Journal of Aquaculture Science October 2021 Vol 6 (2): 99-109
DOI: https://doi.org/10.31093/joas.v6i2.145 Online pada http://joas.co.id

Replacing Fishmeal With Palm Kernel Meal In Formulated Feed For The Pacific White Shrimp (*Litopenaeus Vannamei*)

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Submitted: 20 February 2021 Revised: 11 April 2021 Accepted: 12 July 2021 Publish: 30 October 2021

Abstract

Utilisation of plant proteins to replace fishmeal in shrimp feeds has become an important consideration because fishmeal is becoming more expensive due to increasing demand worldwide. Palm kernel meal (PKM) is the by-product of palm kernel oil extraction and its potential use to substitute fishmeal in the Pacific white shrimp (*Litopenaeus vannamei*) diet was evaluated by conducting a 90-day feeding trial. Shrimp juveniles with an initial average weight of 0.5 g, protein content of 10.74±0.70% were randomly distributed into five treatments in triplicates. Four isonitrogenous (approximately 35% protein) diets were formulated to contain 0% (D0), 25% (D25), 50% (D50) and 75% (D75) of PKM replacement and a commercial feed served as control treatment (Control). Results from this study revealed that shrimp fed D25 were comparable with those fed with Control as there was no significant difference (p>0.05) in weight gain and specific growth rate (SGR) between the groups. However, PKM inclusions above 50% showed detrimental effects on the growth performance. The highest total protein percent was observed in shrimp tissues fed with D25 (67.59±0.87%) and D75 showed the lowest protein among the treatments (57.4±0.63%) (p<0.05). Total lipid content was observed high in shrimp fed with Control (4.33±2.96%) followed by diet D25 (4.32±0.67%). The lowest lipid content was observed in shrimp fed diet D75 (2.03±0.20%). However, there was no significant difference in lipid values among all treatments (p>0.05). Shrimp fed with the control treatment contained 16.04±0.03% of carbohydrate and the lowest was found in shrimp fed with D25 (14.67±0.07%) at p>0.05. When PKM is utilised to replace FM, a limit of 25% level should be recommended.

Keywords: *Elaeis guineensis*, fishmeal, *Litopenaeus vannamei*, palm kernel meal, replacement

INTRODUCTION

The Pacific white shrimp, *Litopenaeus vannamei*, is native to the Pacific coast of central and South America (Briggs et al., 2005). *L. vannamei* is known for its high growth rate, adaptation to numbers of culture systems, and high market value. This white shrimp species has become one of the most important crustacean species for aquaculture production in Malaysia since its first introduction in 2002 (Kua *et al.*, 2018). The production is increasing from time to time and these shrimps have displaced the black tiger shrimp (*Penaeus monodon*) (Liao and Chien, 2011). Fishmeal is the main ingredient in formulated feeds for cultured shrimps due to its high protein content, balanced amount of amino and fatty acids, vitamins, minerals and palatability (Suárez *et al.*, 2009). However, the price of fishmeal is rising enormously throughout the year considering the demand is more likely higher than the production of fishmeal (Olsen and Hasan, 2012). Shrimp consumption has expected to increase continuously and it is important to develop other cost-effective protein sources to reduce the feed cost and to sustain shrimp industry. Providing shrimp with satisfied levels of nutrition is the fundamental part in feed formulation because maintaining and enhancing the shrimp growth and performance leans on optimal nutrition (Cuzon, 1989; Pratoomyot *et al.*, 2010; Molina-Poveda *et al.*, 2013). Palm

kernel meal (PKM) is a common by-product from palm kernel oil extraction process in the palm oil industry. PKM is actually the kernel of palm fruits that has been pressed. The common species of palm fruit in Malaysia is *Elaeis guineensis* or known as the African palm oil originating from West Africa (Awalludin *et al*., 2015). Many studies have been done to recycle palm oil residues including the production of palm kernel loose powder or PKM and used to feed animals, both in poultry and aquaculture industries (Agunbiade *et al*., 1999; El-Sayed, 1999; Ng *et al*., 2002; Perez *et al*., 2000; Alimon, 2004; Ezieshi and Olomu, 2008; Hem *et al*., 2008; Iluyemi *et al*., 2010).

