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Effects of raw and fermented seaweed, *Padina tetrastomatica* on the growth and food conversion of giant freshwater prawn *Macrobrachium rosenbergii*

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Abstract
Raw and fermented seaweed, *Padina tetrastomatica* were incorporated at three levels, 10 %, 20 % and 30 % in diets of giant freshwater prawn *Macrobrachium rosenbergii*. In 15 days digestibility experiment, among the raw and fermented seaweed incorporated diets, the freshwater prawn fed with fermented *Padina tetrastomatica* (FP) at 10 %, showed maximum apparent digestibility coefficients for dry matter (89.05 %), APD (89.61 %) and ALD (89.49 %). In the 45 days growth experiment, prawn fed with FP at 10 % showed maximum mean weight gain (3.0430g), SGR (2.111), mean feed intake (4.4g), and PER (1.2711). The best FCR value of 1.3145 was observed in prawn fed with FP at 10% incorporation. Significant differences (P<0.05) was observed in the growth parameters between treatments. The whole body composition of prawns fed the raw and fermented seaweeds incorporated diets did not show any variations in moisture, protein, lipid and ash. The results of the study suggest that the raw *P. tetrastomatica* could be incorporated in freshwater prawn diets up to 10 % and 20 % respectively and fermented *P. tetrastomatica* could be incorporated up to 30 % level without compromising growth, digestibility and flesh quality. The present investigation has demonstrated that *P. tetrastomatica* can be a potential feed ingredient in the juveniles of freshwater prawn *M. rosenbergii*.

Keywords: giant freshwater prawn, *Padina tetrastomatica*, digestibility, growth.

1. Introduction

The giant freshwater prawn *Macrobrachium rosenbergii* (De Man) popularly known as scampi, is one of the high value aquaculture product emerging from Asia [1]. Freshwater prawns are capable of utilizing less expensive plant based low protein ingredients. Commercial diets are quite expensive due to inclusion of high priced fishmeal. This has stimulated the evaluation of a variety of alternative protein sources for partially or totally replacing the fishmeal protein in aquaculture feeds. Seaweeds are good source of proteins, vitamins and minerals [2]. Seaweeds with good protein level are receiving considerable attention as novel feeds with potential nutritional benefits [3]. However the presence of high crude fiber and low protein content are issues for low inclusion of seaweeds in aquafeeds. Fermentation is a simple and cheap method which might considerably decrease crude fiber content and increase protein value. Fermentation of seaweeds with lactic acid bacteria and yeast enhance the nutritive value by enriching protein, vitamin, mineral, essential amino acids, essential fatty acids and also improves the digestibility of seaweed based feeds [4]. Fermentation of seaweeds with beneficial bacteria like lactic acid bacteria and yeast is known to act as growth promoter, immune enhancer and probiotics in cultivable organisms. The considerable increase in the nutrient level of fermented seaweeds due to microbial synthesis during fermentation process envisages that fermented seaweeds are the possible alternative protein ingredients in aquafeeds. The fermentation process significantly improves nutritive value, acceptability and digestibility and eliminates anti-nutritional factors in plant based ingredients. This provides a promising future for sustainable aquaculture. Fermentation will help feed manufacturers to replace fishmeal to certain levels and help in reducing the feed cost and thereby increasing the profitability of aquaculture systems [5]. In this background, this research was undertaken to study the effects of raw and fermented seaweed, *P. tetrastomatica* on the growth of juveniles of freshwater prawn.

2. Materials and methods

The postlarvae were reared in 250 liter FRP tanks filled with freshwater for 2 months to attain juveniles for the study. After 2 months, the juvenile prawns were transferred to the experimental system and acclimated for 2 weeks before the start of the experiment. The experimental system comprised of a series of 14 cement cisterns. Each 100 L cement tanks were provided with a netting cover (30cm x 20cm) and PVC pipes (10cm x 2 cm) as shelters. Continuous aeration was supplied by Hiblow air blower (Techno- Takatsuki Co. Ltd, Japan) at the airflow rate of 60 L/min to all experimental tanks. In each tank, 90 liters of freshwater was maintained during the course of the experiment. Daily water exchange at the rate of 20 % between 9.00 hrs and 10.00 hrs assured that the water quality parameters maintained were within the acceptable range for *M. rosenbergii*.

