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Research Article

Protein-Enriched Copra Meal as a Substitute for Soybean Meal in the Diet of Nile Tilapia (*Oreochromis niloticus*) Fry

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Abstract | In recent years, considerable research on fish nutrition has focused more on finding and incorporating a cheaper plant-based protein source to the diet without compromising its nutritional value. Protein-Enriched Copra Meal (PECM®) serves as an affordable and sustainable plant-based protein source for aquafeeds. However, its feed value has not been evaluated in most cultured aquatic animals. A 60-day feeding trial was conducted to evaluate the potential of PECM® as a substitute for soybean meal (SM) in the diet of Nile tilapia (*Oreochromis niloticus*) fry. Five diets were formulated containing different PECM® inclusion levels as replacement: Trt0 (0%), Trt25 (25%), Trt50 (50%), Trt75 (75%), and Trt100 (100%) as a replacement for soybean meal. Results indicate that the replacement of SM with PECM® was found to be optimum at 50% which exhibited no negative effects on growth performance and feed conversion ratio. Higher replacement levels in treatments Trt75 and Trt100 significantly ($P < 0.05$) decreased the growth performance of the juvenile tilapia. Including PECM® in the diet had no adverse effects on fish survival or carcass composition. The findings of this study demonstrated that PECM® could substitute up to 50% of soybean meal in tilapia fry diets without compromising overall growth performance, feed utilization, and survival. The use of this ingredient could be a practical and sustainable approach in tilapia aquaculture.

Keywords | Feeds, Nutrition, Coconut, Copra meal, Fermentation

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INTRODUCTION

Aquaculture plays an important role in ensuring global food security by providing a reliable source of protein and supporting livelihoods in many communities. Aquaculture may also assist in alleviating the pressure on wild fish populations, contributing to the conservation of marine ecosystems. By utilizing alternative food sources, such as plant-based proteins, aquaculture can further promote environmental conservation by reducing the reliance on overfished species and minimizing the ecological footprint of fish farming. In aquaculture, 50% of the production cost is accounted for by the capital costs of feed. As requirements for aquaculture feeds increase, shortages in primary feed ingredients have become a limiting factor in the sustainability of aquaculture (Hertrampf and Piedad-Pascual, 2000). To address these concerns, considerable research on fish nutrition has focused on finding and incorporating a cheaper plant-based protein source in the diet without compromising the nutritional availability (Wattanakul *et al*., 2021; Khan *et al*., 2013; Ogello *et al*., 2014; Tantikitti *et al*., 2005). Most of these studies investigated the potential of soybean meal (SBM) to replace fish meal in aquaculture feeds (Wu *et al*., 2021; Egerton *et al*., 2020; Yanti *et al*., 2019; Wang *et al*., 2016). Soybean meal (SBM) is generally utilized as the main plant-derived protein source for fulfilling the essential amino acid needs of commercially grown fish species (Alceste and Jory, 2000). However, its utilization in aquaculture is limited by its high cost, demand for human consumption, and its unpredictable supply as an import commodity in the Philippines due to competition with other agricultural industries (Plaipetch and Yakupitiyage, 2013; Intoy and Traifalgar, 2021). Copra meal, on the other hand, is readily available in mass quantities and cheaper than the imported SBM. The downside is that copra meal is known to be high in non-starch polysaccharides and low in protein as compared to SBM. The University of the Philippines Los Baños-National Institute of Molecular Biology and Biotechnology (BIOTECH-UPLB) recently developed a fermentation technology that converted copra meal into a Protein-Enriched Copra Meal (PECM®) with nutrient contents that are comparable to SBM. Though the nutritive components of this developed feed ingredient are supposedly adequate, its feed value has not been evaluated in most cultured aquatic animals. This study aims to evaluate whether PECM® can serve as an effective alternative to soybean meal in freshwater tilapia culture, determine its effect on fish growth and survival, and identify the optimal inclusion level in tilapia diets.

