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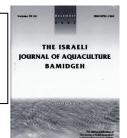
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Fermented Copra Meal as Replacement for Dietary Fish Meal Protein in Grow-Out Culture of Black Tiger Shrimp, *Penaeus monodon* Juveniles

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Keywords: aquaculture; crustacean; feeding; formulated feeds; protein enriched copra meal; protein sources

Abstract

Feeding trials were conducted to assess the effects of diets containing fermented copra (FCM) as an alternative to fish meal (FM) on growth, survival, and feed efficiency, in Black Tiger Shrimp, *Penaeus monodon*. A diet was formulated where 40% of the FM protein was replaced by FCM protein, and compared with a commercial shrimp diet. The feeding experiments were carried out using 10-ton capacity circular outdoor tanks. Three hundred and fifty (350) shrimp were stocked in each tank at a density of 35 shrimp per m³. They were fed the diets at a rate which decreased from 15% down to 4% of average body weight (ABW) per day. The results demonstrated that growth, survival, and feed performance, in *P. monodon* in outdoor tanks were not affected by replacing 40% of the dietary FM with FCM protein. Furthermore, no difference was found in proximate composition of the shrimp carcass fed the commercial and experimental diets. Although protein efficiency ratio (PER) of the commercial and FCM-based diets were similar, protein productive value (PPV) was significantly higher in the FCM than the commercial diet. Hence, we concluded that FCM is a promising alternative protein source for Black Tiger shrimp and can replace 40% of the fish meal protein without adversely affecting growth, survival, and feed efficiency.

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Introduction

Fish meal remains the primary protein source in feeds for farmed marine and other species at the fry or fingerling stages (Hardy 2010). Global aquaculture demand for fish meal (FM) is increasing while production is decreasing (FAO 2016). Consequently, fish meal has become more expensive, threatening the sustainability of the aquaculture industry, since feeds are one of the major aquaculture production costs. A significant increase in the price of FM was reported from 2006 to 2013 (FAO 2016). Nonetheless, producing cheaper feeds is still possible by reducing the amount of dietary FM. Studies show that fish meal in aquafeeds can be substituted by cheaper proteins from plant or animal sources (Fontaínhas-Fernandes et al. 1999; Millamena 2002; Suarez et al. 2009; Ogello et al. 2014). In shrimp, various plant-based meals have been tested as alternative dietary protein sources (Eusebio and Coloso 1998; Peñaflorida 2002; Bautista-Teruel et al. 2003; Kiron et al. 2012).

Copra meal (CM), one of the more abundant alternative plant protein sources in the tropics, has been used traditionally in feeds for livestock, as well as farmed aquatic species. It is often included in fish diets due to abundance of supply and low cost (Kim et al. 2001). Compared to cottonseed meal, the protein quality of copra meal has higher biological value (Gulbransen et al. 1990). On the other hand, copra meal is known to have anti-nutritional properties as it contains high non-starch polysaccharides, phytic acid, tannins, and is notable for its mannan and galactomannan content, (Tacon et al. 2009). Also, the high crude fiber content of copra meal limits its utilization for aquatic species. For example, copra meal negatively affected the feed intake in carp due to poor palatability and difficulty in maintaining feed particle size (Hasan et al. 1997).

Fermentation has long been reported to improve the quality and nutritive value of feedstuffs such as coffee pulp (Peñaloza et al. 1985), linseed (Mukhopadhyay and Ray 2005), soybean (Mathivanan et al. 2006), copra meal (Dairo and Fasuyi 2008), and sesame seed meal (Roy et al. 2014). Fermentation encourages the growth of microorganisms that break down fiber and anti-nutritional substances. Filamentous fungi (e.g. *Aspergillus niger*) are one of the fermenting agents that produce various enzymes such as hemicellulase, pectinase, lipase, and tannase (Zhang et al. 2018, Pinto et al. 2001; Mathivanant et al. 2006). Supplementation of a copra meal-based diet with 1% *Trichoderma* spp. fermented copra meal improved the nutritive value of the diet as exhibited by better growth and feed efficiency in birds (Hatta and Sundu 2009).

