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Growth Performance of *Macrobrachium rosenbergii* Post Larvae Fed with Vegetable Wastes and Palmolein Supplemented Formulated Feeds

A. Anne Rebecca and P. Saravana Bhavan*

Department of Zoology, Bharathiar University, Coimbatore–641046, Tamilnadu, India

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*Corresponding Author

Tel : +91-4222428495
Fax : +91-4222425706

Email:
bhavan@buc.edu.in
psbhavan67@gmail.com
bhavanps1967@yahoo.in

Abstract

The possibility for utilizing massively-available vegetable wastes as one of the feed ingredients for better growth and survival of *Macrobrachium ros*enbergii was checked as these wastes are good sources for antioxidants and other nutrients. Vegetable waste powder (VWP) was prepared from dried potato peel, cabbage thick outer greens, peel of carrot, beetroot and cauliflower. Basal ingredients (BI), such as coconut oilcake, groundnut oilcake and soybean meal (as protein sources), and rice bran and wheat bran (as carbohydrate sources) were powdered separately and taken in equal proportion. The VWP was incorporated with BI in different ratios (5%, 15% and 25%). Egg albumin and tapioca flour were used as binders. Vitamin B-complex was also mixed. The diet also contained Cod liver oil and palmolein in equal proportion as lipid sources. Palmolein, low cost oil was used to replace the high cost fish oil partially. A pinch of table salt was also added. These were subjected to pellet preparation. Feed without addition of VWP was served as control. Feeding trials were conducted for a period of 60 days on *M. rosenbergii* post larvae (PL). Significant increases in weight gain (WG), $P<0.005$, survival rate (SR), $P<0.016$, levels of biochemical constituents (total protein, amino acid, and ash), $P<0.506$, indices of feed quality (feed intake and feed efficiency (FE) and indices of protein (protein intake, protein efficiency ratio (PER), protein conversion ratio (PCR) and protein productive value (PPV) were recorded in PL, particularly fed with 15% VWP incorporated formulated feed when compared with control ($P<0.622$). The digestibility of formulated feeds was found to be lower. This is because of rich fiber content due to addition of VWP. This in turn responsible for the higher fecal output recorded in experimental PL groups. However, the food conversion rate (FCR), $P<0.000$ and protein assimilation rate (PAR), $P<0.122$ were found to be significantly decreased in experimental PL groups when compared with control. The results indicate the fact that the prawn has utilized the VWP effectively for growth. The higher carbohydrate content of VWP ensures sparing of protein for growth. Therefore, VWP can be included in feed formulation for developing sustainable aquaculture practices.

Key Words: Prawn, Growth, Survival, Vegetable waste, Palmolein

Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii* is a commercially important species in India and South Asian countries [1] because of its nutritious delicacy to mankind [2]. Its healthy and sustainable aquaculture is currently the need in India. The supply of fish meal and fish oil is arguably sustainable [3]. Therefore, in order to sustain the rapid growth of global aquaculture industry, it is imperative for the aqua feed industry to find out alternatives to fish oil and fish meal [4, 5]. In this line, studies on the use of palm oil in its various forms (crude palm oil, refined palmolein and palm fatty acid distillates) have been reported to elicit growth and feed utilization efficiency in fishes to comparable levels with dietary marine fish oils [6-8].

Using vegetable protein source as an alternative for fish meal reduces the feed-cost and thereby facilitates sustainable aquaculture [9]. Various cereal grains, pulses, oil seed cakes, rice bran and several other animal husbandry and agro by-products have been reported to be used as ingredients in aqua diets [10-12]. However, prices are soaring for these low-cost feed ingredients. Hence, vegetable wastes which are comparably nutritious to these low-cost feed ingredients and are available in massive quantities [13, 14]. These can be considered as the “better surrogate candidates” for highly priced feed ingredients.

