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Research article

**GROWTH OF *MACROBRACHIUM ROSENBERGII* FED WITH MANGO SEED KERNEL,
BANANA PEEL AND PAPAYA PEEL INCORPORATED FEEDS**P. Aarumugam, P. Saravana Bhavan*, T. Muralisankar, N. Manickam,
V. Srinevasan and S. Radhakrishnan

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

*Corresponding Author E-mail: bhavan@buc.edu.in, psbhavan67@gmail.com

ABSTRACT: The growth promoting potential of fruits wastes, mango seed kernel, banana peel and papaya peel on the freshwater prawn, *Macrobrachium rosenbergii* post larvae (PL) was evaluated. Basal diet equated to 35% protein was prepared by using soybean meal, groundnut oilcake, horse gram and wheat flour. Each fruit waste powder was separately incorporated with basal diet at a proportion of 10%. Sunflower oil was used as lipid source. Egg albumin and tapioca flour were used as binding agents. Vitamin B-complex with Vitamin-C was also mixed. Feed without any fruit waste was served as control. *M. rosenbergii* PL (length: 1.2-1.4 cm; weight: 0.09-0.13 g) was fed with these feeds for a period of 90 days. Significant improvements in the nutritional indices (survival rate, weight gain, biomass index, specific growth rate and condition factor), concentrations of biochemical constituents (total protein, carbohydrate and lipid), levels of non-enzymatic antioxidants (vitamin-C and E), content of minerals (Na⁺ and K⁺), activities of digestive enzymes (protease, amylase and lipase), and profiles of essential amino acids and fatty acids were recorded in fruits wastes incorporated feeds fed PL when compared with control (P< 0.003 – 0.878). The overall results indicated the fact that mango seed kernel incorporated feed was produced the best performance, followed by better performance of banana peel and good performance of papaya peel. These fruits wastes incorporated feeds enhance digestive enzymes activities and act as appetizer, which in turn enhances food utilization and ultimately yielded better survival and growth of *M. rosenbergii* PL. Therefore, these fruits wastes have considerable potentials in sustainable development of *Macrobrachium* culture.

Key words: Prawn, Mango seed kernel, Banana peel, Papaya peel, Growth, Protein, Vitamins, Minerals, Digestive enzymes, Amino acids, Fatty acids.

INTRODUCTION

This study was conducted to evaluate the growth promoting potential of fruits wastes, such as mango seed kernel, banana peel and papaya peel on *Macrobrachium rosenbergii* post larvae (PL) and to recommend the feasibility of incorporation of these fruits wastes in aqua feed formulation. The crustaceans are one of the most numerous and diverse group of non-insect arthropods, comprising approximately 52,000 described species (Martin and Davis, 2001) including prawns, crabs, crayfish, lobster etc. The commercial culture of various shrimp and prawn species for human consumption is one of the fastest growing areas of aquaculture (Rosenberry, 2005). In this connection a large amount of production has taken place in China and there has been rapid expansion in India and Bangladesh (New, 2005). In India, the export of shrimps and prawns earn valuable foreign exchange, they comprise more than 60% of exports among the crustaceans and occupy a unique position in both capture and culture fisheries. After arising serious environmental threats due to shrimp farming and disease outbreak, the freshwater prawn farming got momentums. Among the freshwater prawns, *M. rosenbergii* and *Macrobrachium malcolmsonii* are the two members of the family, *Palaemonidae* potentially important for aquaculture. These prawn species are fetched for high price and have demand in both domestic and export markets, as it provides nutritious delicacy for human being (Radheshyam, 2009). Among these two, *M. rosenbergii* has become the main species for small-scale as well as large-scale commercial farming because of its fast growth, large abdominal size (compared to *M. malcolmsonii*), better meat quality, omnivorous feeding habit, and established domestic and export markets. It is transported to many parts of the world including South America and China (New, 2005).

In recent years, freshwater prawn farming has developed at a fast pace due to more advanced improvement in farming technologies and the increased market demand. This has led to intensification of farming operations in order to achieve higher yields. The intensive prawn culture depends on the use of a well-balanced nutritional and economic diet (Cuzon et al., 2000). Formulated feeds play an important role as major input in aquaculture demand. Even though information regarding the nutritional requirement of *M. rosenbergii* is available the development of economic feed formulation for optimal growth of this species is still needed for cost-effective intense farming (Tidwell et al., 2005). Generally, aquaculture farmers used commercially available feeds prepared from animal and plant sources and their by products. However, such feeds are high cost and non-affordable to small farmers. For sustainable aquaculture, prawn farming based on locally available foodstuffs suited for small-scale farmers are needed. The cereal grains, pulses, vegetable wastes are being used in aquaculture feed for both functional and nutritional rules (Bhavan et al., 2010, 2011a, 2011b; Rebecca and Bhavan et al., 2011). Therefore, in order to include some fruits wastes as feed ingredients/ supplements, which are available free of cost, in the present study, mango seed kernel, banana peel and papaya peel were incorporated with basal diet prepared from locally available ingredients and fed to *M. rosenbergii* PL. The growth performance was assessed, digestive enzymes activities were assayed, carcass quantity in terms of total protein, carbohydrate, lipid, vitamins and minerals was estimated and the quality of amino acid and fatty acid profiles were analyzed.