In an indoor laboratory-scale tank trial, fermented copra meal (FCM) was used to replace up to 40% dietary fish meal protein without detrimental effects on various performance parameters in *P. monodon* (Apines-Amar et al. 2016). The present study was conducted to further evaluate the performance of the abovementioned FCM-based formulated diet against a commercial feed popularly being used by shrimp growers in the black tiger shrimp *P. monodon* grow-out culture system. The present FCM-based feed formulation was assessed in terms of growth, survival, feed conversion ratio, protein efficiency ratio, and productive protein value against the control commercial feed. This study will determine if the FCM feed formulation is comparable to the existing commercial shrimp feed.

Experimental diets.

Materials and Methods

The fermented copra meal (FCM)-based diet was prepared following the formulation of Apines-Amar et al. (2016) with FCM replacing 40% of the fish meal protein (Table 1). The replacement level was based on the result of the previous study which showed that FCM can replace 40% of the FM protein without deleterious effects on the shrimp (Apines-Amar et al. 2016). The FCM produced by solid-state fermentation was obtained from a local supplier. According to the analytical results, FCM has crude protein and crude fat contents of 38% and 7%, respectively. In the present study, the performance of the FCM diet was compared to a commercial shrimp feed. Although the formulation of the commercial feed was not revealed by the feed company for proprietary reasons, its proximate composition was analyzed and reported.

	Commercial diet	FCM diet**	
Dry matter	94.38	95.68	 *Dry matter basis. *Soybean meal 230g, Acetes sp. 115, Fish Meal (Danish) 162g, Fermented Copra Meal (FCM) 213.16g, Bread flour 150, Seaweed (Gracilaria sp.) 25, Cod liver oil 25, Soybean oil 25, Vitamin mix (V-22) 20, Mineral Mix 10, Dicalcium phosphate 20 (Apines-Amar et al. 2016).
Crude protein	44.37	42.21	
Crude fat	6.50	6.57	
Crude fiber	1.52	4.04	
Ash	10.74	12.40	
NFE	36.86	35.59	

Table 1. Proximate composition of the experimental diets (%)*

Feeding trials.

Two feeding trials were conducted to compare the performance of the test diet against the commercial shrimp feed. Good quality hatchery-reared shrimp with average body weight (ABW) of 0.63g (Trial 1) and 0.20g (Trial 2) were distributed in six 10-ton capacity circular outdoor tanks at 35 shrimp m⁻³ or 350 shrimp per tank and were grown for 154 and 150 days for Trial 1 and Trial 2, respectively. The juveniles had undergone screening for White Spot Syndrome Virus (WSSV) by PCR before they were transported from the hatchery source to the experimental facility and were found to be free of the pathogen.

The 10-ton capacity culture tanks were filled with dechlorinated seawater and seeded with microalgae (*Nannochlorum* sp.) at 250-L per tank, to which inorganic fertilizer was applied, and 20 ppm fermented rice bran was thereafter added for 3 consecutive days. After the microalgae had bloomed, White Spot Virus-free shrimp were stocked in the culture tanks.

Each diet was randomly assigned to triplicate tanks. Feeds were initially given at 15% and were then gradually reduced to 4% of the average body weight (ABW) per day as the shrimp increased in size. The daily feed ration was divided in 4 equal parts dispensed at 8:00 H, 11:00 H, 14:00 H, and 17:00 H. Feeding ration was adjusted based on actual feed consumption. Good water quality was maintained by applying 1 ppm probiotics twice a week (BZT[®] Aqua every Monday and BZT[®] Waste Digester every Thursday). The bottom of the tanks were cleaned once a week by siphoning to remove accumulated wastes after which new clean seawater was added to restore the water volume to its original level. Water quality parameters such as temperature, salinity, pH, ammonia, nitrite, and alkalinity, were monitored regularly. Water was changed as necessary depending on the result of the water quality analysis.