PKM shows a potential alternative protein source in poultry and aquaculture feeds other than fishmeal due to its high nutritional values such as carbohydrate and protein (Ng, 2003; Alimon, 2004). The use of PKM has been assessed in the black tiger shrimp, *Penaeus monodon* (Rajaram *et al*., 2010), giant freshwater prawn, *Macrobrachium rosenbergii* (Kader *et al.*, 2018), hybrid red tilapia (Ng and Chong, 2002), nile tilapia (Obirikorang *et al.*, 2015) and the common carp, *Cyprinus carpio L.* (Resan and Obaydi, 2019). Replacement of fishmeal with PKM has its own suitable amount which at a certain level of replacement will defect the growth of cultured organisms (Iluyemi *et al*., 2010; Richard *et al*., 2011). Succeesful replacement of fishmeal with PKM will reduce the operating cost because major expenditure is spent on feeds (Hasan *et al*., 2012). To date, there are only a few studies describing the potential of PKM to substitute fishmeal in aquaculture feeds. Therefore, this study was conducted to find the suitable level of fishmeal replacement with PKM in formulating feeds for the Pacific white shrimp.

MATERIAL AND METHODS

Experimental design and sampling procedure

L. vannamei juveniles with an average weight of 0.5±0.03 g per individual were used in this study. These juveniles were obtained from the Fisheries Research Institute, Pulau Sayak, Kedah, Malaysia. The juveniles were acclimatised for seven days to the new conditions in the laboratory. During the acclimatisation period, they were fed with Cargill™ commercial pellets (≈35% protein) for marine shrimp starter at 7% of their biomass. At the beginning of the feeding trial session, the juveniles were placed in black polyethylene tanks filled with approximately 120 L of saltwater. Each tank contained fifty juveniles with a stocking density of two individuals per litre, which were maintained in a static water system with a 50% weekly water exchange.

A total of twenty tanks were used with four replication treatments for each diet. The water temperature in all tanks was maintained at 24±0.5°C, pH at 7.0 to 8.5 and the salinity was kept at 20±0.5 ppt during the twelve weeks period. Other water quality parameters were also observed during the culture period where ammonia, nitrite and nitrate levels were within 0.0-1.0 mg/L, 0.0-2.00 mg/L and 0.0-6.0 mg/L levels, respectively. Both physical and chemical parameters for water quality were measured once a week. Sampling for growth determination was carried out biweekly. An analytical balance (Shimadzu ELB2000) was used to record the wet weight of the juveniles where the shrimps were placed in a beaker containing saltwater. During the sampling, twenty samples from each tank were weighed individually. Growth

performance is expressed as wet weight, final weight gain (FWG), specific growth rate (SGR), daily weight gain (DWG) and survival (%).

Feeding and feed formulation

Four isonitrogenous (protein at 35%) diets were formulated by replacing fishmeal with PKM at different levels (0%, 25%, 50%, and 75%). Commercial shrimp feed served as the control treatment. The PKM was obtained from FELDA Palm Kernel Crushing Factory, Pandamaran, Klang, Malaysia. Other ingredients including fishmeal, wheat bran, wheat flour, vitamin, minerals and binder were obtained locally. All the dry ingredients were ground and sieved for finest grained output. Then, all the fine textures were weighed to the measurements of 1 kg formulated diets as stated in Table 1. Then, the mixture was blended to homogenous using a Kitchen Aid™ household mixer. The mixture was hydrated with water as cohesive properties and then was extruded (Kitchen Aid™) into "spaghetti-like" strands using a 2 mm die. The finishing strands were dried in an oven at 60°C for six hours. After drying, the strands were cut into approximately 0.2 mm long pellets. The pellets were kept in plastic containers and stored in room temperature.