The price of palm oil is much cheaper than that of other vegetable oils, such as soybean oil and corn oil. For effective cost reduction of feed, 50% fish oil substituted with 50% palmolein does not retard growth [15, 16]. Palm oil is predestined to have no ill-effects in fishes [17]. Considering these facts, palm oil can be an efficient vegetable oil than any other oil for substituting fish oil [7]. Formulation of diets to meet given nutritional specifications by selecting the cheapest available ingredients has been stressed [18]. Therefore, attention was focused to reduce the feed costs by popularizing the uses of vegetable wastes for promoting sustainable culture of *M. rosenbergii*. Further, the expensive fish oil is partially replaced by the in-expensive palmolein. Thus, feeds were prepared by using vegetable wastes and palm oil along with regular ingredients, such as soybean meal, rice bran, wheat bran, coconut oilcake, groundnut oilcake and cod liver oil. These feeds were fed to PL of *M. rosenbergii* and assessments were made on growth, survival, proximate whole carcass biochemical composition, amino acid profile and indices of feed quality and indices of protein.

Materials and Methods

Post larvae of the freshwater prawn, *M. rosenbergii* (PL-5) with an average initial body weight of 0.007-0.01 g) were purchased from the Rosen fisheries (pH, 6.8; 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}), Thrissur, Kerala, India. They were transported to the laboratory in polythene bags filled with oxygenated water and maintained in a cement tank (1000 L) with 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}). The larvae were acclimated to ambient laboratory conditions for 10 days (up to attaining post larval stage of PL 15) and fed *ad libitum* with boiled egg albumin twice a day, and *Artemia* nauplii once a day alternatively at 10% of body weight.

Diet formulation

An experimental control diet was prepared using soybean meal, rice bran, wheat bran, coconut oilcake and groundnut oilcake as basal ingredients (BI) in equal proportions with 0.1% table salt. After steaming at 90-100 °C for 5 minutes (to inhibit anti-trypsin activity) the egg albumin (10 %) and tapioca flour (2 %) were added as binders. Vitamin B-complex (1%) was also mixed. Equal amount of Cod liver oil and palmolein (each 1.5%) were added as sources of lipids. Actually palmolein was added to replace 50% of fish oil. The prepared feed mixture was rapidly squeezed through a hand pelletizer with 3 mm diameter mesh size. The pellets obtained were sundried till no moisture is observed.

The wastes of vegetables, such as potato peel, cabbage thick outer greens, peels of carrot and beetroot and cauliflower all were taken separately, thoroughly washed in running tap water (especially the cauliflower greens, which is considered to have a high risk of pesticide residue). Excessive water is completely drained off. Thick stems in greens were eliminated and only the leafy portions were taken, chopped finely and sun dried till no moisture was observed. Dried vegetable wastes were individually ground to a fine powder. An equal proportion of all of them were taken as “Vegetable Waste Powder” (VWP). The VWP was supplemented with control diet in three different ratios, such as 5%, 15% and 25% by replacing the right quantity of BI. Thus, four feeds (one control with 100% BI (feed-1), and three experimental with 95% BI + 5% VWP (feed -2), 85% BI + 15% VWP (feed -3), and 75% BI + 25% VWP (feed-4) were used. In these feeds, the proximate compositions of biochemical constituents such as total protein [19], lipid [20], carbohydrate [21], and amino acid [22] were estimated. The levels of moisture and ash were also calculated [23].

Growth trial

The prawns used in this study were divided into four groups, each with 120 numbers of PL-15 and each group housed in three aquaria (i.e. in triplicate) of 20 L capacity each with 40 PL. Feeding trials were carried out under laboratory conditions for 60 days. One group was fed with control feed. The remaining three groups were fed with experimental feeds. Water was renewed daily by siphoning. Everyday unfed feed and feces were separately collected and quantified. The PL was provided with continuous aeration. At the end of the feeding trial, growth and survival performances, indices of feed quality and indices of protein were calculated by using following formulae so as to evaluate the efficacy of feeds prepared.

