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Can canola meal and soybean meal be used as major dietary protein sources for kuruma shrimp, *Marsupenaeus japonicus*?

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ABSTRACT

A feeding trial was conducted to utilize canola meal and soybean meal as major dietary protein sources for kuruma shrimp, *Marsupenaeus japonicus*. Four isocaloric diets (19 kJ g^{-1}) were formulated by reducing 0 (FM40), 70 (FM12), 85 (FM6) and 100% (FM0) of dietary fishmeal with a combination (4:6) of canola meal and soybean meal (blend). Based on a series of previous studies, all the plant protein diets (FM12, FM6 and FM0) were supplemented with 1.00% lysine, 0.50% methionine, 0.04% phytase and varying levels of fish soluble to improve the nutritional quality of the diets. Fifteen shrimp with an initial average weight of 1.74 g were randomly stocked in 12, 54 l rectangular tanks in triplicate per dietary treatments. The shrimp were given the respective test diets daily by hand at 8–10% of body weight for 60 days. Final body weight (g) and specific growth rate (% day^{-1}) were not significantly ($P > 0.05$) affected by reducing fishmeal with plant protein blend. Feed intake was also not varied among the dietary treatments. On the other hand, feed conversion ratio was significantly ($P < 0.05$) increased in the FM0 group, while no difference was found among the rests. Protein efficiency ratio had an opposite trend and the FM0 group demonstrated significantly lowest value. Similarly, protein gain (g kg weight $gain^{-1}$) and protein retention (%) were significantly decreased in the FM0 group. Dietary treatments had no negative effects ($P > 0.05$) on the whole body composition. Significant effect was also not found on the protease activity (unit mg^{-1} protein) in the digestive tract of shrimp fed the FM40, FM12 and FM6 diets, while the value was significantly decreased in shrimp fed the FM0 diet. The values for the total hemocyte count (cells ml^{-1}) and viable cells (%) were lowest in the FM0 group, however these parameters were not significantly varied among the dietary treatments. Upon considering the results obtained in the present experimental condition, it has been concluded that canola meal and soybean meal could be effectively utilized as major protein sources by kuruma shrimp. The dietary fishmeal could be reduced to only 6% (85% replacement) with a blend of canola meal and soybean meal, and supplementation of methionine, lysine, phytase and fish soluble without compromising growth, feed utilization, body composition and health of juvenile kuruma shrimp.
Statement of relevance: The research findings will help to develop plant protein based diets for crustaceans.

1. Introduction

Traditionally, kuruma shrimp (kuruma ebi, *Marsupenaeus japonicus*) is one of the most important crustacean species in Japan, because of its delicacy and preference in the expensive and luxury food "sushi" and "tempura" (Alam et al., 2004; Bulbul et al., 2014). Since, wild catch of kuruma shrimp is decreasing, aquaculture is the only means to bridge the gap between demand and supply of this high-valued seafood (Bulbul et al., 2014). Kuruma shrimp is believed to require more dietary protein for growth than other crustacean species and, consequently, inclusion of higher levels of dietary protein from marine sources like fishmeal is necessary for optimum growth (Teshima et al., 2001). However, in addition to sustainability issues, fishmeal is a finite protein source and its demand is increasing day by day which also increases the cost (Tacon and Metian, 2008; Hardy, 2010; Kader and Koshio, 2012). Thus, it is necessary to find alternative sources to make up for the shortage of fishmeal and to secure a stable supply for commercial diets.

Plant proteins are mostly focused as alternative to fishmeal in shrimp feeds because of their lower price, consistent nutrient composition and availability. Different plant proteins have been examined in

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shrimp diets including soybean meals (SBM) (Lim and Dominy, 1990; Saitoh et al., 2000; Paripatananont et al., 2001; Du and Niu, 2003; Alvarez et al., 2007; Amaya et al., 2007; Rahman et al., 2010; Yue et al., 2012; Bulbul et al., 2013, 2015a, 2015b; Chiu et al., in press), canola meal (CM) (Lim et al., 1997; Cruz-Suarez et al., 2001; Bulbul et al., 2014), lupin meal (Sudaryono et al., 1999; Smith et al., 2007), peanut meal (Liu et al., 2011; Yue et al., 2012), *Jatropha curcas* kernel meal (Harter et al., 2011) and *Spirulina* meal (Silva-Neto et al., 2012; Macias-Sancho et al., 2014). A wide variation in fishmeal replacement levels was found in these previous findings; which varied between less than 20% to over 70% depending on the shrimp species and plant proteins used. In most cases, a single source plant protein cannot be effectively utilized by shrimp species because of the negative effects emulated by plant proteins including imbalanced amino acids, specially lysine and methionine, antinutritional and toxic factors, low palatability and indigestible carbohydrates. The use of complementary ingredients or supplementation of specific nutrients such as crystalline amino acid (CAA) or feed additives such as phytase (PT), fish soluble (FS), feeding stimulants is sometimes recommended to achieve higher or complete fishmeal replacement for fish and shrimp diets (Mendoza et al., 2001; Hernández et al., 2004; Samocha et al., 2004; Suarez et al., 2009; Kader et al., 2010, 2012; Kader and Koshio, 2012; Yue et al., 2012; Bulbul et al., 2013, 2015b; Chiu et al., in press).

