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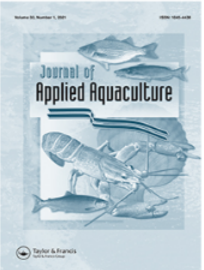

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Efficient utilization of poultry by-product meal-based diets when fed to giant freshwater prawn, *Macrobrachium rosenbergii*

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ABSTRACT

Two feeding trials were conducted to investigate the utilization of poultry by-product meal (PBM) in the post-larvae and juveniles of giant freshwater prawn, *Macrobrachium rosenbergii*. In experiment 1, five diets were formulated using PBM to replace fish meal protein at 0-100% replacement level at 25% interval. The growth performance was highest in diet with 75% replacement level. Feed conversion ratio (FCR) ranged from 1.52 to 1.88 and survival was fairly high (79.37–83.73%). In experiment 2, seven diets with higher replacement levels of fish meal protein with PBM protein (starting at 75% replacement level) were fed to triplicate groups of the juvenile prawn. The best growth performance was obtained in diet without fishmeal. Meanwhile, survival rate (83.33–89.56%) did not show any significant difference among the treatments. The result showed that high-quality PBM is a good candidate to replace fish meal protein in the diet and its utilization improved with the size of giant freshwater prawn.

KEYWORDS
Giant freshwater prawn; *Macrobrachium rosenbergii*; poultry by-product meal; feed development

Introduction

Giant freshwater prawn (*Macrobrachium rosenbergii*) is an important commercial species in Asia, with China being the largest producer in the world (Yang et al. 2012). Unfortunately, the culture of this species has been mostly at a small or medium scale and not much improvement has been made in terms of farming techniques despite inconsistent production since 1970. Recently, there is some interest in exploring ways and means of sustainably developing of this sector. Insufficient number of good quality broodstock, inadequate supply of post-larvae and juveniles, diseases and cost-effective feed for this species have been highlighted as the challenges that need to be addressed (Banu & Christianus 2016; New and Valenti 2000). Unlike the paneaid shrimp, commercial feed for giant freshwater prawn is not easily available in the market, making the farmers to use the

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feed which is readily available for the marine shrimp. This feed is expensive but suitable for high-value marine shrimp, but the market value of freshwater prawn is low because of which the feed reduces the profit margin of entrepreneurs.

Several studies have been conducted to minimize the use of expensive fish meal in aquaculture feed by investigating the potential of other alternative protein sources of terrestrial and aquatic origins. In this context, data were collected on the digestibility, palatability, and acceptability of protein sources and their advantage in aquaculture nutrition. The findings have established advantages of protein-rich poultry by-product meal over plant-based protein in terms of their acceptability by the captive stocks and digestive efficiency (Gunben et al. 2014; Shapawi, Ng, and Mustafa 2007). PBM is a good source of essential amino acids, essential fatty acids (phospholipids and cholesterol), vitamins and minerals, and contains a lower level of ash compared to the fish meal (Cruz-suarez et al. 2007). In some earlier experiments, PBM replaced 50% of fish meal (FM) in the diets for shrimp without affecting the growth performance (Cruz-suarez et al. 2007; Phuong and Yu 2003; Shuyan et al. 2009; Yang et al. 2004). However, there is limited information on the use of PBM as a protein source in the diets of giant freshwater prawns. In our recent study on the early larval stage of giant freshwater prawn, PBM was successfully used as one of the protein sources in the egg custard formulation that comprised 17% of the diet (Nik Sin and Shapawi 2017). In the present study, the PBM was tested on two different stages (post-larvae and early juvenile) of giant freshwater prawns to investigate their performance in terms of growth and feed utilization. The findings of the present study will be able to provide useful information on the suitability of PBM as a major source of protein for the development of species-specific feed for the farming of giant freshwater prawn.

Materials and methods

Experimental designs

Diets were formulated to contain 40% crude protein and 10% crude lipid on dry weight basis. FM, PBM, shrimp meal (SHM), squid meal (SQM), bivalve meal (BVM) and soybean meal (SBM) were used as protein sources while fish oil was used as the main lipid source after taking into consideration the lipid content in other marine protein sources. A recirculating aquaculture system was used in both feeding experiments. Water quality parameters, including temperature, dissolved oxygen, and pH were monitored and recorded weekly using a YSI multiparameter meter (HI 9828, USA) during the experimental period to ensure the water quality remained well within the limit recommended for the culture of prawn.

Preparation of poultry by-product meal (PBM)

PBM was produced from a rendered part of poultry carcasses (inclusive of intestine, liver, skin and some bone and meat with the ratio of 2:1:1:1:1, respectively). Fresh sample was purchased from the local fish market and immediately processed to avoid deterioration and loss of quality. Preparation of meal was carried out at the Aquaculture Feed Laboratory of Borneo Marine Research Institute, UMS.

Fresh samples were thoroughly cleaned in running water. All the unnecessary materials (feces, undigested feed) from the chicken intestine were removed and cleaned before boiled at 100°C using an induction cooker (Philips, Malaysia). The fresh samples were chopped separately into small portion and grinded using a food processor (Panasonic, Malaysia MK-5086M). Ground samples were then spread into a thin layer on the tray and dried using an oven at 50°C for 24 h. Dried samples were then homogenized separately into powder form using a mill grinder (DM-WZ200, China). PBM was then packed in zip-lock plastic bags and temporarily stored in a fridge at −4°C before use.