MATERIALS AND METHODS

Diet Preparation

Five formulated diets were prepared using different inclusion levels of PECM® replacing soybean oil by equivalent weight: Trt25 (25% PECM®), Trt50 (50% PECM®), Trt75 (75% PECM®), and Trt100 (100% PECM®) to substitute soybean meal. Trt0 (without PECM®) served as the control diet. Lipid sources used in the study included Danish fish oil and soybean oil (Table 1). All dry ingredients were ground and sieved to a 100 μm particle size. Sieved ingredients were mixed in a zipper storage bag, transferred to a basin, and liquid ingredients were then added. The ingredients were manually mixed until a clay-like consistency was achieved. The mixture was then processed in a pelletizer (Tasin TS-102AL) to form solid pellets. These pellets were arranged on a tray and oven-dried at 60°C for 24 hours. Once dried, the feeds were stored in a zipper storage bag at 4°C until use.

Proximate Analysis

The proximate composition of the formulated diets (Table 1) and the animal carcass were analyzed to determine how the dietary treatments influenced the carcass composition of cultured tilapia. Crude protein content was measured using the Kjeldahl Total Protein Nitrogen Method (Foss Tecator™ Digestion and Foss Kjeltec™ 8200 153 Auto Distillation). Crude lipid was analyzed using the Foss Soxtec™ 2050 Automatic System, following the Bligh and Dyer (1959) Method. Crude fiber was measured using the Ceramic Fiber Filter Method (AOAC, 1996). Ash content was determined gravimetrically by burning the sample in a muffle furnace at 600°C. Meanwhile, dry matter content was determined using the Mettler Toledo Halogen Moisture 158 Analyzer (AOAC, 1996). All analyses were conducted in triplicate.

Table 1: Dietary and proximate composition of Tilapia diets with different PECM inclusion levels (g/100 g).

Component	Treatment (g/100 g)				
	Trt0	Trt25	Trt50	Trt75	Trt100
Sardine fish meal	16.00	16.00	16.00	16.00	16.00
Soybean meal	35.00	26.25	17.50	8.75	-
PECM	-	8.75	17.50	26.25	35.00
Rice Bran	15.00	15.00	15.00	15.00	15.00
Bread flour	23.00	23.00	23.00	23.00	23.00
Danish fish oil	4.00	4.00	4.00	4.00	4.00
Soybean oil	4.00	4.00	4.00	4.00	4.00
Vitamin	1.50	1.50	1.50	1.50	1.50
Mineral	1.50	1.50	1.50	1.50	1.50
TOTAL	100.00	100.00	100.00	100.00	100.00
Proximate analysis (%DM)					
Crude Protein	30.18	30.22	30.25	30.29	30.32
Crude Lipid	12.70	12.45	12.20	11.94	11.69
Crude Fiber	4.95	4.53	4.11	3.69	3.27
Ash	6.97	6.43	5.89	5.34	4.80
NFE	45.20	46.37	47.55	48.74	49.92

