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The effect of dietary protein on the growth potentials and nitrogen excretion in freshwater prawn *Macrobrachium rosenbergii.*

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Abstract
The effect of dietary protein content on the growth potentials, digestion efficiency of proteins, nitrogen excretion rates were monitored in freshwater prawn *Macrobrachium rosenbergii* after fed with Four Experimental diets with differential amounts of proteins. The Experimental Diets with relatively high protein content capable of including highest growth potentials compared to Experimental diets with low protein quantities. Growth parameters including weight gain, Specific growth rates, Daily growth rates, Feed conversion efficiency, Nitrogen excretion and Nitrogen retentions in the prawns indicate that Experimental diets with more than 40% dietary protein are capable of inducing the best growth potentials and are recommended for use in freshwater prawn Aquaculture.

Keywords: *Macrobrachium rosenbegii*, dietary protein, growth potentials, Nitrogen excretion.

1. Introduction
In recent years, Formulation of Experimental diets inducing best growth potentials for several Crustaceans has progressed remarkably, including for several penaeid shrimp species, [16, 21, 26, 32, 33, 40, 42, 46]. However, in the giant freshwater prawn *Macrobrachium rosenbergii* an artificial diet has not been established precisely. The giant freshwater prawn *M. rosenbergii* is a commercially important species in India and South Asian Countries [41, 42] and its healthy and sustainable Aquaculture is currently the need in India. Though there is a lot of information is available on the nutritional requirements on penaeid prawns [1, 2, 3, 4, 5, 6, 7, 8, 20] especially on dietary protein requirements [6, 9], in the range of 20-62% [29, 30]. But till today the information concerning about dietary protein requirements of tropical freshwater prawn *M. rosenbergii* is Scanty. The studies concerning protein and energy requirements through the feed ingredients for freshwater prawn needs to be investigated for the successful formulation of artificial feeds. Dietary protein is an important component, which intern either trigger or hamper the growth potentials in prawns. Therefore, it is very important to know the fate of dietary protein i.e., Anabolism and Catabolism of proteins in the prawns, and thereby its influence on growth patterns. Ammonia is considered to be the major end product of metabolism of nitrogenous dietary constituents, such as protein [17]. Therefore the rate of Ammonia excretion is very much crucial and will play an important role in the prawns. Moreover, very little is available on how nitrogen excretion is affected by dietary protein content and whether other excretory nitrogenous products, apart from ammonia are sufficient to the prawn species. The Present Study is aimed to determine the effect of dietary protein contents on growth patterns along with nitrogen excretion rates and monitoring of digestibility values of dietary proteins for the assessment of growth patterns in freshwater prawn *M. rosenbergii.*

2. Materials and methods
Juvenile prawns *M. rosenbergii* of equal size (2.35 ± 0.15 g) were obtained from local Aqua farms. They were transported to the laboratory in Polythene Bags filled with Oxygenated water and maintained in a Cement Tanks (1000 Lit Capacity) with ground water (pH 7.0; Total dissolved solids 0.8 g L^{-1}; Dissolved Oxygen 7.2 mg L^{-1}; BOD 30.0 mg L^{-1}, COD 125 mg L^{-1}; Ammonia 0.028 mg L^{-1}).
The juvenile prawns were acclimatized to ambient Laboratory conditions and fed *ad libitum* with commercial diet obtained from CP Aquaculture Ltd, Chennai. The Cement Tanks were continuously aerated with the help of Compressors.

2.1 Diet formulation

Experimental diets were formulated to contain protein contents ranging from 20 to 62% Dextrin, Cod liver oil, Starch and Cellulose were adjusted to maintain similar dietary energy contents and total proportions were adjusted to 100% by adding other ingredients (Table. 1). Diets were prepared by mixing dry ingredients and Water (2:1 W/V) and were squeezed through a hand pelletizer with 3 mm diameter mesh size. The Pellets obtained were sundried till no moisture is observed. Protein content in the

Experimental diets were determined by the Micro Kjeldahl Procedure as described by Koshio *et al* (1992a) [35]. The Total lipid content of the Experimental diets was extracted into Chloroform – Methanol mixture by the method of Bligh and Dyer (1959) [13] and quantified by the method of Bragdon (1950).

