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Efficacy of Cereals and Pulses as Feeds for the Post-larvae of the Freshwater Prawn *Macrobrachium rosenbergii*

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Abstract

Two types of feeds were prepared using cereals (maize, bajra and Italian millet) and pulses (green gram, red gram and cow gram) respectively and fed to the post larvae of *M. rosenbergii* for a period of 60 days. The efficacy of these feeds on growth performance, biochemical constituents and energy utilization were assessed and compared with commercially available standard Scampi feed. Statistically insignificant differences were seen in weight gain, specific growth rate and conversion rate between control and experiments, and between experiments. However, significant differences ($P<0.05$) were recorded in feeding, absorption and metabolic rates between control and experiments. Similarly, significant difference ($P<0.01$) was noted in ammonia excretion rate between experiment groups. As far as feeds are concerned no significant differences were seen in total protein, amino acid, carbohydrate and lipid levels between Scampi feed and pulses. However, significant differences (($P<0.01$) were observed in contents of total protein, amino acid and carbohydrate between Scampi feed and cereals as well as between cereals and pulses. Generally, the trends in biochemical constituents of feeds recorded were also seen in prawns fed with these feeds. However, total protein was found to significantly higher ($P<0.05$) in prawns fed with Scampi feed. Total carbohydrate was significantly higher ($P<0.05$) in prawns fed with cereals. In contrast, total lipid was recorded to significantly higher ($P<0.05$) in prawns fed with pulses. The results indicate the fact that prawns fed with pulses produced equally appreciable growth to that of Scampi feed. Similarly, cereals also produced appreciable growth. Thus, it is evident that protein sparing effect on growth was more in prawns fed with cereals due to its higher carbohydrate content. This suggests that cereals can be included in feed formulation to partially replace the expensive pulses and costly Scampi feed in aquaculture of *M. rosenbergii.*

1. Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii* has received considerable attention as an aquaculture species because of its nutritious delicacy to mankind [1]. Generally, the growth performance and production cost of any aquaculture species are depending upon the quality and types of feeds offered respectively [2]. Artificial feeds constitute a major operating cost (up to 40%) in freshwater prawn culture [3-4]. Protein is the largest and most expensive component in formulation of aquaculture diet. Therefore, it is advisable to formulate diets with optimized non-protein sources for basic energy requirement and maximizing protein sparing on growth alone [5-6]. The usual strategy in formulating aqua diets is that reducing costs by maximizing the inclusion of carbohydrate (up to 50%) to sparing expensive protein, which in turn decreases the accumulation of nitrogen waste in culture ponds [7-10]. Utilization of dietary carbohydrates from plant sources including soybean meal, corn meal and rice bran by aquatic species, particularly crustaceans are vary [11-15].

Knowledge on the growth performance of *M. rosenbergii* on various chiefly available commodities is necessary for developing cost-effective diets that can be formulated with some flexibility in the choice of ingredients [16]. Most modern aquaculture feed formulations contain grain components. Wheat for instance is routinely used as a starch source for binding during the extrusion process. In wheat, both the starch and protein based gluten components contribute to its functional properties. Most grains exerted some functional properties, which is unknown as to whether the protein or the carbohydrate component of the product possesses this property [17-19]. The increasing pressure on global fish meal stocks has generated interest on uses of ingredients, such as cereals, pulses, nuts, vegetables, fruits, green leaves and herbs [20-22]. Therefore, in the present study, certain chiefly available cereals (maize, bajra and Italian millet) and expensive pulses (green gram, red gram and cow gram) were used as ingredients in feed preparations. This is mainly to check whether the inexpensive cereals or the expensive pulses produce better growth performance on *M. rosenbergii* post larvae (PL) when compared with commercially available costly Scampi feed.

