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EFFECT OF PHOSPHOLIPID SUPPLEMENTS TO FISHMEAL REPLACEMENTS ON GROWTH PERFORMANCE, FEED UTILIZATION AND FATTY ACID COMPOSITION OF MUD CRAB, *Scylla paramamosain* (ESTAMPADOR 1949)

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Abstract: Mud crab, *Scylla* sp. aquaculture has gained in popularity due to diminishing wild stocks and high market demand. However, the industry is facing a shortage in quality formulated feed and research on practical feed development remains scarce. We conducted a 2×2 factorial experiment to examine the interactive effects of different levels of fishmeal (FM) and supplementation of dietary phospholipid (PL) on fatty acid composition, carcass composition, feed utilization and growth performance of juvenile mud crab, *S. paramamosain*. Four isonitrogenous experimental diets were prepared by the addition of two levels of FM at 50.0% (Diet 1) and 16.5% (Diet 2); these represented no replacements and two third of the FM protein replaced with fish bone meal, respectively. Two other experimental diets were formulated by supplementing 4% dietary PL to the previous diets and designated as Diet 3 and Diet 4, respectively. Triplicate groups of ten juveniles were fed the experimental diets for 60 days. Findings of the present study indicated that growth performance, feed utilization and body composition of mud crab except for survival were not significantly affected by the addition of different levels of FM. However, growth performance of mud crab significantly (P<0.05) improved with the inclusion of PL. No interactions were found between FM and PL for any of the analyzed parameters. Mud crab fed the experimental Diet 4 showed significantly higher growth performances. Similarly, feed intake, protein efficiency ratio, whole body crude protein and lipid were also highest (P<0.05) in this group. Whole body fatty acid composition showed that replacement of FM significantly decreased ecosapentanoic acid, while total polyunsaturated fatty acid was not affected. However, supplementation of PL significantly increased total polyunsaturated fatty acid. The present study concluded that two third of the FM could be replaced with fish bone meal from mud crab diets. Supplementation of 4% PL could significantly improve the carcass composition, feed utilization and growth performance of mud crab.

Keywords: Fishmeal, fish bone meal, growth, mud crab, phospholipid, Setiu Wetlands

Introduction

Mud crab, *Scylla* sp. is a promising aquaculture species and often seen as an alternative to shrimp because of their unique characteristics of larger size, high meat yield, hardiness, less

prone to disease, more tolerant to environmental stressors, little processing is required during export, and delicate flavor (Macintosh *et al*., 2002; Ut *et al*., 2007). Among the four species of mud crab, *S. paramamosain* is currently the most widely cultured in Indo Pacific region.

However, mud crab aquaculture is facing a number of challenges including hatchery technology, high cannibalism, feed and nutrition (Ut *et al.,* 2007). Moreover, research on feed development for mud crab is scares.

During the last couple of years, few studies have been conducted on different aspects of *S. paramamosain*, including basic nutritional requirements (Zhao *et al*., 2015; 2016), digestibility of feed ingredients (Truong *et al*., 2009), feeding mechanism (Chen *et al*., 2013) culture technique (Nghja *et al*., 2007; Ut *et al*., 2007) and grow-out production (Christensen *et al*., 2004). So far, no study has focused on the use of alternative protein sources or development of sustainable feed for *S. paramamosain*. At present, most farmers are using unwanted fish by-products, molluscan meat and animal viscera as feed in the commercial mud crab aquaculture (Zhao *et al*., 2015). However, the fresh animal byproduct can cause negative impacts on the culture system including deterioration of water quality in a short time, carrying disease organisms to the culture system and infecting the mud crab. In addition, fresh foods are comparatively more expensive than formulated diet and need to be stored in refrigerator, which can increase production costs. Therefore, successful expansion of mud crab farming will largely depend on the development of nutritionally balanced formulated feed in near future (Zhao *et al*., 2016).

Fishmeal (FM) has been considered as the best protein source in aqua-feed due to its unique characteristics such as balanced fatty acids and amino acids, high protein content, attractants, minerals, vitamins, and unidentified growth factor. However, FM is an expensive and limited resource. In addition, FM is produced from fish and thus, there might be a competition of fish consumption between animal feed and food for humans. Replacing FM with alternative protein sources could provide best solution for FM issues in aquaculture. For finfish and crustaceans diets, discard from fish processing industries/by-product and by-catch can be used as promising alternative protein source (Refsite *et al*., 2004; Hernandez *et al*., 2004; Kader *et al.*, 2011; Kader *et al*., 2012; Kader & Koshio, 2012; Bulbul *et al*., 2015). Although no detailed estimation of by-catch is available, a crude estimate suggests that it could be more than 20 million tones globally (FAO, 2009). In addition, a huge amount of proteinaceous by-products including shrimp heads and shells or fish fillet waste, as well as a variety of valuable dissolved and particulate organic compounds that are potentially recoverable for feed use, are also generated through various processing operation by international fish and shrimp industries (Meyers, 1986).