Table 1. Feed formulation (g kg^{-1}) and proximate composition (%) of the experimental diets for *Litopenaeus vannamei*. No significant differences with the same letter within a row (p>0.05).

Feed ingredients (g kg^{-1})	**Control***	**D0**	**D25**	**D50**	**D75**
Fishmeal (57% protein)		980.0	735.0	490.0	245.0
Palm kernel meal (18% protein)		-	245.0	490.0	735.0
Wheat flour (11% protein)		10.0	10.0	10.0	10.0
Wheat bran (15% protein)		10.0	10.0	10.0	10.0
Binder		1.5	1.5	1.5	1.5
De cal phosphate		1.5	1.5	1.5	1.5
Vitamin premix		3.0	3.0	3.0	3.0
Minerals		1.5	1.5	1.5	1.5
Proximate composition (%)					
Protein	35.33±0.15	34.97±0.05	35.21±0.43	34.77±0.08	34.78±0.31
Lipid	9.67±0.16^{a}	6.97±0.27^{b}	5.39±0.17^{c}	4.69±0.30^{d}	5.81±0.25^{e}
Carbohydrate	32.00±0.38^{a}	24.00±0.40^{b}	32.00±0.18^{a}	33.00±0.48^{a}	33.00±0.64^{a}
Moisture	5.67±0.09^{a}	5.56±0.13^{a}	4.70±0.08^{b}	4.16±0.07^{c}	3.90±0.07^{d}
Ash	12.24±0.28^{a}	22.73±0.79^{b}	14.62±0.62^{c}	12.12±0.12^{a}	10.30±0.31^{d}

*Marine shrimp feed from Cargill™ Malaysia

Proximate analysis for experimental diets and shrimp flesh

The juveniles were deprived of feed for 24 hours prior to analysis to remove unnecessary body composition that might affect the results of proximate analysis (Zhang *et al*., 2007). Fifty individuals were collected from the initial stock and all individuals were collected at the end of the feeding trial and peeled. The shrimp tissues were kept frozen at -20°C until further analysis. Proximate content was also determined for the experimental diets. All analyses were performed in triplicate following the standard methods (AOAC, 2005). Moisture content was determined by drying the samples in an oven for 24 hours at 60°C. The dried samples were burned in a furnace at 600°C for 2 hours. The burned samples were cooled to room temperature in a desiccator and the weight differences were determined as ash content. Analyses of protein were conducted following the Kjeldahl method using the K-425 digestion block and the K-350 distillation system

(Buchi, Switzerland). Lipids were extracted using the modified Bligh and Dyer method (1959). Total carbohydrate content was estimated by the difference between 100 and the sum of protein, lipid, and ash contents (FAO, 2003).

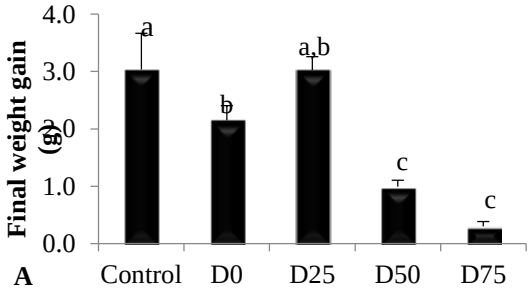
Statistical analyses

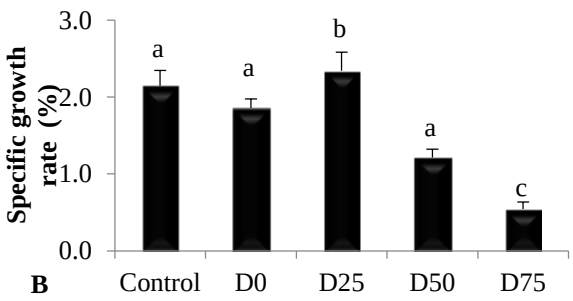
Data on the growth parameters and proximate analysis (both diets and shrimp tissues) were analysed with parametric statistics after all assumptions are met and transformations undertaken, when appropriate. The proportions of growth parameters, survival rates and proximate content were transformed using the arcsine square root transformation before being subjected to one-way analysis of variances (ANOVA). Tukey's HSD Post Hoc tests at $p<0.05$ were conducted to determine the differences between the means of all treatments.

