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Culture of *Macrobrachium rosenbergii* using indigenous floral extract in the hilly terrains of Koraput district of Odisha, India

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ABSTRACT

An experimental design for culturing *Macrobrachium rosenbergii* in four experimental ponds of size 25 m^2 was taken up in Koraput district of Odisha, India. The ponds were filled with water and *Macrobrachium rosenbergii* PL_{20} were released in the ponds with stocking density @ 4 m^{-2}. Ponds 1 and 2 were fed with Conventional Feed (fish meal) and ponds 3 and 4 were fed with Formulated Feed (Soybean + *Cynodon dactylon* + *Ulva lactuca*). Water quality parameters like surface water temperature, pH, dissolved oxygen, transparency, nitrate, phosphate and silicate, chlorophyll *a* and organic carbon were monitored for 195 days. Significant variation ($p < 0.05$) in the water quality parameters were observed during the culture period between ponds respectively. Growth parameters like daily growth rate (DGR), specific growth rate (SGR) and condition index (CI) were monitored simultaneously which showed a discrete growth during the first 90 days and then a stable growth form. Length and weight relationship of prawns were calculated for all the culture ponds which showed highly positive relationship ($p < 0.01$) proving unique contribution of feed towards the growth of prawns. Feed conversion ratio (FCR) values (Ranging from 2.74 ± .05 to 2.95 ± 0.04) also proved the greater acceptance of feed towards the growth of prawns. Biochemical analysis of the feed and prawn (protein, carbohydrate, lipid and astaxanthin) also proved the feed efficiency towards growth and coloration of prawn. Benefit cost analysis (BCA) computed for conventional feed (1.23) and formulated feed (1.62) yielded 637 kg ha^{-1} and 865 kg ha^{-1} respectively.

1. Introduction

The giant freshwater prawn *Macrobrachium rosenbergii* offers high potential for its farming due to its faster growth rate, better survival rate, disease susceptibility, tolerance to wide range of temperature and high international export value [1]. This species has wide acceptance to both plant and animal based diet and its growth performance is a function of water quality, stocking density, feeding system etc. Apart from temperature and good water quality assurance which are necessary for its growth and survival, use of good quality supplementary feed is also ascertained to increase the carrying capacity of culture system and enhance production in many folds [2]. Like other animals, *M. rosenbergii* also requires proteins, lipids, carbohydrates, vitamins and minerals for its growth and hence proper feed composition with optimum culture production ensures the sustainable growth rate, survival rate and breeding efficiency [3].

In a successful prawn culture a great deal of attention is generally given towards feed management. Feed constitute 40–60% of operational cost [4]. Fish meal is generally used as one of the highest quality protein sources although it is most expensive [5], particularly used for finfish and crustaceans [6]. To reduce the production cost of expensive animal protein, less expensive plant protein is desirable to reach the satisfactory growth and feed efficiency. Feed containing 40% protein has been proved to be excellent for sustainable freshwater prawn culture and soybean meal is a promising ingredient to alternative protein sources for many crustaceans [7, 8, 9].

The commercially cultured prawns are generally fed with diet composing of animal origin which deteriorates the aquatic medium and are quite expensive. As the prawns are capable of digesting a wide range of foods (comprising of both plants and animal origin, it is desirable to use the formulated feed prepared from plant origin with required amount of nutrients. The economic nutritional potential of freshwater prawn farming hinges on the availability of seeds, good quality feed and effective pond management [10]. Culturing *M. rosenbergii* in rice field or low land area are very common practices in all over India. However culturing *M. rosenbergii* utilizing the water fall resource as water sources and utilizing the indigenous plant resources from the adjoining villages is a new innovative on our part. Freshwater resources from rivers, lakes, and

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waterfalls are highly unexplored in the hilly terrains, which has immense potentiality for its varying temperature range from 10 - 25 °C. This wide adaptability of temperature has boosted the present research program to be undertaken in the hilly terrains of the northern part of Eastern Ghats. The indigenously available protein sources along with soybean meal have been monitored in preparation of good and viable feed.

The general livelihood of people in Koraput district of Odisha depends on agriculture in valleys or shifting cultivation on the hills. The district is mostly tribal dominated and there is immense pressure on the forest resources. Till date no research or initiative has been taken to explore the vast waterfall resources in utilizing it for aquaculture purposes. The present research program is thus a new initiative in exploring the available water resources in terms of aquaculture practice and find out the viability of such culture in the area.

2. Materials and methods

2.1. Site description

Koraput is a tribal dominated district with a population of 13.80 lakhs (Census, 2011) situated in the southern part of Odisha state ($18^0$13' and $19^0$10' North latitude and $82^0$5' and $83^0$13' East longitude) in India having a geographical area of 8,807 sq km. Physiographically the district is contiguous to the main land of Eastern Ghats high land zone. The general topography of the study area of the district is of broken mountains intercepted by large riverbeds and watercourses. The altitude of the area varies from 500 m near western side to 1600 m on the eastern side with mountain peaks and ridges. The entire district is predominated by laterite soil. The minimum and maximum temperature of the district is 13° and 42 °C in the month of December and May respectively and humidity is generally high especially in the monsoon and post monsoon months. The district receives about 1500 mm rainfall annually. Major portion of the annual rainfall during southwest monsoon occurs between July to September. The district is endowed with 4 major water falls (Bagra, Duduma, Raniduduma and Galigabdar waterfalls) and 6 major rivers (Indravati, Sabari (Kolab), Sileru, Vegavati, Subarnamukhi, Jaryhavati and their tributaries) which are perennial in nature and seem to be unexplored with respect to aquaculture.

The study area was undertaken in the Nandapur village (18^o 33 '35.105" N and 82^o 43' 58.738" E, altitude 891.5 m above MSL) of Koraput district lying between the valleys of the Eastern Ghats hill ranges. A perennial river named Gangasuni arises from the eastern part of the