Keropok, which means fish cracker in Malay, is a famous fish product in the state of Terengganu, Malaysia. It is produced from the fish paste. Different fish species such as *Scolopsis taenioptera*, *Coryphaena hippurus* and *Decapterus maruadsi* are commonly used to produce keropok. Terengganu produces more than a thousand tons of keropok every year (Mr. Raja pers. comms.). In the keropok lekor industry, only the fish flesh is used and rest of the fish body is discarded. The by-products mainly consist of fish bone and small amount of scrap flesh inside the bones (Raes, 2014). The proximate composition of the keropok by-product, which is termed as high ash FM or fish bone meal (FBM) that contains 53.5% crude protein and 11.5% crude lipid (unpublished data, Kader *et al.*). Therefore FBM is considered as a high valued and cheaper alternative protein ingredient for mud crab feed (Toppe *et al*., 2006).

Phospholipids (PL) are important lipid class that maintains structure and functional properties of cellular membranes emulsify

lipids in gut and improve long chain fatty acids absorption in intestine (Kanazawa *et al*., 1983). Crustaceans have an inadequate ability to synthesize PL. Therefore, a stable supply of PL in diet should be maintained to ensure normal growth performance of crab. The significance of dietary supplementation of PL on growth has been reported in a number of fish and crustacean species including kuruma prawn, *Penaeus japonicus* (Teshima *et al*., 1986), Pacific white shrimp, *Litopenaeus vannamei* (Gonzalez-Felix *et al*., 2002), amberjack, *Seriola dumerili* (Uyan *et al*., 2009), Chinese mitten crab, *Eriocheir sinensis* (Wu *et al*., 2011), large yellow croaker, *Larmichthys crocea* (Zhao *et al*., 2013), swimming crab, *Portunus trituberculatus* (Li *et al*., 2014), black tiger prawn, *P. monodon* (Kumaraguruvasagam *et al*., 2005), and blunt snout bream, *Megalobrama amblycephala* (Li *et al*., 2015). Gonzalez-Felix *et al*. (2002) and Kumaraguruvasagam *et al*. (2014) studied the consequence of PL supplementation in diets containing different lipid sources on performances of *L. vannamei* and *P. monodon*, respectively and observed that PL supplementation significantly enhanced the growth performance of shrimp compared to un-supplemented groups. It is also hypothesized that supplementation of PL could improve the growth performance of crab fed low quality alternative protein sources such as FBM. No study was reported on the effects of FM replacement and PL supplementation on the growth performance of crab or any other aquatic species.

Here, we conduct an experiment to examine the interactive effects of different levels of FM and supplementation of dietary PL on fatty acid composition, carcass composition, feed utilization and growth performance of juvenile mud crabs.

Materials and Methods

Mud crab and experimental system

The juvenile mud crabs were collected from hatchery of Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Malaysia (UMT).

The juveniles were acclimated for 10 days to the laboratory condition in a 500 L PVC tank. During this period, a commercial shrimp diet (Cargill Malaysia Sdn. Bhd., Port Klang, Malaysia) was used as feed to the juveniles mud crab. The feeding experiment was conducted in the Institute of Tropical Aquaculture's hatchery, UMT. A total of 120 perforated plastic containers (3 L water volume) were placed in 12, 120 L rectangular PVC tanks with 10 containers in each tank. The plastic containers were covered with a lid and maintained at 2.5 L seawater. All the tanks were supplied with seawater and continuous aeration. All tanks were maintained under natural dark and light regimes.

Experimental diets

Tables 1 and 2 demonstrate the proximate composition of the ingredients and diet formulation, respectively. A 2×2 factorial experiment was designed with four practical diets which were prepared by adding two levels of FM at 50% (Diet 1) and 16.50% (Diet 2). Two other experimental diets were formulated by supplementing 4% dietary phospholipid (PL) to the previous diets, which designated as Diet 3 and Diet 4, respectively.

Table 1: Proximate composition (% dry matter basis) of the test ingredients.

Ingredients	Fishmeal	Fish bone meal
Crude protein	72.74	52.16
Crude lipid	11.70	10.82
Ash	11.93	34.89

* Values are means of triplicate measurements.