Generally, cereals contain 6-12% of protein, 1-7% of fat, 60-79% of carbohydrates, 2-3% of minerals and about 9-15% of moisture. Similarly, pulses contain 17-28% of protein, 0.3-56% of fat, 50-70% of carbohydrates, 2-3% of minerals and about 10-13% of moisture [23]. In crustaceans, ingested food energy is primarily channel into growth, metabolic maintenance, ammonia excretion, feces and moulting. In the present study, *M. rosenbergii* PL was fed with three types of feeds, one was the control, Scampi feed and other two were feeds prepared from cereals and pulses respectively. In addition to nutritional indices, such as total weight gain, specific growth rate and condition factor, the basic biochemical constituents, such as total protein, amino acid, carbohydrate and lipid, and differential amino acids were quantified in order to assess the efficacy of feeds prepared on nutritional quality and growth performance of *M. rosenbergii.* From the perspective of low cost feed formulation in sustainable development of *M. rosenbergii* culture, the present study was conducted to understand whether chiefly available cereals which are rich in carbohydrate can partially be replaced the expensive pulses which are rich in protein and Scampi feed which is very costly and non-affordable to small farmers. This is further to highlights the protein sparing capacity of cereals on growth of *M. rosenbergii.*

2. Materials and Methods

The post larvae of *M. rosenbergii* (PL10) were purchased from Rosen Fisheries, Thrissur, Kerala (pH, 6.7; total dissolved solids, 1.2 g L^{-1}; dissolved oxygen, 6.5 mg L^{-1}; BOD, 42.0 mg L^{-1}; COD, 140.0 mg L^{-1}; ammonia, 1.20 mg L^{-1}). They were safely brought to the laboratory in well-oxygenated plastic bags. They were stocked in a large cement tank (1000 L capacity) and acclimatized for two weeks in ground water (pH, 7; total dissolved solids, 0.9 g L^{-1}; dissolved oxygen, 7.2 mg L^{-1}; BOD, 30.0 mg L^{-1}; COD, 125.0 mg L^{-1}; ammonia, 0.028 mg L^{-1}). During which they were fed *ad libitum* with boiled egg albumin, *Artemia* nauplii and commercially available scampi feed alternatively thrice a day, and the medium was adequately aerated. On daily basis three fourth of the water was renewed by siphoning method causing minimum disturbance to the prawns. The unfed feed if any and the excreta were removed.

In this study, three groups of 90 prawns each (length: 1.2-1.5 cm; body mass: 0.03-0.05 g) were selected. Each group was housed in an aquarium of 75 L capacity, allowed to acclimatize for a week and maintained as described previously. From each group 10 prawns were randomly taken for measurements of initial morphometric data and three such measurements were taken (totally 30 PL were measured). The resulted mean values were taken to calculate an average mean value. After measurement these prawns were re-introduced into the respective aquaria. For the estimation of initial biochemical constituents, such as total protein, amino acid, carbohydrate and lipid, tissues from 30 prawns in each group were pooled separately and taken for analyses. Thus, three such observations were made for each parameter. Total protein content was determined followed by the modified method of Lowry *et al.* [24]. The content of total carbohydrate was estimated by the method of Roe [25]. Total lipid content was assayed by the method of Folch *et al.* [26]. Total amino acid was estimated by the method of Moore and Stein [27].

The remaining 60 prawns in each group were equally divided and housed in three aquaria of 20 L capacity in order to conduct the experiment in triplicate. One group served as control and fed with commercially available standard Scampi feed. Other two groups were fed with practical diets. The feeding trial was conducted for a period of 60 days. The practical diets were prepared using cereals, such as maize, bajra and Italian millet, and pulses, such as green gram, red gram and cow gram. The cereals and pulses were purchased from local market, sun dried and separately powdered using lab mixture grinder and used as basal ingredients. Powders of cereals and pulses were separately mixed in equal proportion (30% each) with tapioca flour (5%) and egg albumin (5%) as binding agents. No vitamin and mineral mixtures were added. A semi solid paste was prepared and then made to form pellets (3-5 mm) using a manual pelletizer. These pellets were sun dried for 48 hrs. Water stability of the feed was assessed and the leaching does not exceed 10% loss of dry matter per hour. Exactly 0.5 g of prepared diet was taken and soaked in water for an hour. After soaking the samples were drained on pre-washed Whatman filter paper and dried in an oven at 40°C for 24 h. The leaching rate was found to 50 mg. On 60th day of feeding trial, 10 prawns from each aquarium were measured for final morphometric data and three such measurements were made for each group and the resulted mean values were taken for calculating nutritional indices, such as survival rate, weight gain, biomass index, specific growth and condition factor followed by the method of Tekinay and Davis [28].

$$\text{Survival Rate (SR)} = \frac{\text{No. of live prawns}}{\text{No. of prawns introduced}} \text{ X } 100$$