Table 1: Composition of Experimental Diets

Ingredients	Diets			
	ED_1	**ED_2**	**ED_3**	**ED_4**
Fish meal	20.0	35.7	50.0	62.0
Dextrin	20.7	17.0	12.0	8.0
Starch	11.0	8.0	6.2	4.0
Cod liver oil	15.0	11.0	7.3	2.7
α-Cellulose	16.0	11.0	7.2	6.0
Cholesterol	0.5	0.5	0.5	0.5
Glucosamine Hydrochloride	1.0	1.0	1.0	1.0
Sodium bicarbonate	1.0	1.0	1.0	1.0
Sodium citrate	0.5	0.5	0.5	0.5
Sodium succinate	0.5	0.5	0.5	0.5
Chromium oxide	0.5	0.5	0.5	0.5
Agar Agar	2.0	2.0	2.0	2.0
Vitamin mixture	8.6	8.6	8.6	8.6
Mineral mixture	2.7	2.7	2.7	2.7
Proximate composition				
Crude Protein (%)	15.1	29.3	38.5	55.5
Crude Lipid (%)	12.82	8.38	7.39	5.13
Energy (Kcal/g)	241.958	271.413	306.400	376.00

2.2 Growth Trail experiments

The prawns used in the present study were divided into Four groups, each with 100 No's of equal size and each group housed in Three Cement Aquatic Tanks with 4-5 cm thick sand bottom. Feeding trail experiments were conducted under Laboratory conditions for 30 days. Water was renewed daily by siphoning method at least 15-20% water exchange was carried out. The Growth trail experiments were conducted under a predominantly dark regime, with occasional lighting when mortality was checked or when feeding or periodic weight measurements were carried out. Prawns were fed with Experimental diets at 6.00 AM on a ration equal to 10% of body weight for 30 days. The ration was adjusted regularly after prawns were removed for each weight measurement. Every day uneaten diet and faeces were separately collected and quantified. The collected materials were oven dried at 100 ^{o}C for 24 h for determination of dry food intake. Generally the prawns were placed on placed on paper towels to remove excess water and then weighed using Electronic Balance. At the end of the feeding trail, growth and survival performances, indices of feed quality were calculated by using the following formulae so as to evaluate the efficiency of feeds prepared.

$$\text{Survival Rate (\%)} = \frac{\text{Number of live prawns}}{\text{Number of prawns at the start as the experiment}} \times 100$$

$$\text{Weight gain (g)} = \text{Final weight} - \text{Initial weight}$$

$$\text{Weight gain (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Specific growth rate (SGR)} = \frac{(\text{Log Final weight} - \text{Log Initial weight})}{\text{Days of Experiment}} \times 100$$

$$\text{Feed conversion efficiency (FCE)} = \frac{\text{Wet weight gain (g)}}{\text{Dry weight of feed offered}} \times 100$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Protein intake}}$$

2.3 Determination of digestibility protein and Ammonia excretion

After the Feeding trail Experiments are over, 10 each Intermolt stage prawns were selected from all the four groups and are used for determination of Diet digestibility studies and Ammonia excretion rates. The prawns were given Four Experimental diets, which were formulated in the present study (Table. 1). For Digestibility studies, individual prawns were allowed to feed on the experimental diets for 1 h in small Aquaria to avoid ingestion sand. Faeces were collected and used for analysis. Chromium Oxide which was already mixed in each diet was used as an inert marker to determine digestibility values.

$$\text{\% Digestibility of Dry matter} = (1 - \frac{\text{\%Cr2O3 in diet}}{\text{\% Cr2O3 in faeces}}) \times 100$$

$$\text{\% Digestibility of Protein} = (1 - \frac{\text{\% Protein in faeces / \% Cr2O3 in Faeces}}{\text{\% Protein in diet / \% Cr2O3 in diet}}) \times 100$$

Faeces and Residual feed, collected are subjected to the estimation of Protein and Chromium contents by following the Methods of Koshio *et al* (1992a) [35] and Furukava and Tsukahara (1966) [25], respectively. For the determination of Ammonia excretion, the prawns were transferred to fresh media and prawns were allowed for feeding for 1 h. Ammonia excretion rates were determined based on the difference in Ammonia Concentration between Initial water sample and water sample after 1 h. Ammonia concentrations were determined continuously with a break of every 1 h up to 5 h by adopting the method of Strickland and Parsons (1977) [49]. For the determination of Ammonia excretion rates of starved prawns, the prawns were starved for 24 h after the prawns were allowed to feed for 1 h and then the measurements were conducted in the same manner as those for fed prawns. Similarly Urea excretion rates were also measured in the same manner as that of Ammonia excretion estimation to determine the relative proportion of Ammonia and Urea excreted over a 24 h period prawns were kept in small Aquarium of 5 lit capacity and prawns were kept in the above mentioned Aquaria 1 h after feeding for 24 h. The Ammonia and Urea contents were measured in Media and Compared with around water samples. Urea content was measured by the method of Strickland and Parsons (1977) [49]. Ammonia – N and Urea – N excretion rates were expressed as µg per wet weight of prawns.