2.1 Seaweed collection and preparation of seaweed powder

Padina tetrastomatica (brown seaweed) was collected from Hare Island, Thoothukudi. The seaweed was washed well in freshwater to remove the foreign particles and sun dried. The dried seaweeds were grounded well in laboratory pulverizer sieved through a 0.3 mm mesh and used as raw seaweed powder and raw material for fermentation. Microbial fermentation of the seaweed was carried out in the fermenter vessel. The dried seaweed powder to seawater in the ratio of 1:9 (seaweed: seawater) was taken in the fermenter vessel. Each 10 ml of *Lactobacillus* spp. and *Saccharomyces cerevisiae* was inoculated at a concentration of 3.10 x 10^4. The sugar substrate, dextrose was added at the rate of 5 % w/v of base material. The fermentation was carried out till the pH reached at 4.00. A pH between 4 and 5 is desired for fermentation of feed ingredients because when the pH is below 4.00 the feed intake decreases and above 5.00, microbial spoilage is likely to occur [6]. The fermented seaweed silage was collected from the fermenter and dried in a hot air oven at 60 ^{0}C for 2 days. The fermented seaweed powder is then used for feed preparation. The proximate composition is given in Table 1.

2.2 Preparation of experimental feeds

Seven isonitrogenous feeds (28 % protein) were prepared for growth experiments. Similarly for digestibility experiment, seven feeds were prepared. The difference between the digestibility and growth experimental diets were digestibility test diets contained 1 % chromic oxide (dietary marker) each. Raw and fermented *Padina tetrastomatica* was incorporated in to the test diets at 10 %, 20 % and 30 % by replacing fishmeal and other ingredients. The raw *Padina* sp. incorporated diets are denoted as RP and the fermented *Padina* sp incorporated diets are denoted as FP. Control feed was prepared without incorporation of raw and fermented seaweeds. The feed ingredients (Table 2) and the seaweed powder were sieved through 350 µm die, mixed with carboxy methyl cellulose (CMC). Hot water was added and ingredients mixed and made in to dough. The dough was then extruded in a noodle making machine through 1mm die. The resulting pellets were dried at 60 ºC for 12 hrs and stored in airtight container.

2.3 Digestibility experiment

The apparent digestibility coefficients (ADCs) of the feeds were measured after incorporation of 1 % chromic oxide in each diet. Seven diets were prepared by incorporating raw and fermented *Padina tetrastomatica* at 10 %, 20 % and 30 % along with one control. Fourteen groups of 10 juveniles (mean initial weight 1.90-1.98 g) were stoked in each 100 liter cement tank and adapted to a new condition for two weeks fed with a commercial feed. Duplicate was maintained for each treatment. Before changing feeds, the prawns were starved for 24 hrs and weighed. The first 2 days was used for acclimation to the feed and no faeces were collected. The time period was deemed sufficient for the prawn to achieve complete evacuation of previous meals. The feed was given at the rate of 10 % of the prawn body weight per day. The digestibility experiment was conducted for 15 days. Feed was given daily at 9.00 am and 6.00 pm and 4 hrs after they consumed their meal, uneaten feed and faeces were removed by siphoning. Faeces collected from replicate treatments were pooled, dried at 60 ºC in an oven and stored for further analysis. The amount of chromic oxide present in the feeds and faecal samples was estimated by digestion with conc.HNO_3 and the absorption was measured in the spectrophotometer at 350 nm [7]. The apparent digestibility coefficients (ADCs) were calculated according to El-Shafai *et al.* (2004) [8].