Table 2: Dietary ingredients and feed formulation

Ingredients (% fed basis)	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal[1]	50.00	16.50	50.00	16.50
Fish bone meal[2]	0.00	44.03	0.00	44.03
Soybean meal[1]	5.00	5.00	5.00	5.00
Shrimp meal[3]	2.00	2.00	2.00	2.00
Squid meal[3]	2.00	2.00	2.00	2.00
Wheat flour[4]	10.00	10.00	10.00	10.00
Corn starch[4]	5.00	2.00	5.00	2.00
Fish oil[1]	2.00	2.00	2.00	2.00
Palm oil[4]	6.00	5.00	2.00	1.00
Soybean lecithin[1]	0.00	0.00	4.00	4.00
Vitamin mixture[5]	2.00	2.00	2.00	2.00
Mineral mixture[6]	2.00	2.00	2.00	2.00
Stay C-35[1]	0.30	0.30	0.30	0.30
CMC[7]	3.00	3.00	3.00	3.00
α-cellulose[1]	10.70	4.17	10.70	4.17
Total	100.00	100.00	100.00	100.00

[1]Sri Purta Trading, Alor Star, Kedah.
[2]Collected from fish flake (locally called keropok) industry in Terengganu, Malaysia, dried and made fish bone meal.
[3]Collected raw material from local market, dried and made shrimp and squid meal in laboratory.
[4]Collected from the local market in Terengganu.
[5]Rovithai, DSM Nutritional Products Ltd. Scotland; composition (IU/g/mg per kg): vitamin A 50 IU, vitamin D3 10 IU; vitamin E130 g, vitamin B1 10g, vitamin B2 25g, vitamin B6 16g, vitamin B12 100mg, biotin 500mg, pantothenic acid 56g, floic acid 8g, niacin 200g, anticake 20g, antioxidant 0.2g and vitamin K3 10g.
[6]Rovithai, DSM Nutritional Products Ltd. Scotland; composition (g per kg): copper 7.50g, iron 125.0g, manganese 25.0g, zinc 125.0g, cobalt 0.50g, iodine 0.175g, selenium 0.300g and anticake 10.0g.
[7]CMC: carboxymethyl cellulose, Sri Putra Trading, Alor Star, Kedah.

Dietary FM levels were maintained by replacing 0 (no FM replacement) and 67% (two third of the FM replacement) FM protein with FBM protein, while soybean lecithin was used in replacement of palm oil as phospholipid source in the diets. The diets were labeled as Diet 1 (FM50.0, PL0), Diet 2 (FM16.5, PL0), Diet 3 (FM50.0, PL4) and Diet 4 (FM16.5, PL4), respectively. FM, FBM and soybean meal were used as major protein sources, while shrimp meal and squid meal were used as attractants as well as protein sources. Palm oil, fish oil and soybean lecithin served as lipid sources; and wheat flour and corn starch as carbohydrate sources.

The diet preparation was adopted from Bulbul *et al*. (2015). In brief, all the dry ingredients were blended to a homogenous mixture in a food mixture. All the lipid sources were premixed and then mixed with the dry ingredients. About 30-40% moisture was added to the premix and blended again to soft consistency for making pellet. Finally, the dough were pelleted with a meat grinder and dried at 50°C for about 4 hours in a mechanical convection oven. Then, the diets were steamed at 100°C for 5 minutes, air dried in room temperature and stored at -20°C until used.

Feeding trial

At the beginning of the feeding trial, 120 homogenous sized and healthy juvenile crabs (0.22 ±0.03 g, mean initial weight ± SD) were randomly and individually stocked in the previously prepared plastic containers. There were four dietary treatments and each treatment had triplicate tanks, stocked with 10 juveniles per replicate. The test diets were supplied at 10–15% of the body weight to feed the juvenile mud crab, twice a day at 0800 and 1700 for 60 days. In order to calculate total feed intake, uneaten test diets were collected by siphoning method in each morning, washed with tap water and finally dried in the oven. The faeces, organic matter and debris were also siphoned from the individual containers and tanks in each morning. Water exchange rate was maintained at 20–30% daily. During the experimental period, dead crabs were collected immediately, weighted and recorded (if any). To determine their growth and health condition, all the crabs were weighted in bulk at every two weeks interval. The water quality such as water temperature, pH, dissolve oxygen (DO) and salinity were measured and recorded every day during feeding trial. The temperature, pH, DO and salinity were varied between 24.6 to 29.5°C, 6.5 to 9.0, 5.0 to 7.1 mg L^{-1} and 10.2 to 11.7 g L^{-1}, respectively.