RESULTS AND DISCUSSION

Growth performance of shrimp juveniles with treatment diets

In general, the growth performance of shrimps after the 90-day feeding trial showed that the final weight gain (FWG) was greatly reduced corresponded to fishmeal replacement as shown in Figure 1A. At the end of the feeding trial, *L. vannamei* juveniles that fed on D25 and Control had the furthermost increment of weight (3.23±0.23 g individual^{-1} and 3.03±0.63 g individual^{-1}, respectively). There was a significant difference in FWG of juveniles after feeding trial ($p<0.05$). The weight gain in juveniles decreased gradually with the increase in replacement levels of PKM in the treatment feeds. It shows that FWG of shrimp fed with D75 illustrated the lowest result among other treatments (0.30±0.08 g individual^{-1}). It is also noted that FWG of juveniles fed with Control, D0, and D25 showed no significant difference (Tukey's tests, $p>0.05$). The fishmeal replacement with PKM was found affecting the specific growth rate (SGR) of *L. vannamei* juveniles significantly. Figure 1B shows clearly that juveniles fed on D25 had the highest SGR (2.37±0.24%) compared to other treatments, followed by juveniles fed on control (2.16±0.20%), D0 (1.86±0.11%), D50 (1.21±0.10%) and D75 (0.54±0.09%). *L. vannamei* had continuously grown during this study as shown in (Figure 1c). The daily weight gain (DWG) of *L. vannamei* during the feeding trial showed a significant difference among the treatments (ANOVA, $p<0.05$) (Figure 1c). Shrimp juveniles fed on Control (0.016±0.004 g day^{-1}), D0 (0.011±0.001 g day^{-1}) and D25 (0.016±0.002 g day^{-1}) showed similar values ($p>0.05$) of weight gain while DWG values for D50 and D75 were significantly different. Shrimps fed on D75 displayed the lowest DWG (0.002±0.004 g day^{-1}).





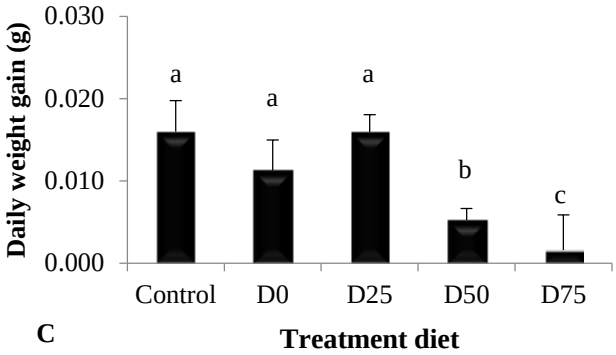


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Figure 1. (A) Final weight gain (FWG), (B) specific growth rate (SGR), and (C) daily weight gain (DWG) of *L. vannamei* juveniles after a 90-day feeding trial. *L. vannamei* juveniles were fed control and treatment diets containing D0 (0%), D25 (25%), D50 (50%) and D75 (75%) of fishmeal replacement with palm kernel meal. *a, b and c denote significant statistical differences at $p<0.05$

Palm kernel meal incorporation had been reported to be possible for diets in *M. rosenbergii* and *Oreochromis* sp. at levels between 20 and 30% inclusion. Kader *et al.* (2018) showed that growth parameters were significantly decreased in *M. rosenbergii* juveniles fed 40% PKM diet. However, supplementation of shrimp meal (2%) and squid meal (2%) in 40% PKM diet was significant in the recovery of depleted growth performance with the diet. Ng and Chong (2002) demonstrated that red tilapia (*Oreochromis* sp.) fed diets with 20% inclusion of PKM had similar growth performance with fish fed the control diet without PKM. Palm kernel by-product, however, could be incorporated only up to 2% in the diet of *P. monodon* (Rajaram *et al*., 2010). In the current study, inclusion levels of PKM at 25% demonstrated no adverse effect on weight gain, daily weight gain, and specific growth rate but deteriorated when FM was replaced with PKM at 50% and 75%. This result suggested that tolerance of the juvenile *L. vannamei* to fishmeal replacement with PKM is up to partial substitution at 25%.