Suarez et al. (2009) reported that a mixture of CM and SBM (3:7) could replace 80% fishmeal from the diets of Pacific white shrimp, *Litopenaeus vannamei* while these plant proteins could individually replace only 33% (Lim et al., 1997) and 40% (Lim and Dominy, 1990), respectively. Similarly, our research group also found that CM and dehulled soybean meal (DSM) can replace only 20 and 45% fishmeal, respectively from the diets of kuruma shrimp (Bulbul et al., 2014, 2015a). While, a combination of CM and DSM (4:6) can accelerate the fishmeal replacement level to 50% of the dietary contents (Bulbul et al., 2013). In a later study by our research group (Bulbul et al., 2015b), it was investigated that the fishmeal replacement level could be further improved by the supplementation of CAA or FS with the same combination of plant proteins (CM:DSM at 4:6 ratio) which allows 60% fishmeal replacement from kuruma shrimp diets. In that study, it was also reported that supplementation of a mixer of CAA, PT and FS with plant protein blend provided numerically higher growth performance of kuruma shrimp compared to those of fishmeal based control group which suggests that appropriate combination of these supplements could further improve fishmeal replacement levels. Based on this hypothesis, a feeding trial was conducted to develop plant protein based diets for kuruma shrimp by gradually eliminating dietary fishmeal with a blend of CM and DSM (4:6), and supplementation of CAA, PT and FS.

2. Materials and methods

2.1. Test diets

The proximate composition and essential amino acid composition of major protein ingredients are shown in Table 1. Based on these nutritional compositions, four diets were formulated to be isonitrogenous (45% crude protein), isolipidic (16% total lipid) and isocaloric (19 kJ g^{-1} gross energy) (Table 2). The dietary components and the basal diet were similar to those used previously (Bulbul et al., 2013, 2014, 2015a, 2015b). In the control diet (FM40), high quality brown fishmeal was used as sole protein source. The remaining three diets were prepared by replacing 70 (FM12), 85 (FM6) and 100% (FM0) of the fishmeal protein with a combination of CM and DSM proteins at a 4:6 ratio (blend). Based on our previous experiments, all the replacement diets (FM12, FM6 and FM0) were supplemented with 1.00% lysine, 0.50% methionine, 0.04% phytase (5000 FTU) and increasing levels (10, 12.5 and 15%, respectively) of FS. Squid liver oil, soybean lecithin, cholesterol and n−3 highly unsaturated fatty acids (HUFA) were supplied as lipid sources. Starch and dextrin were used as the

Table 1
Nutritional composition in the major dietary ingredients[a].

	FM[b]	DSM[c]	CM[d]	KM[e]	SM[f]	FS[g]
Proximate composition (% dry matter basis)						
Crude protein	71.05	50.66	37.33	62.07	83.60	70.59
Total lipid	13.73	3.58	2.90	23.42	9.21	3.98
Ash	12.34	3.52	4.40	9.67	6.39	19.30
Essential amino acid (g 100 g^{-1} dry sample)						
Arginine	4.62	3.14	2.14	2.24	5.80	3.16
Histidine	2.58	1.65	1.31	1.41	1.86	3.31
Isoleucine	2.00	1.51	1.26	1.46	2.18	1.08
Leucine	4.68	3.34	2.47	2.50	4.04	2.09
Lysine	4.07	2.13	1.78	3.02	4.14	4.57
Methionine	1.78	0.52	0.63	1.15	1.55	0.99
Phenylalanine	3.18	3.00	2.15	4.17	3.00	1.12
Threonine	2.74	1.82	1.68	1.51	2.28	1.59
Tryptophan	0.99	0.41	0.81	0.71	1.94	0.27
Valine	2.37	1.75	1.61	1.72	2.59	1.46

[a] Values of means of triplicate measurements.
[b] Fishmeal, Nippon Suisan Co. Ltd., Tokyo, Japan.
[c] Dehulled Soybean Meal, J. Oil Mills, Japan.
[d] Canola meal, J. Oil Mills, Japan.
[e] Krill meal, Nippon Suisan Co. Ltd., Tokyo, Japan.
[f] Squid meal, Nippon Suisan Co. Ltd., Tokyo, Japan.
[g] Fish soluble, Makurazaki Fish Processor Cooperatives, Kagoshima, Japan.

