AACL BIOFLUX

Aquaculture, Aquarium, Conservation & Legislation International Journal of the Bioflux Society

Partial replacement of soybean meal with fermented copra meal in milkfish (*Chanos chanos*, Forsskal) diet

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Abstract. Feeding trials were conducted to determine the optimum partial replacement level of soybean meal (SBM) with fermented copra meal (FCM). Isonitrogenous and isocaloric diets containing 0, 5, 10, 15, 20, and 25% of the locally produced FCM partially replacing SBM protein by 0, 12, 27, 41, 56, and 71%, respectively and fully replacing copra meal were formulated. The diets were fed to the fish with an initial weight of 2.83 ± 0.14g for 12 weeks. Thereafter, the best diet was further tested in a preliminary feeding trial in brackishwater grow-out ponds to verify the performance of the formulated diet against a commercial milkfish feed in an outdoor grow-out system. The results of the indoor tank feeding trial indicated that weight gain of the fish was significantly better in the group fed diet 2, with 5% dietary FCM but further increase in the FCM inclusion level up to 20% of the diet did not exhibit statistical differences against the control. Moreover in the preliminary pond feeding trial, growth and feed conversion ratio (FCR) of the fish fed the FCM diet were significantly higher than the commercial control diet. Survival and nutrient composition of the fish carcass were not adversely affected by the treatments. Hence, optimum dietary FCM inclusion level was determined at 5% of the milkfish diet replacing 100% copra meal and 12% SBM protein. However, in terms of economics, up to 20% FCM can be included in the diet replacing 56% SBM protein may be possible with growth comparable to the FCM-less control.

Key Words: Aquafeeds, protein-enriched copra meal, alternative protein sources, nutrition.

Introduction. At present, there is an increasing emphasis on reducing feed cost in aquaculture by finding cheaper and locally available sources of protein, feed being the most expensive input in the farming of aquatic species. Copra meal (CM), the by-product of coconut oil extraction is abundant in almost all countries in the Asian region with the Philippines as one of the major producers (Mael & Ajuya 2001; FAO 2011; USDA 2013). This byproduct is rich in protein (Yamazaki et al 1988; Thorne et al 1989; Panigrahi 1991) and carbohydrate (Sundu et al 2009). However, the carbohydrate in CM is regarded as unusable fiber which essentially consists of mannan, galactomannan and cellulose (Dusterhoft et al 1992; Bach Knudsen 1997).

CM is a cheap source of protein for other animals such as livestock and poultry (Kim et al 2001; Dairo & Fasuyi 2008) but is also being used as a protein source in diets for fish (Olude et al 2008). However, limitations associated with CM relate to its poor amino acid balance and low digestibility (Thomas & Scott 1962; Lachance & Molina 1974; Thorne et al 1990; Swick 1999; Sundu et al 2009), presence of antinutritional factors (Mukhopadhyay & Ray 1999; Sundu et al 2009), high fiber (Thorne 1992; Swick 1999; Kim et al 2001; Saha 2003; Dairo & Fasuyi 2008; Siebra et al 2008; Diarra et al 2014),

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aflatoxin content (Sutikno 1990; Pue et al 1991; Head et al 1999), and low bulk density (Sundu et al 2004) which drastically restrict its utilization.

Fortifying CM-containing practical diets with some essential amino acids can improve dietary protein quality (Panigrahi et al 1987; Mukhopadhyay 2000). Likewise, physical treatment such as pelleting crumbling, soaking, and grinding has been reported to improve bulk density, and increase utilization and feeding value of the meal (Sundu et al 2009). Also, bio-treatment such as fermentation can improve nutrient value of CM (Panigrahi 1992; Zamora et al 1996; Pluske et al 1997; Hatta et al 2014) making it a cheap and valuable source of energy and protein in the diet. Moreover, fermentation can be employed to reduce aflatoxin levels in CM as has been observed with other feed ingredients as well as beverages (Mokoena et al 2006; Inoue et al 2013). In fish, fermentation resulted in significantly improved performance of groundnut oil cake (GOC) in terms of growth, feed utilization, nutrient digestibility, carcass composition and digestive enzyme activity (Ghosh & Mandal 2015).

