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Evaluation of raw and fermented seaweed, *Ulva lactuca* as feed ingredient in giant freshwater prawn *Macrobrachium rosenbergii*

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Abstract
The evaluation of seaweed, *Ulva lactuca* as feed ingredients in giant freshwater prawn *Macrobrachium rosenbergii* was carried out by incorporating raw and fermented *Ulva lactuca* at three levels, 10 %, 20 % and 30 % in diets. In 15 days digestibility experiment, among the raw and fermented *Ulva lactuca* incorporated diets, the freshwater prawn fed with fermented *Ulva lactuca* (FU) at 30 %, showed maximum apparent digestibility coefficients for dry matter (88.20 %), APD (88.57 %) and ALD (86.56 %). In the 45 days growth experiment, prawn fed with FU at 30 % showed maximum mean weight gain (2.417g), SGR (1.7892) and PER (1.0096). The best FCR value of 1.2691 was observed in prawn fed with FU at 20 % incorporation. The whole body composition of prawns fed the raw and fermented *Ulva lactuca* incorporated diets did not show any variations in moisture, protein, lipid and ash. The results of the study suggest that fermented *U. lactuca* could be incorporated up to 30 % level without compromising growth, digestibility and flesh quality.

Keywords: Freshwater prawn, feed ingredients, *Ulva lactuca*, growth, digestibility.

1. Introduction
Capture fisheries, including large-scale industrial and small-scale artisanal fisheries, are the giant freshwater prawn *Macrobrachium rosenbergii* (De Man) popularly known as scampi, is one of the high value aquaculture product emerging from Asia [1]. Traditionally fishmeal is the foundation for any aquaculture diet formulation. 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 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 [4]. Fermentation can be performed on any kind of seaweeds including rhodophyta, chlorophyta and phaeophyta and fermentation efficiency is relatively high in chlorophyta because of the high content of glucose availability [5]. In this background, this research was undertaken to evaluate raw and fermented seaweeds as feed ingredients in juveniles of freshwater prawn.

2. Materials and methods
Healthy postlarvae of freshwater prawn, *M. rosenbergii* 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

Ulva lactuca (green 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 1.15 x 10^4 cfu/ml and 2.45 x 10^4 cfu/ml. 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 experiment. 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 *Ulva lactuca* was incorporated in to the test diets at 10 %, 20 % and 30 % by replacing fishmeal and other ingredients. The raw *Ulva lactuca* incorporated diets are denoted as RU and the fermented *Ulva lactuca* incorporated diets are denoted as FU. 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 *U. lactuca* 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 *U. lactuca* 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 *Ulva lactuca* (% dry weight)

Seaweed samples	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Fiber (%)	NFE (%)	Gross energy (K cal/g)
Raw seaweed	16.20	21.00	1.11	24.0	19.61	18.08	3.63
Fermented seaweed	16.90	30.43	2.14	20.0	2.10	28.43	4.49

Table 2: Ingredient composition of raw and fermented *Ulva lactuca* incorporated feeds

Ingredients	Control	RU-10 (%)	RU-20 (%)	RU-30 (%)	FU-10 (%)	FU-20 (%)	FU-30 (%)
Fish meal	50.0	44.0	38.0	33.0	42.0	34.0	26.0
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	15.0	12.0	10.0	17.0	14.0	15.0
Cod liver oil	1.0	2.3	2.9	3.3	2.4	3.0	3.4
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	5.7	4.1	0.7	5.6	6.0	2.6

Table 3: Proximate composition of raw and fermented *Ulva lactuca* incorporated feeds

Feeds	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Fiber (%)	NFE (%)	DE (Kcal/g)
RU- 10%	10.57	28.14	7.17	10.21	5.82	38.09	3.45
RU- 20%	11.20	28.63	7.29	10.45	7.83	34.60	3.34
RU -30%	11.30	28.10	7.33	10.51	9.90	32.86	3.34
RU- 10%	10.57	28.14	7.17	10.21	5.82	38.09	3.45
RU- 20%	11.20	28.63	7.29	10.45	7.83	34.60	3.34
RU -30%	11.30	28.10	7.33	10.51	9.90	32.86	3.34
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] 1. The pH and dissolved oxygen was estimated by ECOSCAN water quality analyzer (EUTECH-INSTRUMENTS, Singapore)

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 [1].