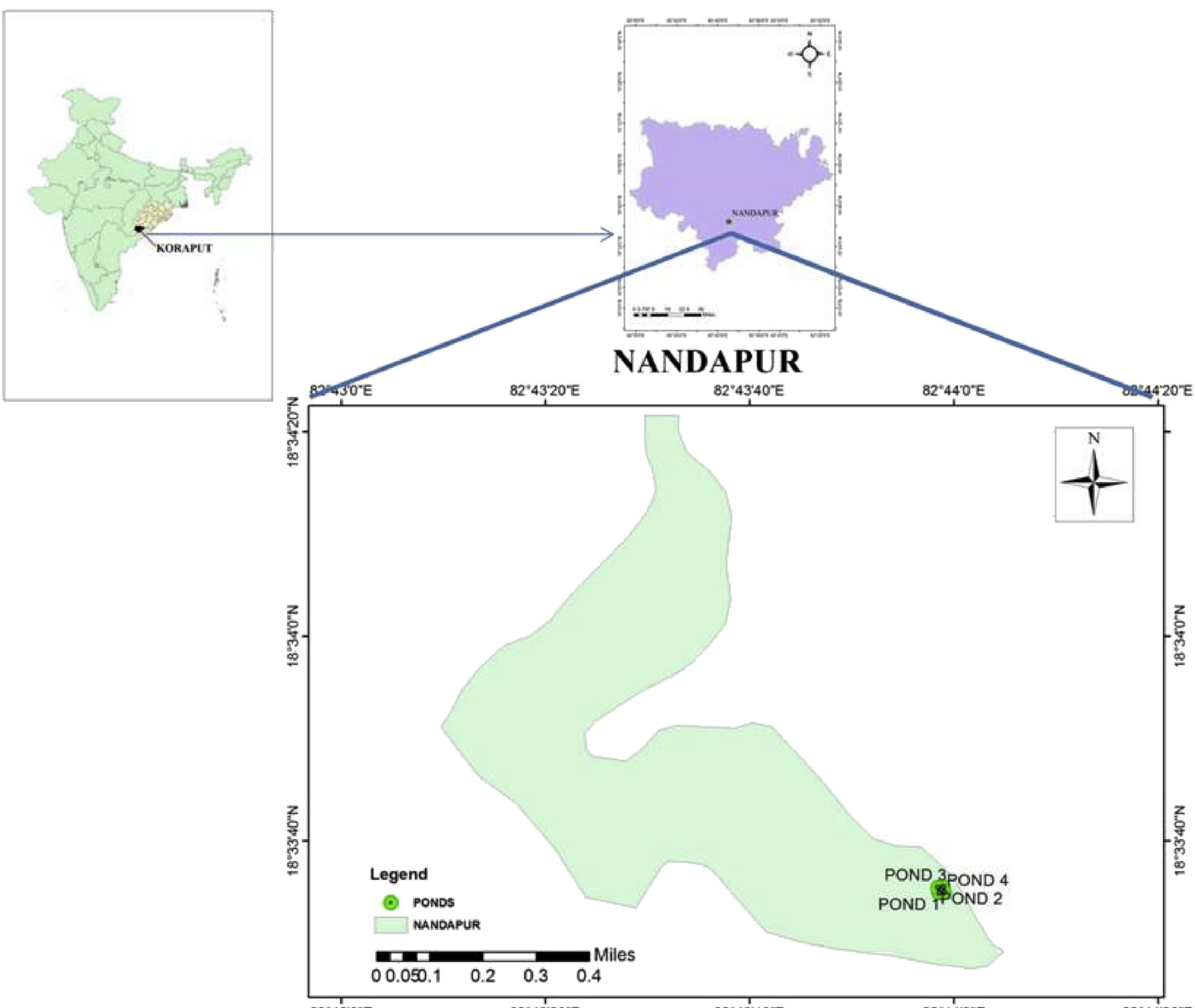


Fig. 1. Map of the study site.

village and facilitates irrigation to huge agricultural lands. The population of Nandapur block is 72,579 (Census-2011) and out of that 43,368 are engaged in different works (61.2% of the workers have earning more than 6 months and remaining 38.8% are involved in marginal activities providing livelihood for less than 6 months), 19,459 are cultivators (owner or co-owner) and 4,412 are agricultural labourers (Fig. 1).

2.2. Feed preparation

In the present study feed was formulated from plant origin ingredients replacing animal protein with soybean meal as principle ingredient and other locally available ingredients like ground nut oil cake, rice bran, *Panicum sumatrense* (Indian millet), liquid extract of grass (*Cynodon dactylon*) based on the recommended nutrient requirements for *M. rosenbergii* [11]. Commercially available conventional feed from CIFA, Bhubaneswar was purchased and used whose ingredients are generally made up of fish meal, wheat flour, soybean meal, groundnut oil cake, vitamins, minerals etc. The ingredients of both conventional and formulated feed have been displayed in (Table 1).

Feed formulation was done taking commercially available conventional feed (CF) as Reference Control feed (Commercial Feed) whose ingredients are generally made up of fish meal, wheat flour, hi pro soybean meal, lecithin, squid liver meal, clam meal, cholesterol, fish oil, phospholipids, vitamins and minerals. Commercially available conventional feed was taken as control. The composition of conventional feed in the present study was wheat flour 10%, rice bran 14%, soybean meal 10%, fish meal 30%, groundnut oil cake 34%, vitamins + minerals 2% and Formulated Feed (FF) ingredients are wheat flour 10%, soybean meal 45%, groundnut oil cake 15%, 8% of liquid extract of *Cynodon dactylon*. Apart from the above ingredients 5% of rice bran, 14% of *Panicum sumatrense*, 2% vitamin and mineral mixtures, 0.5 % binder (Maida or refined white wheat flour) and 0.5% attractant (seaweed *Ulva lactuca* powder) were used to prepare the formulated feed (Table 1).

The feed ingredients were purchased from the local markets viz. soybean meals @ Rs. 68/- per kg, rice bran @ Rs.8/- per kg, ground nut oil cake @ Rs.60/- per kg, wheat flour @ Rs.30/- per kg, vitamins and minerals @ Rs.1/- per 2 gm, maida (super refined wheat flour) @ Rs.20/- per kg and *Panicum sumatrense* (Indian millet) @ Rs.15/- per kg. Seaweed (*Ulva lactuca*) and liquid extract of grass (*Cynodon dactylon*) were collected naturally from wild and hence their costs were not included in the total cost of the feed (Table 1). Formulated feed was prepared from indigenous basal ingredients, such as soybean meal (*Glysine max*), rice bran (*Oryza sativa*), groundnut oil cake (*Arachis hypogaea)*, seaweed (*Ulva lactuca*) etc which were ground separately using a micro pulverizer. For the supplement of vitamins and minerals, Vitamin B-Complex forte with vitamin C manufactured by Pfizer Limited (composed of thiamine mononitrate I.P. 10 mg, riboflavin I.P. 10 mg, pyridoxine hydrochloride I.P. 3 mg, vitamin B_{12} I.P. (as tablets 1:100) 15 mcg, niacinamide I.P.100 mg, calcium pantothenate I.P. 50 gm, folic acid I.P. 1.5 mg, biotin U.S.P. 100 mcg, ascorbic acid I.P. 150 mg) was used to prepare a balanced diet (Table 1). Powdered ingredients were weighed out to prepare feed mix based on Pearson's square method equated to 40% protein. The measured liquid extract of grass (*Cynodon dactylon*) was added as astaxanthin supplement to the feed mix during steam cooking at 95-100 °C for 5 min and allowed to cool at room temperature. Then dough was prepared by adding required amount of binder (Maida flour) and pelletized in a manual pelletizer. The pellets were collected in tin trays and sun dried until the moisture content is less than 10%. The pellets of different sizes were prepared on a smooth surface.

The sun dried feed pellets were physically examined for visual appearance, such as uniformity, colour and fragrant smell and proximate composition was analysed for protein, carbohydrate, lipid and astaxanthin following standard procedure.

2.3. Biochemical analysis of feed and cultured prawns

Conventional feed and formulated feed in addition to the harvested prawns fed with different feeds also analyzed for proximate composition (like protein, carbohydrate, lipid and astaxanthin) by the standard AOAC method [12]. The ethical approval for the experiments was not required because it is already a cultured species and seeds were obtained from Central Institute of Freshwater Aquaculture, Govt. of India, Bhubaneswar, Odisha.

2.4. Experimental design and stocking

Four ponds of size 5 m × 5 m were constructed in the study area and pond preparation was done during the summer months (April–May 2017). The pond bottom was limed and sprinkled with potassium permanganate ($KMnO_4$) to balance the pH and avoid the anoxic condition. Water was filled in the ponds utilizing the natural water sources (waterfall channel) and was fertilized with liquid fertilizer (cow dung @ 0.1 kg m^{-2}) to achieve an algal bloom. The experiment was set up for 195 days during June to December 2017. Water surface area of all experimental ponds was 25 m^2 with an average depth of 1.5 m. One third of the water was exchanged fortnightly and was again refilled from the water

Table 1
Proximate composition of conventional feed, formulated feed and its cost.