Although untreated CM has long been used as a protein source, high replacement levels are normally not attainable because its protein content is not high enough and its fibrous nature results in low nutrient availability. However, for species that can efficiently utilize plant protein sources, coupled with methods to enhance its quality, its use may be a a strategy to increase efficiency and profit. Therefore, the present study was conducted to evaluate the optimum replacement level of soybean meal with fermented copra meal in milkfish diet.

Materials and Method

<u>Formulated diets</u> - Isonitrogenous and isocaloric diets were formulated containing 0, 5, 10, 15, 20, and 25% of the locally produced fermented copra meal (FCM) which partially replaced soybean meal protein by 0, 12, 27, 41, 56, and 71%, respectively and fully replaced copra meal in milkfish diet (Table 1). L-methionine, L-threonine, and L-phenylalanine were added to balance the dietary amino acid levels. The proximate composition of the diets are shown in Table 2.

<u>Tank feeding trial</u> - Ten milkfish juveniles with average body weight (ABW) of 2.83 ± 0.14 g were stocked in 250-L capacity fiberglass tanks. Each dietary treatment was replicated 3 times. The daily feed consumption was divided into 3 equal parts and fed to the fish at 8:00AM, 12:00Noon and 4:00PM at 6% and later adjusted to 3% of the fish ABW. Growth of fish was measured every 3 weeks by bulk weighing all the fish from each tank. Water parameters such as dissolved oxygen (DO), pH, temperature, salinity, ammonia, phosphorus, and nitrite were monitored regularly. The feeding trial was conducted for 12 weeks.

<u>Pond feeding trial</u> – A preliminary pond feeding experiment was conducted to compare Diet 2 (with 5% FCM partially replacing 12% dietary SBM protein) against Diet 1 (without FCM) and the commercial milkfish diet in grow-out pond culture system. The proximate composition of the commercial feed is presented in Table 3. Fish with ABW of 26.98 ± 2.29 g were distributed in 9 units of 500 sq. m. earthen ponds at 350 pcs. per pond. Each diet was fed in triplicate groups at 5% of the fish ABW. The daily feed ration was divided equally into 3 parts for the 8:00AM, 12:00Noon and 4:00PM feeding shedule. Dissolved oxygen (DO), pH, temperature, salinity, ammonia, phosphorus, and nitrite values were measured at weekly intervals. The feeding experiment lasted for 35 days. Milkfish samples (6 pcs./treatment) were collected after the feeding trial for the analysis of the fish proximate composition.

<u>Chemical analysis</u> –Samples for the analysis of proximate compositions were submitted to an analytical laboratory for the determination of moisture, ash, crude protein, crude fat, and crude fiber following the standard AOAC method 950.46, 920.13, 928.08 (using Kjeltec 2300), 991.36 (using Soxtec2055), and 978.10, respectively. Nitrogen free extract was computed by difference.

Statistical analysis - Results were analyzed by one-way ANOVA using SPSS software. Differences between treatments were evaluated by Tukey's test. Values were considered statistically significant at P≤0.05.

Formulation of the experimental diets (g/kg)

Table	-1
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FCM inclusion level	0%	5%	10%	15%	20%	25%
Basal diet ^a	677.60	677.60	677.60	677.60	677.60	677.60
Soybean meal	300.00	265.00	215.00	165.00	115.00	65.00
Copra meal	15.00	0.00	0.00	0.00	0.00	0.00
Fermented copra meal ^b	0.00	50.00	100.00	150.00	200.00	250.00
L-methionine	1.20	0.80	0.55	0.24	0.09	0.00
L-threonine	1.60	1.33	1.35	1.37	1.42	1.47
L-phenylalanine	0.00	0.00	0.00	0.61	1.57	2.49
Rice bran	4.60	5.27	5.50	5.18	4.32	3.44

^a Basal diet – Fish meal 185g, cowpea 200g, corn starch 225g, cod liver oil 30g, soybean oil 30g, vitamin mix 5g, mineral mix 2.5, Asc P (Tiger C) 0.1g. ^b Purchased from a local dealer.