Sample collection and biochemical analysis

Prior to final sampling, crabs were fasted for 24 hours at the end of feeding trial. All the crabs were anaesthetized by using clove oil (100 ppm). Individual body weight and total number of crabs from each replicate were measured and recorded accordingly. For the final whole body proximate and fatty acid analysis, a pooled sample of seven crabs from each replicate was randomly collected and stored at −20°C. Standard methods of AOAC (1990) was used for the analysis of the proximate compositions of the feed ingredients, experimental diets and crab whole body samples. To determine the moisture contents, the samples were dried to a constant weight at 105 °C. Kjeldahl method (2300-Auto-analyzer, FOSS, Denmark) was used to measure nitrogen (N×6.25) for the determination of crude protein content, Soxhlet method (36680-analyer, BUCHI, Switzerland) was used to extract ether for crude lipid content and combustion at 550 °C for 12 h used for determination of ash content. The freeze dried samples of whole body were analyzed for fatty acid compositions by following one-step method of Abdulkadir and Tsuchiya (2008). By combining the extraction and esterification processes, one-step method of fatty acid analysis was principally carried out. Gas chromatography equipped with mass spectrometer (GCMS-QP2010 Ultra) was used to separate and quantify the fatty acids methyl esters (FAMEs). Composition of individual fatty acid was calculated qualitatively (%) by comparing the peak area of each fatty acid with the total peak area of all fatty acid in the sample.

Statistical analysis

Two-way analysis of variance was conducted for all data in order to find the effect of two factors and their interaction. In addition, one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was also applied to find differences among the treatments. The statistical analyses were performed in SPSS Ver. 21.0 for Windows (SPSS. 2012, Inc., Chicago, IL). Probabilities of $P < 0.05$ were considered significant. All the data were normally distributed based on Kolmogorov-Smirnov Test and Levene's Test except for the saturated fatty acid (SFA), n6 fatty acid and ratio of SFA and mono unsaturated fatty acid (MUFA), which were arcsin transformed before ANOVA.

Results

Proximate and fatty acid composition of diets

Table 3 shows the proximate and fatty acid composition of the experimental diets. Similar levels of crude protein (isonitrogenous) and crude lipid (isolipidic) at 50.30–51.25% and 14.11–14.72%, respectively were observed in all diets. However, replacing FM with FBM increases the ash contents. The values were

Table 3: Proximate and fatty acid composition of the experimental diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4
Proximate composition (% dry matter basis)				
Crude protein	50.93	50.47	50.30	51.25
Crude lipid	14.52	14.11	14.29	14.76
Ash	11.66	22.58	12.24	22.76
Fatty acid composition (% total fatty acids)				
∑SAFA[1]	32.69	31.59	28.00	30.00
∑MUFA[2]	29.75	31.82	23.42	22.39
EPA[3]	9.10	8.78	9.48	7.09
DHA[4]	12.73	13.15	10.9	8.8
∑PUFA[5]	34.12	36.39	46.94	46.64
Σ (n-3)[6]	23.64	23.86	24.19	20.10
Σ (n-6)[7]	10.48	12.53	22.75	26.54
(n-3) / n-6) ratio	2.26	1.90	1.06	0.76
DHA / EPA ratio	1.40	1.50	1.15	1.24
SAFA / MUFA ratio	1.10	0.99	1.20	1.34
SAFA /PUFA ratio	0.96	0.87	0.60	0.64
MUFA / PUFA ratio	0.87	0.87	0.50	0.48

*Values are means of triplicate measurements. [1]Saturated fatty acids, [2]Mono-unsaturated fatty acids, [3]Ecosapentanoic acid (C20:5n3), [4]Docosahexanoic acid (C22:6n3), [5]Poly-unsaturated fatty acids, [6]n–3 poly-unsaturated fatty acids, [7]n–6 poly-unsaturated fatty acids.

varied 11.66–22.76% among the treatments. The dietary fatty acid compositions were influenced with both FM replacement and PL supplementation. Supplementation of PL increased total poly unsaturated fatty acid (PUFA) and n-6 PUFA content of the test diets, while n-3 PUFA were similar among the dietary treatments. The ratios of n-3 and n-6 PUFA were decreased with FM replacement and PL supplementation. The DHA/EPA ratios were not influenced with FM replacement, while PL supplementation slightly decreased the ratios.