2.4 Growth experiment

Growth experiment was conducted with raw and fermented *Padina tetrastomatica* incorporated feeds at three different conc. Viz., 10%, 20% and 30%. The prawns were fed with the feeds twice daily at 10.00 am and 6.00 pm at a fixed feeding rate of 10 % wet body weight per day for 45 days. The prawns were weighed every tenth day and the amount of feed adjusted accordingly. The uneaten feed was collected daily by siphoning 4 hrs after each feeding; the collected uneaten feeds were dried in an oven at 60 ^{0}C and weighed for calculating biogrowth parameters.

2.5 Proximate analysis

The moisture, crude protein, lipid, ash, crude fiber, NFE and gross energy in the raw seaweed meal, fermented seaweed meal, raw seaweed incorporated diets, fermented seaweed incorporated diets(Table 3) and prawns of the growth experiments were analyzed according to the standard procedure [9]. Moisture was determined by oven drying at 105 ^{0}C for 24 hrs and protein by Micro Kjeldahl method after acid digestion. Lipid was determined by Bligh and Dyer method by extraction with chloroform methanol mixture. Crude fiber was determined in ELECTRONIC FIBROPLUS (Model-FES 4, Pelican equipments, India) automatic equipment as loss on ignition of dried lipid free residues after digestion with 1.25 % H_2SO_4 and 1.25 % NaOH. Ash was determined by ignition at 600 ^{0}C for 6 hrs in a muffle furnace. The gross energy (GE) was estimated using Digital Bomb calorimeter (Model. No. RSB-3, Rajdhani scientific Inst. Co. New Delhi. India) The nitrogen free extract (NFE) was calculated by using the following formula,

$$NFE = [100 - (\text{crude protein} + \text{crude lipid} + \text{ash} + \text{crude fiber} + \text{moisture})]$$

Table 1: Proximate composition of raw and fermented *Padina tetrastomatica* (% dry weight)

s	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Fiber (%)	NFE (%)	Gross energy (K cal/g)
Raw seaweed	16.40	10.50	1.14	27.0	23.96	21.00	4.12
Fermented seaweed	16.72	15.90	3.23	24.0	3.60	36.55	5.16

Table 2: Ingredient composition of raw and fermented *Padina tetrastomatica* incorporated feeds

Ingredients	Control	RP-10 (%)	RP-20 (%)	RP-30 (%)	FP-10 (%)	FP-20 (%)	FP-30 (%)
Fish meal	50.0	47.0	44.0	41.0	45.0	41.0	38.0
Seaweed meal	20.0	10.0	20.0	30.0	10.0	20.0	30.0
Soya flour	17.0	20.0	20.0	20.0	20.0	20.0	20.0
Corn starch	2.0	10.0	5.0	3.0	10.0	8.0	6.1
Cod liver oil	1.0	2.1	2.5	2.8	2.2	2.3	2.2
Carboxy methyl cellulose	2.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin mineral mixture	8.0	2.0	2.0	2.0	2.0	2.0	2.0
Cellulose	50.0	7.9	5.5	0.2	9.8	5.7	0.7

Table 3: Proximate composition of raw and fermented *Padina tetrastomatica* incorporated feeds

Feeds	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Fiber (%)	NFE (%)	DE (Kcal/g)
RP- 10%	10.70	28.25	7.12	10.90	6.40	36.63	3.37
RP- 20%	11.74	28.14	7.25	10.98	8.80	33.09	3.29
RP- 30%	11.65	28.03	7.35	11.21	11.20	30.56	3.26
FP- 10%	10.39	28.01	7.26	10.82	4.36	39.16	3.50
FP- 20%	11.51	28.06	7.30	10.68	4.72	37.73	3.44
FP- 30%	10.36	28.57	7.22	11.31	5.08	37.46	3.45
Control	10.98	28.35	7.32	10.41	4.80	38.14	3.49

2.6 Water quality analysis
The samples for water quality parameters were taken at weekly intervals prior to water exchange between 8.30 hrs and 9.00 hrs. The temperature, ammonia, nitrite and nitrate were recorded [10].