Experiment 1

FM protein was replaced by PBM protein at 0% (0PBM), 25% (25PBM), 50% (50PBM), 75% (75PBM) or 100% (100PBM) replacement levels (Table 1). These corresponded to 0%, 6.65%, 13.29%, 19.94% and 26.59% of PBM in the diet by dry weight, respectively. Post-larvae of the prawn of average body weight 0.02 ± 0.01 g were used in the feeding trial and were randomly distributed at a stocking rate of 84 specimens per tank (400 L) with three replicate tanks for each treatment (Goda et al. 2011). Prawns were fed by hand three times a day (9 am, 1 pm, and 5 pm). Considering the small size of PL, they were bulk-weighed at 2-week intervals and its average weight was calculated to estimate the weight of PL in each tank (Basri et al. 2015). The feeding rate was adjusted from 20% to 7% based on the average body weight of the captive stock in each tank (Hossain and Islam 2007; Chowdhury et al. 2008). Molted exoskeleton and uneaten feed were removed by siphoning. At the same time, mortality, and number of molting prawns were recorded. The experiment lasted for 90 days.

Experiment 2

In this experiment, replacement of FM protein with PBM protein was tested at higher levels based on the promising findings in Experiment 1 using juvenile giant freshwater prawn. Diets were formulated to replace FM protein with PBM at 0% (0PBM), 75% (75PBM), 80% (80PBM), 85% (85PBM), 90% (90PBM),

Table 1. Diet Formulations of Experiment 1 (% dry weight basis).

Ingredients	0PBM	25PBM	50PBM	75PBM	100PBM
FM[‡]	28.93	21.70	14.47	7.23	0.00
PBM[§]	0.00	6.65	13.29	19.94	26.59
SBM[¶]	19.61	19.61	19.61	19.61	19.61
BVM[§]	4.09	4.09	4.09	4.09	4.09
SHM[§]	4.32	4.32	4.32	4.32	4.32
SQM[§]	3.83	3.83	3.83	3.83	3.83
CMC[¥]	6.00	6.00	6.00	6.00	6.00
Vitamin Premix[†]	4.00	4.00	4.00	4.00	4.00
Mineral Premix[‖]	3.00	3.00	3.00	3.00	3.00
Fish oil[**]	6.52	6.08	5.64	5.20	4.76
Tapioca starch[*]	15.00	15.00	15.00	15.00	15.00
Alpha- Cellulose[']	4.70	5.72	6.75	7.78	8.80

[‡]TripleNine fish meal, Denmark.
[§]Laboratory made.
[¶]Defatted soybean, China (49.85% crude protein; 1.15% crude lipid for % DM basis).
[¥]Carboxymethyl cellulose (Calbiochem, USA).
[†]Vitamin premix. Contained (as g/kg): ascorbic acid, 300; inositol, 125; niacin, 50; riboflavin, 15; pyridoxine. 12; thiamin mononitrite, 15; retinyl acetate, 1.72; cholecalciferol, 0.025; menadione sodium bisulfite, 5; biotin, 0.5; folic acid, 2.5; DL-α-tocopheryl acetate, 50; vitamin B_{12}, 0.025; calpan, 25. Dexchem Industries Sdn. Bhd, Malaysia.
[‖]Mineral premix. Contained (as g/kg): calcium phosphate. H_2O (Mono Di-Calcium Phosphate; MDCP), 397.65; calcium lactate, 327; ferrous sulfate. H_2O, 25; magnesium sulfate. $7H_2O$, 137; potassium chloride, 50; sodium chloride 60; potassium iodide, 0.15; copper sulfate. $5H_2O$, 0.785; manganese oxide, 0.8; cobalt carbonate, 0.1; zinc oxide, 1.5; sodium selenite. $5H_20$, 0.02. Dexchem Industries Sdn. Bhd, Malaysia.
[**]Tuna oil, commercial oil.
[*]AAA Brand, Bake with Me Sdn. Bhd., Malaysia.
[']Sigma-Aldrich, USA.

Table 2. Diet Formulations of Experiment 2 (% dry weight basis).

Ingredients	0PBM	75PBM	80PBM	85PBM	90PBM	95PBM	100PBM
FM	28.93	7.23	5.79	4.34	2.89	1.45	0.00
PBM	0.00	19.94	21.27	22.60	23.93	25.26	26.59
SBM	19.61	19.61	19.61	19.61	19.61	19.61	19.61
BVM	4.09	4.09	4.09	4.09	4.09	4.09	4.09
SHM	4.32	4.32	4.32	4.32	4.32	4.32	4.32
SQM	3.83	3.83	3.83	3.83	3.83	3.83	3.83
CMC	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Vitamin Premix	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Mineral Premix	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Fish oil	6.52	5.20	5.11	5.02	4.94	4.85	4.76
Tapioca starch	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Alpha- Cellulose	4.70	7.78	7.98	8.19	8.39	8.60	8.80

Refer Table 1 for the specification of the ingredients.

95% (95PBM) and 100% (100PBM) (Table 2). The juvenile specimens with an initial weight of 3.77 ± 0.03 g were used in this study and stocked with 16 individuals/tank (dimension: 49 × 49 x 31 cm; volume 70 L). They were fed three times a day at the rate of 5% of their body weight per day for 40 days.

