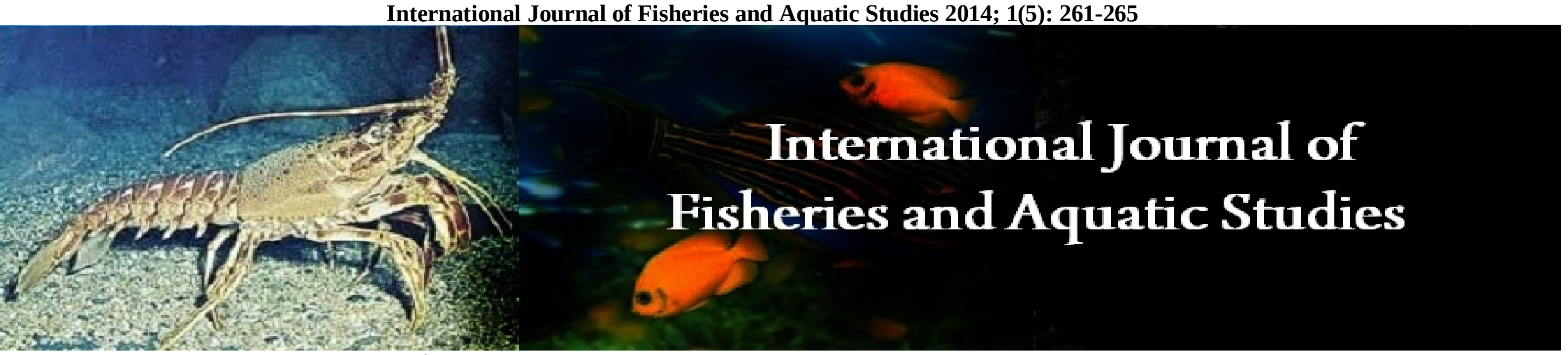
International Journal of Fisheries and Aquatic Studies 2014; 1(5): 261-265



ISSN: 2347-5129
IJFAS 2014; 1(5): 261-265

www.fisheriesjournal.com
Received: 02-03-2014
Accepted: 11-04-2014

N. Felix
Fisheries Research and Extension Centre Tamil Nadu Fisheries University Madhavaram Milk Colony Chennai-600051.

R. Alan Brindo
Fisheries Department Teynampet Chennai – 600018.

Correspondence:
N. Felix
Fisheries Research and Extension Centre Tamil Nadu Fisheries University Madhavaram Milk Colony Chennai-600051.

Substituting fish meal with fermented seaweed, *Kappaphycus alvarezii* in diets of juvenile freshwater prawn *Macrobrachium rosenbergii*

N. Felix, R. Alan Brindo

Abstract
The substitution seaweed, *Kappaphycus alvarezii* by replacing fish meal in giant freshwater prawn *Macrobrachium rosenbergii* was carried out by incorporating raw and fermented *Kappaphycus alvarezii* at three levels, 10 %, 20 % and 30 % in diets. In 15 days digestibility experiment, among the raw and fermented seaweeds incorporated diets, the freshwater prawn fed with fermented *K. alvarezii* (FK) at 10 %, showed maximum apparent digestibility coefficients for dry matter (96.46 %), APD (94.54 %) and ALD (94.69 %). In the 45 days growth experiment, prawn fed with FK at 10 % showed maximum mean weight gain (3.38 g), SGR (2.2326), mean feed intake (4.47 g) and PER (1.4256). The best FCR value of 1.1424 was observed in prawn fed with FK at 10 % incorporation followed by FK at 20 % incorporation (1.1466). 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 *K. alvarezii* could be incorporated in freshwater prawn diets up to 10 % and 20 % respectively and fermented *K. alvarezii* could be incorporated up to 30 % level without compromising growth, digestibility and flesh quality.