2.7 Statistical analysis
Two way analysis of variance (ANOVA) was carried out to find out whether there is any significant difference among the growth related parameters in growth experiments and the digestibility of nutrients in the digestibility experiments [11].

3. Results
3.1 Seaweed fermentation
The fermentation process in the fermenter was observed by measuring the lactic acid production and reduction in the pH level. pH of the fermented seaweeds dropped from 7.0 to 4.0 on the 3rd day. Lactic acid was produced at 0.9101 % on 3rd day. Increasing trend in the propagation of LAB and yeast was observed in fermented seaweeds from 0 to 72 hrs. The *Lactobacillus* sp. and *S. cerevisiae* in fermented *P. tetrastomatica* was 1.50 x 10^8 cfu/ml and 6.20 x 10^8 cfu/ml respectively.

3.2 Proximate composition of fermented seaweeds
There was significant increase in protein levels of *P. tetrastomatica* (from 10.50 % to 15.90 %). The fiber content was drastically decreased to 3.60 % for fermented *P. tetrastomatica.*

3.3 Apparent Digestibility Coefficients of dry matter and nutrients in prawn fed with raw and fermented *P. tetrastomatica* incorporated diets
Apparent digestibility coefficient for dry matter was significantly higher (89.05 %) in FP at 10 % incorporated diet followed by FP at 20 % incorporation (84.39 %). The lowest ADC value of 70.74 % was recorded in prawn fed with RP at 10 % incorporated diet. Significant difference (P<0.01) in dry matter digestibility was observed between the prawns fed with raw and fermented *P. tetrastomatica* incorporated diet. The apparent protein digestibility (APD) value was maximum (89.61 %) in FP at 10 % incorporated diet followed by FP at 20 % incorporation (86.94 %). The lowest APD value of 76.19 % was observed in the prawn fed with RP at 30 % incorporated diet. Significant difference (P<0.01) was observed in APD of prawns fed with raw and fermented *P. tetrastomatica* incorporated diets. The highest apparent lipid digestibility (ALD) value of 89.49 % was recorded in the prawn fed with FP at 10 % incorporated diet followed by FP at 20 % incorporation (83.87 %). The ALD value was minimum in prawn fed with RP at 30 % incorporated diet. (73.16 %). Significant difference (P<0.01) was observed in ALD of prawns fed with raw and fermented *P. tetrastomatica* incorporated diets.

3.4 Effect of raw and fermented *P. tetrastomatica* incorporated diets
The prawn fed with FP at 10 % attained the maximum weight gain of 3.043 g followed by FP at 20 % (2.7185 g).

Significant difference (P<0.01) in mean weight gain was observed between the prawns fed with raw and fermented *P. tetrastomatica* incorporated diets (Table 4 and Fig 1). Prawn fed with FP at 10 % showed the highest SGR of 2.111 followed by FP at 20 % (Table 4 and Fig 1). The minimum SGR value was recorded in prawn fed the diet with RP at 30 % incorporation (1.1499). Significant difference (P<0.05) in SGR was observed between the prawns fed with raw and fermented *P. tetrastomatica* incorporated diets. The mean feed intake was maximum (4.40 g) in prawn fed with FP at 10 % incorporation followed by FP at 20 % incorporation (4.37 g). Significant difference (P<0.01) in mean feed intake was observed between the prawns fed with raw and fermented *P. tetrastomatica* incorporated diets.

Table 4: Growth parameters of prawns fed with raw and fermented *Padina tetrastomatica* incorporated at three different concentrations