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

$$\text{Biomass Index (BI)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \text{ X } 100$$

$$\text{Specific Growth Rate (SGR)} = \frac{\mathit{log} \text{ of Final weight (g)} - \mathit{log} \text{ of Initial weight (g)}}{\text{No. of days}} \text{ X } 100$$

$$\text{Condition Factor (CF)} = \frac{\text{Final weight (g)}}{\text{Final length}^3 \text{ (cm)}} \text{ X } 100$$

Further, at the end of feeding trial, the final concentrations of total protein, amino acid, carbohydrate and lipid, and profiles of amino acids were assessed. For each parameter four prawns from each aquarium were pooled to constitute a single observation and three such observations were made for each parameter (4x5=20x3=60 prawns were sacrificed). The profile of amino acids was done following high performance thin layer chromatographic (HPTLC) method [29]. The prawns were dried (80°C for 3 hrs), digested with 6 M aqueous hydrochloric acid and dried under vacuum. The powdered sample was dissolved in distilled water and 5 µl of sample was loaded on 8 mm thick pre-coated Silica gel $60F_{254}$ TLC plate (20 cm x 15 cm) and processed in CAMAG-LINOMAT 5 instrument. The plate was developed in butane-Ammonia-Pyridine-Water (3.9:1:3.4:2.6) mobile phase. The plate was sprayed with ninhydrin reagent prepared in propan-2-ol and dried. The developed plate was documented using photo-documentation chamber (CAMAG-REPROSTAR 3) at UV 254 nm and UV366 nm lights. Finally, the plate was scanned at 500 nm using CAMAG-TLC SCANNER 3. The peak area of the sample was compared with standard amino acids and quantified.

Similarly feeding trials were conducted with 20 PL in triplicates for calculating food utilization parameters, such as feeding rate, mean absorption, mean conversion and metabolic rate. The energy content of whole prawn, feeds, faeces and exuvia was measured using Parr 1281 Oxygen Bomb Calorimeter. The energy budget was calculated using the equation (C = (P+E) + R + F +U) derived by Petrusewicz & Macfadyen [30]; where, C is the energy consumed in food; P, is the conversion or growth; R, the material lost as heat due to metabolism; F, the energy lost through faeces; U, the energy lost in ammonia excretion and; E, the energy lost through exuvia. The daily excretion of ammonia by the prawn was estimated after feeding as per the phenol hypochloride method of Solorzano [31]. The energy loss occurring by ammonia excretion was calculated using the ammonia calorific quotient, 1 mg NH_3: 5.9 cal. [32]. The food energy consumed was measured as the difference between the energy content of food offered and that of the uneaten food. The quantity of absorbed food energy was calculated by subtracting F from C. Conversion or growth is the sum of energy channelled to somatic growth (P) and exuvia (E). Following the estimations of C, F, U, and P, the metabolism (R=Respiration) was calculated by dividing the respective amount of energy by initial live weight of the prawn per unit time in days

$$\text{Feeding Rate} = \frac{\text{Mean Food Consumption (k.cal/day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{Mean Absorption} = \text{Mean Food Consumption (k.cal/day)} - \text{Mean Food Excreted as Faeces (k.cal/day)}$$

$$\text{Absorption Rate} = \frac{\text{Mean Absorption (k.cal/day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{Mean Conversion} = \text{Mean weight gain (k.cal/day)} + \text{Mean exuvial weight (k.cal/day)}$$

$$\text{Conversion rate, P} = \frac{\text{Mean Conversion (k.cal/day)}}{\text{Initial live weight of the prawn (g)}}$$

$$NH_3 \text{ Excretion rate, U} = \frac{\text{Mean } NH_3 \text{ Excretion (k.cal/day)}}{\text{Initial live weight of the prawn (g)}}$$

$$\text{Metabolic Rate, R} = \text{Absorption rate (k.cal./g/day} - \text{Conversion rate (k.cal/g/day)} + NH_3 \text{ excretion rate (k.cal/g/day)}$$

Metabolic Rate, R = Absorption rate (k.cal./g/day – Conversion rate (k.cal/g/day) + NH3 excretion rate (k.cal/g/day)

All the data were analyzed statistically by adopting 'Student t-test' [33] between control and experiment, and within experiments. The different the levels of significance were indicated.