3. Results and Discussion

The prawns *M. rosenbergii* were subjected to growth trail experiments after feeding with four different formulated diets in the present investigation. The Results obtained in the present investigation associated with growth parameters for *M. rosenbergii* after fed with four Experimental diets were presented in Table. 2. The percent survival values record in the present investigation ranges between 83 to 93%, i.e., not significantly different indicating that differences in dietary protein content in the diet did not affect survival. The crude protein values obtained for the Four Experimental diets recorded as 15.1% for ED_1, 29.3%, 38.5% and 55.5% for ED_2, ED_3 and ED_4, respectively (Table.1). The weight gain in prawns fed with Four different types of diets was significant and showed that weight gain is positively correlated with increased protein contents in the Experimental diets, i.e., with an increase in the crude protein in the diet induced relatively higher growth potentials in prawns. The weight gain and percent weight gain values are relatively lower with prawns fed with ED_1 (109%) and ED_2 (148%), but is relatively higher with ED_3 (196%) and (234%) with ED_4. The Specific Growth rates recorded as 1.01, 1.34, 1.51 and 1.74 for ED_1, ED_2, ED_3 and ED_4, respectively. Minimum SGR recorded with ED_1 containing 15.1% crude protein compared with the maximum recorded SGR with 1.74 ED_4, Which contains crude protein of 55.5%. The Apparent food intake by prawns fed with Four Experimental diets are 1.45 (ED_1), 1.49 (ED_2), 1.53 (ED_3) and 1.54 (ED_4). It is very clear that the values are not significant among each other and also prawns are seems to consume slightly more Protein as dietary protein as dietary protein contents increased in the Experimental diets. Feed Conversion Efficiency (FCE) values, also showed that with an increase in the dietary Protein content, the values also showed a significant increment. The Protein Efficiency Ratio (PER) values showed an increment with an increment in dietary protein up to 38.5% crude protein, but PER values are relatively lower (1.14) with ED_4 containing 55.5% crude protein. The Dry matter digestibility values obtained in the present investigation ranges between 74.18 to 76.54 and not significantly with each other indicates that dietary protein content does not manifest any effect on apparent dry matter digestibility of Experimental diets in prawns. The Digestibility of protein values in prawns fed with experimental diets (ED_1 - ED_4) were ranging between 93.24 to 96.42, indicative of that dietary proteins are efficiently digested by prawns. The values are also shown to increase from ED_1 with lower crude protein (15.1%) 94.32% with ED_2 (29.3 CP), 95.39% with ED_3 (38.5% CP) and 96.42% with ED_4 (55.5% CP) (Table.3). The rate of Ammonia excretion in both fed and starved prawns were determined, i.e., rate of Ammonia excretion in for every hour were determined. The Total Ammonia and Mean Ammonia levels obtained in the present study indicates with an increase in the dietary protein from ED_1 to ED_4 also shown an increase from 81.36 µg with ED_1 to 163.27 µg with ED_4. Similarly Mean Ammonia values obtained were also known to increase with an increase in dietary protein contents in Experimental diets. The rate of Ammonia excretion was relatively higher up to 3 h and later it was decreased in both fed and starved prawns. The Ammonia – N values obtained in the present study also showed to increase with an increase in dietary protein content in Experimental diets. The Excreted Urea levels in the present investigation are relatively very low and are known to increase with an increase in the dietary protein content in the Experimental diets.

Table 2: Growth parameters of fresh water prawn *M. rosenbergii* after fed with Experimental Diets (ED)

Parameter	ED_1	ED_2	ED_3	ED_4
Crude Protein (%)	15.1±0.3	29.3±0.5	38.5±0.5	55.5±0.7
Initial weight (g)	2.35±0.13	2.37±0.14	2.35±0.15	2.36±0.15
Final weight (g)	4.92±0.25	5.89±0.28	6.97±0.33	7.88±0.38
Weight Gain (g)	2.57	3.52	4.62	5.52
Weight Gain (%)	109.36	148.52	196.60	233.89
Specific Growth rate	1.01±0.12	1.34±0.13	1.51±0.14	1.74±0.15
Daily Growth rate co-efficient	2.86±0.18	3.91±0.22	5.13±0.24	6.14±0.29
Feed intake	1.45±0.05	1.49±0.07	1.53±0.08	1.54±0.07
Protein intake	0.38±0.02	0.53±0.05	0.75±0.08	0.92±0.03
Feed Conversion Efficiency	0.34±0.03	0.55±0.07	0.69±0.07	0.78±0.07
Protein Efficiency Ratio	1.39±0.24	1.58±0.25	1.43±0.21	1.14±0.15
Survival (%)	85±3	86±2	93±4	83±3
Crude lipid (%)	12.82±0.22	8.38±0.21	7.39±0.18	5.13±0.12
Energy (Kcal/g)	241.958	271.413	306.400	376.00