Nutrient suppliment	Ingredients	Source	Conventional feed (%)	Formulated feed (%)	Cost of formulated feed (Rs./kg)
Carbohydrate	Wheat flour	Local market	10	10	03.00
	Rice bran	Local rice mill	14	05	00.40
	Panicum sumatrense (Indian millets)	Local market	-	14	02.10
Protein	Soybean meal	Local market	10	45	30.60
	Fish meal	Local fisherman	30	-	-
Lipid	Ground nut oil cake	Local market	34	15	09.00
Vitamins + Minerals mixture		Local market	02	02	10.00
Astaxanthin	% of Liquid extract of grass (*C. dactylon*)	Fringe areas of forest	-	08	0
Binder	Maida (Super refined flour of wheat)	Local market	-	0.5	00.10
Attractant	Seaweed (*Ulva lactuca.)* powder	Visakhapatnam coast	-	0.5	0
Total			100	100	55.20
Proximate composition of feeds					
Protein (%)			28.4 ± 1.05	32.8 ± 0.95	-
Carbohydrate (%)			8.5 ± 1.24	10.6 ± 1.82	-
Lipid (%)			4.6 ± 0.65	4.2 ± 0.75	-
Astaxanthin (ppm)			310.4 ± 7.45	591.2 ± 5.54	-

Each of the value is expressed as Mean ± SD of three individual observations, - means absent.

fall resource. PL_{15} seeds of *M. rosenbergii* were purchased from Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar. Prior to stocking, the seeds were acclimatized in a nursery pond for 15 days. The prawns (PL_{30}) were hand counted and stocked in each pond at a density of 4 m^{-2} on date 1st June 2017. The initial size of prawns during stocking was PL_{30}. The mean stocking weight was determined from the 50 prawns that were blotted free of surface water and individually weighed. Two ponds each were taken as replicate for treatment 1 and treatment 2. Treatment 1 was fed with conventional feed and treatment 2 was fed with self prepared formulated feed.

2.5. Sample collection and water quality monitoring

Throughout the culture period, water parameters like temperature, pH, dissolved oxygen, transparency and water nutrients like nitrate, phosphate, silicate, chlorophyll *a* were monitored fortnightly. Surface water temperature was determined in-situ by a digital thermometer (MEXTECH Multi stem handheld portable LCD digital thermometer with sensor probe -50 °C–300 °C). The pH was measured in the field by pH meter (Oakton eco-testr pH 2 Waterproof pH tester 0.0–14.0 pH range). Dissolved oxygen fixation was carried out in the field in 100–120 ml BOD (biological oxygen demand) bottles. Acidification and titration were accomplished at the laboratory following the standard Winkler method [13]. Transparency was measured in-situ by Secchi discs of 20 cm diameter. The water samples from each pond were collected (usually from 5-10 cm depth from the water surface) in cleaned 1 L tarson bottle and taken to laboratory by covering with black polythene for analysis of water nutrients and chlorophyll *a*. Water nutrients like nitrate, phosphate and silicate were estimated by following the standard procedure of [14]. Chlorophyll *a* was measured spectrophotometrically after 90% acetone extraction [15]. Soil of the pond bottom was analyzed for organic carbon in order to find out the unutilized feed deposition by following the standard procedure of [16]. All the sampling and in-situ measurements were carried out in the same time between 9 am to 10 am in every fifteen days during the culture period.

2.6. Feeding and growth measurement

Supplementary feeds of Treatment 1 (Conventional feed) were applied in ponds 1 and 2, and Treatment 2 (Formulated feed) were applied in ponds 3 and 4 respectively. Feeds were applied with the 20% of the body weight of prawns for first 30 days (1st month), 15% of the body weight for next 30 days (2nd month), 10% of the body weight in next 60 days (3rd and 4th month), 5% of the body weight for next 30 days (5th month) and 2% of the body weight till the end of the experiment. The daily ration was adjusted based on total biomass through monthly sampling with 60% of body weight of feed during night and 40% in the day respectively. The sampling of prawn juveniles was done with the help of cast net for the determination of body weight and length. The experimental data related to growth parameters like gain in weight, gain in length, survival rate (SR), daily growth rate (DGR), specific growth rate (SGR), condition index (CI) and feed conversion ratio (FCR) were determined as per the method outlined by [17].

i) Gain in weight (g): Average initial and final weight was measured by using digital weighing balance. The gain in weight was determined by the following formula

$$\text{Gain in weight} = \text{Mean final weight} - \text{Mean initial weight} \tag{1}$$

ii) Gain in length: Average initial length and final length was measured with the help of measuring scale. The gain in length was determined by using the following formula

$$\text{Gain in length} = \text{Mean final length} - \text{Mean initial length} \tag{2}$$

iii) Survival rate (%): Survival rate (%) was calculated as per the following formula

$$\text{Survival rate } (\%) = \frac{\text{No.of total live prawns harvested}}{\text{Total No.of prawns stocked}} \times 100 \tag{3}$$

iv) Daily growth rate (DGR) (gm day^{-1}): Daily growth rate was calculated using the expression given bellow

$$\text{Daily growth rate (DGR)} = \frac{\text{Wf (final wet weight)} - \text{Wi (initial wet weight)}}{\text{t (in days)}} \tag{4}$$

v) Specific growth rate (SGR) (% day^{-1}): In aquaculture specific growth rate is used to estimate the production of cultured species after a certain period of stocking and rearing. Specific growth rate was calculated after harvesting of prawns as per the expression:

$$\text{SGR } (\% \text{ per day}) = \frac{\ln W2 - \ln W1}{t} \times 100 \tag{5}$$

Where, W1 and W2 are initial and final body weight of prawn and 't' is experimental time period in days.

vi) Condition Index (CI): Condition index reflects the interactions between biotic and abiotic factors in the physiological condition of prawns and it gives the health condition of prawns based on weight at a given length [18]. Biometric measurement for length and weight was calculated from tip of rostrum to tip of telson as per FAO species identification [19]. The mathematical relationship before length and weight was calculated as per conventional formula