Table 2 Proximate composition of fermented copra meal^a and the experimental diets^b,

	Crude	Crude	Crude		
	Protein	Fat	Fiber	Ash	NFE
	(%)	(%)	(%)	(%)	(%)
Fermented copra meal (FCM)	38.27	6.84	6.44	9.61	38.83
Diet 1 (0%)	30.94	8.51	1.50	7.36	48.96
Diet 2 (5%)	30.95	8.70	1.50	7.44	48.82
Diet 3 (10%)	30.67	8.28	1.73	7.54	48.69
Diet 4 (15%)	30.41	8.21	1.95	7.63	48.52
Diet 5 (20%)	30.22	8.13	2.16	7.70	48.30
Diet 6 (25%)	30.03	8.05	2.38	7.78	48.13

^{*} Dry matter basis.

Table 3 Proximate composition (%) of the commercial milkfish diet*

Analysis	(%)
Dry matter	88.44
Crude protein	35.80
Crude fat	3.19
Crude fiber	3.78
Ash	9.71
Nitrogen free extract	47.52

^{*}Dry matter basis.

^a Analyzed values, Apines-Amar et al (in press).

^b Calculated values.

Results and Discussion. This study demonstrated that fermented copra meal (FCM) is a good replacement plant protein source for milkfish diet. The results obtained from this study clearly showed that the optimum inclusion level of FCM in terms of growth was determined to be 5% of the milkfish diet (Figure 1). This amount of FCM replaced 100% dietary copra meal and 12% SBM protein (Table 1). Increasing further the level of dietary FCM resulted in corresponding though not statistically significant decrease in the growth of the fish until it reached 25% dietary inclusion where a significant reduction in growth was observed.

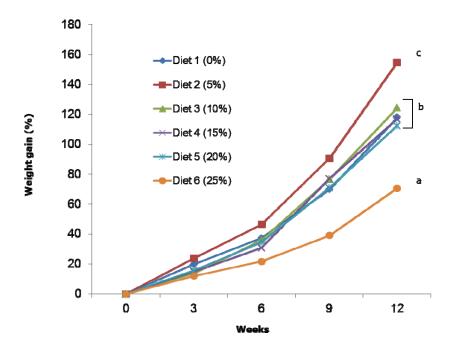


Figure 1. Growth of milkfish in tanks fed the experimental diets for 12 weeks. Means (n=3) at 12 weeks not sharing the same superscript letter are statistically different (P<0.05).

Growth of the fish (Figure 2, Table 4) and feed conversion ratio (FCR) (Table 4) obtained in the pond feeding trial further supported the superior performance of FCM diet (5% FCM inclusion) which was significantly higher than the commercial control diet. High crude fiber, generally associated with plant derived proteins is known to depress apparent protein digestibility coefficients (Olivera-Novoa et al 2002) which directly affects growth. However, as mentioned above, this was not observed in the present study with the use of FCM. Bio-treatment and physical alteration of dietary ingredients can improve the nutritive value and utilization of the diet. Sundu et al (2004) stated that dietary copra meal reduced feed intake in broiler due to high dietary fiber which resulted to a more bulky feed but fermentation was able to lower the bulkiness of the diet. Similarly in the study of Sundu et al (2005), dietary copra meal suppressed growth of broilers while Hatta et al (2014) demonstrated similar growth of birds fed fermented copra meal was similar to the birds fed the control diet indicating the importance of fermentation on copra meal. Comparably, diets containing fermented groundnut oil cake (GOC) performed better than the diet with raw GOC fed to fish (Ghosh & Mandal 2015). Soaking of copra meal at room temperature for a 16-hour period reduced the levels of tannins from 2.4% to as low as 0.9% (Mukhopadhyay & Ray 1999).