Table 2
Formulation and proximate analysis of the experimental diets (% dry matter basis).

Ingredient	Diet groups			
	FM40	FM12	FM6	FM0
Fish meal	40.00	12.00	6.00	0.00
Dehulled soybean meal	0.00	13.39	16.33	19.29
Canola meal	0.00	12.20	14.91	17.59
Krill meal	5.00	5.00	5.00	5.00
Squid meal	10.00	10.00	10.00	10.00
Squid liver oil[a]	2.00	4.50	5.00	5.50
Soybean lecithin[a]	3.00	3.00	3.00	3.00
n−3 HUFA[b]	0.50	0.50	0.50	0.50
Cholesterol[c]	1.00	1.00	1.00	1.00
Starch	7.00	3.00	2.00	1.00
Dextrin	3.00	2.00	2.00	2.00
Vitamin mixture[d]	1.70	1.70	1.70	1.70
Mineral mixture[e]	8.60	8.60	8.60	8.60
Activated gluten	5.00	5.00	5.00	5.00
Amino acid[f]	0.00	1.50	1.50	1.50
Phytase[g]	0.00	0.04	0.04	0.04
Fish soluble	0.00	10.00	12.50	15.00
CMC[h]	1.00	1.00	1.00	1.00
Attractants[i]	1.40	1.40	1.40	1.40
α-Cellulose	10.80	4.17	2.52	0.88
Proximate composition (% dry matter basis)				
Crude protein	45.10	45.74	45.39	45.94
Total lipid	16.57	16.16	16.89	16.95
Ash	14.65	14.61	14.28	13.95
Gross energy (kJ g^{-1})[j]	19.43	19.69	19.59	19.70

[a] Riken Vitamin, Tokyo, Japan.
[b] Poweash A, Oriental Yeast Co, Ltd., Tokyo, Japan.
[c] Takeda Kagaku Siriyo, Japan.
[d] Vitamin mixture (mg 100 g^{-1} diet): ρ-amino benzoic acid, 9.48; *d*-biotin, 0.38; inositol, 379.20; niacin, 37.92; Ca-pantothenate, 56.88; pyridoxine-HCl, 11.38; riboflavin, 7.58; thiamin-HCl, 3.79; L-ascorbyl-2-phosphate-Mg, 132.00; folic acid, 0.76; cyanocobalamin, 0.08; menadione, 3.80; vitamin A-palmitate, 17.85; α-tocopherol, 18.96; calciferol, 1.14.
[e] Mineral mixture (g 100 g^{-1} diet): K_2PO_4, 2.011; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 2.736; $MgSO_4 \cdot 7H_2O$, 3.05; $NaH_2PO_4 \cdot 2H_2O$, 0.795.
[f] Amino acid (g 100 g^{-1} diet): lysine, 1.00; methionine, 0.05.
[g] Ronozyme P 5000, DSM Nutrition Japan K.K., Shizuoka, Japan.
[h] Carboxymethyl cellulose.
[i] Attractants (g 100 g^{-1} diet): glucosamine HCl, 0.80; sodium succinate, 0.30; sodium citrate, 0.30.
[j] Calculated using combustion values for protein, lipid and carbohydrate of 236, 395 and 172 kJ kg^{-1}, respectively. Carbohydrate calculated by difference: 100 − (protein + lipid + ash + moisture).

carbohydrate or nitrogen free extract sources. Diets were prepared as described in our previous study (Bulbul et al., 2015b). Dietary amino acids are shown in Table 3.