Ten percent of the shrimp (35pcs/tank from the 2nd - 8th weeks) and 20% (70pcs/tank from the 10th-22nd weeks) were sampled every 2 weeks to record their periodic growth data and to adjust the feed ration. At the end of the feeding trial, all shrimps were counted to determine survival.

Sample collection and chemical analysis.

Feed samples and shrimp carcass were analyzed for proximate composition before the start of the feeding experiment to obtain initial data. One hundred (100 g) representative samples from both the FCM-based diet and the commercial feed, and ~50g pooled tissues from shrimp samples collected at random were analyzed for moisture content, crude protein, lipid, ash, and NFE. At the end of the feeding trial, 10 shrimp per tank were again sampled to determine proximate carcass composition. Analyses of all samples were done following the methods of the Association of Official Analytical Chemists (AOAC, 2000).

Computations and statistical analysis

Growth and feed performances were evaluated and calculated as follows: SGR (%/day) = ((In Final weight – In Initial weight)/No. of days) x 100 Weight Gain (%) = ((Final weight – Initial weight)/Initial weight)) x 100 Feed conversion ratio (FCR) = Feed consumed (g)/Weight gain (g) Survival (%) = (Nf/Ni) x 100

Where Nf is the number of shrimp at the end of the feeding trial, and Ni is the number of shrimp at the start of the experiment.

PER = Weight gain (g)/Protein intake (g)

PPV = [Final body protein (%) x Final wt. (g) - Initial body protein (%) x Initial wt. (g)] Wt. gain (g) x Total protein intake (g)

Results were analyzed with SYSTAT, SPSS software. Data were transformed (Arcsin or Square root) as appropriate and checked for normality before performing the statistical analysis. Differences in the means of growth, survival, PER, and PPV data between the two diets were analyzed by T-test. Values were considered statistically significant when P \leq 0.05.

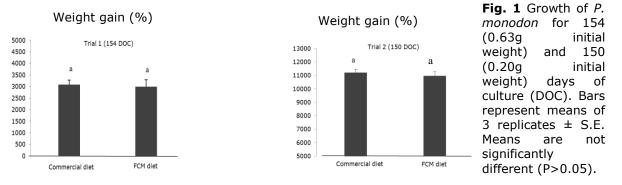
Results

Experimental diets.

The analyzed values for the nutrient compositions of both the FCM and commercial diets are presented in Table 1. Although the formulation of the commercial feed was proprietary and was not divulged, it had a similar proximate composition to the FCM-based diet. The protein, lipid, and energy levels of both diets were comparable and were well within the recommended ranges for shrimp.

Feeding trial.

Growth, feed performance, and survival data are presented in Fig. 1 and Table 2. No significant differences were observed in the growth performances of the shrimp fed the FCM-based or commercial diet in the two feeding experiments as demonstrated by similar weight gains (%) and specific growth rates (SGR). Weight gain was 2989% (FCM) and 3071% (commercial diet) in Trial 1, and 10643% (FCM) and 11208% (commercial diet) in Trial 2. Palatability of the diet was not influenced by the inclusion of dietary FCM in both feeding trials as shown by the immediate acceptance of the diet by the shrimp during weaning with no observable signs of feed rejection. In terms of survival, the diets did not exhibit significant differences in both feeding trials.