Survival Rate (SR) % = No. of live animals / No. of animals introduced X 100

Weight Gained (WG) = Final weight - Initial weight

Daily growth rate (DGR) = Weight Gained/ No. of days X 100

Digestibility (D) % = Amount of food consumed - Amount of feces/ Amount of food consumed X 100

Food Conversion Ratio (FCR) = Feed intake/ Weight gained

Feed Efficiency (FE) % = Wet weight gained/ Dry weight of feed offered X 100

Protein intake = Protein present in consumed feed

Amino acid intake = Amino acid present in consumed feed

Protein absorption = Protein consumed - Fecal protein

Amino acid absorption = Amino acid consumed - Fecal amino acid

Protein Gain = Final protein content - Initial protein content

Amino acid Gain = Final amino acid content - Initial amino acid content

Protein Efficiency Ratio (PER) = Weight gained/ Protein consumed

Protein Conversion Ratio (PCR) = Protein gained/ Protein consumed

Protein Assimilation Rate (PAR) % = Protein consumed - Fecal protein/ Protein consumed X 100

Protein Productive Value (PPV) % = Protein gained/ Protein absorption X 100

The proximate compositions of biochemical constituents (total protein, lipid, carbohydrate, amino acid, moisture and ash) were also calculated. HPTLC profiles of amino acids were also performed [24] and the quantity of individual amino acid was calculated. 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 UV 366 nm lights. Finally, the plate was scanned at 500 nm using CAMAG-TLC SCANNER 3. The Rf value and peak area of each track were observed for comparison with standard amino-acids and quantification. All the data obtained were checked statistically with paired samples t-test [25] by using the IBM software, SPSS, version-11.5.

Cost Analysis

The cost analysis was done for calculating the profit index. The parameter used was, approximate cost (Rs.) of four different feeds prepared/ kg in subjection to the cost and quantity of the feed ingredients obtained from local markets in Coimbatore, excluding the charges paid over tariff, transport, processing etc.

Results and Discussion

Biochemical constituents of feed

The proximate biochemical compositions of feeds formulated are presented in table 1. Concentrations of total protein, amino acid, carbohydrate and ash were slightly increased in VWP supplemented feeds when compared with control. This was found to be the highest in 25% VWP incorporated feed. The reverse trend was observed in the content of total lipid (Table 1). There was no significant alteration in the content of moisture between control and experimental feeds.

Vegetable wastes contain many high value reusable substances [13]. Gradual supplementation of VWP to BI has considerably boosted the protein and carbohydrate proportion in diets, owing to its better nutrient profile [26]. Nevertheless, substitution of VWP to BI has reduced the lipid source ingredients (oilcakes), thereby its share being reduced in the constitutive diets. Therefore, the content of lipid in feed formulated by incorporating VWP was decreased with increasing dietary protein level Similar observation has been reported by Al-Hafedh [27]. Water content is the reciprocal of dry matter and a residual fraction remains after crude protein, fat, ash and fiber. The residual fraction is composed primarily of carbohydrates but other non-carbohydrate compounds may also be present. Ash content represents the sum of minerals in the feed, and greens are better source for minerals [28].

Table 1: Concentrations of biochemical constituents in formulated feeds.

Parameters (%)	Feeds			
	Control	Experimental		
	Feed-1 (100% BI)	Feed-2 (95% BI+5%VWP)	Feed-3 (85% BI+15%VWP)	Feed-4 (75% BI+25%VWP)
Protein	55.69±2.45	56.50±2.57	58.34±2.29	59.43±2.40
Amino acids	40.99±5.97	41.59±3.06	41.71±6.30	41.95±3.40
Carbohydrates	19.74±2.54	24.08±3.24	28.70±4.83	32.40±6.33
Lipids	6.38±1.07	6.32±0.96	6.04±1.03	6.38±0.59
Moisture	5.00±0.53	5.00±0.90	6.17±0.48	6.00±0.72
Ash	8.00±0.41	8.07±1.10	9.00±0.70	10.00±0.60

BI-Basal Ingredients; VWP-Vegetable Waste Powder.
Each value is mean ± SD of triplicate observations.