3. Results

Table 1 represents the morphometric data, nutritional indices and energy utilization in *M. rosenbergii* PL fed with different feeds. The initial body length and weight of PL were recorded to 1.3 cm and 0.05 g respectively (Table 1). The increase in final weight was found to higher in the scampi feed fed PL (0.49 g, 880%) followed by pulses (0.47 g, 840%) and cereals (0.45 g, 800%) fed groups. Similar trend was seen in the increase of final length of PL fed with these feeds (Scampi, 105%; pulses, 102%, and cereals, 98%). Significant differences were recorded in morphometric data between Scampi feed and cereals fed groups, and between cereals and pulses fed groups at 10% level ($P<0.1$), whereas the difference arrived between Scampi feed and pulses fed groups was not significant.

Therefore, growth promotion was in the order of Scampi feed > pulses > cereals (Table 1).

The nutritional indices, such as weight gain (biomass index) and specific growth rate were found to decrease particularly in cereals fed PL (7% and 3% respectively) when compared to that of Scampi feed fed PL. However, the differences were not statistically significant (Table 1). The condition factor was elevated in experimental groups (3% and 2%) when compared with control group. However, this difference was also not statistically significant (Table 1). Survival rate was recorded to statistically elevate particularly in pulses fed PL (P<0.1, 10% level) when compared with Scampi feed fed PL (Table 1). Therefore, among three feeds tested, the survival rate was in the order of pulses (73%) > cereals (72%) > Scampi feed (71%) fed PL (Table 1).

In energy utilization, the feeding rate and absorption rate were found to significantly elevate (P<0.05, 5% level) in experimental groups (10% and 18%; 10% and 17%) when compared to that of control group (Table 1). The differences arrived in these parameters between experimental groups (Cereals fed Vs. Pulses fed) were not statistically significant. Therefore, feeding and absorption were higher in pulses fed PL followed by cereals and Scampi feed fed PL groups (Table 1). The conversion rate was insignificantly decreased in cereals fed PL when compared with Scampi feed fed PL. However, this was insignificantly elevated in pulses fed PL when compared to that of Scampi feed fed PL. Therefore, as far as conversion is concerned there was no significant differences arrived between groups (Table 1). Thus, *M. rosenbergii* fed with Scampi feed, pulses and cereals shown almost similar growth performance. The ammonia excretion was insignificantly lower (6%) in cereals fed PL when compared with Scampi feed fed PL. However, ammonia excretion found to significantly elevated (28%) in pulses fed PL group (P<0.01, 1% level) when compared to that of Scampi feed fed PL group. Similarly, the difference recorded in ammonia excretion between experimental groups was statistically highly significant (P<0.005, 0.5% level). Therefore, ammonia excretion was higher in pulses fed PL followed by Scampi feed and cereals fed PL groups (Table 1). The metabolic rate was significantly higher (P<0.01, 1% level) in experimental groups (14% and 27%) when compared with control. Similarly, significant difference (P<0.1, 10% level) was recorded in metabolic rate between experimental groups. Therefore, the metabolic rate was higher in PL fed with pulses followed by cereals and Scampi feed (Table 1).

Table 2 represents contents of biochemical constituents in feeds and PL fed with these feeds. The protein, amino acid within protein, carbohydrate, and lipid ratio in feeds are as follow: it was 24(14):65:11 for Scampi feed; 8(25):83:9 for cereals and 23(13):66:11 for pulses. The contents of total protein and amino acid were found to significantly lower (P<0.005, 0.5% level) in formulated feeds, particularly with cereals (64% and 42%) when compared with Scampi feed. However, the differences recorded in these parameters between Scampi feed and pulses were not statistically significant (Table 2). Similarly, the differences recorded in these biochemical constituents between pulses and cereals were also found to statistically significant (P<0.005, 0.5%). The level of carbohydrate was significantly (P<0.01, 1%) higher in cereals (40%) when compared with Scampi feed as well as pulses. The level of lipid was found to insignificantly lower in experimental feeds when compared with control feed. The levels of protein, carbohydrate and lipid between pulses and Scampi feed were found to almost equal. The overall proximate composition of these biochemical constituents in Scampi feed and pulses were found in the order of total protein > amino acids > carbohydrate > lipid, whereas in cereals this was in the order of total protein > carbohydrate > amino acid >lipid (Table 2).

Consequently, PL fed with different feeds has acquired total protein, amino acid, carbohydrate and lipid from respective feed (Table 2). The content of total protein was found to significantly lower (P<0.05, 5%) in PL fed with cereals when compared to that of Scampi feed as well as pulses. The protein level between Scampi feed and pulses fed PL showed statistically insignificant difference. When compared with initial protein value its increase was maximum in Scampi feed fed PL (182%) followed by pulses (173%) and cereals (78%).