Survival, growth performances and feed utilization

The survival (%), final body weight (FBW, g), weight gain (WG, %) and specific growth rate (SGR, % day^{-1}) are shown in Table 4. The survival of crab varied between 73.33 to 96.67% which is comparatively higher for this highly cannibalistic aquatic animal. Mud crab fed experimental Diet 2, Diet 3 and Diet 4 showed significantly ($P<0.05$) higher survival compared to Diet 1. FBW was not significantly different ($P>0.05$) between mud crabs fed Diet 1 and Diet 2, indicating that FM can be replaced with FBM at the level of 67% without any negative effects on FBW of crab. Supplementation of PL significantly increased FBW of crabs fed diets containing both 50.0% FM (Diet 3) and 16.5% FM (Diet 4).The FBW in crabs fed Diet 4 was significantly higher than crabs in other dietary treatments. Similarly, FM levels had no significant effects on WG and SGR, while PL supplementation improved ($P<0.05$) these growth performance parameters. However, no interactive effect was found between the two factors on any of the growth performance parameters. The values of feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER) are shown in Table 5. Similar to growth performance, FM levels, and interaction between FM and PL had no significant effects

Table 4: Growth performances and survival of mud crab (n=30)

Diet	Fishmeal level (FM)	Phospholipid level (PL)	FBW (g)[1]	WG (%)[2]	SGR (% day^{-1})[3]	Sur (%)[4]
Diet 1	50.0	0.0	1.75±0.13[a]	695±57[a]	3.44±0.12[a]	73.33±3.33[a]
Diet 2	16.5	0.0	1.86±0.08[a]	744±36[a]	3.55±0.07[a]	96.67±3.33[b]
Diet 3	50.0	4.0	2.56±0.20[b]	1065±93[b]	4.08±0.14[b]	90.00±5.77[b]
Diet 4	16.5	4.0	3.04±0.10[c]	1284±47[c]	4.38±0.06[b]	96.67±3.33[b]
Statistical analysis (two way ANOVA)						
FM	P value		NS	NS	NS	0.0063
	F value		4.659	4.673	3.916	13.50
PL	P value		0.0001	0.0001	0.0001	NS
	F value		54.152	53.876	50.472	4.167
FM×PL	P value		NS	NS	NS	NS
	F value		1.887	1.866	0.825	4.167

*Values are means ± S.E.M. Within a row, means with the same letters are not significantly different (P> 0.05).

[1]Mean final body weight, [2]Weight gain (%), (final weight – initial weight) × 100 / initial weight, [3]Specific growth rate, {ln (final weight) – ln (initial weight) / 45 days} × 100, [4]Survival, final number / initial number × 100

Table 5: Feed utilization of mud crab (n=30)

Diet	Fishmeal level (FM)	Phospholipid level (PL)	FI[1]	FCR[2]	PER[3]
Diet 1	50.0	0.0	3.86±0.27[a]	2.53±0.03[b]	0.78±0.01[a]
Diet 2	16.5	0.0	4.03±0.09[ab]	2.47±0.10[b]	0.80±0.03[a]
Diet 3	50.0	4.0	4.31±0.29[ab]	1.84±0.05[a]	1.08±0.03[b]
Diet 4	16.5	4.0	4.64±0.14[b]	1.64±0.05[a]	1.19±0.03[c]
Sratistical analysis (two way ANOVA)					
FM	P value		NS	NS	0.0424
	F value		1.0365	4.194	5.817
PL	P value		0.0369	0.0000	0.0000
	F value		6.255	137.91	145.42
FMxPL	P value		NS	NS	NS
	F value		0.148	1.130	2.163

Values are means ± S.E.M. Within a row, means with the same letters are not significantly different (P> 0.05).

[1]Feed intake (FI, g crab-1 60 days-1) = (dry diet given – dry remaining diet recovered) / no of crab, [2] Feed conversion ratio (FCR), total dry feed intake / total live weight gain, [3]Protein efficiency ratio (PER), live weight gain / protein intake.

Table 6: Whole body composition of mud crab (% wet basis)

Diet	Fishmeal level (FM)	Phospholipid level (PL)	Moisture	Crude protein	Crude lipid	Ash
Diet 1	50.0	0.0	71.24±1.65	11.78±0.59[a]	1.44±0.24[a]	13.22±0.74
Diet 2	16.5	0.0	72.77±1.20	11.43±0.07[a]	1.37±0.14[a]	12.26±0.68
Diet 3	50.0	4.0	70.68±1.14	12.11±0.27[ab]	2.48±0.23[b]	13.62±0.53
Diet 4	16.5	4.0	70.61±1.69	13.01±0.15[b]	2.25±0.14[b]	13.71±0.70
Statistical analysis (two way ANOVA)						
FM	P value		NS	NS	NS	NS
	F value		0.256	0.702	0.512	0.428
PL	P value		NS	0.0207	0.0033	NS
	F value		0.887	8.268	22.241	1.943
FM×PL	P value		NS	NS	NS	NS
	F value		0.304	3.579	0.151	0.623

*Values are means ± S.E.M. Within a row, means with the same letters are not significantly different ($P > 0.05$).

on these parameters while, supplementation of PL had significant effects. Overall, it was found that FI was significantly improved in crab fed Diet 4compared to Diet 1. Similarly, PER was highest and FCR was lowest ($P<0.05$) in this group (Diet 4).