2.2. Test shrimp and experimental system

The feeding trial was carried out at the Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University, Japan. Juvenile kuruma shrimp was obtained from a commercial hatchery "Matsumoto Suisan", Miyazaki, Japan. The shrimp were acclimatized for one week on a commercial diet (Higashimaru Feeds Ltd.; Kagoshima, Japan) in the laboratory condition. Twelve units of 54-l capacity rectangular polyvinyl chloride (PVC) tanks (60 W × 30 L × 30 H cm^3) were used for the feeding trial. The tanks were equipped with a continuous aeration and water circulating system by filtration through a sand filter covered with a net. Water was circulated at 0.50 l min^{-1} during the experimental period. Each tank was covered with a PVC lid to minimize disturbance and prevent shrimp from jumping out. Natural illumination conditions were followed during the feeding period. After being acclimatized to laboratory conditions, fifteen juveniles with an initial average weight of 1.74 ± 0.02 g (mean ± SD) were stocked randomly in previously prepared 12 tanks in triplicate per dietary treatment. Shrimp were manually fed at a ration size of 8–10% of wet body weight that was divided into 20% at 08.00 and 80% at 17.00 h everyday, for 60 days. Uneaten diets were collected and fecal matter was siphoned from the tanks each morning. Every 10 day interval, sampling was conducted to measure weight gain and survival in order to adjust ration size. Before returning the shrimp to the tanks, all tanks including the sand were cleaned and water was 100% renewed. During the experimental period, water temperature, pH, and salinity were measured daily and the mean values ± SD were 24.7 ± 0.7 °C, 7.9 ± 0.6 and 33.2 ± 0.5 respectively. These values were considered suitable conditions for rearing of kuruma shrimp juvenile.

2.3. Sample collection and biochemical analysis

At the beginning of the experiment, 15 shrimp from the stock were sampled for analysis of whole body composition and stored at −20 °C. At the end of the experiment, all shrimp were fasted for 24 h prior to final sampling. The total number of survivors and individual body weight of shrimp from each tank were measured. Five shrimp from each replicate tank were randomly collected and stored at −20 °C for final whole body analysis. Proximate compositions of feed ingredients, diets and shrimp whole body samples were analyzed in triplicate using the standard AOAC methods (AOAC, 1990). The moisture was determined by drying the sample at 105 °C to constant weight. The ash was analyzed by combustion at 550 °C for 12 h. The crude protein content was determined by measuring the nitrogen content (N × 6.25) using the Kjeldahl method with a Tecator Kjeltec System (1007 Digestion System, 1002 Distilling unit, and Titration unit; FOSS Tecator AB, Högendäs, Sweden). Total lipids were analyzed according to the Bligh and Dyer (1959) method using a 1:1 ratio of chloroform and methanol. Total amino acids in feed ingredients and diet samples were analyzed using high performance liquid chromatography (HPLC, Shimadzu Corp. Tokyo, Japan) according to Teshima et al. (1986). For protease activity (PA) assay, digestive organs from three shrimps were collected and prepared according to Bulbul et al. (2015b). The PA was analyzed by following Sigma's Non-specific Protease Activity Assay (Cupp-Enyard, 2008) and described previously (Bulbul et al., 2015b). The hemolymph was collected from the ventral sinus cavity of individual shrimp using a 1-ml tuberculin syringe (26-gauge) containing chilled (4 °C) anticoagulant solution (10 mM EDTA-Na_2, 45 mM NaCl, 10 mM KCL, 10 mM HEPES, pH 7.3) at a proportion of one part hemolymph to three parts anticoagulant solution (Vargas-Albores et al., 1993). Total hemocyte counts (THC) and viable cell (VC) rates were measured as described by Itami et al. (1998) with slight modification by Tung et al. (2009).

2.4. Statistical analysis

All data from the feeding trial and chemical analysis were statistically analyzed by one-way analysis of variance followed by the Tukey Kramer Test (Super-ANOVA 1.11, Abacus Concepts, Berkeley, CA, USA). Probabilities of $P < 0.05$ were considered significant.

3. Results

Growth performance, nutrient utilization and survival of kuruma shrimp after the 60 day feeding trial are shown in Table 4. Shrimp survival (%) was not significantly ($P > 0.05$) different among the dietary treatments and was greater than 95% in all treatments. Fishmeal free diet (FM0) supported growth equivalent to that of the 100% fishmeal based control diet (FM40) and there were no significant differences in the final body weight (FBW, varied between 3.15 and 3.46 g) and specific growth rate (SGR, varied between 1.04 and 1.24% day^{-1}) of shrimp fed the FM40, FM12, FM6 and FM0 diets. The feed intake (FI) was higher in shrimp fed the FM12 and FM6 diets compared to the FM40 and FM0 diets without any significant difference among treatments. On the other hand, feed utilizations, in terms of feed conversion ratio (FCR) significantly ($P < 0.05$) increased; and protein efficiency ratio (PER) significantly decreased in shrimp fed FM0, while no differences were found among the rest. Similarly, protein gain (PG, g kg weight $gain^{-1}$) and protein retention (PR, % of intake) significantly decreased in the FM0 group and no differences were found among the FM40, FM12 and FM6 groups.