Calculation of growth performance, survival and feed utilization efficiency

At the end of feeding trials, prawns in each tank were individually weighed, measured and counted. The growth performance, survival rate, feed utilization efficiency were determined as follows:

(a) Weight gain (WG; %) = 100 x (final weight – initial weight)/(initial weight)
(b) Specific growth rate (SGR; %/day) = {[Ln (final weight) – Ln (initial weight)]/days} x 100
(c) Dry feed intake (DFI; g) = DFI x (1 – LDM/100), whereLost dry matter (LDM; %) = [(weight of feed before immersion – weightof feed after immersion)/(weight of feed before immersion)] x 100
Dry feed intake (DFI; g) = Σi [(feed intake on ith day)/(number of shrimp on ith day)] Nguyen, Pérez-Gálvez, and Berge (2012)
(d) Feed conversion ratio (FCR) = DFI/weight gain
(e) Survival (%) = 100 x (final count of the prawn)/(initial count of the prawn)

Sample analysis

Protein ingredients, experimental diets and whole-body of the prawn were homogenized using mortar for the proximate analysis following the method described by AOAC (1997). Proximate analysis was performed in triplicate. Nitrogen-free extract was measured by subtracting the protein, fiber, lipid and ash from the dry matter. The gross energy of each experimental diet was estimated based on the physiological fuel value: 5.65 kcal/g for protein, 9.45 kcal/g for lipid, and 4.12 kcal/g for nitrogen-free extract.

Amino acid analysis

Homogenous dried samples of 50 mg were carefully weighed into a hydrolysis tube and 6 mL of 6 N HCI was added to it. The tube was then flushed for 30 s with nitrogen gas and immediately sealed with a Teflon-lined cap. The tube was placed in an electric oven for 24 h at 110°C for sample hydrolysis. The tube was then cooled to room temperature for 30 min.

After sample digestion, the samples were analyzed in High-Performance Liquid Chromatography (HPLC) using a PCX 5200 Post-Column Derivatizer, Pickering Laboratories. It was equipped with fluorescence detector (SHIMADZU RF-10 AXL) with ultraviolet (UV) absorption (wavelength excitation 330 nm, emission 465 nm) and auto-injector (SHIMADZU SIL-10ADvp) equipped with high pH compatible Tefzel ® or PEEK™ seals and liquid chromatography (SHIMADZU LC-10AD VP). The chromatography separation was carried out by using a sodium-ion exchange column (3.0 x 250 nm) which consists of eluent methanol-water (60:40, v/v) at a flow rate of 0.4 mL/min. The temperature of the column and reactor was 53°C and 45°C, respectively. Chromatographic peaks obtained were analyzed with Breeze™ software by comparing it to known standard (Amino Acid Calibration Standard, protein hydrolyzate). Tryptophan, valine, and histidine were not detected as they use different standards (Pickering Laboratories 1999).

Statistical analysis

One way analysis of variance (ANOVA) was applied to test the effect of partial and total replacement of FM with PBM on growth performance, survival, feed utilization efficiency and whole-body composition. Tukey HSD was used to detect the significant differences between the average values of the treatments. The statistical analysis was performed using SPPS v 18.0 for the Windows platform. The level of significance was chosen at $P < .05$ and the results were presented as groups mean (N = 3 per tanks in each treatment ±standard error).

Results

Experiment 1

Table 3 shows the proximate and amino acid composition of FM and PBM of the experimental diets. Laboratory-produced PBM contained higher protein (78.98%) and lipid (15.32%) than the commercial FM (72.58% and 7.99%, respectively). The amino acid composition follows the trend of crude protein content where higher total amount of amino acids was detected in PBM compared to FM. In particular, methionine was identified as the first limiting amino acid in PBM used in the present study.

All the experimental diets have almost a similar level of crude protein (38.42–41.19%) and crude lipid (9.03–9.95%). Crude fiber, crude ash and nitrogen-free extract (NFE) ranged from 4.22% to 7.09%, 7.19–11.53% and 34.03–37.65%, respectively. In this trial, ash content decreased when replacement of FM with PBM increased in diets. The calculated energy value of experimental diets ranged from 458.26 to 3464.52 kcal/100 g diet (Table 4).

The amino acid composition of the diets reflected the composition of the protein sources used and the crude protein content of the diets. Except for 100PBM, the essential amino acid content of the experimental diets was similar across the treatments. It was also observed that the EAA content of diet 75PBM was comparatively better than that of the other diets.

Table 3. Proximate and amino acid compositions (% dry weight basis) of fishmeal (FM) and poultry by-product (PBM) used in the experimental diets.

Ingredients	FM	PBM
Proximate composition		
Moisture	8.65 ± 0.01	6.71 ± 0.01
Crude protein	72.58 ± 0.01	78.98 ± 0.02
Crude lipid	7.99 ± 0.01	15.32 ± 0.01
Ash	15.34 ± 0.04	4.77 ± 0.01
Essential amino acid composition		
Threonine	2.57	3.42
Methionine	1.99	2.09
Isoleucine	2.36	3.72
Leucine	4.48	6.20
Phenylalanine	2.69	3.67
Lysine	4.51	4.94
Arginine	3.63	4.82

Proximate analysis, n = 3; Amino acid analysis, n = 1.

Table 4. Proximate and Amino Acid Compositions (% dry weight basis) of Experimental Diets in Experiment 1.