Table 1: Morphometric data, nutritional indices and energy utilization in *M. rosenbergii* PL fed with different feeds

Parameters			Control	Experiment				
			1. Scampi Feed	P < (%) 1 Vs. 2	2. Cereals	P < (%) 2 Vs. 3	3. Pulses	P < (%) 1 Vs.3
Morphometric Data	Length (cm)	Initial	1.31 ± 0.11		1.31 ± 0.11		1.31 ± 0.11	
			1.34 ± 0.10	--	1.34 ± 0.10	--	1.34 ± 0.10	--
			1.36 ± 0.11		1.36 ± 0.11		1.36 ± 0.11	
			1.33 ± 0.025	--	1.33 ± 0.025	--	1.33 ± 0.025	--
		Final	2.71 ± 0.11		2.60 ± 0.14		2.67 ± 0.11	
			2.68 ± 0.17	--	2.62 ± 0.17	--	2.73 ± 0.17	--
			2.74 ± 0.18		2.67 ± 0.18		2.67 ± 0.23	
			2.72 ± 0.045	5	2.63 ± 0.036	10	2.69 ± 0.034	NS
			105%↑		98%↑		102%↑	
	Weight (g)	Initial	0.049 ± 0.008		0.049 ± 0.008		0.049 ± 0.008	
			0.052 ± 0.007	--	0.052 ± 0.007	--	0.052 ± 0.007	--
			0.051 ± 0.008		0.051 ± 0.008		0.051 ± 0.008	
			0.050 ± 0.001	--	0.050 ± 0.001	--	0.050 ± 0.001	--
		Final	0.51 ± 0.087		0.45 ± 0.052		0.48 ± 0.078	
			0.46 ± 0.069	--	0.43 ± 0.048	--	0.45 ± 0.052	--
			0.51 ± 0.099		0.47 ± 0.048		0.50 ± 0.105	
			0.49 ± 0.028	10	0.45 ± 0.020	10	0.47 ± 0.025	NS
			880%↑		800%↑		840%↑	
Nutritional Indices	Weight gain		0.429 ± 0.050 BI = 858	NS	0.399 ± 0.020 BI = 798 7%↓	NS	0.426 ± 0.025 BI = 852 1%↓	NS
	Specific growth rate		1.62 ± 0.096	NS	1.57 ± 0.046 3%↓	NS	1.62 ± 0.046 0%	NS
	Condition factor		2.40 ± 0.198	NS	2.47 ± 0.08 3%↑	NS	2.45 ±0.21 2%↑	NS
	Survival rate		71.0 ± 2.0	NS	72.0 ± 2.0 1%↑	NS	73.0 ±1.0 2%↑	10
Energy Utilization (k.cal./g/day)	Feeding rate		1.071 ± 0.065	5	1.173 ± 0.031 10%↑	NS	1.262 ± 0.120 18%↑	5
	Absorption rate		0.961 ± 0.061	5	1.053 ± 0.027 10%↑	NS	1.126 ± 0.116 17%↑	5
	Conversion rate		0.444 ± 0.051	NS	0.430 ± 0.022 3%↓	NS	0.472 ± 0.027 6%↑	NS
	NH_3 Excretion rate		0.155 ± 0.006	NS	0.145 ± 0.005 6%↓	0.5	0.199 ± 0.009 28%↑	1
	Metabolic rate		0.672 ± 0.030	1	0.767 ± 0.031 14%↑	10	0.853 ± 0.079 27%↑	1

Each value of morphometric data is mean ± SD of 10 individual observations. An average also obtained by pooling these three mean values.

Each value of nutritional indices and energy utilization parameters is mean ± SD of three individual observations.

Values in % are increase or decrease over control.

Values are significant between 0.5-10% levels. NS, not statistically significant.

BI, Biomass Index.