MATERIALS AND METHODS

Post larvae (PL) of the freshwater prawn, *M. rosenbergii* were procured from Happy Bay Aqua Nova Hatchery, Mugaiyur, Marakanam Taluk, Kancheepuram District, Tamilnadu, India. They were safely transported to the laboratory in plastic bags half filled with hatchery water and well-oxygenated. They were acclimatized in large cement tank (6'×4'×3') for 2 weeks before commencement of the experiment. During acclimatization, prawns were alternatively fed with boiled egg albumin, live *Artemia* nauplii and commercially available scampi feed. The unfed feed, exuviae and dead PL if any were all removed daily. The one third of the water medium was renewed every day and artificially aerated to ensure supply of sufficient oxygen and to avoid accumulation of metabolic wastes.

The branded feed basal ingredients, such as soybean meal, groundnut oilcake, horse gram, wheat flour, tapioca flour, sunflower oil and egg were purchased from local merchants. Soybean meal, groundnut oilcake and horse gram were extra sundried, grounded to a powder forms and separately stored at room temperature. Vitamin B-complex with vitamin-C (Pfizer Ltd., Mumbai, India) was purchased from local medical shop. In addition, fruit wastes, such as mango (*Mangifera indica*) seeds (MS), banana (*Musa sapientum*) peels (BP) and papaya (*Carica papaya*) peels (PP) were collected in good condition from local fruit stall at free of cost and immediately processed to avoid degradation. These fruits wastes were taken separately and sun dried until no moisture is observed. Dried fruits wastes were individually ground to fine powders and stored at room temperature.

Pelletized feed preparation

Powdered form of soybean meal, groundnut oil cake, horse gram, wheat flour and tapioca flour were taken in different proportion (Table 1) and thoroughly mixed. The mix was steam cooked in a stainless steel vessel at 95-100°C for 5 minutes to inactivate trypsin inhibitors, and allowed to cool at room temperature. The selected fruits wastes powder was added individually in a desired concentration (10%) to the steam cooked basal ingredients. Sunflower oil, vitamin B-complex and egg albumin were also added and thoroughly mixed. Sterilized water was adequately added for maintaining the mix in moist and paste form. The dough was pelletized in a manual stainless steel pelletizer fixed with 3 mm diameter mesh. The pellets were sun dried until they reach constant weight. The diet without incorporation of any vegetable waste powder was served as control. These feeds were subjected to estimation of concentrations of total protein, carbohydrate, and lipid by following standard methods, Lowry *et al.*, 1951, Roe, 1955, and Folch *et al.*, 1957 respectively. Ash content was also calculated (APHA, 2005).

Water stability of the feeds prepared was also tested. 1g of sample in triplicate was placed in 100 cc water in a glass bowl. The feed leaching was checked at 2, 4 and 6 hours separately by filtering with No.30 blotting silk cloth and the residue was dried in a hot air oven at 65°C until they attain a constant weight. The mean weight before immersion and after drying was used to calculate the percentage of dry matter loss, which is the measure of the water stability of the feeds for the corresponding time intervals (Leaching % (dry wt.) = Weight before immersion – Weight after immersion). The mean percentage of leaching of dry matter was calculated to each feed prepared (Table-1).

Experimental procedures

Four groups of *M. rosenbergii* PL (one control and three experimental groups) in triplicate ranging from 1.2 to 1.4 cm length and 0.09 to 0.13 g weight were used in this experiment. Each group consisted of 30 PL in an aquarium and maintained with 40 L of ground water (Temperature, 28°C; TDS, 1200.00 mg/l; DO₂, 7.20 mg/l; BOD, 30.00 mg/l; COD, 125.00 mg/l; Ammonia, 18 mg/l (APHA, 2005). The water medium was renewed daily by siphoning method without severe disturbance to the PL and aerated adequately. The control group was fed with formulated feed prepared from basal ingredients described previously without addition of any fruit waste. The three experimental groups were fed with experimental feeds prepared with addition of mango seed, banana peel and papaya peel respectively. All the groups of prawns were allowed to take the respective feeds at 10 % of initial body weight. The feeding trial was conducted for a period of 90 days. On 91st day, the final morphometric data were noted. The nutritional indices, such as survival rate, weight gain, biomass index, specific-growth rate and condition factor were calculated by adopting the following formulae. Concentrations of basic biochemical constituents, such as total protein (Lowry et al., 1951), carbohydrate (Roe, 1955) and lipid (Folch et al., 1957) were estimated in the PL on initial and final day of feeding trial. The content of ash in the muscle tissue of prawn was also calculated (APHA, 2005).

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

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

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

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

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

The PL was subjected to feeding trial as described previously in a separate simultaneous experimental set-up. Concentrations of vitamin-C (ascorbic acid) and vitamin-E (α -tocopherol) in the muscle of PL were estimated by following the method of Roe and Kuether (1943) and Baker et al., (1980) respectively. Contents of minerals, such as Na⁺ and K⁺ were estimated in the muscle tissues of PL by following the simple flame photometric method (Jeffery et al., 1989). The values are calculated by adopting the following formula.

$$\text{Na}^+ \text{ (or) } \text{K}^+ \text{ Content (mg)} = \frac{\text{Sample reading}}{\text{Standard reading}} \times \frac{\text{Standard concentration}}{\text{Sample concentration}} \times \text{Purity of NaCl/ K}$$