Diets	0PBM	25PBM	50PBM	75PBM	100PBM
Proximate composition					
Protein	41.19 ± 0.01	39.56 ± 0.10	39.2 ± 0.08	39.06 ± 0.08	38.42 ± 0.04
Lipid	9.03 ± 0.01	9.76 ± 0.02	9.09 ± 0.04	9.95 ± 0.02	9.65 ± 0.03
Ash	11.53 ± 0.07	10.16 ± 0.05	9.12 ± 0.06	8.39 ± 0.12	7.19 ± 0.06
Fiber	4.22 ± 0.11	5.21 ± 0.09	5.72 ± 0.31	6.24 ± 0.07	7.09 ± 0.07
Moisture	6.98 ± 0.11	7.78 ± 0.09	7.29 ± 0.14	7.27 ± 0.22	7.54 ± 0.02
NFE	34.03	35.31	36.87	36.36	37.65
Gross energy (kcal/100g)	458.26	461.22	459.28	464.52	463.38
Essential amino acid composition					
Threonine	1.24	1.18	1.18	1.30	1.24
Methionine	0.77	0.72	0.70	0.74	0.65
Isoleucine	1.27	1.22	1.25	1.37	1.23
Leucine	2.47	2.38	2.40	2.57	2.38
Phenylalanine	1.32	1.29	1.33	1.45	1.36
Lysine	2.58	2.49	2.33	2.26	2.26
Arginine	2.08	2.10	2.08	2.21	1.67

Proximate analysis, n = 3; Amino acid analysis, n = 1
Mean values with different superscripts in the same column are significantly different from each other (Tukey test, $P < .05$).

The final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival of the prawn are shown in Table 5. FBW of post-larvae fed with 75PBM (2.28 ± 0.03 g) was the highest ($P < .05$), followed by 25PBM (2.12 ± 0.01 g), 50PBM (2.10 ± 0.02 g), 0PBM (1.91 ± 0.06 g) and 100PBM (1.78 ± 0.02 g). Similarly, the percentage of WG (11,301.85 ± 165.24%) and SGR (5.06 ± 0.00%d^{-1}) of post-larvae fed with 75PBM were significantly higher ($P < .05$) than in other treatments, whereas the percentage of WG and SGR (8806.93 ± 113.20% and 4.78 ± 0.00% d^{-1}; 9438.14 ± 275.24% and 4.81 ± 0.01%d^{-1}, respectively) of post-larvae fed with 100PBM and 0PBM respectively, was significantly lower ($P < .05$) than other diets. The FCR during

Table 5. Growth performance, survival and feed utilization efficiency of giant freshwater prawn post-larvae fed with different experimental diets in Experiment 1.

Diets	FBW (g)	WG (%)	SGR (%d^{-1})	DFI adj. (g/prawn)	FCR	Survival (%)
0PBM	1.91 ± 0.06^a	9438.14 ± 275.24^a	4.81 ± 0.01^a	3.33 ± 0.04^a	1.77 ± 0.05^b	79.37 ± 1.43^a
25PBM	2.12 ± 0.01^b	$10,482.15 \pm 74.71^b$	4.97 ± 0.01^b	3.34 ± 0.01^a	1.60 ± 0.01^a	82.54 ± 1.43^a
50PBM	2.10 ± 0.02^b	$10,377.35 \pm 102.95^b$	4.96 ± 0.01^b	3.39 ± 0.03^a	1.63 ± 0.01^a	82.54 ± 1.43^a
75PBM	2.28 ± 0.03^c	$11,301.85 \pm 165.24^c$	5.06 ± 0.00^c	3.43 ± 0.02^a	1.52 ± 0.01^a	83.73 ± 1.43^a
100PBM	1.78 ± 0.02^a	8806.93 ± 113.20^a	4.78 ± 0.00^a	3.32 ± 0.05^a	1.88 ± 0.00^b	82.94 ± 1.05^a

Proximate analysis, n = 3; Amino acid analysis, n = 1.
Mean values with different superscripts in the same column are significantly different from each other (Tukey test, $P < .05$).

Table 6. Whole-body proximate (% wet body weight basis) and amino acid (% dry body weight basis) compositions of giant freshwater prawn post-larvae fed with different experimental diets in Experiment 1.

Diets	0PBM	25PBM	50PBM	75PBM	100PBM
Proximate composition					
Moisture	73.44 ± 0.87[a]	73.58 ± 0.48[a]	73.64 ± 0.36[a]	75.15 ± 0.17[ab]	76.04 ± 0.64[b]
Crude protein	16.59 ± 0.19[a]	17.43 ± 0.13[b]	17.37 ± 0.15[b]	16.60 ± 0.06[a]	16.53 ± 0.06[a]
Crude lipid	1.27 ± 0.05[c]	1.18 ± 0.03[bc]	1.13 ± 0.01[bc]	1.04 ± 0.03[b]	0.68 ± 0.07[a]
Ash	16.36 ± 1.13[ab]	17.44 ± 1.66[ab]	18.67 ± 1.11[b]	14.96 ± 0.92[ab]	14.72 ± 0.64[a]
Fiber	7.60 ± 0.30[a]	9.30 ± 0.15[b]	9.46 ± 0.18[b]	9.46 ± 0.13[b]	9.44 ± 0.17[b]
Essential amino acid composition					
Threonine	1.97	1.86	1.87	2.05	2.07
Methionine	1.36	1.27	1.30	1.44	1.43
Isoleucine	2.28	2.16	2.20	2.43	2.47
Leucine	3.90	3.74	3.77	4.08	4.00
Phenylalanine	2.59	2.50	2.52	2.74	2.72
Lysine	4.54	4.41	4.34	4.24	3.96
Arginine	4.09	3.96	3.94	4.16	4.05

Proximate analysis, n = 3; Amino acid analysis, n = 1.
Mean values with different superscripts in the same column are significantly different from each other (Tukey test, $P < .05$).

the feeding trials ranged from 1.52 to 1.88, following the growth trend, with 75PBM yielding the best FCR among all the treatments. However, there was no significant different ($P > .05$) found in daily feed intake (DFI) of post-larvae prawn. The survival of post-larvae in the present study was high (79.37–83.73%) and without any significant differences ($P > .05$) detected among the dietary treatments.