Growth and survival

The initial length and body mass of the PL was 0.8 ± 0.1 cm and 0.01 ± 0.009 g respectively (Table 2). After feeding trial, morphometric measurement showed significantly increase ($P<0.01$) in weight gain (WG) and daily growth rate (DGR) in PL fed with experimental feeds when compared with control. The increase was found to be the highest in 15% VWP supplemented feed (Table 2). Therefore, the elevation in WG and DGR found to decrease in 25% VWP supplemented feed fed PL group when compared to that of 15% VWP supplemented feed fed PL group (Table 2). The survival rate was also significantly increase in experimental groups with maximum increase in 25% VWP supplemented feed fed PL when compared with control.

A diet with insufficient nutrition will induce cannibalism. High cannibalism consequently might influence not only on survival rate, but also protein efficiency and growth [29]. Weight gain is affected by the quantity and quality of protein in the diet [30]. In the present study, it is ascertained that the control diet was of inferior quality when compared to that of VWP incorporated feeds. Therefore, WG and SR were recorded to be poor in control feed fed PL.

Table 2: Growth performance and concentrations of biochemical constituents in *M. rosenbergii* PL fed with formulated feeds.

Category	Parameters	Feeds			
		Control	Experimental		
		Feed-1 (100% BI)	Feed-2 (95% BI+5% VWP)	Feed-3 (85% BI+15% VWP)	Feed-4 (75% BI+25%VWP)
Growth and survival	Length (initial) cm	0.80±0.10	0.80±0.10	0.80±0.10	0.80±0.10
	Length (final)	1.79±0.19	2.33±0.23 (0.002)	3.34±0.32 (0.002	3.05±0.22 (0.000)
	Weight (initial)	0.016±0.005	0.016±0.005	0.016±0.005	0.016±0.005
	Weight (final)	0.036±0.005	0.044±0.003 (0.020)	0.068±0.004 (0.000)	0.061±0.007 (0.002)
	WG (g)	0.027±0.003	0.035±0.002 (0.004)	0.058±0.004 (0.000)	0.051±0.005 (0.005)
	DGR (%)	0.044±0.007	0.058±0.004 (0.015)	0.097±0.008 (0.000)	0.085±0.006 (0.000)
	SR (%)	61.33±6.11	81.18±9.64 (0.010)	80.7±5.48 (0.000)	81.96±1.59 (0.016)

Feed quality	Feed intake (g/g)	1.60±0.21	1.69±0.17 (0.060)	1.88±0.13 (0.026)	1.7±0.18 (0.029)
	Fecal output (mg/g)	7.26±0.96	16.36±0.79 (0.000)	25.6±1.66 (0.000)	30.75±2.79 (0.002)
	Food absorption (g/g)	1.59±0.26	1.67±0.19 (0.186)	1.85±0.18 (0.030)	1.67±0.17 (0.264)
	Digestibility (%)	99.38±2.35	98.82±2.1 (0.060)	98.4±1.78 (0.097)	98.24±2.19 (0.007)
	FCR	59.26±1.05	48.29±1.15 (0.000)	32.41±0.98 (0.000)	33.33±1.12 (0.000)
	FE (%)	1.8±0.15	2.33±0.17 (0.000)	3.87±0.14 (0.000)	3.40±0.21 (0.000)
Protein indices	Protein intake (mg/g)	89.1±2.15	109.41±2.83 (0.000)	145.4±3.12 (0.000)	140.13±2.18 (0.000)
	Protein absorption (mg/g)	88.55±1.1	108.12±1.25 (0.000)	143.1±3.45 (0.001)	137.66±2.85 (0.000)
	Protein gain (mg/g)	40.6±1.11	50.77±1.6 (0.001)	76.16±2.12 (0.000)	69.95±1.25 (0.000)
	Amino acid intake (mg/g)	65.84±1.34	78.74±1.4 (0.000)	108.49±2.67 (0.000)	103.79±3.23 (0.001)
	Amino acid absorption (mg/g)	65.17±2.3	77.81±1.89 (0.000)	106.76±2.16 (0.000)	101.95±2.78 (0.000)
	Amino acid gain (mg/g)	34.75±0.55	41.59±0.77 (0.000)	51.85±1.13 (0.000)	42.95±0.98 (0.001)
	PER	0.30±0.04	0.32±0.03 (0.074)	0.4±0.05 (0.003)	0.36±0.04*
	PCR	0.45±0.02	0.46±0.05 (0.622)	0.52±0.06 (0.094)	0.50±0.03 (0.013)
	PAR (%)	99.39±1.25	98.82±1.63 (0.122)	98.42±1.57 (0.034)	98.24±1.98 (0.112)
	PPV (%)	45.85±0.87	46.96±1.12 (0.016)	53.22±1.37 (0.002)	50.81±1.46 (0.005)