Table 5 represents the whole body proximate composition of kuruma shrimp at the end of the feeding trial. There was no significant difference in whole body moisture, crude protein, total lipid and ash contents among the treatments at the end of the feeding trial. The PA (unit mg^{-1} protein) in the digestive tract of kuruma shrimp is presented in Fig. 1. No difference was found in the PA of shrimp fed the FM40, FM12 and FM6 diets. On the contrary, significantly lowest PA was observed in shrimp fed the FM0 diet compared to the FM40 and FM12 diets.

Table 3
Amino acid content of the diets (AA g 100 g^{-1} dry sample)[a].

Ingredients/diet	Diet groups				EAA requirement[b]
	FM40	FM12	FM6	FM0	
Essential amino acid					
Arginine	3.27	3.11	3.17	3.10	1.40–1.80
Histidine	1.59	1.22	1.31	1.46	0.50–0.70
Isoleucine	1.62	1.42	1.52	1.46	1.10–1.40
Leucine	2.94	2.64	2.73	2.71	1.70–2.10
Lysine	2.40	2.75	2.80	2.77	1.70–2.00
Methionine	1.00	1.12	1.05	1.04	0.60–0.80
Phenylalanine	1.99	1.83	1.84	1.88	1.30–1.60
Threonine	1.46	1.36	1.35	1.36	1.10–1.40
Tryptophan	tr[c]	tr	tr	tr	0.30–0.40
Valine	1.83	1.72	1.84	1.77	1.20–1.50
Non-essential amino acid					
Taurine	0.39	0.41	0.42	0.42	–
Aspartic acid	2.53	2.69	2.83	2.87	–
Glutamic acid	7.03	6.90	7.29	7.56	–
Serine	1.50	1.52	1.52	1.67	–
Proline	2.17	1.93	2.05	2.09	–
Glycine	2.13	1.93	2.05	2.14	–
Alanine	1.84	1.98	2.10	2.09	–
Tyrosine	0.68	0.86	0.89	0.89	–
Hydroxyproline	0.10	0.20	0.21	0.21	–

[a] Values of means of triplicate measurements.
[b] Teshima et al. (2002).
[c] Trace.

Table 4
Growth parameters and nutrient utilization in kuruma shrimp fed test diets for 60 days*.

Parameters	Diet groups			
	FM40	FM12	FM6	FM0
FBW[1]	3.46 ± 0.14	3.39 ± 0.11	3.34 ± 0.08	3.15 ± 0.09
SGR[2]	1.24 ± 0.09	1.18 ± 0.06	1.16 ± 0.04	1.04 ± 0.05
FI[3]	2.67 ± 0.33	2.81 ± 0.04	2.80 ± 0.05	2.66 ± 0.08
FCR[4]	1.55 ± 0.03^{a}	1.72 ± 0.10^{ab}	1.76 ± 0.08^{ab}	1.92 ± 0.06^{b}
PER[5]	1.47 ± 0.03^{b}	1.33 ± 0.07^{ab}	1.30 ± 0.06^{ab}	1.18 ± 0.04^{a}
PG[6]	198 ± 4^{b}	199 ± 9^{b}	191 ± 6^{ab}	169 ± 4^{a}
PR[7]	27.11 ± 0.88^{b}	26.60 ± 0.97^{b}	24.80 ± 0.75^{ab}	20.57 ± 1.16^{a}
Survival[8]	100	100	100	97

* Values are means ± pooled S.E.M. from triplicate groups. Means in each row with different letters are significantly different ($P < 0.05$). Absence of letters indicates no significant difference between treatments.
[1] Mean final body weight.
[2] Specific growth rate = {ln (final weight) − ln (initial weight) / 60 days} × 100.
[3] Feed intake (g shrimp^{-1} 60 days^{-1}) = (dry diet given − dry remaining diet recovered) / no of shrimp.
[4] Feed conversion ratio = `dry feed intake (g) / live weight gain (g).
[5] Protein efficiency ratio = live weight gain (g) / protein intake (g).
[6] Protein gain (g kg weight gain^{-1}) = {(final weight (g) × final whole body protein content (%) / 100) − (initial weight (g) × initial whole body protein content (%) / 100)} / (weight gain (g)) × 1000.
[7] Protein retention (% of intake) = {protein gain (g kg weight gain^{-1}) × 100} / protein intake (g kg weight gain^{-1}).
[8] Survival (%) = 100 × (final number of shrimp / initial number of shrimp).