In an another simultaneous experimental set-up the PL was subjected to feeding trial for assaying the activities of digestive enzymes, such as protease, amylase and lipase on initial and final day of feeding trial. The whole flesh except eye stalk and exoskeleton was homogenized in ice cold distilled water and centrifuged at 10,000 rpm under 4^oC for 20 minutes. The supernatant was used as crud enzyme source. Protease enzyme activity was estimated by the method of Furne et al., (2005). One unit of enzyme activity represents the amount of enzyme required to liberate one μg of tyrosine min^{-1} under assay conditions. Amylase enzyme activity was assayed followed by the method of Bernfeld (1955) in which the increase in reducing power of buffered starch solutions is measured. The specific activity of amylase was calculated as mg of maltose liberated/ g of protein/ h (mg/g/h). The lipase activity was assayed by the method of Furne et al., (2005). The amount of free fatty acid released per unit time was estimated by the amount of NaOH required to maintain pH constant and represented as mille equivalents of alkali consumed.

Similarly, the PL was subjected to feeding trial for the analyses of profiles of amino acids and fatty acids. The high performance thin layer chromatographic (HPTLC) method of Hess and Sherma (2004) was performed and the profiles of amino acids in formulated feeds and the PL fed with these feeds was studied. The feeds/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 60F254 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. Four groups of standard amino acids were also run in parallel. Group-I: Proline, Serine, Asparagine, Glutamine and Methionine; Group-II: Aspartic acid, Glutamic acid, Alanine, Valine and Phenyl alanine; Group-III: Lysine, Glycine, Threonine, Isoleucine and Tyrosine; Group-IV: Arginine, Cystine, Histidine, Leucine and Tryptophan.

The profiles of fatty acids in formulated feeds and the PL fed with these feeds were performed following the gas chromatographic (GC) method (Nichols et al. 1993). Fatty acids were obtained from lipids by saponification using NaOH dissolved in methanol H₂O mixture (hydrolysis with alkali). They were methylated into fatty acid methyl ester using HCl and methanol mixture, which can be easily identified by gas chromatography. The fatty acid methyl ester was separated using mixture of hexane and anhydrous diethyl ether. For the organic phase aqueous NaOH was used as base wash and the upper organic layer was separated. 2 µl of sample was injected and analyzed using Chemito 8610 Gas chromatography, with BPX70 capillary column and flame ionization detector. Nitrogen was used as carrier gas. The chromatogram was used for calculation. Standard fatty acids were analyzed simultaneously. Based on the retention time and peak area of the standard fatty acids, each fatty acid in the unknown sample was identified. The data obtained were all analyzed statistically by adopting Student t-test (Zar, 1984) using SPSS software (version, 16.0) of IBM Company, USA.

RESULTS

In this study, feeds were formulated with plant ingredients, excluding ingredients of animal origin (Table 1). The basal ingredients (soy bean meal, ground nut oil cake and horse gram) were used as protein sources, wheat bran as carbohydrate source, and tapioca flour was used as a binder. It also contributes to carbohydrates. Egg albumin was also used as a binder. It also contributes to protein. Sunflower oil was used as lipid source. Fruits wastes (mango kernel, banana peel, and papaya peel) were supplemented to promote growth and wellness of *M. rosenbergii* PL. The leaching of formulated feeds in water was found to be between 18-19% after 6 hr of immersion. Therefore, incorporation of fruits wastes, particularly mango seed kernel and banana peel have no negative influence on water stability of formulated feeds (Table 1). The formulated feeds contained more or less 35% protein, 23-25% carbohydrate, 10 % lipid, and 8-9% ash. However, as far as carbohydrate is concerned, the feeds incorporated with banana peel, and papaya peel showed higher level of total carbohydrate when compared with control and mango seed kernel incorporated feed (Table 1).

Nutritional indices

The nutritional indices parameters, such as survival rate, weight gain/ biomass index, and specific growth rate were found to be significantly increased ($P < 0.828$) in mango seed incorporated feed fed PL followed by banana peel when compared with control. These parameters were found to be significantly decreased ($P < 0.878$) in papaya peel incorporated feed fed PL when compared with control (Table 2). The condition factor was found to be significantly decreased ($P < 0.225$) in mango seed incorporated feed fed PL followed by banana peel when compared with control ($P < 0.419$). The decrease in condition factor over control indicates the superior quality of feeds formulated (Table 2). This parameter was found to be significantly increased ($P < 0.003$) in papaya peel incorporated feed fed PL when compared to that of control. The increase in condition factor over control indicates its inferior quality (Table 2).

Biochemical constituents, vitamins, minerals and digestive enzymes

The basic biochemical constituents, such as total protein, carbohydrate and lipid, and ash contents were found to be significantly ($P < 0.205$) increased in experimental groups, particularly mango seed incorporated feed fed PL followed by banana peel incorporated feed fed PL when compared with control. Whereas in papaya peel incorporated feed fed PL, these parameters were found to be significantly decreased ($P < 0.072$) when compared to that of control (Table 3).