Whole body proximate composition

Proximate compositions of whole body of mud crab are presented in Table 6. Moisture and ash content of whole body were not affected by any of the factors. Crude protein and crude lipid were significantly influenced by PL supplementation. The highest crude protein and crude lipid of whole body were found in crab fed Diet 4 and Diet 3, respectively. However, differences ($P>0.05$) were not observed in crude protein and crude lipid contents in crab fed Diet 3 and Diet 4.

Whole body fatty acid composition

The composition of major fatty acids of whole body of mud crab is demonstrated in Table 7A & B. Both FM and PL levels had significant effects on fatty acid composition of whole body, while interaction between two factors was only found for n-6 PUFA. PUFA content of whole body increased with PL supplementation, while n-3 PUFA decreased with the replacement of FM. The n-6 PUFA increased with both FM replacement and PL supplementation. Significantly higher n-6 PUFA was found in crab fed diet replacing 67% FM with FBM and supplemented with 4% PL (Diet 4). Additionally, ratios of n-3 and n-6 PUFA had opposite trend and the lowest value was observed with the group fed Diet 4. Docosapentanoic acid (DHA) was not affected by any of the factors, while ecosapentanoic acid (EPA) decreased and the DHA/EPA ratio increased with FM replacement.

Discussion

At present, commercial diet is not available for mud crab species in Malaysia. Farmers commonly use shrimp diet or trash fish for mud crab farming. Therefore, development of a suitable formulated feed is a priority research for mud crab (William & Abdullah, 1999; Zhao *et al*., 2016). The present research is an initiative to develop cost effective diet for mud crab by replacing costly FM with underutilized sea food industry by-products (FBM) and supplementation of PL. It is clearly obvious that 67% FM could be replaced with FBM and supplementation of 4% PL could

Table 6: Whole body composition of mud crab (% wet basis)

(A)

Diet	Fishmeal level (FM)	Phospho-lipid level (PL)	ΣSAFA[1]	ΣMUFA[2]	EPA[3]	DHA[4]	ΣPUFA[5]
Diet 1	50.0	0.0	27.37±2.35^{b}	33.15±1.53^{b}	14.93±0.68^{b}	16.94±2.30^{a}	40.70±1.61^{ab}
Diet 2	16.5	0.0	26.24±0.26^{ab}	33.87±0.07^{b}	8.40±0.03^{a}	14.70±1.34^{a}	34.03±1.26^{a}
Diet 3	50.0	4.0	22.23±0.87^{a}	24.00±0.77^{a}	13.42±0.01^{b}	16.71±3.65^{a}	46.26±3.42^{b}
Diet 4	16.5	4,0	23.15±0.37^{ab}	25.66±0.78^{a}	8.08±1.36^{a}	13.70±1.26^{a}	43.28±2.67^{b}
Sratistical analysis (two way ANOVA)							
FM	P value		NS	NS	0	NS	NS
	F value		0.007	1.598	60.877	1.251	4.040
PL	P value		0.012	0	NS	NS	0.015
	F value		10.415	85.301	1.439	0.068	9.535
PMxPL	P value		NS	NS	NS	NS	NS
	F value		0.644	0.248	0.601	0.027	0.592

(B)

Diet		Σ (n-3)[6]	Σ (n-6)[7]	(n-3) / n-6) ratio	DHA / EPA ratio	SAFA / MUFA ratio	SAFA / PUFA ratio	MUFA / PUFA ratio
Diet 1		33.22±1.62^{b}	7.47±0.08^{a}	4.45±0.22^{c}	1.15±0.21^{a}	0.82±0.03^{a}	0.67±0.07^{bc}	0.82±0.06^{b}
Diet 2		4.70±1.29^{a}	9.33±1.29^{b}	2.65±0.15^{b}	1.75±0.16^{a}	0.78±0.01^{a}	0.77±0.03^{c}	1.00±0.04^{c}
Diet 3		32.57±3.63^{ab}	13.69±0.51^{c}	2.39±0.32^{b}	1.25±0.27^{a}	0.93±0.01^{b}	0.49±0.05^{a}	0.53±0.05^{a}
Diet 4		24.28±2.63^{a}	19.00±0.23^{d}	1.28±0.13^{a}	1.75±0.16^{a}	0.90±0.01^{b}	0.54±0.03^{ab}	0.59±0.04^{a}
Sratistical analysis (two way ANOVA)								
FM	P value	0.009	0	0	0.028	NS	NS	0.025
	F value	11.597	154.73	43.725	7.135	3.392	2.426	7.559
PL	P value	NS	0	0	NS	0	0.002	0
	F value	0.047	760.99	60.471	0.048	36.623	19.895	61.959
FMxPL	P value	NS	0	NS	NS	NS	NS	NS
	F value	0.002	35.927	2.400	0.055	0.377	0.321	1.545