The values for THC and VC in kuruma shrimp presented in Table 6. Although, significant differences were not found in THC and VC among the dietary treatments, the values had decreasing trends with the reducing levels of fishmeal in diets. It is also noted that FM0 represented the lowest values for both the parameters.

4. Discussion

The aquaculture business is facing a serious problem of scarcity of its finite protein source fishmeal. Therefore, replacement of fishmeal with cost-effective alternative protein sources, even in minor quantities from a feed formulation is desirable as it will obviously reduce the feed cost as well as farm production costs (Amaya et al., 2007). CM and SBM are two important plant proteins for shrimp. Most of the previous studies showed a partial fishmeal replacement with these plant proteins. In the case of kuruma shrimp, it has been reported that CM and DSM alone could replace 20% and 45% of dietary fishmeal, respectively (Bulbul et al., 2014, 2015a); while combination of these two plant proteins (blend) provided better nutritional quality and could replace half of the dietary fishmeal (Bulbul et al., 2013). The fishmeal replacement level could be further improved by the supplementation of either CAA or FS with the blend (Bulbul et al., 2015b). In this recent study, it has also been suggested that the appropriate ratio of CAA, PT and FS mixture could facilitate higher or even complete fishmeal replacement with plant proteins in kuruma shrimp diets. In line with this hypothesis, the present study showed that a blend of CM and DSM with supplementation of CAA, PT and FS could replace 85% dietary fishmeal from the diet of kuruma shrimp without compromising growth, feed utilization, body composition, enzyme activity and general health condition. This report is the first time to reach such higher level of fishmeal replacement in kuruma shrimp diets. This study also confirmed our previous findings that supplementation of appropriate feed additives such as CAA, PT, and FS is beneficial to formulate high plant protein based aquafeed. The results of this study is in agreement with Kader et al. (2012) who developed fishmeal free diet for red sea bream by utilizing DSM and a combined supplementation of FS, krill meal and squid meal.

Although numerous studies have been conducted to evaluate the effect of replacing fishmeal with plant proteins and other alternative sources, few were successful in reducing higher or complete fishmeal from shrimp diets. It was found that 40% fishmeal could be replaced with SBM in the diets of Pacific white shrimp (Lim and Dominy, 1990) and speckled shrimp, *Metapenaeus monoceros* (Rahman et al., 2010). Paripatananont et al. (2001) reported that 50% fishmeal could be replaced with soy protein concentrate in black tiger shrimp, *Penaeus monodon*. An inclusion of 15% CM was recommended by Lim et al. (1997) for Pacific white shrimp. Growth performance and nutrient utilization were not significantly affected by the inclusion of 20% CM in the diets of tiger shrimp (Buchanan et al., 1997). Cruz-Suarez et al. (2001) reported that extruded CM could replace a portion of SBM, fishmeal and wheat flour (1:2:3) from the diets of blue shrimp, *Litopenaeus stylirostris*. In contrast, comparatively higher fishmeal replacement (75% or more) was also reported with dehulled lupin meal for *P. monodon* (Sudaryono et al., 1999); SBM for *Penaeus schmitti* (Alvarez et al., 2007) and *Spirulina* meal for *L. vannamei* (Macias-Sancho et al., 2014). The combination of two or more protein sources is sometime beneficial to improve the nutritional quality of the blend and provide higher degree of fishmeal replacement. A mixture of either CM and SBM (3:7) or fermented SBM and earthworm meal (4:1) could replace 80% fishmeal from Pacific white shrimp diet (Suarez et al., 2009; Chiu et al., in press). Similarly, Davis and Arnold (2000) reported that coextruded soybean poultry by-product meal could replace 80% marine protein mix. Fishmeal can be completely eliminated with the coextruded soybean poultry by-product meal (Samocha et al., 2004) and the combination of solvent extracted soybean meal, poultry by-product