Table 2: Concentrations of biochemical constituents in formulated feeds and M. rosenbergii PL fed with these feeds

Parameters		Initial	Final Control		Experiment			
			1.Scampi Feed	P < (%) 1 Vs. 2	2.Cereals	P < (%) 2 Vs. 3	3.Pulses	P < (%) 1 Vs. 3
Feed (% in dry wt. basis)	Protein	--	11.12 ± 1.40 24% --	0.5	3.96 ± 0.29 8% 64%↓	0.5	10.65 ± 1.10 23% 4%↓	NS
	Amino acid	--	3.26 ± 0.26 14% --	0.5	1.88 ± 0.18 25% 42%↓	0.5	3.04 ± 0.35 13% 7%↓	NS
	Carbohydrate	--	30.60 ± 2.77 65% --	1	42.80 ± 3.83 83% 40%↑	1	30.60 ± 2.77 66% 0%	NS
	Lipid	--	5.30 ± 0.30 11% --	NS	4.80 ± 0.60 9.3% 9%↓	NS	5.20 ± 0.50 11% 2%↓	NS
PL (% in wet wt. basis)	Protein	19.38 ± 2.86	54.70 ± 3.70 -- (182%↑)	5	34.50 ± 2.70 37%↓ (78%↑)	5	53.00 ± 4.80 3%↓ (173%↑)	NS
	Amino acid	14.30 ± 0.70	30.00 ± 1.81 -- (110%↑)	0.5	16.40 ± 0.70 45%↓ (15%↑)	0.5	26.80 ± 1.93 11%↓ (87%↑)	10
	Carbohydrate	11.20 ± 0.58	12.40 ± 0.70 -- (11%↑)	0.5	27.80 ± 2.78 124%↑ (148%↑)	0.5	19.60 ± 1.30 58%↑ (75%↑)	0.5
	Lipid	0.25 ± 0.02	0.27 ± 0.03 -- (8%↑)	10	0.31 ± 0.03 15%↑ (24%↑)	5	0.40 ± 0.04 48%↑ (60%↑)	0.5

Each value is mean ± SD of three individual observations.
Values in % are their proportion in respective feed.
Values in % with mark are increase or decrease over control.
Values in % within parentheses are increase or decrease over initial.
Values are significant between 0.5-10% levels. NS, not statistically significant.

Similar to total protein, the content of total amino acid was also found to significantly lower ($P<0.005$, 0.5%) in PL fed with cereals when compared to Scampi feed as well as pulses. The amino acid level in pulses fed PL also showed statistically significant decrease with Scampi feed ($P<0.1$, 10%). When compared with initial value the increase in total amino acid level was maximum in Scampi feed fed PL (110%) followed by pulses (87%) and cereals (15%)(Table 2).

In contrast to total protein and amino acid levels, the increase in the content of total carbohydrate was maximum in cereals fed PL (148%) followed by pulses (75%) and Scampi feed (11%). The differences in total carbohydrate level between control and experiment as well as between experiments were found to statistically significant ($P<0.005$, 0.5%). As far as lipid is concerned, the PL fed with pulses acquired more level of total lipid (60%) followed by cereals (24%) and Scampi feed (8%). The difference observed between Scampi feed and pulses was highly significant ($P<0.005$, 0.5%), the difference recorded between pulses and cereals was less significant ($P<0.05$, 5%), and least significant differences was noted between Scampi feed and cereals fed PL ($P<0.1$, 10%). Therefore, Scampi feed fed PL acquired more protein (182%), cereals fed PL acquired more carbohydrate (148%), and pulses fed PL acquired more lipid (60%) (Table 2).

The HPTLC analysis of amino acid profile showed elution of eleven essential amino acids, such as phenylalanine, leucine, tyrosine, isoleucine, tryptophan, methionine, valine, threonine, arginine, histidine and lysine, and eight non-essential amino acids, such as alanine, cysteine, proline, glutamic acid, serine, aspartic acid, glutamine and glycine (Table 3). The total quantity of essential amino acids in experimental groups was found to lower (cereals, 12%; pulses, 9%) when compared with control, the Scampi feed fed group. However, the differences arrived for tyrosine, isoleucine, methionine, valine and histidine between Scampi feed and cereals fed PL were statistically significant ($P<0.1$, 10%). Similarly, the difference arrived for leucine between Scampi feed and pulses fed prawns alone found to statistically significant ($P<0.1$, 10%). In the non-essential amino acids category, the total quantity in experimental groups was also found to lower (cereals, 8%; pulses, 6%) when compared with Scampi feed fed group. In this category none of the amino acid showed statistically significant difference between control and experimental groups. The proportion of essential (64-65%) and non-essential (35-36%) amino acids in each feed was almost similar. The overall decrease in total quantity of amino acids in experimental groups was falls between 8-10% over control group (Table 3).