This could be associated with the feeding habit of *L. vannamei.* This shrimp species is known as omnivore which habitually feeds on both animal and plant as diets. According to Rønnestad *et al*. (2013), omnivorous species are considered to have longer intestine than carnivorous and herbivorous species. These anatomy differences are related to the differences in the abundance of amylase digestive enzymes. High amount of digestive enzyme can be found in omnivores that influence the digestibility activity in the stomach and efficiently digest plant carbohydrate in their diets into energy (Hidalgo *et al*., 1999). This is because amylase enzyme is typically known as a dynamic carbohydrate digester enzyme and spare protein as muscle and lipid as fat (Warren *et al*., 2015). High survival percent after 90-day feeding trial among all treatments for Pacific white shrimp was observed between 98-100% (Figure 2). The highest survival of shrimps after fed on diets with different replacement levels was found in Control, D50 and D75 (100%) followed by those fed with D25 treatment diet (99%) and the lowest survival (98%) found in D0. However, there was no significant difference in survival among all treatment diets (ANOVA, $p>0.05$). Molina-Poveda *et al.* (2013) also found no differences and no relationship with treatment diets in survival of *L. vannamei* when fed with diets using corn gluten meal as protein sources.

Similarly, López-Vela *et al.* (2014) observed there was no significant difference in survival (83-87%) of *L. vannamei* fed with a mixture of animal and plant protein. Survival of *L. vannamei* fed with the treatment diets in the current study is higher than those displayed by other studies; 71-89% with combination of soybean meal and distiller's dried grains with soluble (Cummins *et al*., 2013), 77-90% with corn gluten meal (Molina-Poveda *et al*., 2013) and 71-99% with soy protein concentrate and soybean meal (Xie *et al*., 2016). Even though the growth of shrimp deteriorated with treatments D50 and D75, however there was no relationship between mortality and treatment diets could be observed. Furthermore, the experimental conditions for this study were suitable for *L. vannamei* as confirmed by reported high survival percent (98-100%) (p>0.05).

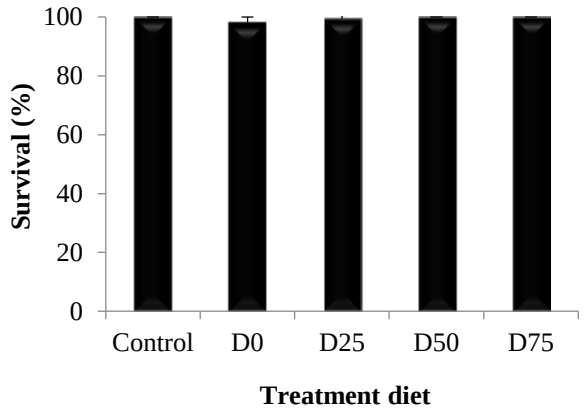

Figure. 2. Final survival (%) of *L. vannamei* juveniles after 90-day feeding trial. *L. vannamei* were fed Control, and treatment diets containing D0 (0%), D25 (25%), D50 (50%) and D75 (75%) of fishmeal replacement with palm kernel meal. There was no significant difference among the treatments (p>0.05).