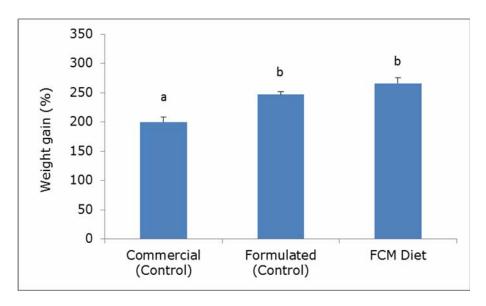


Figure 2. Growth of milkfish fed the experimental diets for 35 days. Means \pm S.E. (n=3) not sharing the same superscript are statistically different (P<0.05).

Table 4 Growth and survival of milkfish in ponds fed the experimental diets for 35 days

Experimental Diets	FCM inclusion level (%)	Initial Weight (g)	Final Weight (g)	FCR ¹	SGR² (%/day)	Survival (%)
Commercial (Control)	0	27.38ª	81.67ª	0.91 ^b	3.13ª	100
Formulated (Control)	0	26.28ª	91.00 ^b	0.74ª	3.55 ^b	100
FCM Diet	5	27.29ª	94.00 ^b	0.69ª	3.70 ^b	100

Means (n=3) not sharing the same superscript letters are statistically different (P<0.05);

Survival (Table 4) and proximate composition of the fish carcass (Table 5) were not affected by the replacement of SBM with FCM. Water parameters such as dissolved oxygen (DO), pH, temperature, salinity, ammonia, phosphorus, and nitrite for both the tank and pond trials did not vary among treatments and fall within the tolerable limits for milkfish (data not shown). This indicate that water quality in the culture system did not influence the performance of the diets.

The present results demonstrated that FCM can be used as SBM replacement for milkfish diet. As FCM is way cheaper than SBM, the highest inclusion level without adverse effect on performance parameters would be beneficial. In this sense, up to 20% inclusion level may be used since growth data indicated that FCM was well-utilized by milkfish at this level (Figure 1.)

However, more studies especially in ponds are needed to verify these preliminary results, which could lead to better utilization of FCM and widespread adoption for use in aquaculture.

¹ Feed conversion ratio = feed consumed (g)/weight gain (g);

² Specific Growth Rate = ((In Final weight - In Initial weight)/No. of days) x 100.

Table 5 Proximate composition of the fish carcass fed the experimental diets in ponds for 35 days

Experimental Diets	FCM inclusion level (%)	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	NFE (%)
			Dry Matter Basis				
Commercial Control)	0	63.63	57.94	33.56	0.05	8.29	0.30
Formulated (Control)	0	63.48	57.81	32.25	0.06	8.97	1.25
FCM Diet	5	63.89	56.50	34.00	0.04	7.55	3.45

Conclusions. Under the conditions prevailing in our preliminary investigation, it was demonstrated that, with the present feed formulation, FCM can partially replace 12% dietary SBM protein in milkfsh. Better growth and FCR were observed in milkfish fed the FCM formulated diet compared to the commercial milkfish feed. However, up to 20% inclusion level replacing 56% SBM protein may be possible with performance comparable to the FCM-less control. Since copra meal is available locally as by-product of the coconut industry, reduction in feed cost might become more tenable with the use of FCM. Nonetheless, larger feeding studies with longer culture periods including economic analysis in grow-out ponds and cages are needed to verify the effects of FCM and evaluate its prospects as an important feed ingredient in milkfish diets.

Acknowledgements. This study was fully supported by the Department of Science and Technology (DOST) through the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD). The authors are also grateful to the University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Brackishwater Aquaculture Center (UPV-CFOS-IA-BAC) and the Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC AQD) for the use of research facilities. Likewise, we are thankful to Alex Gustillo and James David Lopez for their assistance during the conduct of this study.

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Received: 15 November 2015. Accepted: 17 December 2015. Published online: 21 December 2015.

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How to cite this article: Apines-Amar M. J. S., Coloso R. M., Jaspe C. J., Salvilla J. M., Murillo M. N. A., Saclauso C. A., 2015 Partial replacement of soybean meal with fermented copra meal in milkfish (Chanos chanos, Forsskal) diet. AACL Bioflux 8(6):1019-1026.