All values are Mean ± SD of six individual observations

Table 3: Apparent digestibility of dry matter and protein for prawns fed with experimental diets

Diet No	Dietary protein (%)	Digestibility (%)	
		Dry matter	Protein
ED1	15.1	75.23±2.12	93.24±2.15
ED2	29.3	74.18±2.14	94.32±2.18
ED3	38.5	74.89±2.13	95.39±2.32
ED4	55.5	76.54±2.14	96.42±2.41

Table 4: Ammonia excretion by fresh water prawn *M. rosenbergii* during feeding and starvation

Fed	1h	2h	3h	4h	5h	Total	Mean
ED1	20.18±1.12	16.24±1.02	16.05±1.08	12.95±1.13	15.95±1.18	81.36±2.14	16.272±1.24
ED2	22.45±1.18	24.32±1.24	25.77±1.28	21.42±1.18	17.95±1.05	111.90±3.44	22.382±1.12
ED3	28.34±1.19	46.24±1.71	33.42±1.13	22.14±1.18	22.74±1.18	152.88±3.85	30.576±1.13
ED4	49.22±1.75	37.11±1.78	24.34±1.14	29.18±1.03	23.42±1.03	163.27±4.18	32.654±1.34
Starved	**1h**	**2h**	**3h**	**4h**	**5h**	**Total**	**Mean**
ED1	16.34±1.13	17.38±1.12	14.88±1.02	16.04±1.12	24.35±1.32	88.99±3.75	17.798±1.12
ED2	21.33±1.24	21.04±1.11	19.32±1.05	19.38±1.08	16.35±1.13	97.42±3.58	19.484±1.34
ED3	20.14±1.18	23.14±1.18	21.85±1.21	16.45±1.32	17.39±1.18	98.77±4.52	19.794±1.54
ED4	22.45±1.32	20.25±1.34	20.57±1.18	16.77±1.18	13.99±1.02	94.03±5.04	18.806±1.04

Table 5: Excretion rates of Ammonia and Urea by fresh water prawn *M. rosenbergii* after fed with experimental diets

Diet	Ammonia	Urea
ED1	658±25	45±2
ED2	959±42	54±3
ED3	1043±56	89±3
ED4	1210±55	92±4

4. Discussion

Freshwater Prawns generally known to require relatively higher dietary proteins compared to other crustaceans including penaeid prawns including *Penaeus monodon*, *P. indicus* and *P. vannamei*. Freshwater prawns are known to require dietary protein contents around 50 – 55% [10, 31, 36, 39, 45, 50]. In the present investigation the protein requirement in freshwater prawn appears to be more than 40% and above 40% dietary protein could sustain good growth and survival of prawns during feeding trail experiments. Several authors emphasized that more than 40% dietary proteins also sustained good growth and survival of freshwater prawns, which were fed on Experimental diets containing Fish Meal, Soybean Meal and other Protein rich ingredients [11, 27, 38, 44]. The discrepancies in reported optimum dietary protein content among studies may be due to the use of different digestible energy contents rather than species dependent protein requirements. Deshmaru and Kurokhi (1974), Deshimaru and Yone (1978), Habashy (2009), Gomez (1988) [23, 24, 28, 31] reported that Casein was the main protein source in feeding trail experiments in penaeid prawns and also Freshwater prawns, but the role of Casein in the dietary energy was not studied. But Boghen *et al*., (1982) Castell *et al*., (1989), Morrissy (1989), Koshio *et al*., (1990) [14, 15, 34, 37] reported that crab protein is one of the best protein sources for several crustaceans including penaeid prawns and freshwater prawns. Crab Protein is likely to contain amino acid profile, which is very efficiently absorbed and utilized by the prawns. It is very clear from the results obtained that minimum dietary protein content is hampering the achievement of maximum growth rates, may be due to reduced energy production rates of the Experimental diets with low protein quantity. The results also indicate that prawns may require less protein, but their energy requirement is being met from the Non – protein nutrients substances including Carbohydrates included in the feed formulation. From the results obtained it is very clear that the minimum protein requirement for achieving maximum growth rates by freshwater prawns appears to be above 40% and around 50% and may not differ significantly from that of other species of freshwater prawns and also penaeid prawns. But in the case of certain penaeid shrimps the protein requirement for including maximum growth rates was reported to be 30 to 40% [19, 48], the lower range of protein requirement may be due to the incorporation of ingredients with high amino acid profiles or amino acids which are efficiently assimilated into the stuff of the body.