Treatment	Mean initial weight (g)	Mean final weight (g)	Mean weight gain (g)	SGR	PER	FCR	Mean feed intake (g)	Survival (%)
Control	1.9655±0.4031	3.8255±0.04949	1.860±0.3535	1.4907±0.0436	0.7862±0.0148	1.7814±0.03352	3.352±0.0256	95.00±3.5355
RP-10%	1.9265±0.2758	4.1950±0.7072	2.2685±0.0502	1.7785±0.0040	0.9476±0.0210	1.9106±0.0212	4.305±0.007	100
RP-20%	1.9000±0.0001	3.9550±0.4949	2.055±0.0212	1.6302±0.0119	0.8584±0.0089	2.122±0.0219	4.360±0.004	100
RP-30%	1.9820±0.0636	3.3305±2.68740	1.3485±0.2623	1.3249±0.1723	0.5633±0.1096	2.3266±0.4526	3.128±0.0707	100
FP-10%	1.9280±0.1131	4.9710±0.0989	3.0430±0.0113	2.111±0.00042	1.2711±0.0046	1.3145±0.0004	4.40±0.3291	100
FP-20%	1.9665±0.3323	4.6850±0.0020	2.7185±0.0332	1.9293±0.0375	1.1356±0.0139	1.5635±0.0190	4.37±0.2100	100
FP-30%	1.9400±0.0566	3.9740±0.1202	2.034±0.0297	1.5936±0.0197	0.8497±0.0124	1.5046±0.0219	3.26±0.5166	100

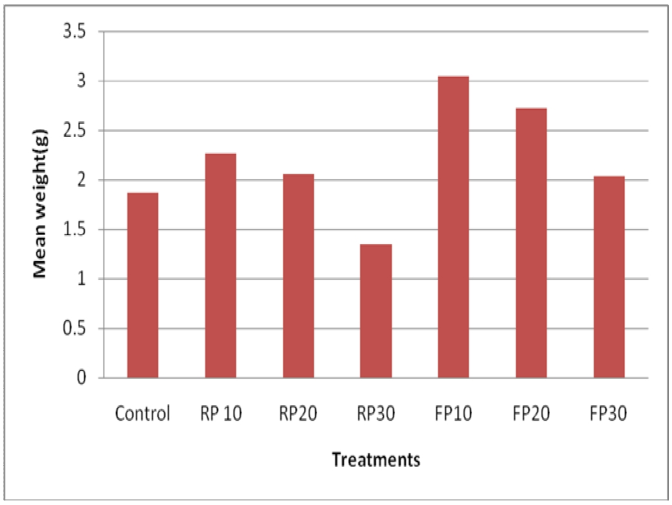

Fig 1: Effect of raw and fermented *P. tetrastromatica* on the growth of freshwater prawn

The highest FCR of 1.3145 was obtained in the prawn fed with FP at 10 % incorporation followed by 1.5046 in FP at 30 % incorporation. FCR value was poor (2.3266) in the prawn fed with RP at 30 % incorporation. The PER value was maximum of 1.2771 in prawn fed with FP at 10 % followed by 1.1356 in prawn fed with FP at 20 % incorporation. The minimum PER value of 1.1356 was recorded in prawn fed with RP at 30 % incorporation. Significant difference (P<0.05) in PER was observed between the prawns fed with raw and fermented *P. tetrastomatica* incorporated diets.

The whole body composition of prawns fed the seaweed *P. tetrastomatica* incorporated diets did not show significant differences. The moisture content of prawn fed the control feed showed higher value of 75.01 %. The protein and lipid levels were higher (17.61 % and 1.95 %) in the prawn fed the FP at 30 % incorporated diet. The ash content was higher in prawn fed the RP at 30 % incorporated diet.