Keywords: Freshwater prawn, *Kappaphycus alvarezii, Macrobrachium rosenbergii.*

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 assess the replacement level of fish meal by seaweed in 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 (30 cm x 20 cm) 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

Kappaphycus alvarezii (red seaweed) was purchased from Pepsi-Co Company, 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 seaweed to sea water at 1:9 ratio was introduced in fermenter. Each 10 ml of *Lactobacillus* spp. and *Saccharomyces cerevisiae* was inoculated at a concentration of 1.93 x 10^4 and 2.14 x 10^4 respectively. 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 *Kappaphycus alvarezii* was incorporated in to the test diets at 10 %, 20 % and 30 % by replacing fishmeal and other ingredients. The raw *Kappaphycus* sp. incorporated diets are denoted as RK and the fermented *Kappaphycus* sp. incorporated diets are denoted as FK. Control feed was prepared without incorporation of raw and fermented *Kappaphycus alvarezii*. 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 *Kappaphycus alvarezii* 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 *Kappaphycus alvarezii* 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 Kjeldhal 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 *Kappaphycus alvarezii* (% dry weight)

Seaweed samples	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Fiber (%)	NFE (%)	Gross energy (K cal/g)
Raw seaweed	16.00	14.00	1.16	34.0	18.48	16.36	3.88
Fermented seaweed	16.96	23.86	3.64	27.0	5.20	23.34	4.98

Table 2: Ingredient composition of raw and fermented *Kappaphycus alvarezii* incorporated feeds

Ingredients	Control	RK-10 (%)	RK-20 (%)	RK-30 (%)	FK-10 (%)	FK-20 (%)	FK-30 (%)
Fish meal	50.0	46.0	42.0	39.0	43.0	37.0	31.0
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	6.2	5.0	3.0	9.2	5.0	11.0
Cod liver oil	1.0	2.4	2.7	3.0	2.3	2.6	2.7
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	12.4	7.3	2.0	12.5	12.4	2.3

Table 3: Proximate composition of raw and fermented *Kappaphycus alvarezii* incorporated feeds

Feeds	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Fiber (%)	NFE (%)	DE (Kcal/g)
RK- 10%	10.98	28.21	7.25	10.25	5.85	37.46	3.47
RK- 20%	10.87	28.07	7.29	10.36	7.70	35.71	3.44
RK- 30%	10.69	28.32	7.38	10.45	9.55	33.61	3.36
FK- 10%	11.31	28.03	7.21	10.15	4.52	38.78	3.49
FK- 20%	10.90	28.11	7.28	10.25	5.04	38.42	3.47
FK- 30%	11.36	28.18	7.36	10.31	5.56	37.23	3.42
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]. 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 [11].

3. Results

3.1 Proximate composition of fermented seaweeds

There was significant increase in protein levels of *K. alvarezii* (from 14.00 % to 23.86 %) was observed. The fiber content was drastically decreased to 5.20 % for fermented *K. alvarezii*.

3.3 Digestibility studies

Apparent digestibility coefficient for dry matter was higher (96.46 %) in FK at 10 % incorporated diet followed by FK at 20 % incorporation (Table 4). Significant difference (P<0.01) in dry matter digestibility was observed between the prawns fed with raw and fermented *K. alvarezii* incorporated diets. The apparent protein digestibility (APD) value was maximum (94.54 %) in FK at 10 % incorporated diet followed by FK at 20 % incorporation (92.36 %). Significant difference (P<0.01) in APD was observed between the prawns fed with raw and fermented *K. alvarezii* incorporated diets. The highest apparent lipid digestibility (ALD) value of 94.69 % was recorded in the prawn fed with FK at 10 % incorporated diet followed by FK at 20 % incorporation (92.83 %). Significant difference (P<0.01) in APD was observed between the prawns fed with raw and fermented *K. alvarezii* incorporated diets.