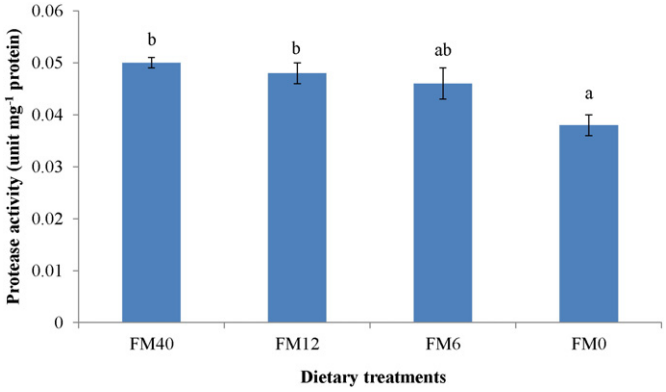

Fig. 1. Protease activity in digestive tract of kuruma shrimp fed test diets for 60 days.

Table 5
Proximate analysis (%) of whole body in kuruma shrimp fed test diets for 60 days*.

Parameters	Diet groups			
	FM40	FM12	FM6	FM0
Dry matter	24.85 ± 0.33	23.86 ± 0.95	23.70 ± 0.49	21.89 ± 0.93
Crude protein	15.76 ± 0.26	14.98 ± 1.06	15.33 ± 0.29	14.09 ± 0.66
Total lipid	1.64 ± 0.20	1.66 ± 0.17	1.50 ± 0.09	1.51 ± 0.21
Ash	4.34 ± 0.15	4.19 ± 0.22	4.55 ± 0.17	4.29 ± 0.11

* Values are means ± pooled S.E.M. from triplicate groups. Means in each row with different letters are significantly different ($P < 0.05$). Absence of letters indicates no significant difference between treatments. Crude protein, lipid and ash are expressed on a wet weight basis.

Table 6
Total hemocyte count and viable cell in kuruma shrimp fed test diets for 8 weeks*.

Parameters	Diet groups			
	FM40	FM12	FM6	FM0
THC[1]	6.49 ± 0.27	6.70 ± 0.29	6.07 ± 0.27	5.48 ± 0.37
VC[2]	85 ± 1.3	81 ± 5.2	79 ± 8.5	67 ± 3.8

* Values are means ± pooled S.E.M. from triplicate groups. Means in each row with different letters are significantly different ($P < 0.05$). Absence of letters indicates no significant difference between treatments.
[1] Total hemocyte count (10^5 cells ml^{-1}).
[2] Viable cell (%) = (Viable cells / THC) × 100.

meal and corn gluten meal (Amaya et al., 2007) from the diets of Pacific white shrimp.

It is interesting to note that the growth performance of shrimp in all the treatments in the present study was satisfactory without any significant difference among them (Alam et al., 2004; Alam et al., 2005; Traifalgar et al., 2010; Bulbul et al., 2015b). This growth promotion effect of shrimp even with diet devoid of fishmeal can be attributed to the similar FI among treatments. In general, FI has an inverse relationship to higher fishmeal replacement levels in fish and shrimp (Lim and Dominy, 1990; Kader et al., 2010; Kader and Koshio, 2012; Bulbul et al., 2013, 2014, 2015a, 2015b; Chiu et al., in press). Recovering depleted FI is therefore one of the great challenges for the utilization of higher levels of plant proteins in aquafeed (Kader et al., 2012). In the present study, kuruma shrimp successfully maintained the similar FI among treatments with gradually replacing 70, 85 and 100% of the dietary fishmeal. In our previous studies, it was found that FI significantly decreased by 60% fishmeal replacement with plant protein blend which was attributed for the reduced performance of shrimp (Bulbul et al., 2013, 2015b). The depleted FI was recovered by the supplementation of either CAA, FS or the mixture of CAA, PT and FS (Bulbul et al., 2015b). Similarly, in the present study, supplementation of CAA, PT and FS might appear for the comparable FI among treatments. Yue et al. (2012) also found similar FI in *L. vannamei* fed higher SBM and peanut meal based diets (more than 80% fishmeal replacement) supplemented with crystalline methionine and lysine to optimize dietary amino acid requirement of shrimp. Both CM and DSM contained lower levels of amino acids compared to fishmeal, specially lysine and methionine which are considered as the first limiting amino acids in plant proteins (Bulbul et al., 2013). The values for lysine and methionine contents in fishmeal were 4.1 and 1.8 g 100 g^{-1}, respectively; in contrast the values in CM and DSM were 1.8 and 0.6; and 2.1 and 0.5 g 100 g^{-1}, respectively. The gradual elimination of fishmeal with plant proteins obviously caused an imbalance in dietary amino acid contents (Bulbul et al., 2015b). Thus, dietary amino acids were adjusted by the supplementation of 1.00% lysine and 0.50% methionine in the present study. Since, FS contained well-balanced amino acids (Kader et al., 2010), supplementation of incremental levels of FS also elucidates the amino acid contents in replacement diets which was reflected in the analyzed values of dietary amino acid contents. It has been showed that all the diets contained similar levels of amino acids, including methionine and lysine, and these amounts might appear to meet the requirement for kuruma shrimp (Saitoh et al., 2000; Alam et al., 2002; Teshima et al., 2002; Bulbul et al., 2013, 2014, 2015a, 2015b).