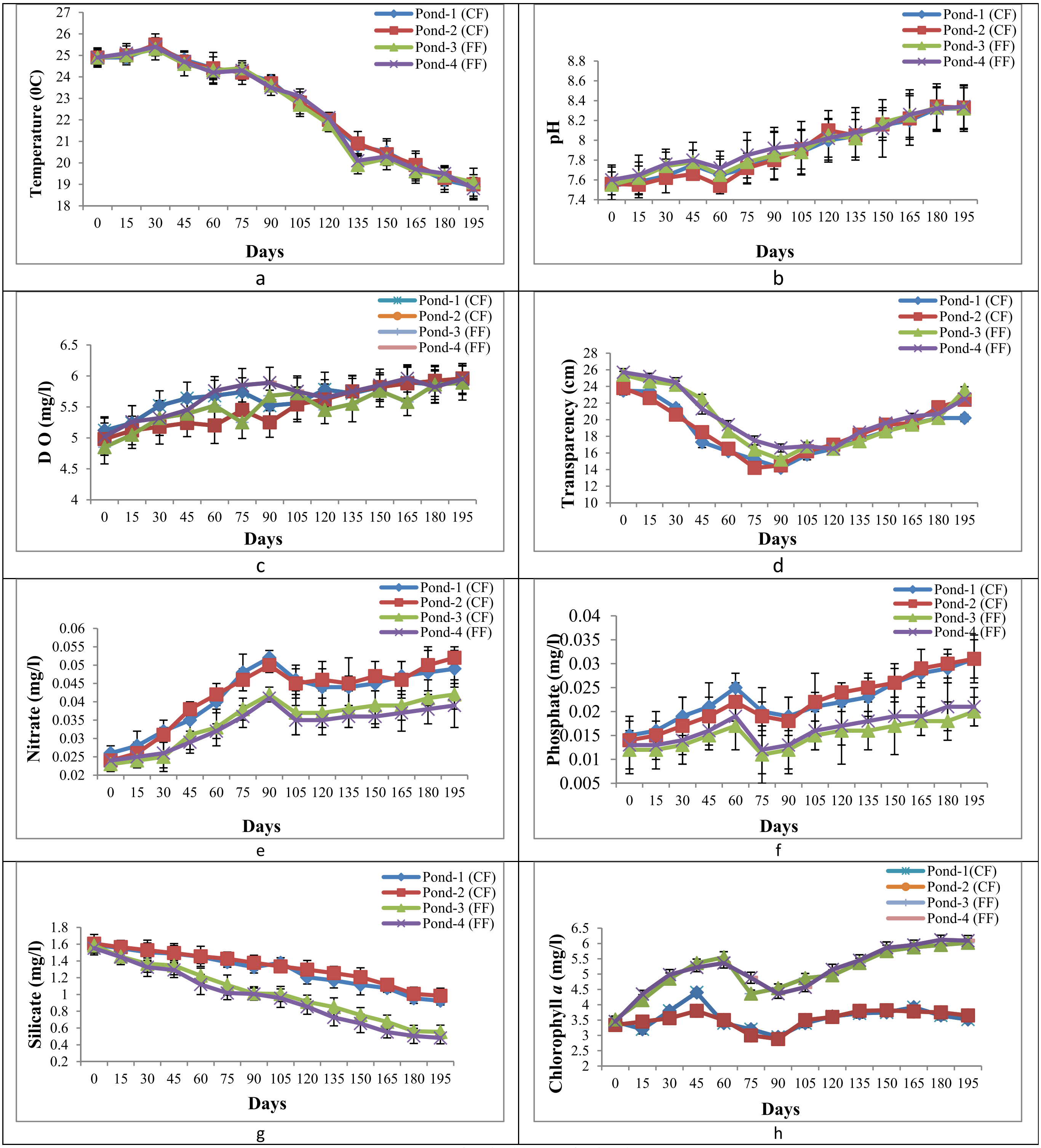
$$W = a\,.TL^{b} \tag{6}$$

Where, W = total weight, TL = Total Length, a = proportionality constant, b = isometric exponent [20].

vii) Feed Conversion Ratio (FCR): It gives the mathematical relationship between the input of the feed that has been fed to an organism and the weight gained by that organism. The lower the

Table 2
T-test for biochemical parameters of conventional and formulated feed.

Parameters	Combinations	t	df	Sig. (2-tailed)	Mean diff.	95% confidence interval of difference	
						Lower	Upper
Protein	Conventional	48.21	2	0.000	28.43	25.89	30.97
	Formulated	32.68	2	0.001	32.81	28.49	37.13
Carbohydrate	Conventional	20.88	2	0.002	8.47	6.72	10.21
	Formulated	25.46	2	0.002	10.60	8.81	12.39
Lipid	Conventional	11.55	2	0.007	5.26	3.30	7.22
	Formulated	6.67	2	0.002	3.12	1.11	5.13
Astaxanthin	Conventional	72.23	2	0.000	112.50	105.80	119.21
	Formulated	53.17	2	0.000	150.60	138.55	162.65



CF= Conventional Feed and FF= Formulated Feed

(a=Temperature, b=pH, c=Dissolved oxygen, d=Transparency, e=Nitrate, f=Phosphate, g=Silicate and h=Chlorophyll *a*)

Fig. 2. (a–h) Variation in physico-chemical parameters of culture ponds.

FCR value the higher the weight gain obtained from the feed. Feed Conversion Ratio was calculated after harvesting of the prawns as per the expression [21].

$$FCR = \frac{\text{Total feed consumed (gm)}}{\text{Total yield (gm)}} \quad (7)$$

2.7. Benefit cost analysis (BCA)

Benefit Cost Analysis (BCA) and Benefit Cost Ratio (BCR) of the cultured species were determined as per the method outlined by [22]. One day prior to harvest the water level in the ponds were lowered to approximately 0.5 m. On 12th December 2017 pond was seined using cast net. After complete draining of the ponds the prawns were manually harvested from the pond bottom and purged water. A sample of 50 prawns were randomly collected and individually weighed. Benefit cost analysis was calculated in order to find out the profit or loss from the production as per standard arithmetic calculations. The economic analysis was carried out on the basis of variable cost of inputs like prawn juveniles, feed, lime, pond management and hired labours. Pond construction cost was excluded in the calculation. Cost of production was estimated based on local market price during 2017 and the harvested prawns were sold locally. Gross margin was estimated by subtracting the total variable cost and total returns. The benefit cost analysis was estimated by

$$\text{BCR (Benefit cost ratio)} = \frac{\text{Total return}}{\text{Total variable cost}} \tag{8}$$

The net production was calculated for treatment 1 and treatment 2 and the benefit cost analysis were performed based on the two types of treatments.

2.8. Statistical analysis

For the statistical analysis of the data, two way ANOVA (Analysis of Variance) was used to determine the effect of different feeds on the growth of prawns. Data analysis was done by using the SPSS software version 13 to identify the significance level of variance among the different treatments.

3. Results and discussion

3.1. Proximate composition of feeds

The proximate composition of conventional feed, formulated feed as well as its cost per 100 gm is given in Table 1. The percentage of protein (32.8 ± 0.95%), carbohydrate (10.6 ± 1.82%) and astaxanthin (591.2 ± 5.54 ppm) contents were found to be highest in formulated feed where as lipid (4.6 ± 0.65%) content was found to be highest in conventional feed. The conventional feed was purchased from the local market at the rate of Rs. 65.00 per kg. The cost of formulated feed was estimated by combining the price of individual ingredients which cost around Rs. 5.52/- per 100 gm or Rs.55.20 per kg (Table 1). The diets (conventional and formulated) information about the ingredients and about their sources are mentioned in Table 1. Keeping the conventional feed as reference the formulated feed was prepared by balancing the important ingredients. The use of fish meal and soybean meal in the ratio of 1:3 in conventional feed has been completely replaced by soybean meal. In the similar way the use of wheat flour in conventional feed has been replace by the liquid extract of grass (*C. dactylon*), binder and attractant in the ratio of 8:1:1. The seaweed extract is used only as attractant in order to give a fishy smell to the feed, whereas the astaxanthin ingredient has played a positive effect on productivity and profitability of prawn farming because of its anti-oxidative property which has made the prawns hardy and disease resistive for its farming in the adverse climatic condition of the hilly terrains of Koraput district. Hence, the protein efficiency of the formulated feed using soybean meal by the total replacement of fish meal is proved. T-test for biochemical analysis of two different feeds were performed (conventional and formulated feed) and significant variation was found between them (Table 2).

3.2. Physico-chemical parameters

For culturing *M. rosenbergii,* water quality is one of the important parameters because it plays a vital role in governing the health of the prawns [23]. In the present study 4 ponds were taken for culturing *M. rosenbergii* with a stocking density of 4 m^{-2}. Overall the productivity performances in all the 4 ponds were quite satisfactory and the results of the study support the improved traditional method of prawn culture in the present study area which is located in the hilly terrains of Koraput district of Odisha. The average surface water temperature in the culture ponds varied from 18.8 ± 0.32 °C in pond 4 during December 2017 to 25.5 ± 0.44 °C in pond 2 during June 2017 (Fig. 2a). The variation in the water temperature among the 4 ponds was quite significant ($p < 0.05$) between days; however there was not much disparity in water temperature between ponds, being located in the same geographical locale (Table 3). The value of pH which is a major parameter for prawn moulting also showed a rapidly rising trend from June 2017 to December 2017. The values among the 4 ponds varied between 7.54 ± 0.16 during June 2017 in pond 1 and 8.34 ± 0.28 in pond 4 during December 2017 respectively (Fig. 2b). The increase in pH might probably be due to the supplied feed in the culture ponds and also due to heavy precipitation during the culture period. The pH values were constant after September to December 2017 where there is a steady gain of pH from 7.9 ± 0.36 to 8.34 ± 0.28 respectively (Fig. 2b). This has also been proved from the F_{cal} values in Table 3. The dissolved oxygen (DO) which is a major parameter

Table 3
ANOVA analysis of physico-chemical and growth parameters ($p < 0.05$).