Table 4: Apparent digestibility coefficients (ADCs) of dry matter and nutrients for prawns fed with raw and fermented *Kappaphycus alvarezii* incorporated diets

Treatment	Dry matter (%)	Protein APD (%)	Lipid ALD (%)
Control	80.34 ± 0.0213	80.64 ± 0.0252	82.81 ± 0.0361
RK-10%	78.13 ± 0.0541	78.37 ± 0.0365	73.28 ± 0.0963
RK-20%	75.47 ± 0.0254	76.82 ± 0.0874	70.39 ± 0.0875
RK-30%	71.58 ±0.0214	74.88 ± 0.0525	68.14 ± 0.0254
FK-10%	96.46 ± 0.0254	94.54 ± 0.0254	94.69 ± 0.0754
FK-20%	94.36 ± 0.0247	92.36 ± 0.0853	92.83 ± 0.0842
FK-30%	90.65 ± 0.0326	91.15 ± 0.0354	91.91 ± 0.0741

3.3 Effect of raw and fermented *K. alvarezii* incorporated diets on the growth of prawn

The prawn fed with FK at 10 % attained the maximum weight gain of 3.389 g followed by FK at 20 % incorporation (Table 5). The minimum weight gain of 1.3485 g was observed in prawn fed with RK at 30 % inclusion. Significant difference (P<0.05) in mean weight gain was observed between the prawns fed with raw and fermented *K. alvarezii* incorporated diets. Prawn fed with FK at 10 % showed the highest SGR of 2.2326 followed by FK at 20 % (2.1545). The minimum SGR value was recorded in prawn fed the diet with RK at 30 % incorporation (1.1809). The mean feed intake was maximum (4.47 g) in prawn fed with FK at 10 % incorporation followed by FK at 20 % incorporation (3.90 g). The minimum mean feed intake was recorded in prawn fed with RK at 30 % incorporation (3.212 g). Significant difference (P<0.01) in mean feed intake was observed between the prawns fed with raw and fermented *K. alvarezii* incorporated diets. The highest FCR of 1.1424 was obtained in the prawn fed with FK at 10 % incorporation followed by FK at 20 % incorporation (1.1466). FCR value was poor (2.4299) in the prawn fed with RK at 20 % incorporation. The PER value was maximum of 1.4256 in prawn fed with FK at 10 % followed by 1.2569 in prawn fed with FK at 20 % incorporation. The minimum PER value of 0.5695 was recorded in prawn fed with RK at 20% incorporation.

Table 5: Growth related parameters of prawns fed with raw and fermented *Kappaphycus alvarezii* 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
RK-10%	1.9420±0.0848	4.070±0.25541	2.1280±0.1188	1.6439±0.0599	0.8890±0.0495	2.1081±0.0235	3.665±0.0778	100
RK-20%	1.9820±0.2333	3.3455±1.6263	1.3635±0.1393	1.1809±0.1078	0.5695±0.0581	2.4299±0.0047	3.075±0.0070	100
RK-30%	1.9840±0.0354	3.4755±0.7778	1.4915±0.0813	1.2458±0.0538	0.6230±0.3390	2.1241±0.1158	3.212±0.0735	100
FK-10%	1.9580±0.2829	5.3470±0.6364	3.3890±0.0354	2.2326±0.0057	1.4256±0.0070	1.1424±0.0148	4.470±0.6211	100
FK-20%	1.9230±0.1273	4.9320±0.1980	3.0090±0.0070	2.1581±0.0977	1.2569±0.0029	1.1466±0.0026	3.900±0.0991	100
FK-30%	1.9265±0.0636	4.8450±0.7072	2.9185±0.0790	2.0491±0.03946	1.2191±0.0322	1.15605±0.0028	3.700±0.0331	100

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

The moisture content of prawn fed the RK at 30 % showed higher value of 75.24 %. The protein (16.78 %) and lipid levels (1.90 %) were higher in the prawn fed the RK at 30 % incorporated diet and control diet respectively. The ash content was higher (1.59 %) in prawn fed the RK at 30 % incorporated diet. However, there was no significant difference in its values among the prawns fed the raw and fermented *K. alvarezii* incorporated diets.