BI-Basal Ingredients; VWP-Vegetable Waste Powder; WG-Weight Gain; SR-Survival Rate; PER-protein Efficiency Ratio; PPV-Protein Productive Value; PCR-Protein Conversion Ratio; PAE-Protein Assimilation Efficiency; FE-Feed Efficiency; D-Digestibility; DGR-Daily Growth Rate; FCR-Food Conversion Rate.
Each value is mean ± SD of triplicate observations.
Significance (P<) of paired samples t-test are given in parentheses (* the correlation and t cannot be computed because the SE of the difference is '0').

Feed quality indices and protein indices

Feed intake was found to slightly increase in experimental feed fed PL when compared with control. This was found to be the highest in 15% VWP incorporated feed fed PL (Table 2). The progressive increase in fecal output was recorded in PL fed with experimental feeds when compared with control (Table 2). The increase in fecal output indicates progressive decrease in digestibility of feed by experimental groups. The decrease in digestibility was found to be the highest in 25% VWP supplemented feed fed prawns (Table 2). Therefore, fecal output was found to be higher in 25% VWP supplemented feed fed prawns. The FCR was found to significantly decrease in VWP supplemented feed fed PL groups when compared to that of the control group (Table 2). A maximum decrease in FCR was recorded in 15% VWP supplemented feed fed prawns. The recorded higher FCR in 25% VWP supplemented feed fed prawns when compared to that of 15% VWP supplemented feed fed prawns can be correlated with increased fecal output and decreased digestibility (Table 2). Lower FCR recorded in 15% VWP supplemented feed fed prawns indicates better growth performance. Which was further confirmed through the increased FE observed in15% VWP supplemented feed fed prawns when compared to that of control as well as 25% VWP supplemented feed fed prawns (Table 2).

Protein intake was increased in PL fed with VWP supplemented feeds with the maximum increase in 25% VWP supplementation when compared to that of control (Table 2). Protein absorption and protein gain were recorded to increase in PL fed with VWP supplemented feeds, but with the maximum increase in 15% VWP supplementation when compared to that of control. The decrease recorded in protein absorption and protein gain in 25% VWP supplementation when compared to that of 15% VWP supplementation is due to the increased fecal output. The amino acid absorption and amino acid gain were found in the similar trend. The amino acid intake was also in the similar trend in contrast to the protein intake. This is because of the increased fecal output (Table 2). The PER, PCR, PAR and PPV were all found to be higher in PL fed with VWP supplemented feeds, particularly in 15% VWP supplementation when compared with respective controls (Table 2). The better protein indices recorded in 15% VWP supplementation indicates better growth when compared to that of other experimental groups and control group.

Feed intake can be affected by palatability [30]. Moreover, prawns consume more feed to overcome energy insufficiency. In the present study, the experimental feeds appeared to be more palatable because of VWP supplementation. This is not the case for feed-4, which is incorporated with 25% VWP. This may be because of rich fiber content. Therefore, a low calorie diet results in a higher food conversion ratio. However, the lower feed efficiency implying that prawns consume more food to overcome energy insufficiency, but this is depending upon the palatability of the diet. Hence, control of food consumption through dietary energy density is possible. An inverse relationship between dietary energy density and ingestion rate has been recorded [31]. Hence consumer's needs could be regulated through food ingestion adjusted with quality food. The growth differences observed were not only due to differences in digestible energy content in the diets but also due to differences in dietary protein content [32]. Diet with high quality protein produces higher apparent digestibility [33]. The higher protein intake has resulted in higher protein gain and improved growth rate of *M. rosenbergii*. This hypothesis is supported by [34].