Physico-chemical and growth parameters		*df*	F_{crit}	F_{cal}
Temperature (°C)	Between days	13	1.981	769.72
	Between ponds	3	2.845	1.619
pH	Between days	13	1.981	207.913
	Between ponds	3	2.845	7.550
Dissolved oxygen (mg/l)	Between days	13	1.981	16.572
	Between ponds	3	2.845	7.061
Transparency (cm)	Between days	13	1.981	43.534
	Between ponds	3	2.845	12.002
Nitrate (mg/l)	Between days	13	1.981	61.530
	Between ponds	3	2.845	80.865
Phosphate (mg/l)	Between days	13	1.981	23.135
	Between ponds	3	2.845	75.515
Silicate (mg/l)	Between days	13	1.981	41.401
	Between ponds	3	2.845	66.211
Chlorophyll *a* (mg/l)	Between days	13	1.981	16.074
	Between ponds	3	2.845	67.680
Organic carbon (%)	Between days	13	1.981	59.421
	Between ponds	3	2.845	36.862
Condition Index of prawn	Between days	13	1.981	3.283
	Between ponds	3	2.845	3.200
Specific growth rate of prawn (% day^{-1})	Between days	13	1.981	5.102
	Between ponds	3	2.845	5.911
Daily growth rate of prawn (gm day^{-1})	Between days	13	1.981	255.587
	Between ponds	3	2.845	69.089
Protein of prawn (%)	Between ponds	3	9.277	17.913
	Between feeds	1	10.128	284.81
Carbohydrate of prawn (%)	Between ponds	3	9.277	56.1
	Between feeds	1	10.128	228.15
Lipid of prawn (%)	Between ponds	3	9.277	0.137
	Between feeds	1	10.128	0.312
Astaxanthin of prawn (ppm)	Between ponds	3	9.277	271.365
	Between feeds	1	10.128	148035.2

particularly with respect to prawn health and growth has also varied from 4.85 ± 0.45 mg/l in pond 3 during June 2017 to 5.96 ± 0.38 mg/l in pond 4 during November 2017 respectively (Fig. 2c). The overall trend of DO showed more or less fluctuation in its value because of heavy precipitation during culture period. Significant difference in DO was observed both between ponds as well as days (Table 3) proving the culture period to be most congenial for the growth of prawns in the study area. The use of aerators has actually helped in maintaining the DO levels during the culture period (July–September 2017). Transparency which is a major parameter of water column was measured in all culture ponds and the values varied from 14.2 ± 0.18 cm in pond 2 during August 2017 to 25.7 ± 0.30 cm in pond 4 during June 2017 respectively (Fig. 2d). The transparency value decreased from June to August owing to heavy precipitation during this period and increased there after till December. This has been proved from the significant F_{cal} value between days and between ponds (Table 3). Significant positive correlation was obtained between treatment 1 and treatment 2 ponds which showed that with the increase in transparency there is greater level of efficiency of photosynthesis in phytoplankton which has led to increased DO level in the culture ponds (Tables 4 and 5).

Nutrition in the culture ponds is a very essential criteria which governs the primary productivity of any aquaculture ecosystem particularly in the aquaculture ponds. In the present study nutrient levels were measured with respect to nitrate, phosphate and silicate levels. The nitrate values in the 4 ponds ranged from 0.02 ± 0.003 mg/l in pond 3 during June 2017 to 0.052 ± 0.005 mg/l in pond 1 during August 2017 (Fig. 2e). Similarly the phosphate value ranged from 0.011 ± 0.005 mg/l in pond 3 during August 2017 to 0.031 ± 0.003 mg/l in pond 1 and 2 during December 2017 (Fig. 2f) and silicate values ranged from 0.482 ± 0.05 mg/l in pond 4 during December 2017 to 1.606 ± 0.02 mg/l in pond 2 during June 2017 respectively (Fig. 2g). The amount of nitrate and phosphate in the culture ponds showed an increasing trend where as for silicate values it showed a decreasing trend proving the fact that nitrate and phosphate are basically contributed by the decomposition of organic waste like the algal biomass as well as the uneaten remaining foodstuffs. This also holds a very good relationship in increasing trends in pH levels (Tables 4 and 5). In the present study the nitrate and phosphate levels are in almost normal levels as per the earlier studies [24, 25].

Chlorophyll *a* which is a measure of primary production in culture ponds is a critical parameter as it is considered as the building block of any aquatic ecosystem. In the studied culture ponds chlorophyll *a* values ranged from 3.34 ± 0.28 mg/l in pond 1 during June 2017 to 6.12 ± 0.35 in pond 4 during November 2017 respectively (Fig. 2h). Significant variation ($p < 0.05$) in the nutrient level (nitrate, phosphate and silicate) has proved that there was significant variation in pond water owing to the consumption of feeds by the cultured prawns. It is quite evident that over 195 days of culture period the nutrient concentration has varied significantly, which has proved the application of feed in maintaining the water quality of the culture ponds and also the phytoplankton bloom. The month of November being the second blooming period of phytoplankton has showed higher values than December. During the study period chlorophyll *a* values has shown a rising trend over the culture periods similar to nitrate and phosphate levels (Fig. 2 h) which proves that increasing nutrient level has helped the phytoplankton to bloom (Tables 4 and 5). Furthermore, the significant negative relationship at 1% level of significance with silicate has shown the presence of diatoms in

Table 4
Correlation between physicochemical parameters and Condition Index of prawns fed with conventional feed.

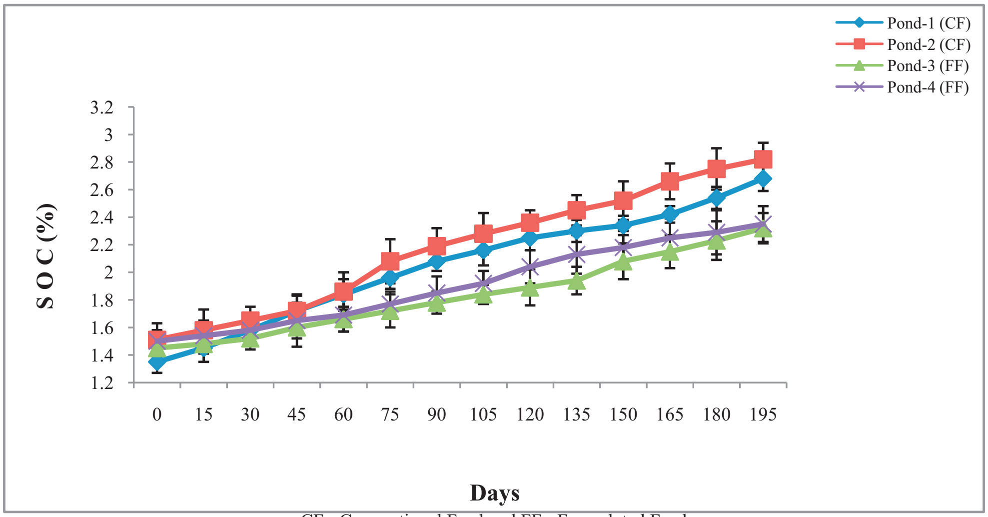
	Temperature (⁰C)	pH	D O (mg/l)	Transparency (cm)	Nitrate (mg/l)	Phosphate (mg/l)	Silicate (mg/l)	Chlorophyll *a* (mg/l)	Organic Carbon (%)	Condition Index
Temperature (°C)	1									
pH	-0.981**	1.000								
D O (mg/l)	0.520*	-0.574**	1.000							
Transparency (cm)	-0.075	0.011	0.717**	1.000						
Nitrate (mg/l)	-0.698**	0.743**	-0.944**	-0.612**	1.000					
Phosphate (mg/l)	-0.918**	0.907**	-0.618**	-0.036	0.731**	1.000				
Silicate (mg/l)	0.977**	-0.983**	0.625**	0.018	-0.786**	-0.943**	1.000			
Chlorophyll *a* (mg/l)	-0.882**	0.888**	-0.492*	0.101	0.613**	0.957**	-0.911**	1.000		
Organic Carbon (%)	-0.949**	0.963**	-0.738**	-0.213	0.880**	0.913**	-0.974**	0.845**	1.000	
Condition Index	0.610**	-0.549*	-0.089	-0.574**	-0.067	-0.647**	0.578**	-0.763**	-0.429	1

*$p < 0.05$; **$p < 0.01$.