Experimental Design and Feeding Trial

Nile tilapia (*Oreochromis niloticus*) obtained from SEAFDEC in Tigbauan, Iloilo, were raised at the Multi-Species Hatchery Complex under the management of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, UP Visayas. Tilapia fingerlings (mean size: 0.47 ± 0.02 g) were randomly distributed in a 50 L rectangular tank with a stocking density of 20 individuals per tank. The tank assignment for each treatment was based on a completely randomized design (CRD) and was carried out in triplicates. Replacement fish were kept in a 1000 L tank with continuous aeration for seven days before starting the feeding trial. During the acclimation process, fish were fed with the control diet (Trt0). Siphoning of feces and excess feeds were done every morning. The feeding trial was carried out in a closed recirculating system with a mechanical filter operating at a flow rate of 50 mL per minute. Freshwater volume inside each tank of about 40 liters was maintained. The initial weight of the fish was taken after the acclimation period and dead individuals were replaced. The fish were hand-fed to satiation three times daily (0900, 1300, and 1600 h). Biomass sampling was conducted every 10 days, and the feeding rate was adjusted. Water change was done twice a week, by which about 50% of the water volume in each tank and 100% in the reservoir were replaced. Aeration was continuously supplied, and water quality was kept within the optimal range, with a temperature of 25-28°C, dissolved oxygen levels between 5-8 mg/L, a pH of 7-8, and salinity ranging from 0.02 to 0.04 g/L. Ammonia levels were monitored once a week (Molins-Legua *et al*., 2006): 0.04 ± 0.00 - 0.12 ± 0.01 ppm. At the end of the feeding trial, growth and feeding parameters, and survival of the fish were calculated using the following formulae (Traifalgar *et al*., 2010):

$$Percent\ Weight\ Gain\ (\%WG) = \frac{final\ weight\ (g) - initial\ weight\ (g)}{initial\ weight\ (g)} \times 100$$

$$Specific\ Growth\ Rate\ (SGR) = \frac{\ln\ [weight\ gain\ (g)]}{culture\ period\ (days)} \times 100$$

$$Feed\ Conversion\ Ratio\ (FCR) = \frac{total\ feed\ intake\ (g)}{weight\ gain\ of\ fish\ (g)}$$

$$Protein\ Efficiency\ Ratio\ (PER) = \frac{weight\ gain\ (g)}{total\ protein\ intake\ (g)} \times 100$$

$$Percent\ Survival\ (\%S) = \frac{final\ no.of\ fish}{initial\ no.of\ fish} \times 100$$

Statistical Analysis

The data gathered in the study were examined using IBM SPSS Version 26. A one-way ANOVA was conducted, followed by Tukey's HSD post-hoc test, to identify significant differences ($P < 0.05$) between data sets.

RESULTS

Growth and Feeding Performance

After the 60-day feeding trial, growth performance, survival, and utilization of the provided feeds to *O. niloticus* fingerlings were determined. The growth performance of tilapia-fed diets containing different PECM inclusion levels is demonstrated in Table 2. R The results showed a significant difference ($P < 0.05$) between treatments in terms of growth performance indicators. Trt25 and Trt50 exhibited consistent results in regard to weight gain (%WG) (Figure 1) and specific growth rate (SGR), which did not exhibit significant difference from Trt0. A similar effect was also observed in feed utilization. The highest protein efficiency ratio (PER) value was recorded in Trt25 while Trt100 showed the significantly ($P < 0.05$) lowest value (Table 3). The feed conversion ratio (FCR) value of Trt100 was also significantly ($P < 0.05$) higher when compared to other treatments. Feed intake (FI), exhibited the same downward trend observed in Trt75 and Trt100. Survival rates were high across all treatments, and increased inclusion levels of PECM as a substitute for soybean meal had no adverse effects on the survival of cultured tilapia.

Table 2: Growth performance and survival of Nile Tilapia (*Oreochromis niloticus*) fed different treatments for 60 days.

Treatment	IBW	FBW	SGR (% d-1)	S (%)
Trt0	0.48 ± 0.07^{a}	2.16 ± 0.37^{b}	2.31 ± 0.20^{a}	92.50 ± 2.89^{a}
Trt25	0.59 ± 0.07^{a}	2.34 ± 0.07^{c}	2.29 ± 0.05^{a}	98.33 ± 2.89^{a}
Trt50	0.38 ± 0.07^{a}	2.11 ± 0.68^{b}	2.37 ± 0.11^{a}	82.50 ± 9.57^{a}
Trt75	0.50 ± 0.02^{a}	1.46 ± 0.27^{ab}	1.68 ± 0.04^{b}	91.25 ± 6.29^{a}
Trt100	0.42 ± 0.10^{a}	1.08 ± 0.20^{a}	1.23 ± 0.24^{c}	87.50± 11.90^{a}

Data represented as means ± SD. Means with the same superscripts are not significantly different (One-way ANOVA, Tukey's HSD test, $P > 0.05$). BW (g), initial body weight; FBW (g), final body weight; SGR (% d-1), specific growth rate; S (%), survival rate.