At the end of the feeding trial post-larvae fed diets, 25PBM and 50PBM contained higher whole-body protein (17.43 ± 0.13% and 17.37 ± 0.15%, respectively) than post-larvae of the other groups (16.53–16.60%). Meanwhile, post-larvae fed 0PBM diet were found to contain the highest whole-body lipid (1.27 ± 0.05%) among all the dietary treatments (Table 6). The whole-body ash and fiber contents of post-larvae in the present study ranged between 14.72% and 18.67% and 7.60–9.46%, respectively. Regarding the results on the amino acid, slightly better amino acid profile was observed in 75 PBM and 100PBM diets compared to 0PBM, 25PBM, and 50PBM.

During the experimental period, all water quality parameters including the temperature, dissolved oxygen, and pH were within the acceptable range in the giant freshwater prawn rearing tanks, averaging 28.38 to 28.47 C, >5.0 mg/L and 8.06–8.18, respectively (Table 10) (Chowdhury et al. 2008). There were no significant differences observed in any of these parameters among the treatments.

Experiment 2

The proximate and amino acid compositions of the experimental diets used in Experiment 2 are shown in Table 7. The calculated crude protein values of all

Table 7. Proximate and essential amino acid (EAA) compositions (% dry weight basis) of different experimental diets in Experiment 2.

Components	0PBM	75PBM	80PBM	85PBM	90PBM	95PBM	100PBM
Protein	40.62 ± 0.14	40.44 ± 0.21	40.67 ± 0.09	40.02 ± 0.01	40.85 ± 0.07	40.62 ± 0.31	40.51 ± 0.09
Lipid	10.42 ± 0.03	10.36 ± 0.05	9.42 ± 0.25	9.41 ± 0.08	10.20 ± 0.05	9.76 ± 0.07	9.55 ± 0.08
Ash	11.93 ± 0.07	8.78 ± 0.08	8.55 ± 0.04	7.92 ± 0.01	7.67 ± 0.08	7.71 ± 0.04	7.59 ± 0.02
Fiber	5.71 ± 0.09	7.43 ± 0.29	7.53 ± 0.20	7.76 ± 0.38	7.67 ± 0.09	7.55 ± 0.06	8.20 ± 0.02
Moisture	7.74 ± 0.06	7.74 ± 0.12	7.54 ± 0.02	7.45 ± 0.18	7.49 ± 0.21	7.74 ± 0.06	7.50 ± 0.18
NFE	31.32	32.99	33.83	34.89	33.61	34.36	34.15
Gross energy (kcal/100g)	457.01	462.31	458.18	458.78	465.67	463.30	459.83
Essential amino acid composition							
Threonine	1.20	1.35	1.37	1.34	1.27	1.40	1.58
Methionine	0.70	0.80	0.73	0.72	0.73	0.83	0.72
Isoleucine	1.20	1.45	1.57	1.48	1.48	1.47	1.79
Leucine	2.41	2.65	2.70	2.60	2.53	2.59	2.89
Phenylalanine	1.25	1.50	1.40	1.35	1.27	1.41	1.54
Lysine	2.40	2.35	2.68	2.66	2.34	2.31	2.43
Arginine	2.00	2.28	2.24	2.16	2.01	2.16	2.27

Proximate analysis, n = 3; Amino acid analysis, n = 1
Values are expressed as mean ± standard error.

the diets were 40% and the result from analyzed value was in the range of 40.02–40.85%. The observed lipid content of the diets corresponded with the calculated value (9.41% to 10.42%). The crude fiber, crude ash, moisture, and nitrogen-free extract (NFE) contents were in the range of 5.71–8.20%, 7.59–-11.93%, 7.45–7.74% and 31.32–34.89, respectively. The estimated energy value of the experimental diets varied from 344.76 to 348.50 kcal/100 g. Dietary methionine and lysine in the diets ranged from 0.70% to 0.83% and 2.31% to 2.68%, respectively.

The initial body weight (IW), final body weight (FW), weight gain (WG), specific growth rate (SGR), dry feed intake (DFI), feed conversion ratio (FCR) and survival of the juvenile prawn fed with different experimental diets are shown in Table 8. The juvenile prawn of initial average weight of 3.71–3.90 g attained a final weight ranging from 7.62 to 9.66 g. The weight gain was significantly influenced by the inclusion level of PBM in the diets. There is a trend of increasing body weight with the increase of PBM inclusion in the diets. The 100PBM diet yielded the highest value of weight gain (160.64 ± 7.38%), followed by 95PBM (137.86 ± 0.75%), 80PBM (129.70 ± 7.88%), 75PBM (119.82 ± 10.77%), 85PBM (118.07 ± 5.22%), 90PBM (108.22 ± 4.12%) and 0PBM (104.01 ± 4.82%). The SGR of prawn fed 100PBM (2.06 ± 0.07%d^{-1}) was significantly higher (P < .05) compared to prawn fed 0PBM (1.32 ± 0.12%d^{-1}). Juvenile prawn fed diet 100PBM and 95PBM exhibited a significantly better FCR (1.50 and 1.70, respectively) (P < .05) compared to juvenile fed diet 0PBM (2.20), 75PBM (1.98), 80PBM (1.84), 85PBM (1.96) and 90PBM (2.18). DFI did not exhibit any significant difference (P > .05) among the treatments. The survival of juvenile prawn in the present study ranged from 83.33% to 89.58% and these values were not significantly different (P > .05) from each other.