Table 3: Amino acid profiles in *M. rosenbergii* PL fed with different feeds

Amino Acid Type	Amino Acid	Concentration of amino acid (mg g^{-1} dry wt.)		
		Control	Experiment	
		Scampi Feed	Cereals	Pulses
Essential	Phenylalanine	1.40 ± 0.14	1.10 ± 0.10	1.10 ± 0.12
	Leucine	1.39 ± 0.10	1.09 ± 0.10	1.13 ± 0.10*
	Tryrosine & Isoleucine	3.60 ± 0.43	3.12 ± 0.29*	3.20 ± 0.30
	Trphtophan	3.40 ± 0.31	3.06 ± 0.20	3.20 ± 0.20
	Metheonine	3.39 ± 0.24	3.03 ± 0.25*	3.23 ± 0.25
	Valine	3.40 ± 0.21	2.99 ± 0.18*	3.13 ± 0.25
	Threonine	1.13 ± 0.13	1.10 ± 0.10	1.10 ± 0.16
	Argenine	1.40 ± 0.12	1.30 ± 0.12	1.31 ± 0.13
	Histidine	0.64 ± 0.04	0.56 ± 0.02*	0.59 ± 0.02
	Lycine	3.53 ± 0.25	3.25 ± 0.25	3.29 ± 0.23
	Quantity	23.28 (65%) --	20.60 (64%) 12%↓	21.28 (64%) 9%↓
Non-essential	Alanine	1.42 ± 0.14	1.28 ± 0.12	1.29 ± 0.10
	Cystine	1.13 ± 0.13	1.10 ± 0.10	1.10 ± 0.16
	Proline	1.40 ± 0.12	1.29 ± 0.13	1.29 ± 0.14
	Glutamic acid	1.40 ± 0.14	1.30 ± 0.10	1.30 ± 0.12
	Serine	1.40 ± 0.20	1.30 ± 0.30	1.30 ± 0.10
	Aspartic acid	1.16 ± 0.12	1.12 ± 0.10	1.14 ± 0.10
	Glutamine	3.41 ± 0.30	3.10 ± 0.40	3.30 ± 0.26
	Glycine	1.39 ± 0.13	1.29 ± 0.13	1.30 ± 0.12
	Quantity	12.75 (35%) --	11.78 (36%) 8%↓	12.02 (36%) 6%↓
Total Quantity		36.03 --	32.38 10%↓	33.30 8%↓

Each value is mean ± SD of three individual observations.

*Value significant at $P < 0.1$ (10%). All other values are not statistically significant.

Values in % within parentheses represent respective proportion in total quantity.

Values in % are increase or decrease over control.

4. Discussion

The content of protein, carbohydrate and lipid are expression of an animal's adaptive characteristics. Many biotic (e.g., maturation, reproduction, food availability and food quality) and abiotic factors (e.g., photoperiod, temperature, pH and oxygen in water) can strongly affect the biochemistry and physiology of decapods crustaceans [34-36]. The optimal protein, carbohydrate and lipid for growth vary among species.

Protein is essential for growth and development. It is essential to provide the body with energy and is needed for the production of hormones, antibodies, enzymes etc. [21]. The protein requirement is dependent on many nutritional factors, such as lipid, carbohydrate contents or energy level. In juveniles of *Penaeus japonicus* the optimal level of protein requirement is between 45.5 - 52% for good growth and survival, and the level of protein exceeding 60% lead a clear depressing effect on growth [8, 37-38]. Dietary protein is the source of nitrogenous waste products. Consequently, optimization of dietary protein levels along with increasing other nutrients retention could reduce nitrogen loading and positively influence production cost [39]. In the present study, ammonia excretion rate was found to significantly higher ($P<0.01$, 1% level) in PL fed with pulses when compared to that of Scampi feed. In contrast ammonia excretion rate in PL fed with cereals was lower than that of Scampi feed (Table 1). Therefore, the presence of nitrogen source was higher in pulses, less in Scampi feed and least in cereals. The gross dietary protein requirement is not influenced directly by the amino acid composition of the diet [40]. But the growth is strongly influenced by the digestibility and essential amino acid composition of protein sources [41-43]. In the present study, protein and amino acid ratio of Scampi feed (24%:14%) and pulses (23%:13%) were almost similar (Table 2). The PL fed with these feeds also showed similar pattern of growth (Table 1). As far as feed prepared from cereals is concerned, the protein amino acid ratio was 8%: 25%). This feed also produced appreciable growth. This may be because of its higher amino acid ratio. Further, the higher carbohydrate ratio of cereals may aid to increase the protein sparing effect on growth. Therefore, the PL fed with cereals also resulted in appreciable growth.