*Values are means ± S.E.M. Within a row, means with the same letters are not significantly different ($P > 0.05$).
[1]Saturated fatty acids, [2]Mono-unsaturated fatty acids, [3]Ecosapentanoic acid (C20:5n3), [4]Docosahexanoic acid (C22:6n3), [5]Poly-unsaturated fatty acids, [6]n–3 poly-unsaturated fatty acids, [7]n–6 poly-unsaturated fatty acids.

enhance the growth performance of mud crab, *S. paramomsain*.

Although replacement of dietary FM with alternative protein sources for many fish and crustacean's species are well studied phenomena in aquaculture nutrition, research on crab species is scarce. Replacement of FM with different vegetable protein sources are reported for *S. paramamosain* (Suwirya *et al*., 2009), *S. serrata* (Nguyen *et al*., 2014), *E. sinensis* (Luo *et al*., 2011; Jiang *et al*., 2013). In our experiment, it was found that FBM could replace 67% dietary FM without negotiating body composition, feed utilization and growth performances of *S. paramamosain*, which were in line with the study of Toppe *et al*. (2006). In their research, FBM was used to replace0, 15, 30 and 45% dietary FM for Atlantic cod *Gadus morhua*. The proximate composition of FBM was similar to that used in the present research. They found no substantial differences in SGR and WG of fish fed FM based control and FBM based test diets and it was concluded that FBM is a potential dietary protein source which could replace 45% FM from the diet of Atlantic cod. To our best knowledge, no research has been reported to replace FM with FBM or any other

animal proteins for *S. paramamosain*. Suwirya *et al*. (2009) stated that 20–40 % FM could be replaced with corn gluten meal and soybean meal for this species. In another study, it was found that 40% FM could be replaced with a combination of rapeseed meal and soybean meal in the diets of *E. sinensis* (Luo *et al*., 2011). Our research also supported the finding of Jiang *et al*. (2013) who found that 64% FM could be replaced with a mixture of soybean meal and cottonseed meal and supplementation of crystalline amino acid for *E. sinensis*. In our study, we observed that the comparatively higher level of FM could be replaced, which might be due to the inclusion of animal protein source. It is well documented that animal proteins are superior over plant proteins because of higher protein contents, well balanced amino acids and fatty acids, palatability, and less anti-nutritents or toxins. Fisheries by-catch and by-products meal can replace 25–50% FM from the diets of red drum (Li *et al*., 2004). In red sea bream diet, soybean meal and fermented sea food by-products could replace as high as 80% FM (Kader & Koshio, 2012).