Proximate composition of shrimps after feeding trial

The results of proximate composition in shrimp tissues after the feeding trial (Table 2). Protein levels increased in shrimp tissues for all treatment diets. Shrimps with the highest protein were those fed with D25 (67.6±0.87%) followed by D0 (67.1±0.10%), D50 (66.5±0.17%) and Control (65.6±0.30%). The least amount of protein was found in shrimps fed on D75 treatment diet (57.4±0.63%). Total protein concentration in shrimp tissues yielded a significant variation among all treatment diets (ANOVA, p<0.05). However, Lim *et al.* (1997) found that *L. vannamei* fed with formulated diets from canola meal showed no significant difference in their body protein content (p>0.05). Similarly, Bulbul and co-workers (2013) also found total protein composition in *M. japonicus* displayed no significant difference when fed with diets containing soybean meal (p>0.05). In the current study, the body protein content observed in shrimp tissues decreased when fed with high content of PKM. Ng et al. (2002) also observed the deterioration in the body protein content of red hybrid tilapia fed on fermented PKM based diets. On the other hand, Lim and Dominy (1990) found the increment of total protein in *L. vannamei* was corresponded with the increasing levels of soybean meal in the

diets. Protein retention is considered as an important indicator for optimal supply and efficiency in nutrient utilisation (Deng *et al*., 2006). Reduction in nutrient utilisation of diets with PKM might be due to the presence of anti-nutritional factors (ANFs) such as phytic acid and tannins and oxalate (Akinyeye *et al*., 2011) which present bitter taste (Thakur *et al*., 2019; Vikram *et al*., 2020) and reduce the feed intake in shrimps. Therefore, it describes the lower growth performance of the shrimps in the present study when the shrimp juveniles were fed on D75.

The highest lipid content in body composition of shrimps was displayed by those fed on Control (4.33±2.96%) followed by D25 (4.32±0.67%), D0 (4.04±1.21%) and D50 (2.10±0.14%). Lipid content in shrimps fed on D75 showed the lowest concentration of 2.03±0.20%. There was no significant difference (ANOVA, $p>0.05$) in lipid content among all treatments. A similar trend has been observed by Harter et al. (2011), Chiu et al. (2016) and Sun et al. (2016) in which *L. vannamei* were fed with Barbados nut (*Jatropha curcas*) kernel meal, fermented cottonseed meal and fermented mixture of soybean meal and earthworm. The substitutions of fishmeal with the above-mentioned plant meals did not show significant variation in the total lipid content of shrimp body. Bulbul *et al.* (2013) also found that there was no significant effect in whole body lipid on *M. japonicus* fed diets with soybean meal ($p>0.05$). After the 90-day feeding trial, shrimps fed on D75 exhibited the highest accumulation of carbohydrate (16.75±0.04%) followed by D50 (16.65±0.04%), Control (16.04±0.03%) and D0 (14.72±0.01%) with slight variation. The lowest concentration of carbohydrate was observed in shrimps fed on D25 (14.67±0.07%). However, one way variance analysis showed no significant difference in carbohydrate content among treatments (ANOVA, $p>0.05$). Total carbohydrate contents were found generally low in all treatments. The current study showed similar results as in a sample of collected wild African river prawn, *Macrobrachium vollenhovenii* (16.1%) (Adeyeye & Adubiaro, 2004). A previous study by Ravichandran *et al.* (2009) found a lower amount of carbohydrate in the flesh of wild Indian white shrimp, *Penaeus indicus* (2.4%). Similarly, Gunalan *et al*. (2013) also found a lower carbohydrate in cultured *L. vannamei* (3.2%) compared to the current study. The present study also observed high content of total carbohydrate and low total protein fed on D50 and D75, and vice versa when fed on Control, D0 and D25 in the *L. vannamei* tissues. This finding was supported earlier by Gunalan *et al.* (2013) who also found that total carbohydrates tend to have inverse relationship with protein content in shrimp tissues. These results suggested that shrimps have limited ability in digesting carbohydrate in plant protein-based diets (Sun *et al*., 2016).