Another interesting finding in the present investigation is, that even though the dietary protein was reduced from 62% to 20% and consequent increase in Non – Protein nutrients such as Carbohydrates and Lipid sources, did not show much influence on the growth rates i.e., the growth rates of ED_1 & ED_2 were considered to be in one range, whereas the growth rates of ED_3 & ED_4 are falling in one range. The SGR values obtained in the present investigation also did not differ much for all the Experimental diets and indicating that the required energy was derived from Non – Protein sources of feed ingredients and hence proteins are spared only for induction growth potentials in prawns. Several authors also reported that, the protein requirement of 60% was reduced to 40% with incorporation of suitable Carbohydrate sources thereby formulating isocaloric diets with relatively high Carbohydrate contents [10, 27, 28, 31, 44, 45].

Similarly Sedwick (1979) [47] reported that, the growth of *Penaeus merguiensis* did not change when fed diets, in which the protein content was reduced from 51% to 34%.Which maintaining an isocaloric dietary energy content with the substitution of Non – protein nutrients, capable of providing sufficient energy for the prawns. Relatively little information is available on digestible energy values of dietary ingredients used for the formulation of diets in several crustaceans including prawns and crabs (Koshio *et al* 1992) [35] obtained apparent energy digestibility values for American lobster after feeding with crab – Protein based experimental diets. The dry matter digestibility values were lower than the energy digestibility values, clearly reveals the involvement of carbohydrate ingredients for the production of energy in prawns [10, 38, 44]. The digestible energy values are falling in the same range for all the Experimental diets. But the growth rates shown to be different in prawns after feeding with Experimental diets. In the present investigation the uptake of feed and increased ingestion rates facilitate to meet the energy requirements in prawns and hence protein sparing action differs, thereby the growth rates also varies in prawns fed with different Experimental diets.

Protein breakdown and utilization are very well established in several crustaceans including crabs and prawns [17, 22]. Protein catabolism results in the production of Ammonia, Urea and Uric acid, dependent on water availability. But information about the production of Nitrogenous end

products is somewhat available in penaeid prawns and crabs, and no data is available about freshwater prawns. In the present investigation relatively more quantities of Ammonia is excreted compared to Urea in the freshwater prawns and hence the freshwater prawns are basically Amminotelic. Similar kinds were reported in penaeid prawns *P. japonicas, P. aztcus, P. monodon, P. esculentus, Macropetasma africanus* [18, 22]. So from the results obtained that Ammonia is the major end product of Nitrogen Catabolism in freshwater prawn *M. rosenbergii* and therefore, the rate of Ammonia excretion may be taken as an indicator for prawn catabolism. In the present investigation, it is very clear that prawns after fed with diets containing high protein content excreted more the amounts of Ammonia compared to starved groups indicative of higher intake and catabolism of proteins of dietary protein. The growth rates of prawns are also shown to be maximum after 40% dietary protein or more than it. The higher rate of Ammonia production also reveals that the excess dietary protein was subjected to catabolism and excreted as Ammonia, leading to the efficient use of protein for growth. But the relationship between Growth rates and Ammonia excretion needs to be studied thoroughly. In the present study different dietary protein contents were tested as the main Variables, but additional parameters such as different sources of protein, energy and other related ingredients must be tested to confirm the influence of protein catabolism and relationship between growth and Ammonia excretion rates needs to be unraveled.

The present investigation may be concluded that 40% and above protein is required for induction of maximum growth potentials. The Experimental diets formulated in the present study are capable of meeting in protein and energy requirements and hence highest growth potentials are resulted in the freshwater prawns. The ingredients incorporated in the formulation of Experimental feeds for freshwater prawns are catering the needs of the animals, thereby inducing the best growth potentials. Therefore the Experimental feeds formulated ED_3 and ED_4 i.e., with more than 40% are more than 40% are most ideal for culture activity of freshwater prawn *M. rosenbergii*.

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