A blend of canola meal and soybean meal supplemented with fish soluble (a fish processing by-product) could replace 60% FM from the diet of kuruma shrimp (Bulbul *et al*., 2015). Similarly, Davis and Arnold (2000) reported that 80% marine protein mix could be replaced with coextruded soybean poultry by-product meal for Pacific white shrimp. Co-extruded soybean poultry by-product meal (Samocha *et al*., 2004) and the combination of solvent extracted soybean meal, poultry by-product meal and corn gluten meal (Amaya *et al*., 2007) could completely eliminate dietary fishmeal for Pacific white shrimp. Supplementation of 4% PL significantly improved growth performance of crab fed both FM (Diet 3) and FBM (Diet 4) based diets, which is in agreement with Li *et al*. (2014) who investigated that up to 4% PL supplementation significantly improved FBW, WG and SGR of juvenile swimming crab compared to the un-supplemented group. Kumaraguruvasagam *et al*. (2005) studied the supplemental effects of dietary PL to diets replacing fish oil with different vegetable oils for black tiger shrimp and found that supplementation of 2% PL could significantly enhanced WG of shrimp fed vegetable oil based diets. Similarly, Gonzalez-Felix *et al*. (2002) found that an inclusion of 3% PL significantly increased the growth performance of Pacific white shrimp. Previous studies also showed that inclusion of 1–6.5% soybean lecithin as dietary PL source has beneficial effects over growth performance of different crustacean species, such as 1–2% for *P. monodon* (Paibulkichakul *et al*., 1998), 6.5% for *L. vannamei* (Coutteau *et al*., 1996), 3.5–6% for *M. japonicas* (Kanazawa *et al*., 1985) and 2% for *Fenneropenaeus chinensis* (Kanazawa, 1993). Although, growth performances of crabs were improved ($P<0.05$) with the supplementation of PL in groups fed Diet 3 and Diet 4, differences in the performance parameters were also found between these two treatments. This might be due to the level of FM and FBM in Diet 3 and Diet 4 which were also evident between Diet 1 and 2. Although two third of the FM was replaced with FBM, similar growth was observed in crab fed Diet 1 and Diet 2 which might be due the similar or even slightly higher feed intake in crab fed Diet 2 (Toppe *et al*., 2006). Since FBM is prepared from basically fish bones, it contains higher levels of calcium, phosphorus and ash. Quantitative measurements of calcium and phosphorus were not conducted in our study - this needs further investigation, the ash contents in FBM and diets were analyzed. It was found that FM and FBM based diets contained 11.66–11.24% and 22.58–22.76% ash, respectively.

Similarly, Toppe *et al*. (2006) stated that increasing addition of FBM increased the dietary calcium, phosphorus and ash contents which were positively correlated with the growth performance and feed intake of Atlantic cod. In our study, supplementation of PL also increased FI in both FM and FBM based diets (Diet 3 and Diet 4) and it might be liable for higher growth performance of crab in these groups. Li *et al*. (2015) explored the consequence of dietary PL

on growth performance of blunt snout bream and found that inclusion of 4% PL significantly enhanced the feed intake of fish which might be liable for higher growth performance of fish. The PER is considered as a suitable parameter to assess protein quality in aqua-feed ingredients (Goytortua-Bores *et al*., 2006; Luo *et al*., 2011). In our study, FCR decreased and PER increased with the supplementation of PL, which are the indicators of better feed utilization as well as growth performance of crab. Similarly, it was found that inclusion of PL enhanced the feed utilization of swimming crab (Li *et al.,* 2014), kuruma shrimp (Michael *et al.,* 2008), black tiger shrimp (Kumaraguruvasagam *et al*., 2005) and blant snout bream (Li *et al*., 2015).

Replacement of FM with FBM had no noteworthy effects ($P>0.05$) on the composition of whole body, however supplementation of PL significantly improved crude protein and crude lipid content of whole body which might be correlated to the higher growth performance of crabs in these groups. Similarly, Li *et al*. (2015) reported that whole body crude protein and crude lipid contents were increased in blant snout bream fed diets containing higher level of dietary PL. Uyan *et al*. (2009) stated that dietary PL increasingly improved the protein retention in juvenile amberjack (*Seriolla dumerilli*). Although, FBM based diets (Diet 2 and Diet 4) contains higher level of ash, it was not revealed in the whole body composition. Similarly, Toppe *et al*. (2005) found that dietary FBM has no effect on whole body ash composition of Atlantic cod. Similar to the previous studies by Gonzalez-Felix *et al*. (2002), Kumaraguruvasagam *et al*. (2005) and Li *et al*. (2015), the trends of dietary fatty acid compositions were closely matched with the whole body fatty acid composition of crab. It is well known that PL of soybean lecithin is rich in linoleic acid which is the precursor of n-6 fatty acid (Li *et al*., 2015). Therefore, dietary supplementation of PL increased the n-6 PUFA, as well as total amount of PUFA in Diet 3 and Diet 4, which also reflected the whole body n-6 PUFA and total amount of PUFA. The dietary n-3:n-6 ratio decreased with the replacement of FM and supplementation of PL in diets which were also reflected in whole body composition. Similarly, Hamza *et al*. (2008) and Gao *et al*. (2014) reported that supplementation of PL in expense of fish oil or palm oil resulted in decrease values of n-3:n-6 ratio in diets and subsequently whole body composition ($P<0.05$).

Conclusion

This study suggests that two third of the fishmeal could be replaced with fish bone meal from mud crab diet formulation. Supplementation of 4% phospholipid could significantly improve the whole body composition, feed utilization and growth performance of mud crab. Since fish bone meal can be prepared from the by-products and waste products of fish processing industry, it is a promising alternative protein source for aquatic animals as it can be effectively included in aqua-feed with appropriate feed additives such as phospholipid.

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