Table 1. Proportion of ingredients in formulated feeds, water stability and concentrations of biochemical constituents

Parameters/ Ingredients		Control (g/100g)	Experiment (g/100g)		
		Feed-1 (BI only)	Feed-2 (BI+MS)	Feed-3 (BI+BP)	Feed-4 (BI+PP)
Basal ingredients (protein and carbohydrate sources)	Soybean meal	30	30	30	30
	Groundnut oilcake	20	18	18	18
	Horse gram	20	16	16	16
	Wheat flour	20	16	16	16
Binding agents	Tapioca flour	5	5	5	5
	Egg albumin	3	3	3	3
Lipid source	Sunflower oil	1	1	1	1
Vitamin mix	B-complex with vitamin-C	1	1	1	1
Fruits wastes	Mango seed kernel	-	10	-	-
	Banana peel	-	-	10	-
	Papaya peel	-	-	-	10
	Total	100	100	100	100
Water stability	Leaching (%)	18.0	18.0	18.0	19.0
Concentrations of biochemical constituents (g/100g)	Protein	34.9	35.2	34.7	34.7
	Carbohydrate	23.2	24.0	25.2	23.8
	Lipid	9.6	9.6	9.6	10.2
	Ash	8.0	8.6	8.4	8.2

BI, Basal ingredients; MS, Mango seed; BP, Banana peel; PP, Papaya peel

Table 2. Nutritional indices of *M. rosenbergii* PL fed with fruits wastes incorporated feeds

Parameters	Control	Experiment			P<
	Feed-1 (BI)	Feed-2 (BI+MS)	Feed-3 (BI+BP)	Feed-4 (BI+PP)	
Initial length (cm)	1.26±0.25	1.26±0.25	1.26±0.25	1.26±0.25	--
Final length (cm)	3.71±0.31	4.25±0.37	3.85±0.21	3.15±0.44	0.136
Initial weight (g)	0.13±0.03	0.13±0.03	0.13±0.03	0.13±0.03	--
Final weight (g)	0.86±0.09	1.08±0.19	0.91±0.07	0.71±0.03	0.062
Survival rate (%), SR	86.76±4.37	93.33±3.65	86.66±3.67	80.00±3.38	0.828
Weight gain (g), WG	0.73±0.08	0.95±0.10	0.78±0.11	0.58±0.09	0.102
Specific growth rate (%), SGR	1.88±0.33	2.19±0.53	1.98±0.25	1.90±0.13	0.878
Condition factor (%), CF	1.58±0.14	1.40±0.32	1.51±0.26	2.27±0.21	0.419

Each value is mean ± SD of three individual observations.

BI, Basal ingredients; MS, Mango seed; BP, Banana peel; PP, Papaya peel.

Concentrations of vitamins (vitamin-C and E), mineral salts (Na⁺ and K⁻), and activities of digestive enzymes (protease, amylase and lipase) were found to be significantly (P< 0.132) increased in experimental groups when compared with control (Table 3). The elevation in these parameters was the maximum in mango seed incorporated feed fed PL (P< 0.005), followed by banana peel incorporated feed fed PL group (P< 0.049). In the case of papaya peel incorporated feed fed PL group these elevation was found to be very least (P< 0.132) when compared with control (Table 3).

Profiles of amino acids

The essential amino acids present in the formulated feeds are lysine, arginine, threonine and valine (Table 4). However, valine was below detectable level in the control feed. These essential amino acids also detected in PL fed with these formulated feeds (Table 4).

The content of lysine was found to be higher ($P < 0.622$) in experimental PL except papaya peel incorporated feed fed PL when compared with control. The concentrations of arginine, threonine and valine were recorded to be slightly altered ($P < 0.368$) in experimental PL when compared with control (Table 4). The semi essential amino acid, glycine and the non-essential amino acid alanine were observed to be present in both feeds and the PL fed with these feeds. The semi essential amino acid, tyrosine was only detected in mango seed incorporated feed. Whereas, this amino acid was not detected in any of the feeds fed PL (Table 4). The contents of cystine, leucine and serine were below detectable levels in both feeds formulated and the respective PL group fed with these feeds.

Table 3. Concentrations of basic biochemical constituents, vitamins, minerals and activities of digestive enzymes in *M. rosenbergii* PL fed with fruits wastes incorporated feeds

Aspect	Parameter	Initial	Final				P<
			Control	Experiment			
			Feed-1 (BI)	Feed-2 (BI+MS)	Feed-3 (BI+BP)	Feed-4 (BI+PP)	
Biochemical constituents (mg/g wet wt.)	Protein	32.74±1.98	71.50±3.70	76.00±4.19	72.00±4.03	63.20±3.23	0.120
	Carbohydrate	7.28±2.02	11.67±1.17	12.34±1.45	10.17±1.20	7.91±1.07	0.054
	Lipid	4.32±0.92	9.17±1.20	9.88±1.32	9.02±1.06	8.09±0.67	0.205
	Ash (%)	4.30±0.20	9.50±0.33	11.28±0.27	10.21±0.26	8.12±0.47	0.003
Vitamins (µmol/mg protein)	Vitamin-C	26.72±2.12	37.23±3.53	57.97±3.40	50.11±2.63	41.49±2.22	0.030
	Vitamin-E	1.56±0.12	9.24±0.89	14.49±1.37	14.01±1.24	12.82±1.01	0.003
Minerals (mg/g wet wt.)	Sodium	0.08±0.001	0.16±0.002	0.21±0.006	0.20±0.007	0.17±0.006	0.049
	Potassium	0.09±0.001	0.11±0.003	0.19±0.005	0.17±0.003	0.15±0.007	0.003
Digestive enzymes (U/ mg protein)	Protease	0.36±0.019	0.58±0.028	0.67±0.039	0.61±0.040	0.59±0.035	0.132
	Amylase	0.32±0.022	0.49±0.031	0.58±0.028	0.56±0.021	0.53±0.017	0.038
	Lipase *U×10 ⁻³	0.61±0.038	2.41±0.129	3.02±0.186	2.78±0.147	2.56±0.131	0.003

Each value is mean ± SD of three individual observations.

