mg l ⁻¹)	4.66 ± 0.77	5.47 ± 0.12
Nitrate (µgat l ⁻¹)	19.5 ± 1.21	16.6 ± 1.01
Phosphate (µgat l ⁻¹)	2.22 ± 0.05	2.19 ± 0.29
Silicate (µgat l ⁻¹)	64.01 ± 2.23	64.32 ± 2.67
Chlorophyll <i>a</i> (mg m ⁻³)	1.74 ± 0.51	1.92 ± 0.16
Soil organic carbon (%)	1.18 ± 0.16	0.97 ± 0.10

Values are the mean ± SD

The replacement of dietary fish meal in the diets of fish and shrimp by another protein sources derived from animal, plant and biology has been considered as a practical solution for sustainable aquaculture development. Thus, the finding of alternatives of fish meal is absolutely in compliance with the trend of using another protein sources to replace fish meal in the diets of aquatic animal. Recent studies proved that several plant and animal protein sources can be used for partial and totally fish meal replacement in the diets for many aquatic species. In which, PBM and SM have been commonly used as the suitable sources to replace FM and other animal protein in the diets for fish and shrimp. Yesilayer *et al.*, (2011) found that it had no adverse effects on growth performance of koi juveniles fed the diets containing 0% FM and 16% FM, correspondingly with 45% SM and 22.5% SM in the diet. Ismail *et al.*, 2013 reported that 100% fish meal in the diet of Malaysian mahseer (*Tor tambroides*) can be replaced by poultry offal meal without adversely effecting survival and growth and body composition. Tri and Davis (2009) asserted that dietary FM can be totally replaced in commercial diets for tilapia juveniles by de-hulled soybean meal (solvent extracted) and expelled soybean meal (pressed) without adversely effecting growth and feed utilization.

Yang *et al.* (2004) recommended that PBM could replace up to 500 g kg⁻¹ of FM protein in diets for gibel carp (*Carassius auratus gibelio*) without negative effects on growth. Likewise, Saadiah and Abol-Munafi (2011) found 100% dietary FM can be replaced by PBM in the diets for cobia, *Rachycentron canadum* without adversely affecting growth performance, but an optimal replacement level at approximately 60% was recommended for better growth and efficient feed utilization. Whereas, the replacement of FM with PBM in the diets for mirror carp fingerling (*Cyprinus carpio*) with the range of PBM from 12-36% showed significant differences and as increasing the PBM content in the diets resulted in lower WG and higher feed utilization (Emre *et al.*, 2003). Not only SBM (Soybean meal) and PBM (poultry by-product meal), but also the other protein sources derived from plant and biology have been used as potential protein sources to replace dietary fish meal for reducing feed cost as well as exhausted exploitation of wild fish. Korkmaz and Cakirogullari, (2011) found that Dried Baker's Yeast can be used to replace up to 30% dietary fish meal in the diets for koi carp fingerlings that did not show significant differences in growth compared to those at the control diet without FM replacement. Similarly, Mazurkiewicz (2009) reported that replacement of dietary FM by plant-derived protein with the ration of plant protein (legume seeds and extracted rapeseed meal) up to 26% in the diets (35% protein, 22% FM) for carp fingerlings and early juveniles without adversely affecting the rearing results. Kim *et al.*, (2013) indicated that spirulina can replace dietary fish meal up to 26% in the diet for parrot fish (*Oplegnathus fasciatus*) without negative effects in weight gain and feed efficiency.

CONCLUSIONS

Aquaculture has become a peak industry in the present millennium, which involves seafood farming with shrimp, cuttle fish, squid, lobster and other such culinary delights actually "cultivated" in aquatic enclosures under scientifically controlled conditions (Rajkhowa, 2005). The use of nutrient-rich feed continues to gain wide acceptance in the aquaculture industry in order to boost up the quality of the aquacultural products. The use of quality feed results in substantial reduction in the overall variable cost of an operation through improved animal performance, better FCR and improved water quality due to a reduction in the amount of nutrients and solids (*i.e.*, feces and uneaten food) in the waste water effluent. *P. coarctata* based formulated feed showed better growth performance of the cultured species with respect to condition index values and survival rate. Body pigmentation improved in the cultured species of experimental pond and showed significantly higher astaxanthin level than the controlled pond. A series of experiments are still needed for time testing the results and make the programme sustainable for the poor island dwellers of lower Gangetic delta.

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