	Initial wt. (g)	Final wt. (g)	SGR (%/day)ª	FCR ^b	Survival (%)
Trial 1 (154 DOC*)					
Commercial diet	0.61 ± 0.03	20.4 ± 1.38	2.2 ± 0.04	2.1 ± 0.08	82.0 ± 3.27
FCM diet	0.65 ± 0.02	20.0 ± 0.90	2.2 ± 0.06	2.1 ± 0.05	81.6 ± 4.79
Trial 2 (150 DOC)					
Commercial diet	0.20 ± 0	21.6 ± 0.39	3.3 ± 0.02	2.1 ± 0.14	89.4 ± 0.87
FCM diet	0.20 ± 0	20.0 ± 0.33	3.2 ± 0.08	2.2 ± 0.11	88.9 ± 1.43

*Days of culture

^aSpecific Growth Rate = ((In Final weight – In Initial weight)/No. of days) x 100

^bFeed Conversion Ratio = Feed consumed (g)/Weight gain (g)

Means \pm S.E. within a column in each Trial are not significantly different P>0.05.

Nutrient compositions of the shrimp fed the two diets were comparable (Table 3). Likewise, carcass composition analysis revealed that the shrimp dry matter, crude protein, crude fat, crude fiber, ash, and the nitrogen free extract had similar values regardless of the type of feed consumed. Moreover, protein efficiency ratio (PER) did not differ significantly between the diets (Table 4). On the other hand, protein productive value (PPV) was significantly higher in FCM than in commercial diet in both feeding trials.

Diets	Dry matter	Crude protein	Crude fat	Crude fiber	Ash	NFE
Trial 1						
Commercial	24.9±0.62	74.1±1.67	3.6±0.32	6.4±0.40	14.8±0.40	1.6±0.36
FCM	25.6±0.87	73.6±0.56	3.3±0.34	6.7±0.23	15.6±0.79	1.7±0.25
Trial 2						
Commercial	25.3±0.03	74.9±0.38	3.2±1.08	6.4±0.03	14.6±0.18	1.0±0.53
FCM	24.1±0.62	73.3±0.23	3.4±0.93	6.6±0.09	15.4±0.20	1.2±0.40
*Dry woight basis						

Table 3. Proximate composition of the shrimp carcass $(\%)^*$

*Dry weight basis.

Means \pm S.E. within a column in each Trial are not significantly different P>0.05.

Table 4. Protein efficiency ratio and protein productive value in *P. monodon* fed the experimental diets for 150 and 154 days

	PER	PPV
<i>Trial 1 (154 DOC*)</i>		
Commercial diet	0.81 ± 0.04^{a}	2.65 ± 0.01^{a}
FCM diet	0.81 ± 0.04^{a}	3.31 ± 0.06^{b}
Trial 2 (150 DOC)		
Commercial diet	0.92 ± 0.04ª	2.63 ± 0.17ª
FCM diet	0.98 ± 0.05 ^a	3.47 ± 0.21 ^b

*Days of Culture *Means \pm S.E. (n=3) within a column in every trial not sharing the same superscript letter are significantly different (P<0.05).

Water quality between the groups fed either the commercial or FCM-based diets was similar and was within the optimum range for shrimp (Table 5).

Dietary	Temp.	Salinity	Ammonia	Nitrite	рН	Alkalinity	
treatment	(°C)	(ppt)	(ppm)	(ppm)		(mg/li CaC0 ₃)	
Trial 1							
Commercial	30±1.4	20±2.8	0.4±0.7	0.1 ± 0.1	8.4±0.4	143±29.3	
FCM diet	30±1.2	21±3.1	0.5±0.6	0.1±0.2	8.3±0.6	138±30.5	
Trial 2							
Commercial	29±1.0	20±3.3	0.4±0.5	0.1 ± 0.1	8.2±0.5	129±35.4	
FCM diet	29±1.2	20±3.3	0.3±0.5	0.2 ± 0.1	8.0±0.4	140±33.1	