The proximate and essential amino acid compositions of the whole-body of the juvenile prawn are shown in Table 9. The moisture and crude protein contents of the shrimp fed experimental diets ranged from 73.20% to 73.56% and 16.76– 18.00%, respectively. Crude lipid content of whole body in the present study was 1.35– 1.58%. Meanwhile, the crude ash and crude fiber were 16.24–19.41% and 7.98– 9.91%, respectively. The highest whole-body methionine and lysine were observed in prawn fed with a control diet (1.23% and 4.34%, respectively), while those fed with 90PBM diet attained the lowest levels of these amino acids.

During the experimental period, water temperature ranged from 28.12 to 28.41°C, dissolved oxygen 5.74 to 5.90 mg/L and pH 7.39 to 7.94 (Table 10). The water parameter showed no significant difference among all treatments during the culture period and the values were within the suggested range for freshwater prawn culture.

It is interesting to note that spawning has occurred only in 100PBM treatment involving 4 out of 48 individuals (8.3%).

Table 8. Growth performance, survival and feed utilization efficiency of giant freshwater prawn juvenile fed with different experimental diets in Experiment 2.

Diets	IW (g)	FW (g)	WG (%)	SGR ($\%d^{-1}$)	DFI adjust (g/prawn)	FCR	Survival (%)
0PBM	3.74 ± 0.02^{a}	7.62 ± 0.16^{a}	104.01 ± 4.82^{a}	1.32 ± 0.12^{a}	8.50 ± 0.20^{a}	2.20 ± 0.15^{c}	83.33 ± 2.08^{a}
75PBM	3.76 ± 0.10^{a}	8.25 ± 0.20^{ab}	119.82 ± 10.77^{ab}	1.63 ± 0.12^{ab}	8.78 ± 0.26^{a}	1.98 ± 0.18^{ab}	87.50 ± 0.00^{a}
80PBM	3.79 ± 0.09^{a}	8.71 ± 0.51^{abc}	129.70 ± 7.88^{abc}	1.68 ± 0.05^{ab}	8.96 ± 0.32^{a}	1.84 ± 0.09^{ab}	85.42 ± 2.08^{a}
85PBM	3.90 ± 0.06^{a}	8.50 ± 0.12^{abc}	118.07 ± 5.22^{ab}	1.61 ± 0.04^{ab}	9.01 ± 0.28^{a}	1.96 ± 0.08^{ab}	87.50 ± 3.61^{a}
90PBM	3.78 ± 0.09^{a}	7.87 ± 0.12^{ab}	108.22 ± 4.12^{ab}	1.56 ± 0.05^{ab}	8.91 ± 0.24^{a}	2.18 ± 0.05^{c}	89.58 ± 2.08^{a}
95PBM	3.74 ± 0.12^{a}	8.89 ± 0.25^{bc}	137.86 ± 0.75^{bc}	1.83 ± 0.01^{bc}	8.72 ± 0.12^{a}	1.70 ± 0.05^{ab}	87.50 ± 0.00^{a}
100PBM	3.71 ± 0.14^{a}	9.66 ± 0.21^{c}	160.64 ± 7.38^{c}	2.06 ± 0.07^{c}	8.88 ± 0.40^{a}	1.50 ± 0.09^{a}	87.50 ± 0.00^{a}

Mean values with different superscripts in the same column are significantly different from each other (Tukey test, $P < .05$).

Table 9. Whole-body proximate (% wet body weight) and essential amino acid (% dry body weight) compositions of giant freshwater prawn juvenile at the end of experiment.

Components	0PBM	75PBM	80PBM	85PBM	90PBM	95PBM	100PBM
Moisture	73.38 ± 0.12^{a}	73.55 ± 0.06^{a}	73.20 ± 0.03^{a}	73.26 ± 0.08^{a}	73.56 ± 0.08^{a}	73.28 ± 0.09^{a}	73.27 ± 0.16^{a}
Crude protein	17.72 ± 0.10^{cd}	17.38 ± 0.07^{bc}	16.76 ± 0.07^{a}	18.00 ± 0.14^{d}	17.22 ± 0.03^{b}	17.3 ± 0.03^{b}	16.82 ± 0.02^{a}
Crude lipid	1.35 ± 0.01^{a}	1.58 ± 0.02^{b}	1.44 ± 0.05^{a}	1.47 ± 0.01^{ab}	1.58 ± 0.03^{b}	1.47 ± 0.01^{ab}	1.47 ± 0.03^{ab}
Fiber	8.29 ± 0.08^{ab}	8.88 ± 0.12^{b}	9.81 ± 0.12^{cd}	7.98 ± 0.09^{a}	9.05 ± 0.11^{bc}	9.91 ± 0.27^{d}	8.94 ± 0.22^{b}
Ash	17.74 ± 0.24^{ab}	17.32 ± 0.09^{a}	19.41 ± 0.25^{b}	16.24 ± 0.21^{a}	17.27 ± 0.28^{a}	18.89 ± 0.03^{ab}	17.58 ± 0.28^{ab}
Essential amino acid composition							
Threonine	2.10	1.94	1.88	2.29	2.16	1.94	1.81
Methionine	1.23	1.13	1.09	1.14	0.72	1.16	1.08
Isoleucine	2.30	2.12	1.98	2.52	2.28	2.07	1.95
Leucine	3.96	3.70	3.50	4.24	3.87	3.62	3.41
Phenylalanine	2.64	2.47	2.35	2.97	2.76	2.43	2.29
Lysine	4.34	4.14	3.95	4.00	3.88	3.90	3.84
Arginine	3.81	3.67	3.58	3.43	2.46	3.51	3.41

Proximate analysis, n = 3; Amino acid analysis, n = 1.
Mean values with different superscripts in the same column are significantly different from each other (Tukey test, $P < .05$).