The PT is also an effective supplement for better utilization of plant proteins in aquafeeds (Rodehutscord et al., 1995; Jackson et al., 1996; Biswas et al., 2007; Hien et al., 2015). Hien et al. (2015) reported that supplementation of 0.002% phytase or 0.10% taurine can replace 40% FM with SBM compared to only 30% without any supplementation from the diets of snakehead (*Channa* sp.). FS is a potent feeding stimulant commonly used in fish diets (Kousoulaki et al., 2009; Kader et al., 2010, 2012). In the previous study (Bulbul et al., 2015b), it was also found that supplementation of FS significantly improved the feed intake as well as the growth of shrimp. Glycine and alanine are known as effective feeding stimulants in shrimp diet (Coman et al., 1996; Sudaryono et al., 1999; Xie et al., 2014). Recently, Suresh et al. (2011) reported that taurine is another important amino acid for the feeding stimulation in shrimp diet. All these stimulating agents are rich in FS (Kousoulaki et al., 2009; Kader et al., 2010, 2012) and, therefore similar levels were found in all the diets in the present study which are believed to trigger the feed stimulating effects and maintained the similar FI among all the treatments, even in FM free diet. In this context, the present study provides very useful and promising results that supplementation of appropriate feed additives would be beneficial for the effective utilization of plant proteins in shrimp diets.

Feed utilization, protein utilization, protein retention and protease activity were significantly decreased in the fishmeal free group (FM0), which might be the reason for the numerically lowest growth and whole body protein content of shrimp in this group among the dietary treatments. Similar result was also reported by Cummins et al. (2013) who found that complete fishmeal replacement with SBM had no significant effects on final weight and weight gain of *L. vannamei*; while FCR was significantly decreased. Fishmeal was completely eliminated from the FM0 diet which might lower the quality of diet protein and digestibility; and thus decreased the feed utilization (Yue et al., 2012). Although, dietary amino acids were adjusted by CAA supplementation, juvenile shrimp might not be capable enough to effectively utilize the CAA compared with protein bound amino acids derived from fishmeal. The decreased value of protein retention suggested a higher rate of protein catabolism in shrimp fed FM0, which further supported the lower feed utilization in this group (Yue et al., 2012). In crustaceans, hemocytes are considered as primary effectors of immunological defense. The number of circulating hemocytes has been reported to influence disease resistance (Persson et al., 1987) and has been proposed as a potential indicator of immune status in shrimp (Bach'ere, 2000). Although, the THC and VC were not significantly different among treatments, however shrimp fed the FM0 diet showed the lowest values among treatments which are the signs of poor health condition compared to the rest. Therefore, it is evident that kuruma shrimp cannot effectively utilize an all plant protein based diet and a small amount of fishmeal is necessary in the diet formulation in order to ensure the diet quality, notably micronutrients or unknown growth promoting factors (Davis and Arnold, 2000; Suarez et al., 2009; Yue et al., 2012).

It can be concluded that kuruma shrimp are able to utilize plant proteins such as CM and SBM at higher levels in diets. A combination of CM and DSM at a 4:6 ratio can replace 85% high quality fishmeal without affecting growth performance, feed utilization, protein gain, protein retention, protease activity and general health condition of juvenile kuruma shrimp. However, a supplementation of 1.00% lysine, 0.50% methionine, 0.04% phytase and 12.5% fish soluble with the plant protein mixture is necessary to maintain the diet quality. In this replacement level, dietary fishmeal could be reduced down from 40% to only 6% which might be a significant achievement in developing cost-effective and sustainable feeds for shrimp. Since feed cost is the highest operational cost in shrimp production, reducing feed cost will also reduce the production cost, which ultimately increases the profit from shrimp aquaculture. Therefore, appropriate combination of different protein sources and specific feed additives might be an effective approach to formulate cost-effective and sustainable diets for shrimp in the near future.

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