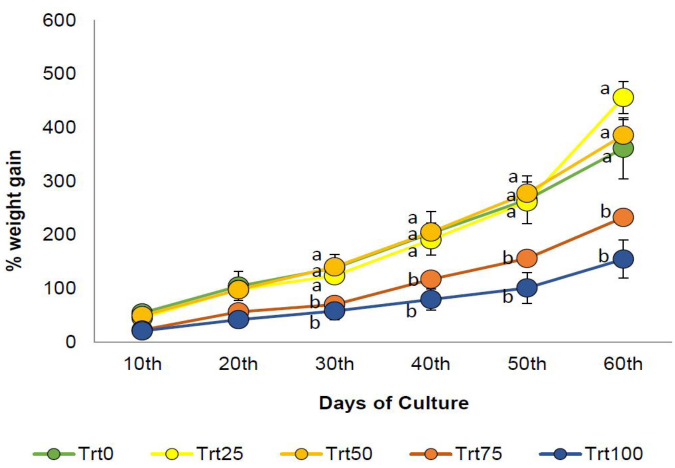

Figure 1: Percent weight gain (%WG) of Nile Tilapia Oreochromis niloticus fed different treatments for 60 days. Data represented as means ± SD. Means with the same superscripts are not significantly different (One-way ANOVA, Turkey's HSD test, $P>0.05$).

Table 3: Feed utilization of Nile Tilapia (*Oreochromis niloticus*) fed different treatments for 60 days.

Treatment	FCR	FI (g/fish)	PER
Trt0	0.54 ± 0.03^{ab}	0.91 ± 0.15^{b}	6.15 ± 1.18^{ab}
Trt25	0.40 ± 0.01^{a}	1.07 ± 0.03^{b}	8.24 ± 0.29^{b}
Trt50	0.60± 0.08^{ab}	0.98 ± 0.21^{b}	5.84 ± 1.72^{ab}
Trt75	0.59 ± 0.06^{ab}	0.60 ± 0.13^{a}	5.60 ± 1.10^{ab}
Trt100	0.76 ± 0.21^{b}	0.47 ± 0.04^{a}	4.56 ± 1.21^{a}

Data represented as means ± SD. Means with the same superscripts are not significantly different (One-way ANOVA, Tukey's HSD test, $P > 0.05$). FCR, feed conversion ratio; FI (g/fish), feed intake; PER, protein efficiency ratio.

Carcass Composition

The carcass moisture content, crude protein, and crude lipid levels showed no significant differences among treatments suggesting that these were not influenced by elevated PECM® inclusion levels in the diet. However, the ash content was significantly (P < 0.05) different with Trt75 and Trt100 (Table 4).

Table 4: Carcass proximate composition of Nile Tilapia (*Oreochromis niloticus*) fed different treatments for 60 days.

Treatment	Moisture	Crude Protein	Crude Lipid	Ash
Trt0	70.34± 1.00[a]	61.83 ± 1.55[a]	6.94 ± 0.27[a]	5.34 ± 0.66[ab]
Trt25	70.42± 0.48[a]	62.50 ± 1.48[a]	6.92 ± 0.11[a]	5.02 ± 0.52[ab]
Trt50	70.45± 0.85[a]	62.81 ± 1.63[a]	6.92 ± 0.03[a]	5.88 ± 0.23[ab]
Trt75	70.45± 0.86[a]	62.92 ± 0.99[a]	6.98 ± 0.22[a]	6.03 ± 0.43[b]
Trt100	70.45± 0.87[a]	63.27 ± 1.63[a]	7.24 ± 0.14[a]	4.76 ± 0.35[a]

Data represented as means ± SD. Means with the same superscripts are not significantly different (One-way ANOVA, Tukey's HSD test, P > 0.05).