Table 5
Correlation between physicochemical parameters and Condition Index of prawns fed with formulated feed.

	Temperature (⁰C)	pH	D O (mg/l)	Transparency (cm)	Nitrate (mg/l)	Phosphate (mg/l)	Silicate (mg/l)	Chlorophyll *a* (mg/l)	Organic Carbon (%)	Condition Index
Temperature (⁰C)	1									
pH	-0.955**	1.000								
D O (mg/l)	0.285	-0.222	1.000							
Transparency (cm)	0.258	-0.287	0.592**	1.000						
Nitrate (mg/l)	-0.738**	0.788**	-0.607**	-0.732**	1.000					
Phosphate (mg/l)	-0.827**	0.792**	0.027	-0.087	0.521*	1.000				
Silicate (mg/l)	0.948**	-0.972**	0.366	0.449	-0.879**	-0.783**	1.000			
Chlorophyll a (mg/l)	-0.877**	0.923**	-0.169	-0.462*	0.832**	0.839**	-0.953**	1.000		
Organic Carbon (%)	-0.975**	0.985**	-0.338	-0.355	0.832**	0.814**	-0.990**	0.931**	1.000	
Condition Index	0.843**	-0.774**	0.496*	0.335	-0.646**	-0.574**	0.758**	-0.608**	-0.805**	1

*$p < 0.05$; **$p < 0.01$.



CF= Conventional Feed and FF= Formulated Feed

Fig. 3. Variation in soil organic carbon (SOC) percentage in the culture ponds.

the constituent of phytoplankton which has up-taken silicate levels in the culture ponds (Tables 4 and 5). Significant variation ($p < 0.05$) over the days and between the ponds was also observed for Chl *a* which proves the above stated explanation (Table 3).

Organic carbon which is a measure of left over feed accumulation in the pond bottom as well as the left over carcasses and dead phytoplankton remains, is an important parameter which governs the acidity and alkalinity of a pond. The organic carbon values ranged from 1.35 ± 0.02% in pond 1 during June 2017 to 2.82 ± 0.03% in the pond 2 during December 2017 (Fig. 3). The overall increasing trend of organic carbon during the culture period speaks that with the increasing days of culture the organic carbon load increased. This is quite natural in a culture medium which indirectly speaks of the growth of the prawns with the change in time. The positive relationship between organic carbon and pH and chlorophyll *a* speaks in favour of changing pH levels over the time as well as the effect of this change on phytoplankton concentration. ANOVA analysis (Table 3) has proved the change in organic carbon levels over the days and between the ponds ($p < 0.05$).

Table 6
Growth and biochemical parameters of *M. rosenbergii* fed with conventional and formulated feed.

Growth parameters	Conventional feed		Formulated feed	
	Pond 1	Pond 2	Pond 3	Pond 4
Survival rate (%)	62.0 ± 1.05	65.0 ± 1.13	72.0 ± 0.89	71.0 ± 1.12
Initial average weight (gm)	0.24 ± 0.02	0.24 ± 0.05	0.24 ± 0.04	0.24 ± 0.03
Initial average length (cm)	2.0 ± 0.95	2.0 ± 0.65	2.0 ± 1.05	2.0 ± 0.85
Final average weight (gm)	51.45 ± 1.15	48.92 ± 0.84	58.85 ± 1.40	62.08 ± 1.12
Final average length (cm)	14.8 ± 0.91	14.5 ± 1.45	16.2 ± 0.87	16.5 ± 0.75
Gain in Weight (gm)	51.21 ± 0.99	48.68 ± 0.98	58.61 ± 1.02	61.84 ± 1.08
Gain in Length (cm)	12.8 ± 1.00	12.5 ± 0.99	14.0 ± 0.96	14.5 ± 1.13
Daily growth rate (DGR) (gm day^{-1})	0.143 ± 0.02	0.156 ± 0.03	0.203 ± 0.05	0.218 ± 0.02
Specific growth rate (% day^{-1})	3.08 ± 1.07	3.74 ± 1.02	4.62 ± 0.79	4.91 ± 1.17
Condition Index (CI)	2.18 ± 0.06	2.77 ± 0.09	3.29 ± 0.05	3.62 ± 0.04
FCR	2.95 ± 0.04	2.77 ± 0.02	2.74 ± .05	2.91 ± 0.05
Proximate analysis of prawn				
Protein (%)	17.2 ± 1.05		23.6 ± 0.96	
Carbohydrate (%)	6.65 ± 0.45		10.5 ± 0.57	
Lipid (%)	5.26 ± 0.29		3.12 ± 0.22	
Astaxanthin (ppm)	112.5 ± 8.25		150.6 ± 9.64	

Each of the value is expressed as Mean ± SD of three individual observations.