There was a significant difference in moisture concentration in shrimp tissue fed on all treatment diets (ANOVA, $p<0.05$). The highest total moisture was found in shrimps fed on D75 (13.66±0.31%) and the lowest was in D25 (6.07±0.09%). Meanwhile, D0, D50 and Control total moisture contents are 6.50±0.04%, 6.30±0.09% and 6.14±0.15%, respectively. A post hoc Tukey's test also showed the shrimps fed with D75 differed significantly in moisture content compared to other treatments ($p<0.05$). The present study displays a high body moisture content when fishmeal replacement levels with PKM increased. Iluyemi *et al.* (2010)

also observed the same trend in whole body moisture of red tilapia fed on diets with palm kernel cake (PKC). Body moisture of red tilapia increased when PKC level in diets increased. Similar pattern was observed in *L. vannamei* fed on diets when fishmeal was totally replaced with canola meal (Lim *et al*., 1997). They reported that the augmentation of body moisture of the shrimp was related to the increased levels of canola meal in the diets. Total ash content in shrimps is highly distributed in shrimps fed on D75 diet (10.12±0.12%) followed by D50 treatment (8.40±0.40%), Control (7.81±0.81%) and D0 (7.61±0.61%). Based on the one-way analysis of variance (ANOVA), ash distribution in shrimps showed significant difference in all treatment diets (p<0.05). Post hoc Tukey's tests interpreted that ash in shrimps fed Control, D0 and D25 were not significantly different at p>0.05. Ash in shrimps fed D50 and D75 showed a significant difference (Tukey's test, p<0.05). Total body ash content of *L. vannamei* in the current study was higher in shrimp fed D75 than in shrimp fed control and D0 diets. It is comparable with the study by Ng *et al.* (2002) who discovered that ash content of red hybrid tilapia was higher in PKM based diets compared to Control diets. In an earlier study, Iluyemi *et al*. (2010) also found the ash content of red tilapia increased when fed with PKC based diets. A previous study by Soltan *et al.* (2008) reported likewise, where whole body ash of nile tilapia escalated with the increment of cottonseed, sunflower, canola, sesame and linseed mixture in the diets. However, nearly none of the previous studies had reasoned out the positive correlation between plant protein-based diets with the body ash of cultured animals.

Table 2. Proximate composition of shrimp tissues (*L. vannamei*) before and after a 90-day feeding trial.

	Protein	Lipid	Carbohydrate	Ash	Moisture
Initial	10.74±0.70	3.24±0.12	73.04±0.06	9.08±0.14	3.90±0.07
Control	65.67±0.30^{a}	4.33±2.96	16.04±0.03	7.81±0.81^{a}	6.14±0.15^{a}
D0	67.13±0.10^{b}	4.04±1.21	14.72±0.005	7.61±0.61^{a}	6.50±0.04^{b}
D25	67.59±0.87^{b}	4.32±0.67	14.67±0.07	7.34±0.34^{a}	6.07±0.09^{a}
D50	66.54±0.17^{a,b}	2.10±0.14	16.65±0.04	8.40±0.40^{b}	6.3±0.09^{a,b}
D75	57.44±0.63^{c}	2.03±0.20	16.75±0.04	10.12±0.12^{c}	13.66±0.31^{c}

*^{a,b,c,d} denote significant statistical differences at p<0.05 in the same column.

Conclusions

The results obtained in this study have shown that 25% inclusion of palm kernel meal do not negatively affect the growth performance of *L. vannamei*. The addition of PKM above 50% significantly impaired the growth of the shrimp. However, the survival *of L. vannamei* seemed to not be affected by PKM inclusion in the diet. Higher PKM diets (D50 and D75) resulted in lower protein and lipid contents in shrimp. Prior treatments of PKM may be necessary to increase protein content and eliminate anti-nutritional compounds in the meal. PKM can be a suitable alternative ingredient for *L. vannamei* feeds, with great potential to partially replace fishmeal at 25% level without deteriorating the growth. The outcomes of this study are useful in providing better understanding on nutrients required by *L. vannamei* and proposing the use of PKM in their formulated feeds.

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