Table 5. Water quality in *P. monodon* culture tanks*

Discussion

The performance of the formulated diet in the present study using fermented copra meal (FCM) to replace 40% of the fish meal protein was comparable to that of a commercial shrimp feed available in the market. Detrimental effects were not observed in the shrimp fed the FCM diet. It was previously reported that a fermented product can possibly replace fish meal in *P. monodon* diet (Vijayakumar et al. 2009). The replacement level of 40% FCM that we obtained in this study was notably higher compared to studies in other species that used only non-fermented copra meal. In *Sarotherodon mossambicus*, a diet containing 25% protein from copra meal supported growth similar to the control diet but yielded lower growth rates when inclusion level reached 50% (Jackson et al. 1982). In Nile tilapia fingerlings, soaked copra meal can be included up to 30% with the same growth performance, nutrient utilization, and

feed conversion ratio as the control diet (Olude et al. 2008; Santos et al. 2009). Similarly, in carp, use of fermented linseed meal increased the inclusion level of the meal in the diet formulated for the fish (Mukhopadhyay and Ray 2005). The present results clearly demonstrate that inclusion levels of plant protein sources can be increased with the use of a fermented product without compromising growth or feed performance.

Bio-treatment of copra meal such as by fermentation enhances the quality of the meal to be more competitive in the market particularly for use in livestock and aqua feeds. This is commonly done by a process known as solid state fermentation (SSF) whereby a plant substrate is subjected to the action of enzymes produced by filamentous fungi such as *Aspergilus niger*, deemed to be among the best enzyme-producing microorganisms (McCleary 1988). The enzyme "coprase" produced by *A. niger* through this process was reported to be superior than the enzyme from *Trichoderma* spp. in terms of improving growth in poultry which could possibly be due to the greater capacity of the filaments of *Aspergilus* to permeate the copra meal particle and break its nutrients into more bioavailable forms (Sundu and Hatta 2009).

Generally, when a product undergoes pre-treatment such as fermentation, this improves its quality by increasing its crude protein level, improving its amino acid profile, and decreasing its crude fiber content (Ghosh et al. 2004; Dairo and Fasuyi 2008). Fermentation significantly reduced the anti-nutritional factors such as tannins and phytic acid and enhanced the available free amino acids, free fatty acids, and mineral concentration of sesame seed meal as well as improving the digestibility of the diet (Mukhopadhyay and Ray 2005; Roy et al. 2014). Indeed, the above studies clearly indicate how poor-quality plant feedstuffs can benefit from fermentation.

The growth, feed efficiency, and carcass protein content of shrimp fed the diet in which 40% of the fish meal protein was replaced with FCM in the present study were comparable to those of the commercial diet. The high performance results of FCM could be attributed to the beneficial effects of fermentation mentioned above. Similar favorable results in terms of growth, feed utilization efficiency, apparent digestibility of protein, lipid, and minerals, and protein deposition were observed in carp fed diets containing fermented oilseed meal compared to fish fed diets containing raw meal (Roy et al. 2014). In addition, the use of the fermented coffee pulp in poultry had a feed efficiency comparable to the standard value and was better than the unfermented product in terms of higher total amino acid content and lower cell wall component value (Peñalosa et al. 1985). Interestingly, the fermented ingredients can also be used directly as an enzyme source to increase the digestibility and availability of nutrients (Pandey et al. 1999). The similar protein efficiency ratio (PER) in shrimp fed either diets in the present study indicated that both the commercial and the FCM diets have equivalent protein quality. Furthermore, protein retention was considerably higher in shrimp receiving the FCM-based diet as expressed by the protein productive value (PPV). Certainly, these attributes could make FCM an ideal replacement for fish meal protein.

In conclusion, the present results demonstrated the benefits of using fermented copra meal as alternative to fish meal in the shrimp diet. With the present formulation, FCM can replace 40% of the fish meal protein without detrimental effects on shrimp performance. The FCM-based formulated diet in the present study compared favorably with commercial shrimp feed in terms of growth, survival, and feed efficiency. Thus, a 40% replacement of fish meal protein by FCM can be recommended for the grow-out culture of *P. monodon*.

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