3.3. *Growth performance of M. rosenbergii*

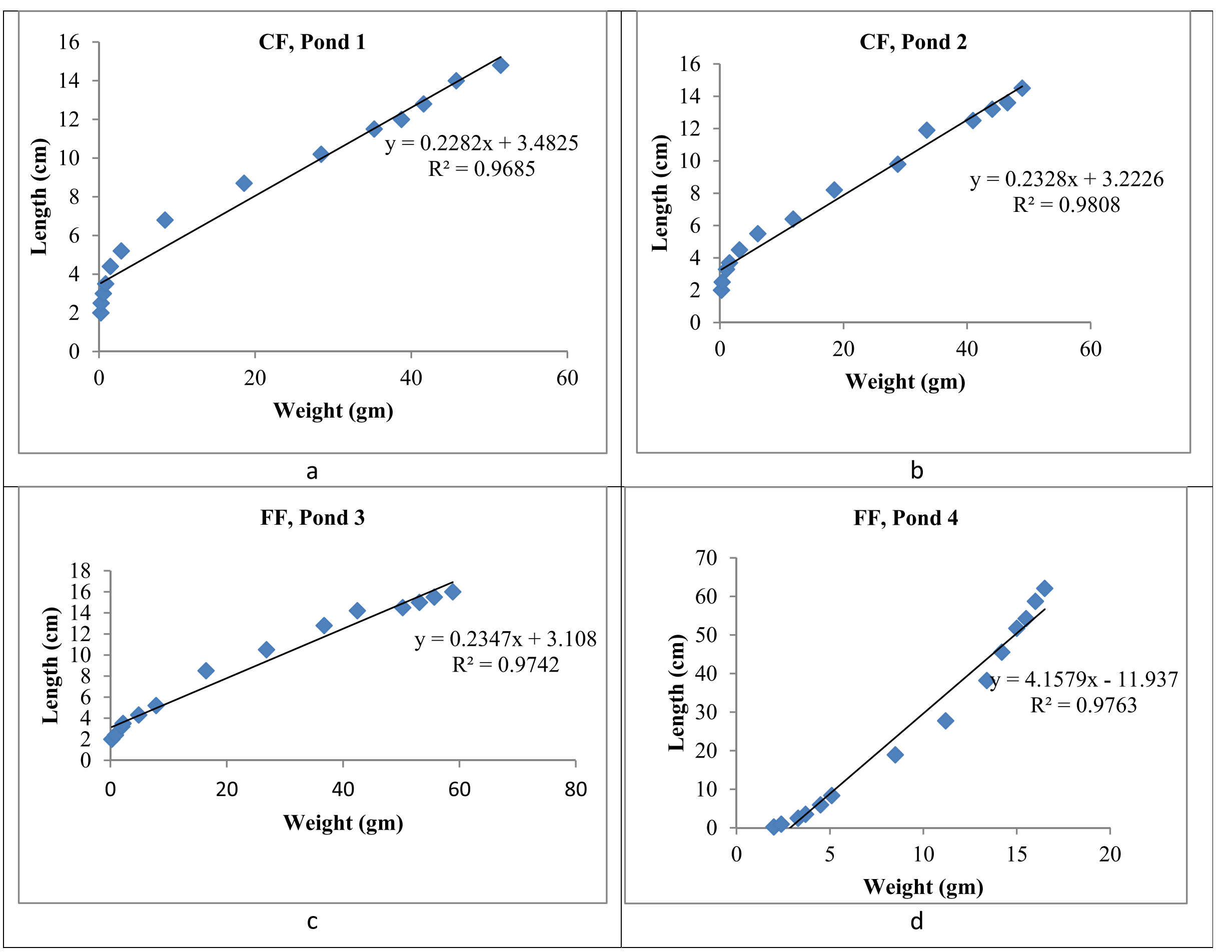
The growth parameters of the target species *M. rosenbergii* is given in Table 6 and Fig. 4 (a-e). The survival rates varied from 62.0 ± 1.05% to 72.0 ± 0.89% among the two different treatments of conventional feed and formulated feed respectively. The highest survival rate was observed in pond 3 and lowest was in pond 1 (Table 6). Morphometric measurements particularly with respect to length and weight of prawns were measured during the initial and final stages which varied from 2.0 ± 0.65 and 0.24 ± 0.02 gm to 16.5 ± 0.75 cm and 62.08 ± 1.12 gm respectively (Table 6 and Fig. 4a). On an average, the gain in length of prawn varied from 12.5 ± 0.99 cm to 14.5 ± 1.13 cm and average gain in weight varied from 48.68 ± 0.98 gm to 61.84 ± 1.08 gm respectively (Table 6 and Fig. 4b). It was observed that the DGR of the prawns varied from 0.143 ± 0.02 gm day^{-1} to 0.218 ± 0.02 gm day^{-1}, which was found to be higher in formulated feed than the prawns fed with conventional feed (Table 6 and Fig. 4c). The SGR for the treatment 1 and treatment 2 ponds varied between 3.08 ± 1.07% day^{-1} to 4.91 ± 1.17% day^{-1} respectively (Table 6 and Fig. 4d). Similarly the Condition Index of the prawns as measured in conventional and formulated feed varied from 2.18 ± 0.06 to 3.62 ± 0.04 which proved a better growth of prawns with respect to formulated feed than the conventional feed (Table 6 and Fig. 4e). The FCR value varied from 2.74 ± .05 to 2.95 ± 0.04 for treatment 2 and treatment 1 respectively (Table 6). In order to prove the relationship of length and body weight of prawns, about 50 samples were randomly weighed and measured which proved a significant relationship in all the 4 ponds at $p < 0.01$ (Fig. 4 a-d). Such type of relationship has also been confirmed by previous workers on *M. rosenbergii* [26, 27, 28, 29]. It is to be noted that length and weight relationship is a vital parameter which establishes relationship between two variables and helps to determine the possible differences among the different stocks of the same species and also to compare the growth performance of the same species [30, 31, 32, 33, 34]. Condition Index is also a reflection of the growth performance of prawns with the physico-chemical parameters of ambient media. Hence, correlation coefficient was computed between condition index and physico-chemical variables where significant relationship with temperature at 1% level of significance (Tables 4 and 5) has proved the ambient environment to be quite congenial for the growth of *M. rosenbergii*. A significant negative relationship was observed in pH and organic carbon with condition index of prawn which has proved that with the increase in pH and organic carbon the growth of the prawn was hindered and vice versa (Tables 4 and 5). Significant negative relationship of Condition Index with chlorophyll *a* at 1% level of significance was observed in both the treatment ponds fed with conventional feed and formulated feed, which has proved the uptake of phytoplankton by the prawns for its growth, apart from the artificial feed provided to the pond (Tables 4 and 5). ANOVA results have also proved significant difference ($p < 0.05$) between ponds and between the culture days for condition index, daily growth rate and specific growth rate which has proved the improved condition of the health of prawns over the culture days (Table 3).

The present study confirms a significant difference in prawn length, weight, condition index, daily growth rate and specific growth rate between the selected ponds which may be attributed to difference in feed components. The growth of *M. rosenbergii* shows a relatively continuous growth up to 75 days in ponds 1 and 2 and relatively discrete growth pattern in ponds 3 and 4. After 75 days there is a continuous trend of normal growth in all the 4 ponds. Similar trend is followed in case of specific growth rate also [35, 36, 37, 38]. The prawn length, weight and daily growth rate has shown an exponential growth pattern after 75 days which shows a better aquatic environment of the culture ponds and proper diet of the prawns (Fig. 5 a-e).

3.4. Biochemical parameters

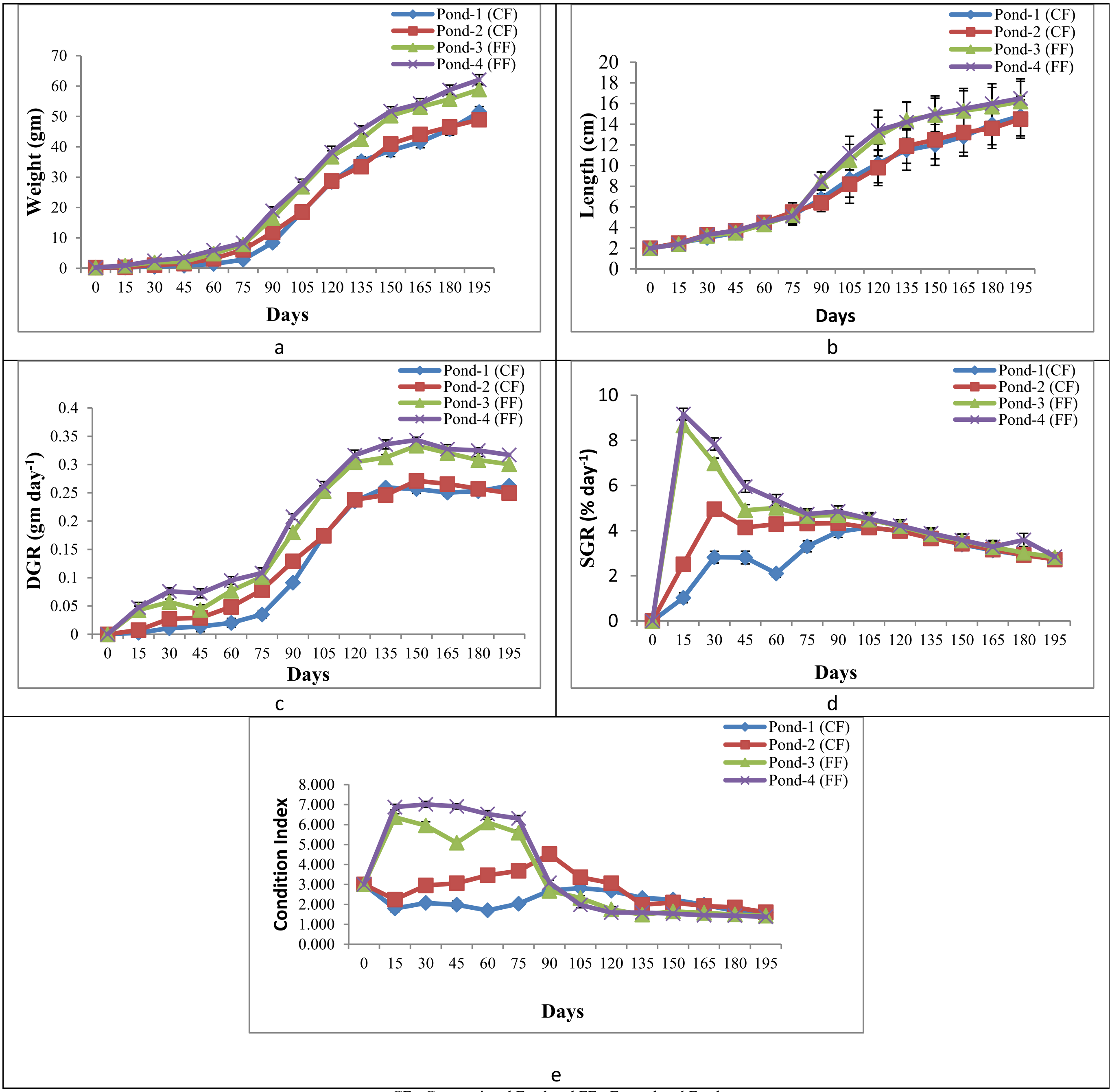
There is a fairly good amount of information on nutrient requirement of freshwater prawn. Since prawns are omnivorous, hence they are capable of ingesting wide range of food comprising of both animal and plant origin. Proximate composition of conventional and formulated feed was monitored with respect to protein, carbohydrate, lipid and astaxanthin (Table 1). It has been known from the earlier studies that feed with 35–40% protein are generally considered suitable for growth of *M. rosenbergii* in clear water system that do not have any natural food supply. In the present research program since there was use of *Cynodon dactylon* (a perennial species in Koraput district of Odisha) as well as seaweed powder (*Ulva* sp. collected from the nearby coast of Visakhapatnam) along with soybean meal, hence the feed has a specialized constituent for better feed efficiency. The study revealed that protein, carbohydrate and astaxanthin content in the formulated feed were comparatively more than the conventional feed (Table 1).