A higher FCR can be explained by a higher energy requirement for assimilation at higher food consumption, which is usually accompanied by reduced nutrient absorption and growth [35, 36]. There is a correlation between feed efficiency and the amount of crude protein in the diets. Higher PCR and PPV in PL fed with 15% vegetable wastes suggests that the protein consumed was utilized for muscle construction and not for energy sparing, for which carbohydrates and lipids have been metabolized rather than getting stored, which are evident from their proportion being reduced in experimental prawns. In the present study, protein assimilation rate was increased when the relative carbohydrate and lipid proportion of the diet is increased. This is also due to amino acid composition of protein source. This hypothesis has been proved by Hajra *et al.,* in *Penaeus monodon* [37].

Biochemical constituents of PL

Concentrations of total protein, amino acid and ash were significantly higher (P<0.02) in PL fed with experimental feeds when compared to that of the control (Table 3). Among three experimental feeds, 15% VWP incorporated feed has produced maximum elevation in these biochemical constituents (Table 3). Concentration of total carbohydrate was found to decrease in PL fed with experimental feeds even though carbohydrate content of these feeds increased proportionately with the concentration of VWP (Table 1 and 3). This indicates the fact that protein sparing was enhanced due to higher carbohydrate source of VWP. In this study, total lipid was also found to decrease in PL fed with experimental feeds. However, the feed itself contain lower lipid level corresponding to higher VWP incorporation (Table 1). Moreover, absorptions of carbohydrate and lipid may be reduced because of excess fecal output due to higher content of undigested fiber.

Table 3: Concentrations of biochemical constituents in PL of *M .rosenbergii* fed with formulated feeds.

Parameters (mg/g Wet. Wt.)	Initial	Feeds: Control Feed-1 (100% BI)	Experimental Feed-2 (95% BI+5% VWP)	Feed-3 (85% BI+15% VWP)	Feed-4 (75% BI+25% VWP)
Protein	64.34±7.56	104.94±4.26	115.38±2.90 (0.006)	140.50±3.00 (0.000)	134.29±6.24 (0.002)
Amino acids	35.85±5.18	70.59±4.29	77.43±3.91 (0.001)	87.69±4.50 (0.000)	78.79±4.90 (0.002)
Carbohydrates	15.34±3.23	31.43±0.66	28.28±0.75 (0.000)	19.25±3.08 (0.013)	16.36±3.38 (0.011)
Lipids	3.11±0.62	9.77±0.62	9.44±0.55 (0.015)	8.39±0.40 (0.008)	8.08±0.45 (0.003)
Moisture (%)	83±2.00	73.48±2.45	73.35±2.17 (0.506)	67.78±6.84 (0.153)	71.93±2.41(0.000)
Ash (%)	7.07±0.40	9.06±0.20	10.24±0.49 (0.020)	21.40±0.73 (0.001)	15.33±0.81(0.003)

BI-Basal Ingredients; VWP-Vegetable Waste Powder.
Each value is mean ± SD of triplicate observations.
Significance (P<) of paired samples t-test are given in parentheses.

Generally, dietary fiber slows down the nutrients flow in the gastro-intestinal tract, causing increased time for nutrient absorption. However, one can restore decreases in growth rate of prawn, even at excessive levels of protein, by increasing dietary fiber (vegetable) content. As the gastro-intestinal tract of prawns is short the passage of food is rapid and the time for digestion is limited [38]. Since VWP is a good source of fiber content, it regulates carbohydrate and lipid metabolism [26]. Therefore, carbohydrate and lipid proportions in VWP supplemented feed fed prawns were found to decrease (Table 3). Hence, the PL fed with poor lipid diet has limited lipid storage. No significant difference was recorded in the percentage of moisture between experimental and control feed fed PL group.