BI, Basal ingredients; MS, Mango seed; BP, Banana peel; PP, Papaya peel.

Profiles of fatty acids

In the present study, none-of the feeds formulated, except mango seed incorporated feed contain detectable level of highly unsaturated fatty acids (HUFA). Whereas HUFA (linolenic, eicosapentanoic (EPA) and docosahexanoic (DHA) acids) were found to be present in PL fed with both control and all the three experimental feeds (Table 5). Moreover, the levels of these fatty acids in experimental groups were found to be significantly elevated ($P < 0.269$) when compared with control (Table 5). The poly unsaturated fatty acids (PUFA), linoleic acid and the mono unsaturated fatty acid (MUFA), oleic acid were present in all the feeds formulated and the respective PL fed with these feeds (Table 5). However, linoleic acid level was found to be significantly lower in experimental groups, particularly PL fed with banana peel and papaya peel incorporated feeds only ($P < 0.002$) when compared with control group (Table 5). The mono unsaturated fatty acid, oleic acid was observed to be slightly increased in mango seed incorporated feed fed PL ($P < 0.758$) when compared to that of control. However, its level was found to be significantly decreased in banana peel ($P < 0.004$) and papaya peel ($P < 0.001$) incorporated feeds fed PL (Table 5).

Generally, saturated fatty acids were present in formulated feeds as well as in PL fed with these feeds except some differences in lauric acid, stearic acid, arachidic acid and lignoceric acid (Table 5). The lauric acid present in the feeds was utilized by the PL therefore its level was not detected in PL fed with any of the feeds (Table 5). Similarly, utilizations occurred in lignoceric acid (applicable to feed-1, feed-2 and feed-3, and respective PL groups fed with these feeds), and arachidic acid as well (applicable to feed-1 and respective PL fed with this feed). In the case of stearic acid just the reverse was recorded, and its synthesis from other fatty acids might have been occurred in PL fed with all the formulated feeds (Table 5). Similarly, synthesis of lignoceric acid as well might have been occurred (applicable only to feed-4, papaya peel incorporated feed and respective PL group).

Table 4. Profiles of amino acids in *M. rosenbergii* PL fed with fruits wastes incorporated feeds

Amino acids (%)	Feed					PL						
	Control	Experiment				P<	Control	Experiment				P<
	Feed-1 (BI)	Feed-2 (BI+MS)	Feed-3 (BI+BP)	Feed-4 (BI+PP)	Feed-1 (BI)		Feed-2 (BI+MS)	Feed-3 (BI+BP)	Feed-4 (BI+PP)			
Lysine*	1.59±0.15	1.37±0.13	1.61±0.17	1.44±0.17	0.22	1.59±0.11	1.75±0.21	1.61±0.17	1.54±0.21	0.622		
Arginine*	2.33±0.24	1.78±0.17	2.67±0.21	2.12±0.26	0.005	3.32±0.33	3.37±0.51	3.34±0.28	3.21±0.33	0.11		
Isoleucine*	ND	ND	ND	ND	--	2.62±0.19	ND	ND	ND	--		
Tyrosine**	ND	0.94±0.14	ND	ND	--	ND	ND	ND	ND	--		
Threonine*	0.60±0.03	0.61±0.06	0.58±0.07	0.65±0.08	0.622	1.08±0.09	0.96±0.16	0.96±0.16	1.06±0.12	0.368		
Glycine**	1.27±0.17	1.03±0.11	1.18±0.13	1.15±0.12	0.089	1.85±0.23	1.75±0.24	1.80±0.29	1.83±0.18	0.56		
Asparagine	0.91±0.10	ND	1.18±0.15	1.03±0.12	0.009	ND	ND	ND	ND	--		
Alanine	1.27±0.18	1.03±0.12	1.18±0.16	1.15±0.19	0.020	1.85±0.27	1.75±0.21	1.80±0.18	1.83±0.12	0.83		
Valine*	ND	0.43±0.02	0.51±0.08	1.03±0.15	0.008	1.03±0.12	1.03±0.16	1.00±0.09	1.06±0.11	0.11		

Each value is mean ± SD of three individual observations. ND, not deducted.

* Essential, **Semi essential, and the remaining are non-essential amino acids.

BI, Basal ingredients; MS, Mango seed; BP, Banana peel; PP, Papaya peel.