4. Discussion

In the present study the effects of raw and fermented *Padina tetrastomatica* on the digestibility and growth of *M. rosenbergii* was undertaken. The present investigation, considerable increment in the protein and lipid contents of *P. tetrastomatica* (protein from 10.50 % to 15.90 % and lipid from 1.14 % to 3.23 %) was observed. During fermentation, an increase in the nutrient level through microbial synthesis is expected [12]. Similar reports on the improvement of nutritional value of fermented plant based ingredients viz., sesame seed meal in rohu [13], shrimp head waste meal in African cat fish [14], palm kernel meal in red hybrid tilapia [15] and prawn shell waste in Indian white shrimp [16, 17] were recorded. The fiber contents of fermented *Padina tetrastomatica* showed drastic reduction (from 23.96 % to 3.60 %). There are other reports showed similar decrease in fiber content of shrimp head silage meal[14] , fermented duck weed meal from 11.0 % to 7.5 % [18], yeast fermented water hyacinth from 19.0 % to 16.2 % [19] and fermented grass pea seed meal (from 9.6 % to 10 % reduced to 4.9 – 6.5 %). The increasing concentration of raw and fermented *P. tetrastomatica* resulted in reduction in mean weight gain and feed efficiency. The mean weight gain and feed efficiency of prawn fed the fermented seaweed incorporated diets and raw *Padina tetrastomatica* at 10 % and 20 % were higher than control. The prawn fed the raw *Padina tetrastomatica* at 30 % level showed mean weight gain and feed efficiency lower than control. The results obtained in this study with respect to raw seaweeds suggest that raw *Padina tetrastomatica* at 10 % and 20 % can be included in the diet for freshwater prawn without any compromise in growth performance and feed utilization efficiency. It was reported that *G. cervicornis* could be effectively used as a partial substitute for industrial feeds in shrimp *Litopenaeus vannamei* [20]. Inclusion of brown seaweeds such as *Undaria pinnatifida* and *Ascophyllum nodosum* enhanced the growth and feed efficiency of red sea bream [21]. It was reported that *G. cervicornis* could be effectively used as a partial substitute for industrial feeds in shrimp *Litopenaeus vannamei* [20]. Inclusion of brown seaweeds such as *Undaria pinnatifida* and *Ascophyllum nodosum* enhanced the growth and feed efficiency of red sea bream [21]. It was suggested that the inclusion of *G. bursa-pastoris* and *U. rigida* up to 10 % can be considered in diets for European sea bass (*Dicentrarchus labrax*) [22]. The Indian white shrimp fed with seaweed incorporated diet (*Ulva lactuca* and *Sargassum wightii*) improved the survival and resulted in higher SGR [23]. The utilization of several seaweed meals was evaluated in snakehead fry and found that among the seaweeds tested, *Ulva* sp provided the highest relative growth performance [24]. The green macro algal species such as *Ulva* sp., *Enteromorpha* and *Chaetomorpha* sp. have been used with some success as artificial feed supplement in mullet production [25]. The inclusion of seaweeds like *Ulva pertusa, Ascophyllum nodosum* and *Porphyra yezoensis* at a level of 5 % increased the body weight, feed utilization and muscle protein deposition in red sea bream [26].The reduced growth of the prawn fed the diets containing higher levels of raw *Padina tetrastomatica* appeared to be due to increasing fiber content of seaweeds in the diets. Seaweeds are the cheapest protein sources but their utilization is limited by the presence of high amount of crude fiber which can be eliminated by fermentation process [5]. The prawns fed the fermented *Padina tetrastomatica* incorporated diets showed higher growth and feed utilization efficiency than control and all the three raw seaweeds incorporated diets. The results demonstrated that even higher level incorporation of 30% fermented *Padina tetrastomatica* did not affect the growth. The inclusion of seaweeds like *Ulva pertusa, Ascophyllum nodosum* and *Porphyra yezoensis* at a level of 5% increased the body weight, feed utilization and muscle protein deposition in red sea bream [26]. The reduced growth of the prawn fed the diets containing higher levels of raw seaweed appeared to be due to increasing fiber content of seaweeds in the diets. Seaweeds are the cheapest protein sources but their utilization is limited by the presence of high amount of crude fiber which can be eliminated by fermentation process [5].

It suggests that fermentation was not required when *P. tetrastomatica* was included at lower levels of 10% and 20% level in the diets. Fermentation was found necessary only when seaweed was included at higher levels. Further research on the inclusion of above 30% level fermented *Padina tetrastomatica* in freshwater prawn diets may result in further replacement of fishmeal. Fermented *P. tetrastomatica* may be incorporated up to 30% level. The raw materials selected for this study are less costly than fishmeal and mass production of fermented seaweeds would pave way for replacing high cost fishmeal in freshwater prawn *M. rosenbergii*.

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