Table 10. Water quality parameters during the feeding trials for giant freshwater prawn post-larvae and juvenile culture.

Diets	Temperature (C)	Dissolved Oxygen (mg/L)	pH
Experiment 1			
0PBM	28.38 ± 0.02	5.87 ± 0.03	8.18 ± 0.07
25PBM	28.41 ± 0.05	5.81 ± 0.05	8.06 ± 0.01
50PBM	28.38 ± 0.01	5.83 ± 0.03	8.09 ± 0.02
75PBM	28.42 ± 0.03	5.90 ± 0.02	8.13 ± 0.02
100PBM	28.47 ± 0.03	5.93 ± 0.08	8.11 ± 0.01
Experiment 2			
0PBM	28.14 ± 0.03	5.89 ± 0.09	7.39 ± 0.04
75PBM	28.18 ± 0.10	5.89 ± 0.12	7.56 ± 0.02
80PBM	28.27 ± 0.03	5.76 ± 0.03	7.88 ± 0.10
85PBM	28.20 ± 0.04	5.88 ± 0.01	7.94 ± 0.05
90PBM	28.41 ± 0.09	5.74 ± 0.04	7.65 ± 0.03
95PBM	28.31 ± 0.04	5.90 ± 0.03	7.84 ± 0.07
100PBM	28.12 ± 0.02	5.85 ± 0.02	7.60 ± 0.14

Discussion

The inclusion of PBM up to 75% replacement of FM protein (Experiment 1) resulted in significantly better performance of the prawn compared to dietary treatments without PBM or 100PBM diet. It also appeared that the replacement of FM protein with PBM protein did not cause a significant reduction or deficiency in any of the EAA and this is probably because the amino acid profile of PBM used in the present study compares favorably with that of FM. PBM has been widely studied and it seems to be a promising protein source to replace fishmeal in the diets for cultured fishes and crustaceans. Several aquaculture species have been reported to tolerate the replacement of FM in the diets with up to 100% if high-quality PBM is used (Kureshy, Davis, and Arnold 2000; Webster et al. 2000). The PBM used in the present study was prepared using fresh raw materials which obviously resulted in good quality PBM.

It was revealed in the present study that 19.94% of PBM in the diet was optimal for the giant freshwater prawn, without any adverse effects on growth or productivity. Similarly, inclusion of 20.1% PBM in the diet of white shrimp produced the best performance (Shuyan et al. 2009). Other studies on different PBM inclusion level (% of diet) had been studied in the crustacean diets without affecting the growth, including 27.7% in *P. monodon* (Phuong and Yu 2003), 29.82% in *M. nipponense* (Yang et al. 2004) and 21.2% in *C. quadricarinatus* (Saoud et al. 2008).

Feed intake was not affected by the dietary treatments and FCR values of PBM-based diets were either better than or equal to the control diet. In the present study, growth performance (SGR) and FCR were better than those reported by Hossain and Islam (2007) (1.73–1.93) when post-larvae of the prawn fed formulated diets using meat and bone meal (MBM). Another similarity was also observed when FM was replaced with PBM at up to 60-80% in the diets of Pacific white shrimp, *L. vannamei* (Davis and Arnold 2000) with no negative effects on the palatability of the feeds.

The reduced performance of 100PBM diet as compared to other PBM-based diets was probably due to the lower digestibility of the diets. Even though apparent digestibility coefficient of the diets in the present study was not measured, reduced growth performance of many fish has been reported due to poor digestibility of diets containing a high level of poultry by-product meal (Rawles et al. 2009; Yang et al. 2006, 2004). Studies on gilthead seabream (Nengas, Alexis, and Davies 1999) and humpback grouper (Shapawi, Ng, and Mustafa 2007) found that diets with 75% and 100% FM protein replaced by PBM protein indicated that the decreased growth performance was correlated with the decreased digestibility of dietary protein. Digestibility of PBM is highly variable among the different grades (Cheng and Hardy 2002; Thompson et al. 2008) and compositions of the raw materials used to make this product (Nengas, Alexis, and Davies 1999).

The better growth performance of post-larvae in 75PBM might also be related to the better EAA profile, particularly of methionine, threonine, isoleucine, leucine, phenylalanine, and arginine. However, methionine and lysine contents in 75PBM diet (0.74% and 2.26%, respectively) were slightly lower compared with 0PBM diet (0.77% and 2.58%, respectively). The methionine content in all experimental diets was in the range of 0.65–0.77%, which was higher than the suggested value of 0.25% for giant freshwater prawn post-larvae. Similarly, the lysine content in all the experimental diets (2.26–2.58%) exceeded the recommended values (1.57–1.76%) (Hossain and Islam 2007). Other than that, little is known about the amino acid requirement for the giant freshwater prawn post-larvae. Attempts to define the essential amino acid requirements and potential interaction among amino acids are needed to provide a better understanding of the nutritional requirements of the species. To summarize, up to 19.94% of PBM (75% replacement of FM protein) can be included in pelleted diets for post-larvae of giant freshwater prawn from the onset of exogenous feeding up to 90 days of intensive rearing without impairing growth or feed conversion.