The ability to spare protein differs among species, and an imbalance in the ratio of dietary protein to other energy sources leads to either wasted protein, or produced lower-value 'fatty' animals [44]. Most diets formulated for larvae and juvenile crustaceans contain 30-50% crude protein [45-46]. However, higher dietary protein levels have been used successfully for larvae of *Penaeus monodon* [47] and *P. japonicus* [48]. An effective dietary protein source must satisfy an animal's requirement for both essential and non-essential amino acids [49]. In the present study, the percentage of total amino acid was found to lower in cereals (42%) as well as pulses (7%) when compared with Scampi feed (Table 2). Therefore, total amino acid content of cereals was 35% lower than that of pulses. However, the proportion of total amino acids with total protein was higher in cereals (25%) when compared with other two feeds (Table 2). The proportion of essential and non-essential amino acids in PL fed with respective feeds also found to almost equal (Table 3). However, cereals fed PL showed 12% lower essential amino acid than that of Scampi feed fed PL, while this was 3% when compare with pulses fed PL (Table 3). Similarly, the non-essential category as well the similar trend with 8% and 2% respectively was recorded. As cereals possess higher amino acid proportion when compared with its protein level, and higher carbohydrate content as well may contributed to appreciable production of PL in competence with Scampi feed and pulses.

Dietary lipids are known to play a vital role in nutrition as they provide energy, maintain the structural integrity of biological membranes and function as precursors for important steroids [50]. The optimum level of dietary lipid required for crustacean generally ranged from 2 to 10% [51-56]. The diet deficient in lipid affects moulting frequency and weight gain due to insufficient lipid utilisation [56]. Similarly, diet with excessive lipid also affects the growth due to inefficient lipid utilization and results in lipid accumulation and lowers meat quality [57]. This is particularly common when other energy sources are available in right proportion. However, diets with higher lipid level have a protein-sparing effect on growth [6]. In the present study, the proportion of total lipid content of feeds falls between 9-11% (Table 2). However, lipid accumulation was found higher in PL fed with pulses (60%) followed by cereals (24%) and Scampi feed (8%). This indicates the fact that pulses and cereals generated lipid synthesis in PL, which in turn reflects energy generating capacity and protein sparing effect of these commodities.

Carbohydrate plays an important role in balancing the utilization of protein and lipid for energy production [58]. They are the first to be exhausted when energy is required, followed by lipid and then protein. Most crustaceans do not have a specific requirement for dietary carbohydrate [59]. It has been reported that carbohydrates are used for short-term energy requirements in prawns [60-61]. According to Shiau & Peng [14], the protein-sparing effect was more obvious in *P. monodon* when the dietary protein level was reduced from 40 to 30% by increasing the dietary corn starch level from 20 to 30%.

5. Conclusion

In the present study, among three feeds used the PL fed with Scampi feed resulted in accumulation of protein, the PL fed with cereals showed accumulation of carbohydrate and the PL fed with pulses recoded accumulation of lipid. Therefore, combinations of cereals and pulses are required to attain prawns with balanced nutrients in right proportions. From feed formulation perspective, it should be noted that some responses to dietary protein source seem to be independent of their amino acid balance. For example, Squid protein has a biological value that is higher than would be expected from its amino acid profile and improves growth in shrimps [62-63]. This growth enhancing effect is related to improved palatability and increased food intake [64]. These phenomena may be more applicable to feed prepared with cereals as well as pulsed in this study. Overall result indicated the fact that in addition to costly Scampi feed and expensive pulses, the feed prepared from low-cost cereals also produced compatible growth in *M. rosenbergii* because of more protein sparing capacity on growth owing to its higher carbohydrate content. This promising factor can be considered. However, to attain maximum growth with right proportion of nutrient accumulation, combinations of cereals in major proportion and pulses in moderate proportion would be required for arriving more meaningful conclusion that costly Scampi feed can be replaced with low cost cereals. At the outset this study encourages the uses of cereals in sustainable development of aquaculture of *M. rosenbergii.*

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