Vegetables and their products are highly valued for their nutrient content and they are rich sources of non-glycemic carbohydrates, the dietary fiber. Leafy vegetables composed primarily of the polysaccharides, cellulose, hemicelluloses and pectin, gum and mucilage and non-carbohydrate components, lignins [26]. Carbohydrates derived from vegetable sources are relatively degradable than those obtained from the basal ingredients. Simple carbohydrates are considered to be inferior to complex carbohydrates like starch in promoting growth in many crustaceans [39]. It has been reported that prawns with increased body length has high moisture content [40]. An increase in ash content with increase in size has been reported in juveniles of *P. monodon* [41]. In the present study, higher ash content recorded in PL with increase of VWP represent attaining good proportion of minerals.

Profiles of amino acid

The results of HPTLC analyses of amino acids present in the PL are presented in table 4. The following individual as well as combined amino acids, such as lycine, histidine, aspartic acid, asparagine+glutamic acid+glycine (Aspg+GluA+Gly), glutamine+alanine (Glu+Ala), serine+arginine+cystein (Ser+Arg+Cys), valine+proline (Val+Pro), methionine+tyrosine (Met+Tyr), isoleucine+tryptophane (Iso+Tryp) and phenylalanine were identified. On the whole, the PL fed with feed containing 15% VWP holds a greater proportion of these amino acids than that of the other two experimental feeds (containing 5% and 25% VWP) and control feed. Concentration wise these amino acids appeared in the following order: Aspg+GluA+Gly > Val+Pro > Ser+Arg+Cys > Histidine > Glu+Ala > Iso+Tryp > Lycine > Aspartic acid > Phenylalanine > Met+Tyr. When individual amino acid is concerned, histidine was found to be present in higher level followed by lycine, aspartic acid and phenylalanine. Among the essential category histidine was found to be in higher level followed by isolucine and tryptophane, lycine, phenylalanine, and methionine and tyrosine (Table 4).

Table 4: HPTLC profile of amino acid concentration in different groups of *M. rosenbergii* PL fed with formulated feeds.

Amino acids (%)	Feeds: Control Feed-1 (100% BI)	Experimental Feed-2 (95% BI+5% VWP)	Feed-3 (85% BI+15% VWP)	Feed-4 (75% BI+25% VWP)
Lycine	2.58±0.14	2.72±0.21 (0.074)	3.08±0.09 (0.003)	2.93±0.08 (0.010)
Histidine	2.59±0.06	2.87±0.12 (0.015)	3.14±0.07 (0.000)	2.98±0.13 (0.011)
Aspartic acid	2.51±0.25	2.54±0.08 (0.789)	2.97±0.21 (0.003)	2.63±0.18 (0.097)
Aspg+GluA+Gly	3.30±0.11	3.38±0.16 (0.109)	3.83±0.12 (0.000)	3.40±0.12 (0.003)
Glu+Ala	2.80±0.33	3.06±0.06 (0.237)	3.21±0.17 (0.047)	3.03±0.16 (0.144)
Ser+Arg+Cys	3.22±0.56	3.45±0.21 (0.373)	3.66±0.15 (0.204)	3.42±0.24 (0.392)
Val+Pro	3.38±0.32	3.60±0.15 (0.154)	3.75±0.17 (0.051)	3.50±0.21 (0.199)

Met+Tyr	1.81±0.66	1.84±0.15 (0.928)	2.03±0.11 (0.560)	1.77±0.12 (0.910)
Iso+Tryp	2.46±0.18	3.07±0.11 (0.004)	3.10±0.12 (0.003)	2.47±0.13 (0.762)
Phenylalanine	1.71±0.63	2.05±0.07 (0.403)	2.06±0.11 (0.364)	1.91±0.13 (0.560)

BI-Basal Ingredients; VWP-Vegetable Waste Powder; Aspg-Asparagine; GluA-Glutamic acid; Gly-Glycine; Glu-Glutamine; Ala-Alanine; Ser-Serine; Arg-Argenine; Cys-Cystine; Val-Valine; Pro-Proline; Met-Metheonine; Tyr-Tyrosine; Iso- Isoleusine; Tryp-Tryphtaphan.
Each value is mean ± SD of triplicate observations.
Significance (P<) of paired samples t-test are given in parentheses.