Table 5. Profile of fatty acids in *M. rosenbergii* PL fed with fruits wastes incorporated feeds

Fatty Acids (%/ µl methylated fatty acid)	Feed					PL						
	Control	Experiment				P<	Control	Experiment				P<
	Feed-1 (BI)	Feed-2 (BI+MS)	Feed-3 (BI+BP)	Feed-4 (BI+PP)	Feed-1 (BI)		Feed-2 (BI+MS)	Feed-3 (BI+BP)	Feed-4 (BI+PP)			
Lauric acid	1.42±0.02	2.21±0.03	2.17±0.01	5.99±0.04	0.000	ND	ND	ND	ND	--		
Myristic acid	1.78±0.04	1.70±0.04	1.71±0.02	2.63±0.06		0.71±0.004	0.46±0.22	0.69±0.15	0.911±0.01	0.835		
Palmitic acid	15.44±0.85	18.77±0.21	16.16±0.07	17.88±0.56	0.251	17.52±0.56	17.04±0.57	15.16±0.57	16.22±0.32	0.011		
Stearic acid	ND	ND	ND	ND	--	9.22±0.05	9.00±0.04	10.28±0.06	10.50±0.16	0.002		
Arachidic acid	1.66±0.05	0.28±0.08	0.58±0.03	0.21±0.04	0.000	ND	0.79±0.21	0.92±0.05	0.20±0.28	--		
Behenic acid	2.63±0.07	5.24±0.02	10.44±0.02	5.65±0.21	0.001	0.71±0.03	0.66±0.02	1.31±0.06	0.36±0.19	0.063		
Lignoceric acid	0.37±0.006	0.30±0.01	0.09±0.04	ND	0.005	ND	ND	ND	2.133±0.08	--		
Oleic acid*	34.28±0.54	32.38±0.18	33.77±0.34	32.09±0.06	0.048	31.75±0.57	31.85±0.08	29.06±0.87	25.39±0.32	0.758		
Linoleic acid**	38.63±0.08	33.14±0.25	26.22±0.06	28.92±0.03	0.000	30.46±0.34	29.04±0.04	26.22±0.04	25.47±0.57	0.015		
Linolenic acid***	ND	1.88±0.05	1.22±0.05	1.71±0.06	--	0.33±0.20	0.66±0.17	0.80±0.43	0.47±0.04	0.269		
EPA***	ND	0.07±0.02	ND	ND	--	0.52±0.02	2.03±0.03	1.93±0.47	2.56±0.07	0.032		
DHA***	0.33±0.04	0.46±0.02	0.28±0.01	ND	0.102	1.69±0.08	2.20±0.45	2.54±0.02	4.52±0.09	0.140		

Each value is mean ± SD of three individual observations. ND, not deducted.

*MUFA (n-9), ** PUFA (n-6), *** HUFA (n-3), and all others are SFA.

BI, Basal ingredients; MS, Mango seed; BP, Banana peel; PP, Papaya peel.

DISCUSSION

Many by-products including fruits wastes contain polyphenols with potential application as food antioxidants and polyunsaturated fatty acids (Baydar et al., 2006). According to Seleim et al., (1999), although mango seed kernels have a low content of protein, the quality of protein is good. It is a good source of antioxidants, including ascorbic acid, carotenoids, polyphenols and phytosterols, campesterol, β-sitosterol and α-tocopherols (Kim et al., 2007; Okonogi et al., 2007). It also contains good amount of unsaturated fatty acids (Mohamed and Girgis, 2005; Nzikou et al., 2010). Banana peel is rich in gallic acid than the pulp, thus possesses stronger antioxidant activity (Someya et al., 2002). It is a rich, low-cost source of dietary fiber, mainly hemicelluloses and pectin polysaccharides (Zhang et al., 2005; Anhwange, 2009). Papaya contains carotene and a pigment, caricaxanthin, and vitamin-A, vitamin-C, niacin, potassium, calcium, phosphorus, sodium and iron. Papaya peel contains cysteine proteases (papain, chymopapain, glycy l endopeptidase and caricain), which contribute 69 to 89% of total protein (Chaiwut et al., 2007). The above mentioned properties of fruits wastes definitely should have added nutritious quality, digestibility and palatability to the feeds formulated.

Nutritional indices

Prawns are slow and continuous feeders and hence the feeds should be of water stable relatively for long durations to enable consumption without waste. In the present study, no difference was seen in the water stability between control and experimental feeds except the feed formulated with incorporation of papaya peel (Table 1). Feeds with poor water stability disintegrate rapidly, cause feed waste and give poor growth (Sanhotra, 1994). This is valid as for as papaya peel incorporated feed fed PL is concerned as poor growth performance was recorded (Table 2). The better growth recorded in PL fed with mango seed followed by banana peel incorporated feeds and elevations noted in activities of digestive enzymes suggest that these fruits wastes were acted as appetizers (Table 3). Moreover, the palatability of these feeds may be enhanced due to incorporation of respective fruit wastes. This is attributed to their essential and amino acids, lysine, arginine, threonine, valine and glycine (Table 4), and fatty acids, linoleic, linolenic, EPA and DHA (Table 5) contents. Commonly recognized feeding stimulants are certain amino acids (taurine, glycine, arginine, glutamic acid, alanine etc.), betaine nucleotides and organic acids (Grey et al., 2009). It have been reported that better growth was achieved in marine as well as freshwater prawns when practical protein sources are supplemented with amino acids (Murai et al., 1985; Costa-Pierce and Laws, 1985; Pittet et al., 1996; Guillaume, 1997).