4. Discussion

The present study assesses the replacement of fish meal by seaweed in juveniles of *M. rosenbergii.* Considerable increment in the protein and lipid contents of the fermented seaweed (protein from 14.00 % to 23.86 % and lipid from 1.16 % to 3.64 %) was recorded. Microbial protein is believed to contribute significant to the protein content of fermented product. During fermentation, an increase in the nutrient level through microbial synthesis was recorded [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] and palm kernel meal in red hybrid tilapia [15] were recorded. The fiber contents of all the three fermented seaweeds showed drastic reduction 18.48% to 5.20 % in *K. alvarezii*) There are other reports showed similar decrease in fiber content of shrimp head silage meal [14] and fermented duck weed meal from 11.0 % to 7.5 % [16]. It is evident from the present study that seaweed can be utilized as feed ingredients in the diets for juveniles of *M. rosenbergii*. The increasing concentration of raw and fermented *K. alvarezii* resulted in reduction in mean weight gain and feed efficiency. The prawn fed the raw *Kappaphycus alvarezii* at 20 % and 30 % levels showed mean weight gain and feed efficiency lower than control. The results obtained in this study with respect to raw seaweed suggest that raw *Kappaphycus alvarezii* at 10 % 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* [17] The Indian white shrimp fed with seaweed incorporated diet (*Ulva lactuca* and *Sargassum wightii*) improved the survival and resulted in higher SGR [18] . The higher levels of incorporation (20 % and 30 % for RK) did not perform well. The reduced growth of the prawn fed the diets containing higher levels of raw seaweeds 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 prawn fed even higher level incorporation of 30 % fermented seaweeds did not affect the growth. The best performance of prawn in terms of SGR, PER and FCR, was observed in the diets containing fermented seaweed than raw seaweed. The mean feed intake was also higher in prawns fed the fermented seaweeds. Best performance in fish (rohu) fed sesame seed meal fermented with *L. acidophilus* [13]. 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 [19]. 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 [20].

The dry matter, APD and ALD values were higher for the diets containing fermented seaweed meals in comparison to those containing raw seaweed meals and control feed. This may be due to high protein and low fiber contents of fermented seaweeds which are highly digestible. Similar declining trends in APD and ALD values have also been reported with higher levels of inclusion of raw sesame seed [13] and leaf meals [20] in carp diets. Higher APD values in fermented meals containing diets were observed in rohu with duckweed leaf meal [20], grass pea [19] and sesame seed meal [13]. 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 [21].

The whole body composition of prawn fed the raw and fermented seaweeds incorporated diets did not show any variations in moisture, protein, lipid and ash. In contrast the values of carcass protein and lipid contents were found to be higher in the fish fed diets containing fermented sesame seed and leaf meal [13, 20]. 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 [22]. The carcass crude protein levels are reported to vary only with varying levels of dietary protein [23]. 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 the seaweed did not affect the carcass nutrients. The results of this study suggest that the seaweed based diets could be used efficiently for *M. rosenbergii* without compromising flesh quality.

The present investigation has demonstrated that seaweed can be a potential feed ingredient in the juveniles of freshwater prawn *M. rosenbergii*. It suggests that fermentation was not required when *K. alvarezii* was included at lower levels of 10% and 20 % in the diets. Fermentation was found necessary only when seaweeds were included at higher levels. Superior growth performance, feed utilization efficiency and apparent protein and lipid digestibility could be achieved with the incorporation of 30 % fermented *K. alvarezii* in the prawn diets by replacing 19 % fishmeal. Further research on the inclusion of above 30 % level fermented seaweeds in freshwater prawn diets may result in further replacement of fishmeal. The results showed that raw *K. alvarezii* may be incorporated in freshwater prawn diets up to 10% and 20% respectively. Fermented *K. alvarezii* 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*.

5. Acknowledgement

The authors thank the Tamil Nadu Veterinary and Animal Sciences University, Chennai for the facilities provided to carry out the research in Fisheries College and Research Institute, Thoothukudi.

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