The results obtained in Experiment 2 showed improved utilization of PBM when tested on the prawn juveniles. Growth performance of the juveniles in the present study was very encouraging, indicating that the diets containing a high inclusion level of up to 100% PBM protein was well accepted by the juvenile prawn. Evidently, it is possible to use FM free diets for larger giant freshwater prawn without a negative effect on growth performance. Phuong and Yu (2003) reported that meat and bone meal (MBM) and poultry by-product meal (PBM) can be successfully used as the sole animal protein source in the diet for juvenile black tiger shrimp (*Penaeus monodon*). In Pacific white shrimp, good quality PBM can be used without amino acid supplementation to replace 80% of the fish meal in the diets (Cruz-suarez et al. 2007), and up to 70% in Pacific white shrimp diets (Shuyan et al. 2009) without a significant reduction in growth performance. In the past, PBM of different quality was successfully tested (75-100%) to replace FM in the diets of marine fish species such as gilthead seabream (Nengas, Alexis, and Davies 1999), red seabream (Takagi et al. 2000) and humpback grouper (Shapawi, Ng, and Mustafa 2007).

In Experiment 2, the amount of PBM in juvenile prawn diets can be increased up to 26% of the diet on a dry weight basis (100% PBM protein replacing FM protein) as compared to 19.94% in experiment 1. Apparently, larger sized specimens of giant freshwater prawn have a better ability to utilize PBM when compared to the post-larvae stage. The less efficient PBM utilization in Experiment 1 might be due to the lower digestibility by the less developed digestive system of the post-larvae. The utilization of PBM in Pacific white shrimp was also better in the juvenile stage (up to 80% replacement level) (Cruz-suarez et al. 2007) compared to the post-larvae stage (only about 70% replacement level) (Shuyan et al. 2009). The present study showed that freshly prepared PBM with the combination of skin, liver, intestine and some bone and meat can be used as a sole source of protein in the juvenile prawn diets without adverse effects on growth performance. According to Basri et al. (2015), utilization of green watermeal (GWM) in larger juvenile was better in terms of growth performance and feed utilization than the early juvenile stages of the white shrimp.

The composition of PBM depends on processing condition and the source of raw materials (Johnson and Parsons 1997). Good grade PBM normally contains very high protein (75-90%), relatively low content of ash (less than 10%) and moderate lipid level (<15%). Other PBM is of lesser quality, with protein content of 55-75%, higher levels of ash (up to 15%) and amount of fat in the range of 15%- 30%. Some poultry by-products are deficient in one or more essential amino acids (Srour et al. 2016). The laboratory-made PBM used in the present study contained very high protein content (78.98%), moderate fat (15.32%) and low content of ash (4.77%).

The essential amino acid content of the experimental diets in Experiment 2 did not differ appreciably. The dietary methionine and lysine requirements are reported to be 0.26% and 1.76%, respectively, in post-larvae of the prawn where meat and bone meal were used as protein sources (Hossain and Islam 2007). Methionine and lysine content in all the experimental feeds in the present study was above 0.70% and ranged between 2.31% and 2.68%, respectively. There was no amino acid supplementation in these experimental diets. However, it is important to note that bivalve, shrimp and squid meals (a total of 12.24%) were also incorporated in the formulation which contributed to the nutritional quality of the experimental diets. Thus, it is apparent that the amino acid content of the experimental diets was sufficient to support the normal growth of the prawn.

The prawn in Experiment 2 did not demonstrate palatability issues when PBM was used to replace 100% FM as indicated in DFI. Our result is also in line with those reported by Davis and Arnold (2000) and Samocha et al. (2004) with 80% and 100% replacement levels, respectively. The favorable response of the prawn to PBM is probably due to the good quality of the meal in terms of nutrient profile, digestibility, and palatability (Davis and Arnold 2000).

In this study, it is interesting to note that prawn fed 100PBM diet not only achieved the highest weight gain but was also the first to produce berried female, indicating early maturity was attained as a result of dietary treatment. Based on the positive results achieved in this study, further research on evaluating the replacement of marine by-products with PBM in broodstock stage of giant freshwater prawn is recommended. Generally, giant freshwater prawn broodstock culture is dependent on marine protein resources (squid, bivalve, and shrimp) for reproductive performance (egg production, fertilization rate, egg quality, embryo development) and offspring quality (Nik Sin, Yong, and Shapawi 2016). However, these resources are limited due to increased demand and not economical for the farming of prawn because of high cost (Davis and Arnold 2000). Undoubtedly, the commercial viability of aquaculture will require the replacement of expensive marine protein with lower cost and sustainable ingredients in feed production. PBM seems to meet these criteria.

The PBM used in the present study was of high quality due to the fresh raw materials used and hygienic processing. It is also important to note that other protein sources (BVM, SHM, SQM, and SBM) were also incorporated in the dietary formulation. Apparently, the combination of these ingredients was able to provide a balanced amino acid profile of the diets. Cost-effectiveness of substituting FM with PBM or any ingredients will depend on the cost of ingredients. Similar to FM, PBM can also vary considerably in quality and should be evaluated before incorporated in the prawn diet. In terms of economic benefits, PBM is estimated to be about 30% cheaper than FM. Even though cost analysis was not performed in the present study, the use of a significant amount of PBM to replace FM will definitely be able to decrease the diet cost.

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Disclosure statement

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Efficient utilization of poultry by-product mealbased diets when fed to giant freshwater prawn, Macrobrachium rosenbergii

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