Concentrations of basic biochemical constituents

Protein is one of the most important components of food because, after digestion, it supplies amino acids needed to construct various body tissues of an organism, which is essential for growth. It is also needed for the production of hormones, antibodies, enzymes etc., (Gimenez et al., 2009). Dietary protein is the primary energy source for crustaceans. Its sparing can be directed towards growth by optimizing dietary carbohydrate and lipid levels. In general, larval and juveniles have greater protein requirement than adults, because the formers usually have faster growth rates and higher metabolic rates. Feed protein level up to 57% is recommended for suitable growth of *Penaid* shrimps (Shiau, 1998; Kureshy and Davis, 2002). For *M. rosenbergii*, generally it is 30-45%. In the present study, the formulated feeds were seemed to be iso-nitrous due to their almost equal proportion of protein level, about 35% (Table 1). In this study, the PL were found to be effectively utilized the protein sources available in the feed incorporated with mango seed followed by banana peel and papaya peel, which was accordingly reflected on growth (Table 1-3). Previous studies on *Macrobrachium* revealed that feeds formulated with cereals, pulses, groundnut oilcake, feeds incorporated with vegetable wastes, and herbals have yielded sustainable growth (Bhavan et al., 2010, 2011a, 2011b; Rebecca and Bhavan et al., 2011).

Carbohydrates are the most economical and inexpensive source of energy. It together with proteins and lipids form dietary sources of energy, and are important in synthesis of chitin, steroid, fatty acids and glycogen (Mukhopadhyay et al., 2003). It is used as precursor for various metabolic intermediates (dispensable amino acids) necessary for growth. However, if the amount is too high, energy utilization tends to be less efficient, resulting in poor digestibility. Carbohydrate associated with the protein in the diet play an important role in improving the survival (Bages and Sloane, 1981). It has been reported that artificial diets with increasing proportion of carbohydrate (5 to 25%) concomitantly decreasing the protein requirement (55 to 35%) in *P. japonicus* larvae without compromising the survival rate (Teshima and Kanazawa, 1984). The requirement of carbohydrate for *M. rosenbergii* has been reported to be 25-35% as optimum in the diet (Raj, 1993). It has also been reported that soluble starch is the most suitable form of carbohydrate for *M. rosenbergii* (Gomez Diaz and Nakagawa, 1990). In the present study, the PL was seemed to be effectively utilized the available natural carbohydrate (30-33%) with formulated feeds and aid for growth without any negative influence.

Dietary lipids are known to play a vital role in providing essential fatty acids as they provide energy, maintain the structural integrity of biological membranes, functions as precursors for important steroids, act as carriers of fat soluble vitamins like A, D, E and K, and essential for growth, moulting and reproduction (Yepiz-Plascencia et al., 2000; Richardo et al., 2003; Vasagam et al., 2005). The optimum level of dietary lipid required for crustacean generally ranged from 2 to 10% (Deshimaru et al., 1979; Ponat et al., 1980; D'Abramo, 1997). Lipid inclusion in ration reduces the protein denaturation, the removal of nitrogen from amino acids for use as energy. Diets with higher lipid level have a protein-sparing effect on growth (Anderson et al., 2003). However, excessive lipid source in diet will lower food consumption due to its accumulation in the mid-gut gland and ultimately retard growth (Ponat et al., 1980; Gonzalez-Felix et al., 2002). The diet deficient in lipid affects moulting frequency and weight gain due to insufficient lipid utilization (Sheen et al., 1999). In the present study, the total lipid content was optimum (about 10%) in formulated feeds (Table 1). Therefore, it also favours growth of *M. rosenbergii* PL. In this study, since the levels of both carbohydrate and lipid were optimum in formulated feeds, it is obvious that they have enhanced the protein sparing on growth.

Vitamins

Most of the B group vitamins are essential in the diet of crustacean, as vitamin-C and vitamin-E (New, 1980). Fish and crustaceans are incapable of biosynthesizing ascorbate since they lack l-gulonolactone oxidase, the enzyme responsible for conversion of glucose to ascorbic acid. Vitamin-C is plays an important role in animal health as antioxidant and increases immune resistance to bacterial and viral infections (Kontara et al., 1997). It enhances tolerance to hypoxic stress (Ishibashi et al., 1992). In the present study, in addition to fruits wastes, 1% vitamin B-complex with vitamin-C was added in formulated feeds (Table 1). In the present study, no symptoms of vitamin-C deficiency, melanisation (collagenous tissue underlying the exoskeleton), chronic soft shelling, opaque whitish musculature, flapped gill cover, incomplete moulting and increased rates of mortality (Shiau and Hsu, 1994) was recognized in PL fed with formulated feeds. The fruits wastes incorporated feeds should have supplied additional quantity of vitamin-C and therefore, its content was higher in experimental PL and it favours growth (Table 3). Vitamin-C associated promotion of growth, survival, feed efficiency, moulting, stress resistance, and immune response in juveniles of penaeid shrimp have been reported (Shiau and Hsu, 1994; Merchie et al., 1997; Lee and Shiau, 2002). Similarly, dietary supplementation of ascorbic acid and n-3 fatty acids are known to increase the survival and stress tolerance in *M. rosenbergii* post larvae (Devresse et al., 1990; D'abramo et al., 1994). Vitamin-E is a well-known antioxidant due to its role in inhibiting lipid peroxidation and possessing immune stimulatory property (He et al., 1992; He and Lawrence, 1993). It involves in the metabolism of arachidonic acid and other unsaturated fatty acids (Rice and Kennedy, 1988). The elevation in vitamin-E recorded (Table 3) suggests that the PL fed with fruits wastes incorporated feeds were in good health. In this study, vitamin-C and E levels also favour for better survival and growth of PL.