DISCUSSION

This study demonstrates the feasibility of replacing soybean meal with Protein-Enriched Copra Meal (PECM®) at varying inclusion levels in the diet of Nile tilapia (*O. niloticus*). PECM® is made using a solid-state fermentation bio-processing system utilizing the fungus *Aspergillus niger* to improve the nutritive content of copra meal (Pham, 2017). Our results in the growth of the fish and utilization of the feed showed significant differences (P < 0.05) among dietary treatments. Tilapia exhibiting the highest %WG values were recorded in Trt25 and Trt50, indicating that soybean meal can be replaced with PECM® up to 50% (17.5% dietary inclusion) in a diet. This finding recommends that soybean meal, when combined with fermented copra meal in the diet, results in better growth. However, increasing the inclusion levels to 75% and 100% decreased the growth performance of the fish, suggesting that a total replacement of SBM with PECM® is not ideal. The apparent inhibition of growth at the higher dietary inclusion levels hints at the presence of anti-nutritional factors which can cause the unavailability of nutrients. This can limit the assimilation of nutrients, thus disenabling the attainment of optimum growth (Intoy and Traifalgar, 2021). Laining *et al*. (2017) reported similar studies showing that completely substituting soybean meal with fermented copra meal hinders rabbitfish growth and is not suitable for siganid diets, with only 13.7% inclusion being feasible. In milkfish, *Chanos chanos*, fermented copra meal was also incorporated in the diet (Apines-Amar *et al*., 2015) - at 5% inclusion level, this showed significant improvement of weight gain but at 20% inclusion, there was no significant difference to the control treatment. Further, its incorporation in the diet of *Penaeus monodon* (Apines-Amar *et al*., 2016) suggested that inclusion by as much as 40% did not affect feed efficiency, survival, and growth of the animal. In contrast, Mamauag *et al*. (2019) demonstrated the 100% replacement of SBM with FCM (16% dietary inclusion) upon supplementation of crystalline amino acids and this did not negatively affect the growth performance and digestive health of the grouper *Epinephelus fuscoguttatus*.

In the present study, the lowest FCR was also observed in Trt50, and values were significantly higher in treatments with elevated PECM® inclusion levels (Trt75 and Trt100). Significantly higher FI and PER values were also recorded in Trt50. In addition, a significant decline in feed utilization indices were consistently observed in Trt75 and Trt100. Most often, the high tannin concentration in copra meal, results in negative outcomes such as poor growth, feed utilization, and impaired feed palatability, this reduces feed efficiency and nutrient absorption. Due to its high fiber and anti-nutrient content, unprocessed copra inclusion levels in aquafeed are limited to 15% for omnivorous fish and 10% for carnivorous fish species (Obirikorang *et al*., 2016). Dileep *et al*. (2021) replaced fish meal in tilapia diet with a mixture of yeast fermented guar and copra meal. Findings of the study suggest that fermentation with yeast considerably improved the protein and amino acid content of guar and copra meal. Due to the presence of natural phytase enzymes and hydrolytic capacity of yeast, antinutritional factors (ANFs) such as saponin, tannin, trypsin inhibitor, and phytic acid showed a substantial drop in quantity following fermentation (Shiu *et al*., 2013; Greiner and Konietzny, 2006). The production of extracellular enzymes during yeast fermentation aids the synthesis of plant components and has also been highlighted in similar studies (Dawood and Koshio, 2020). However, Intoy and Traifalgar (2021) noted in their research that other components, including non-starch polysaccharides (NSP), may still be present in copra meal even after fermentation has removed major ANFs (Mukhopadhyay, 2000). This results in a decrease in the feed value of the plant ingredient (Sundu *et al*., 2009). Additionally, it has been demonstrated that raw copra meal contains 42.2% NSPs, which includes water-soluble galactomannans and water-insoluble mannan oligosaccharides (Knudsen, 1997). Galactomannan in the diets was associated with decreased feed utilization efficiency and growth retardation in tilapia (Hossain *et al*., 2003) and increased gastrointestinal viscosity which inhibits efficient nutrient absorption in carp (Hossain *et al*., 2001).