Proximate composition for the harvested prawns were also done simultaneously with respect to protein, carbohydrate, lipid and astaxanthin where protein contents in prawns ranged from 17.2 ± 1.05% to 23.6 ± 0.96%, carbohydrate content ranged from 6.65 ± 0.45% to 10.5 ± 0.57%, lipid content ranged from 3.12 ± 0.22% to 5.26 ± 0.29% and astaxanthin ranged from 112.5 ± 8.25 ppm to 150.6 ± 9.64 ppm



(a= Length-Weight relationships of prawns fed with conventional feed (CF) of Pond 1, b= Length-Weight relationships of prawns fed with conventional feed (CF) of Pond 2, c= Length-Weight relationships of prawns fed with conventional feed (FF) of Pond 3, d= Length-Weight relationships of prawns fed with conventional feed (FF) of Pond 4).

Fig. 4. (a–d) Length-Weight relationships of cultured prawns in the 4 ponds.



CF= Conventional Feed and FF= Formulated Feed

(a=Weight of prawns fed with conventional and formulated feeds of 4 Ponds, b= Length of prawns fed with conventional and formulated feeds of 4 Ponds, c=DGR of prawns fed with conventional and formulated feeds of 4 Ponds, d= SGR of prawns fed with conventional and formulated feeds of 4 Ponds and e= Condition index of prawns fed with conventional and formulated feeds of 4 Ponds)

Fig. 5. (a–e) Fortnightly recorded growth parameters for *M. rosenbergii* fed with conventional and formulated feed.

respectively (Table 6). Lipids are primarily included in the formulated feed to maximize their protein sparing effect [39] by being a source of energy. The proximate composition showed a lesser lipid content in the formulated feed as well as in prawns (less than 5%) which is a more acceptable diet in terms of aquatic standard [40]. Low lipid content in the formulated feed is a symbol of greater binding efficiency and more pellet water stability. It has been reported that dietary carotenoids are responsible for flesh pigmentation in aquatic organisms [41, 42]. It is to be noted that crustaceans are capable of transforming ingested carotenoids usually astaxanthin in their tissues [43, 44] which is a vital factor for the commercial value of the prawns.

There was significant difference ($p < 0.05$) in the FCR of prawn among the ponds ($F_{cal} = 6.499$ and $F_{crit} = 4.066$). The FCR of the feeds (2.74 ± 0.05–2.95 ± 0.04) in the present study was better than the FCR reported by [45] and FCR (2.99–3.18) reported by [46] using feed prepared with trash fish and other plant protein containing 34.26 ± 0.20%.

Table 7
Benefit cost analysis (BCA) of the culture of *M. rosenbergii.*

Investment cost	Culture with Conventional Feed	Culture with Formulated Feed
Pond area	10 m^2/0.001ha	10 m^2/0.001ha
Stocking density @ 4 m^{-2}	200 PLs	200 PLs
Seed	@ 0.80/ seed,0.80*200 = Rs.160.00	@ 0.80/seed,0.80*200 = Rs. 160.00
Feed	@65/kg, 65*13.887 = Rs.903.27	@Rs.55.20/ kg,55.20*17.89 = Rs.987.53
Pond management (lime, cow dung, labour charges per hour basis, etc.)	Rs. 1030.00	Rs. 1030.00
Misc. (generator, aerator etc.)	Rs. 500.00	Rs. 500.00
Total	Rs.2593.27	Rs.2677.53
Benefit from the production		
Total yield (kg)	6.37	8.65
Total yield (kg m^{-2}/kg ha^{-1})	0.637/637	`0.865/865
Selling price (@ Rs.500/- kg^{-1})	Rs. 3185.00	Rs. 4325.00
Profit (Selling Price-Investment Price)	Rs. 591.73	Rs. 1647.47
Profit in Rupees m^{-2}/Rupees ha^{-1}	Rs. 59.17/Rs.59170	Rs. 164.75/Rs.164750
Benefit cost ratio	1.23	1.62

3.5. Economic viability

The productivity and economy of prawns from 4 culture ponds fed with conventional feed and formulated feed is given in Table 7. After a culture period of 195 days, although the stocking density of the prawns of all the 4 ponds were equal but owing to the difference in the water quality parameters, consumption of feed and chlorophyll *a* concentration the quantity of production in conventional feed and formulated feed were different which accounts to 6.37 kg in conventional feed and 8.65 kg in formulated feed respectively (Table 7). The present results coincide with the findings of [47, 48, 49, 50]. In the present study a better production profit was noted in ponds fed with formulated feed which ranged from Rs. 591.85/- in conventional feed to Rs. 1489.18/- in formulated feed within 6 months of culture period. It can further be stated that a stocking density of 4/m^2 was economically attractive as has been stated by [51, 52, 53].

The growth performance of prawns in the present culture yielded 637 kg ha^{-1} in ponds fed with conventional feed to 865 kg ha^{-1} from ponds fed with formulated feed which was quite higher than the average annual yield of prawns (425–440 kg ha^{-1}) from south western region of Bangladesh and 505–573 kg ha^{-1} in Khulna district in Bangladesh [54, 55, 56].

4. Conclusion

The result of the current experiment demonstrated that total replacement of fish meal with soybean meal had a positive effect on productivity and profitability of the prawn farming in village ponds of Koraput district of Odisha. Koraput being a tribal district in the high lands of Eastern Ghats has basically an agro-based economy with large amounts of agricultural wastes. In the present study locally available feed ingredients were selected to utilize these agricultural wastes and byproducts which will otherwise effectively manage the freshwater resource of the area. A good growth of the prawns (*Macrobrachium rosenbergii*) has lit the lamp of aquaculture initiative in the area which can act as alternative livelihood for the local inhabitants by harnessing the freshwater resources leading to increased employability through this sector.

Declarations

Author contribution statement

Gopal Raj Khemundu: Performed the experiments.

Kakoli Banerjee: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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