Minerals

In crustaceans minerals are necessary for maintenance of osmotic pressure, and acid-base balance. They are essential components of exoskeleton, soft tissues, enzymes, vitamins, hormones and respiratory pigments. They are also essential for muscle contraction and transmission of nerve impulses. Minerals in crustaceans are primarily of CaCO_3 and minor quantities of magnesium, phosphorus and sulfur. Minerals, such as phosphorus, calcium, potassium, magnesium, iron and sodium are essential for the growth of prawns (Kanazawa et al., 1984; McNamara et al., 1990; Lee and Shiau, 2002). However, no data are available on qualitative or quantitative mineral requirements for *M. rosenbergii*. In the present study, the elevation in Na^+ and K^+ levels in fruits wastes incorporated feed fed PL indicates its general health status and suggest that these salts dependent activities were performed well.

Digestive enzymes

Digestion of food is one of the most important functions in the physiology of organism to obtain nutrients for growth, maintenance, motion and reproduction. In crustaceans, digestion is both intracellular and extracellular, and the hepatopancreas is the primary site for the final digestion and absorption of nutrients as well as the secretion of digestive enzymes (Barker and Gibson, 1977, 1978). Actually, enzymes are synthesized in the hepatopancreas, particularly by the F cells and released into the foregut, where they occur in a relatively high concentrations compared to the hepatopancreas (Ceccaldi, 1997). Digestive enzymes accomplish breakdown of complex foodstuffs into its components. Crustaceans digest a variety of food materials and possess high concentration of protein, carbohydrate and lipid digestive enzymes, such as pepsin, trypsin, chymotrypsin, carboxypeptidases-A and B, leucine, aminopeptidase, amylase, collagenase, esterase and lipase have been reported in prawns (Tsai et al., 1986; Biesiot and McDowell Capuzzo, 1990; Fang and Lee, 1992; Lemos et al., 1999; Hidalgo et al., 1999; Gonza lez-Baro et al., 2000; Fernandez et al., 2001; Gamboa-Delgado et al., 2003; Lopez-Lopez et al., 2003; Debnath et al., 2007). Digestive enzyme activity varies with feeding, size, molting stage, environmental stress, and other factors (Lee et al., 1984). In the present study, the activity of protease, amylase and lipase was higher in fruits wastes incorporated feed fed PL (Table 3). The increased secretions of digestive enzymes increases the appetite, which consequently may lead to more food consumption, digestion and absorption of nutrients, which in turn ultimately resulted in better growth of *M. rosenbergii* PL. Increase in activities of protease, amylase and lipase has also been reported in *P. monodon* PL fed with *Z. officinalis* enriched *Artemia* (Venkataramalingam, 2007) and *M. rosenbergii* PL fed with *ocimum sanctum* and *withania somnifera* (Bhavan et al., 2011b), *Andrographis paniculata*, *Cissus quadrangularis* and *Eclipta alba* (Shanthi et al., 2012), garlic, ginger, turmeric and fenugreek (Poongodi et al., 2012), and *Murraya koenigii*, *Coriandrum sativum* and *Mentha arvensis* (Bhavan et al., 2012).

Profiles of amino acids and fatty acids

Crustaceans use amino acids as a source of energy. Therefore, supplementations of essential amino acids in commercial diets have been stressed (Mitra *et al.*, 2005). Crustaceans contain high concentrations of free amino acid, particularly glycine, prolin, arginine, glutamine and alanine, and are involved in osmoregulation (Fang *et al.*, 1992; Fox *et al.*, 1995; Wilson, 2002). In the present study, fruits waste supplemented diets appear to provide appreciably good quantity and quality of dietary amino acids. Therefore, levels of essential amino acids were improved in PL fed with these feeds (Table 4). There are four essential fatty acids (EFA), linoleic, linolenic, EPA and DHA that are essential for the growth of prawns (Merican and Shim, 1996, 1997; Glencross *et al.*, 2002). Several authors have studied the individual and combined requirements of these fatty acids for better growth of prawns (Xu *et al.*, 1994; Merican and Shim, 1997; Gonzalez-Felix *et al.*, 2002; D'Abramo and Sheen, 1993). In the present study, appreciable levels of HUFA and PUFA, linoleic have been detected in PL fed with fruits wastes incorporated feeds (Table 5). Since the feed formulated with incorporation of fruits wastes contained considerable quantity of essential amino acids and fatty acids, it is emphasized that these fruits wastes have potential for enhancement of growth of *M. rosenbergii* PL.

CONCLUSION

In this study, the experimental feeds prepared with incorporation of fruits wastes, particularly mango seed kernel and banana peel incorporate feeds were alone acted as appetizers even though papaya peel contains proteases, thereby increasing the secretion of digestive enzymes, which facilitates better digestion and absorption of nutrients, which in turn ultimately promotes the growth of *M. rosenbergii* PL. Moreover, the quality of PL in terms of total protein, essential amino acids and fatty acids, vitamin-C and E, and sodium and potassium levels was improved due to these fruits wastes incorporation. In this study, the PL fed with papaya peel incorporated feed did not showed appreciable growth performance. This may be due to lower feed stability and associated reason. However, this needs further clarification. The overall performance of fruits wastes incorporated feeds, particularly mango seed kernel and banana peel on survival, growth, nutritional and general health of *M. rosenbergii* PL was appreciable. Therefore, these fruits wastes can be taken as ingredients in on-farm feed management for promoting sustainable aquaculture of freshwater prawns.

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