A decline in protein deposition in fish carcasses has often been observed in fish fed higher amounts of plant-based protein meals. However, in the present study, carcass proximate analysis showed that dietary treatments with

PECM® inclusion did not significantly influence carcass composition. Similar findings were also observed in the grow-out culture of black tiger shrimp fed with fermented copra meal as a replacement for fish meal (Apines-Amar *et al.*, 2019). In juvenile black tiger shrimp, a significant reduction in carcass protein was observed in a plant protein-based diet (PECM®: 10 g/100 g diet) with squid by-product hydrolysate as fish meal replacement (Pan and Traifalgar, 2022). No negative effects on carcass composition also supported the replacement of soybean meal with fermented palm kernel meal for sex-reversed red tilapia (Wattanakul *et al*., 2021), and soybean meal with fermented copra meal (FCM) for saline-tolerant Nile tilapia (Intoy and Traifalgar, 2021) but the highest replacement level resulted in decreased carcass protein content. The decline in growth performance and carcass protein content associated with increased inclusion levels of most plant protein components in fish diets has been linked to the imbalance profile and a lack of essential amino acids, which stimulate protein catabolism rather than anabolism (Lim and Lee, 2011). Tilapia is an omnivorous species, and the findings of this study indicate that its carcass composition, apart from ash content, remained unaffected by different PECM® inclusion levels. This suggests the species' ability to tolerate high amounts of plant-based ingredients in its diet. Significantly higher value of ash content in carcass samples fed with Trt75 was observed. Intoy and Traifalgar (2021) also found that the ash content of tilapia carcass was significantly increased with elevated FCM inclusion. In contrast, carcass samples fed with Trt100 exhibited significantly lower ash content than the control. Magbanua and Ragaza (2022) reported that ash composition may be linked to the nutrient and mineral content of other feed materials, excluding the protein source. Furthermore, the survival rate of tilapia in this study remained unaffected by the varying levels of PECM® inclusion in the diet.

CONCLUSIONS AND RECOMMENDATIONS

The findings of this study indicate that Protein-Enriched Copra Meal (PECM®) performed best at a 25% inclusion level in the diet of Nile tilapia. However, replacing 50% of soybean meal with PECM® could be a more cost-effective option, as there were no notable differences in growth parameters among the control group, fish fed with 25% PECM®, and those receiving a 50% PECM® replacement. Dietary treatments in the present study compared favorably with soybean meal protein sourced diet in terms of growth, feed efficiency, and survival. PECM® can provide a sustainable and cost-effective source of feed protein since it is a cheaper and more readily available ingredient.

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NOVELTY STATEMENTS

The study introduces Protein-Enriched Copra Meal (PECM®) as a novel plant-based protein source in tilapia nutrition, demonstrating its potential to replace up to 50% of soybean meal without compromising growth or health. This highlights PECM® as a practical alternative that supports sustainable aquaculture while addressing feed cost challenges.

AUTHOR'S CONTRIBUTIONS

VC designed the experimental study, culture facility, and feeding protocol of Nile Tilapia (Oreochromis niloticus). RT and CdC contributed to data analysis, interpretation, and manuscript preparation. RRC conducted the experiments proper; collected the data, conducted the initial result analysis, and drafted the manuscript.under the guidance of VC, CdC, and RT,. All listed authors contributed to the article and have given thumbs up to the submitted version.

Conflict of Interest

The authors have declared no conflict of interest.

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