Essential amino acids like histidine, isolucine, tryptophane, lycine, phenylalanine, methionine, tyrosine, arginine and valine are (and other essential amino acids like leucine, and threonine) not only vital for prawns, but for human consumers as part of protein rich aquatic-food. Fish meal contains a high amount of lysine and methionine, which is less in plant sources [42]. Nevertheless, it is ascertained that, *M. rosenbergii* do not require much protein when compared to marine and carnivorous aquatic species [34, 43]. Arginine and glutamine holds great promises for growth and health management in aquaculture [44].

As amino acids are building blocks for protein a considerable proportion of essential amino acids could have taken part in growth promotion [44]. Essential amino acids cannot be synthesized in sufficient quantities in the animal body, and must therefore be included in the diet. In the present study, results indicate the fact that VWP contained considerable quantity of essential amino acids as their profile (histidine, isolucine, tryptophane, lycine, phenylalanine, methionine and tyrosine) were increased in PL fed with VWP incorporated feeds. Non essential amino acids can be synthesized in the animal body at higher rates, either from intermediates of cellular metabolism or by transamination of other amino acids, and are therefore not individually required in specific dietary concentrations. However, all 20 common amino acids are essential for protein synthesis and other functions, whether they are derived from the diet or are synthesized within the body. In the present study, VWP contained considerable quantity of non-essential amino acids. It has been reported that diets completely lacking non essential amino acids have been shown to result in reduced growth rate in fish [45]. It follows that, for a given animal species, there must be an optimum dietary ratio of essential to non essential amino acids, which will achieve maximum protein utilization for growth [46]. In this line this study needs further clarification.

Feed cost

In aquaculture, food and feeding usually represent the largest single operating cost item [47]. The preparation cost of experimental feed is lower when compared with the cost of control feed. The estimated cost is reduced to about 10% with the feed prepared by incorporating 25% VWP (Table 5). As vegetable wastes are relatively inexpensive it has contributed much in cost reduction.

Table 5: Estimated feed cost excluding incidental cost and charges paid over tariff, transport, processing etc.

Ingredients	Price Rs./ Kg	Control		Experimental		
		Feed - 1 (100% BI) Rs./ Kg		Feed – 2 (95% BI + 5% VWP) Rs./ Kg	Feed – 3 (85% BI + 15% VWP) Rs./ Kg	Feed – 4 (75% BI + 25%VWP) Rs./ Kg
Coconut oilcake	17.00	3.40				
Groundnut oilcake	25.00	5.00				
Soybean meal	30.00	6.00	19.40	18.05	16.50	14.55
Rice bran	8.00	1.60				
Wheat bran	17.00	3.40				
Palm oil (L)	40	0.60				
Cod liver oil (L)	1200	1.80				
Egg albumin (L)	250	25.00				
(10.0 ml/egg, Rs.2.50)			37.80	37.80	37.80	37.80
Tapioca flour	20	0.40				
Vitamin-B complex (1000 capsule)	1000	10.00				
VWP	Free of cost	--		--	--	
Total Feed Cost (Rs./ Kg)		57.20		55.85	54.30	52.35

The utilization of dietary protein is mainly affected by its amino acid composition, calorific content of the diet, digestibility of the protein, physiological state and age of the species and water temperature [48]. For efficient utilization of dietary protein, while maintaining the total energy level of food, protein content of the diet may be spared by carbohydrate or lipid [49]. As the VWP contained quite good amount of carbohydrates it can surely be supplemented with any protein based ingredients. The result suggests that 15% VWP inclusion has produced better growth with appreciable proportion of protein and amino acid but not with 25% VWP as it retard digestion because of high fiber content. However, not the case may be provided that the digestibility is improved. Therefore, vegetable wastes can be taken as an ideal supplementary material to incorporate as a feed ingredient in low cost feed formulation for development of sustainable aquaculture of freshwater prawns.

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