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 *U. lactuca* was 6.30 x 10^8 cfu/ml and 6.10 x 10^9 cfu/ml respectively at the end of 72 hrs. There was significant increase in protein levels of *U. lactuca* (from 21.0 % to 30.43 %). The fiber content was drastically decreased to 2.10% for fermented *U. lactuca.*

3.2 Apparent Digestibility Coefficients of dry matter and nutrients in prawn fed with raw and fermented *U. lactuca* incorporated diets
Apparent digestibility coefficient for dry matter was higher (88.20 %) in the FU at 30 % incorporated diet followed by FU at 20 % incorporation (83.07 %). The lowest ADC of dry matter value (70.89 %) was recorded in prawn fed with RU at 30 % incorporation. Significant difference ($P<0.05$) in dry matter digestibility was observed between the prawn fed the raw and fermented *U. lactuca* incorporated diets. The apparent protein digestibility (APD) values were maximum (88.57 %) in FU at 30 % incorporated diet followed by FU at 20 % incorporation (85.62 %). Significant difference ($P<0.01$) was observed in APD of prawns fed with raw and fermented *U. lactuca* incorporated diets. The highest apparent lipid digestibility (ALD) value of 86.56 % was recorded in the prawn fed with FU at 30 % incorporated diet followed by FU at 20 % incorporation (82.84 %). Significant difference ($P<0.01$) was observed in ALD of prawns fed with raw and fermented *U. lactuca* incorporated diets.

3.3 Effect of raw and fermented *U. lactuca* incorporated diets on the growth of freshwater prawn
Prawns fed with FU at 30 % attained a maximum weight gain of 2.417 g followed by FU at 20 % (Table 4 and Fig 1). The highest SGR of 1.7892 was recorded in prawn fed the diet with FU at 30 % incorporation. The lowest SGR of 0.8394 was observed in prawn fed the diet with RU at 30 % incorporation. The mean feed intake was maximum (3.007 g) in prawn fed the diet with FU at 10 % incorporation followed by FU at 20 % incorporation (2.679 g). The lowest mean feed intake of 2.1535 g was observed in prawn fed the diet with RU at 30 % incorporation. Significant difference ($P<0.01$) in mean feed intake was observed between the prawns fed with raw and fermented *U. lactuca* incorporated diets. The prawn fed the diet with FU at 30 % showed good FCR of 1.1738. The prawn fed the diet with RU at 30 % showed poor FCR of 2.3537. The PER value was highest (1.0096) in prawn fed the diet with FU at 30 % incorporation followed by FU at 20% incorporation (0.8818). The lowest PER was observed in prawn fed the diet with RU at 30 % incorporation (0.3760). Significant difference ($P<0.05$) in PER was observed between the prawns fed with raw and fermented *U. lactuca* incorporated diets.

Table 4: Growth related parameters of prawns fed with raw and fermented *U. lactuca* 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	1.986±0.1202	3.850±0.4246	1.864±0.3253	1.47085±0.0135	0.7786±0.0136	1.7433±0.0304	3.250±0.00141	95.00±3.5355
RU-10%	1.9590±0.4455	3.3105±5.7276	1.3515±0.2100	1.1143±0.0513	0.5332±0.0434	2.1716±0.0322	2.935±0.0494	95.00±3.5355
RU-20%	1.9190±0.4101	3.0455±0.7778	1.1265±0.1195	0.9820±0.0421	0.4453±0.0141	2.3271±0.0741	2.480±0.0141	95.00±3.5355
RU-30%	1.9620±0.0495	2.9120±0.2487	0.950±0.0736	0.8394±0.00374	0.3759±0.0012	2.3736±0.0055	2.1535±0.0233	95±3.5355
FU-10%	1.9550±0.6223	3.9545±0.3748	1.9995±0.0997	1.5632±0.0919	0.8331±0.0417	1.50956±0.0755	3.007±0.1251	95.00±3.5355
FU-20%	1.9450±0.2263	4.0460±0.3536	2.101±0.0127	1.6515±0.0193	0.8818±0.0053	1.2691±0.0076	2.679±0.0252	100
FU-30%	1.9540±0.0849	4.3710±0.0849	2.417±0.0169	1.7892±0.00141	1.0096±0.0071	1.7375±0.0082	2.837±0.4310	100

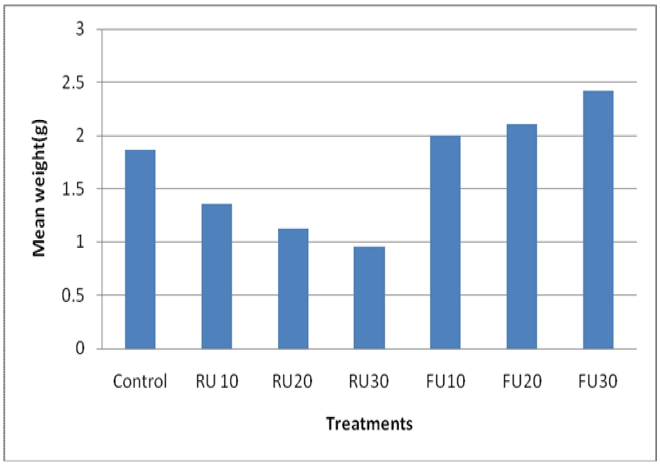

Fig 1: Effect of raw and fermented *U. lactuca* on the growth of *M. rosenbergii*

3.4 Effect of raw and fermented *U. lactuca* on the body composition (% wet weight) of freshwater prawns

The moisture content of prawn fed the control feed showed higher value of 75.89 %. The protein and lipid levels were higher (17.61 % and 1.95 %) in the prawn fed the FU at 30 % incorporated diet. The ash content was higher in prawn fed the FU at 20 % incorporated diet. However, there was no significant difference in its values among the prawns fed the raw and fermented *U. lactuca* incorporated diets.

4. Discussion

The present study evaluated the nutritional value of raw and fermented *U. lactuca* in juveniles of *M. rosenbergii* by assessing the digestibility and growth performance. In the present investigation the pH of fermented *U. lactuca* dropped from 7.0 to 4.0 on the 3rd day. The rapid and sharp drop in the pH values indicated good fermentation of *U. lactuca*. This pH value is within the recommended value of successful silage Fermentation [12]. During the present investigation, considerable increment in the protein and lipid contents of the fermented *U. lactuca* (protein from 21 % to 30.43% and lipid from 1.11 % to 2.14 %) was observed. During fermentation, an increase in the nutrient level through microbial synthesis was observed [13]. Similar reports were recorded on the improvement of nutritional value of fermented plant based ingredients viz., sesame seed meal in rohu [14], shrimp head waste meal in African cat fish [15] , palm kernel meal in red hybrid tilapia [16] and prawn shell waste in Indian white shrimp [17, 18] . The fiber contents of fermented *U. lactuca* showed drastic reduction (from 19.61 % to 2.01 %. There are other reports showed similar decrease in fiber content of shrimp head silage meal [15] , fermented duck weed meal from 11.0 % to 7.5 % [19] , yeast fermented water hyacinth from 19.0 % to 16.2 % [20] and fermented grass pea seed meal (from 9.6 % to 10 % reduced to 4.9 – 6.5 %). The mean weight gain and feed efficiency of prawn fed the fermented *U. lactuca* showed increasing trend with the increasing incorporation in the diet. The prawn fed the raw *Ulva lactuca* at all levels showed mean weight gain and feed efficiency lower than control. It was reported that *G. cervicornis* could be effectively used as a partial substitute for industrial feeds in shrimp *Litopenaeus vannamei* [21]. Inclusion of brown seaweeds such as *Undaria pinnatifida* and *Ascophyllum nodosum* enhanced the growth and feed efficiency of red sea bream [22] (Yone *et al*., 1986). It was interesting to note that raw *U. lactuca* at all levels showed inferior performance than control feed. The utilization of several seaweed meals were evaluated in snakehead fry and among the seaweeds tested, *Ulva* sp provided the highest relative growth performance [23] 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 [24]. 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 [25]. 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 [4]. The prawns fed the fermented *U. lactuca* incorporated diets showed higher growth and feed utilization efficiency than control and the raw *U. lactuca* incorporated diets. The results demonstrated that even higher level incorporation of 30 % fermented *U. lactuca* did not affect the growth. The best performance of prawn in terms of percentage weight gain, SGR, PER, FCR, was observed in the diets containing fermented *U. lactuca* than raw *U. lactuca*. The mean feed intake was also higher in prawns fed the fermented *U. lactuca*. Similar results were observed in fish (rohu) fed sesame seed meal fermented with *L. acidophilus* [14]. Fish fed the diet containing 40 % fermented grass pea recorded better weight gain, SGR and PER than fish reared on a reference diet containing 40 % fishmeal as the protein source [26]. Leaf meals of *Lemna* sp. and *Leucaena* sp. fermented with *Bacillus* sp. were successfully used to replace fishmeal in diets for rohu fingerlings up to 30 % level [19] (Bairagi *et al*., 2002).

APD and ALD were increasing for increasing levels of raw and fermented *U. lactuca* incorporation was observed. The dry matter, APD and ALD values were higher for the diets containing fermented *U. lactuca* meals in comparison to those containing raw *U. lactuca* meals and control feed. Similar declining trends in APD and ALD values have also been reported with higher levels of inclusion of raw sesame seed [14] and leaf meals [19] in carp diets. Higher APD values in fermented meals containing diets were observed in rohu with duckweed leaf meal [19], grass pea [26] and sesame seed meal [14]. High levels of fiber in the raw seaweeds may have been responsible for the observed low APD and ALD values. The apparent digestibility coefficients of dry matter and lipid were significantly lower in fish fed diet *G. cornea* at 10 % level relative to those fed the control diet [27].

The whole body composition of prawn fed the raw and fermented *U. lactuca* incorporated diets did not show any variations in moisture, protein, lipid and ash. The diets with variable protein sources i.e. fishmeal and soy flour did not affect the carcass protein content of the prawn [28]. Feeding freshwater prawn, *M. rosenbergii* juveniles with isocaloric and isonitrogenous diets prepared with fishmeal, acid fish silage and fermented fish silage did not affect their carcass protein [29]. The carcass crude protein levels are reported to vary only with varying levels of dietary protein [30].

The present study also confirms that isonitrogenous diets with seaweed protein sources did not affect the carcass nutrients. The results of this study suggest that fermented *U. lactuca* at 30% level could be used efficiently for *M. rosenbergii* without compromising flesh quality.

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