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Efficacy of Poultry By-product Meal as an Effective Alternative to Fish Meal in Aquaculture Feed for Milkfish *Chanos chanos*

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Abstract

We verified the efficiency of poultry by-product meal (PBM) as a substitute for fish meal (FM) in feed for juvenile milkfish (*Chanos chanos*). Juveniles (mean 48.0 g) were fed for 12 weeks with two experimental feeds containing different levels of PBM (8.0% and 12%), FM (10% and 5.0%), and cod liver oil (fish oil or FO, 4.0% and 3.8%). A feed without PBM having higher levels of FM and FO (20% and 4.5%, respectively) was used as control. Weight gain, specific growth rate, and feed conversion ratio were not significantly affected by the levels of dietary PBM. In addition, no significant differences were detected among the dietary groups in plasma triglyceride, total cholesterol, phospholipid, glucose, or total protein concentrations. Furthermore, crude protein, crude fat, moisture, and ash contents in the whole body, liver, and dorsal muscle were not significantly influenced by the dietary treatments. The results of organoleptic examinations that included tests of smell, flavor, and texture were almost the same among the dietary groups. These results indicated that PBM is the applicable substitute for FM, with performance of the high PBM feed (PBM-FM-FO = 12%-5.0%-3.8%) being comparable to that of the control feed.

Discipline: Fisheries
Additional key words: feed conversion ratio, fish oil, specific growth rate, taste, weight gain

Introduction

Milkfish (*Chanos chanos*) is the national fish and top aquaculture species of the Philippines, with a total production of 416,360 t in 2017 (Philippine Statistics Authority 2018a). Aquaculture production of this species is still increasing and the amount produced in 2017 was 3.40% higher than that in 2016, more than 1.3 times that of tilapias (*Oreochromis niloticus* and *Oreochromis mossambicus*)—the country's second major aquaculture species. In 2016, milkfish production in the Philippines was the second highest in the world after Indonesia (FAO 2018). This demonstrates the national importance of milkfish in the Philippines.

Milkfish culture using ponds, pens, and net cages has existed for centuries in the Philippines, Indonesia, and Taiwan. Various examinations of nutritional requirements in this species have already been made (Borlongan 1992a,b, Borlongan & Coloso 1993, Borlongan & Satoh 2001). According to Yap et al. (2007), feed costs represent over three-quarters of the total management expenses in milkfish culture using net cages. This suggests that reducing feed costs is a prerequisite to improving the cost of aquaculture management. Aquaculture feed is generally costlier than livestock feeds (e.g., cow, pig, chicken feeds), with the essential difference being the protein content. The crude protein content of aquaculture feed is generally more

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than 30%, which is markedly higher than that of livestock feed (at around 18%). The high protein content of aquaculture feed is the major cause of its high cost. In aquaculture feed, fish meal (FM) is the main protein source, which substantially increased in price from 2006 to 2013, with a peak value of USD 1,747/t in 2013. Since then, it has slightly decreased, but remains high (FAO 2016). The high price of FM increases the price of aquaculture feed, and hence the need for a low FM feed in order to reduce overall costs.

There are several alternative protein sources to FM for aquaculture feed, such as oil seed by-products (i.e., soybean meal (SBM), canola meal, cotton seed meal, peanut meal) and corn gluten meal. These are plant-based and are more economical than FM and other animal-based protein sources (Tridge 2019). However, these protein sources reportedly have several problems as fish feed components, such as amino acid imbalance, low palatability, and the inclusion of anti-nutritional factors (NRC 2011). In order to mitigate such negative aspects, crystal amino acids and enzyme supplements are efficient, as well as extrusion processing (Watanabe 2009), but such treatments are also costly and increase feed costs. Therefore, other animal-based protein sources are considered as more efficient alternatives to FM.

Poultry by-product meal (PBM) is one of the major animal protein sources for aquaculture feed alternatives to FM, as well as cow/pork meat and bone meal, blood meal, and feather meal (Watanabe 2009). PBM is produced from chicken waste products that mainly include the esophagus, lung, gall bladder, and rectum, which are considered inedible for humans. As large quantities of chickens are produced in the Philippines (1,746,000 t in 2017) (Philippine Statistics Authority 2018b), the availability of PBM is thus potentially large. Although the nutritional efficiencies of PBM as protein sources for fish feed have been evaluated in several fish species (Sato et al. 1997, Booth et al. 2012, Hartviksen et al. 2014, Quangen et al. 2014, Chun et al. 2016, Mohanta et al. 2016), milkfish has yet to be evaluated.

Taking the aforementioned points into consideration, we conducted culture trials of milkfish by using feeds in which FM was partially replaced by PBM in order to evaluate the replacement effect by analyzing growth performance and biochemical compositions.

Materials and methods

1. Experimental feeds

Table 1 lists the compositions of the experimental feeds. The control feed (CTF) contained 20.0% FM, 20.0% SBM, 4.5% cod liver oil (FO), and 19.4% wheat flour as the Main Protein, fat, and carbohydrate sources, respectively. Low PBM feed (LPF) and high PBM feed (HPF) contained 8.0% and 12.0% PBM, respectively. The FM contents of LPF and HPF were 10.0% and 5.0%, respectively (i.e., 50% and 75% replacements by PBM). As the crude fat content of PBM is higher than that of FM, the FO contents of LPF and HPF were maintained at 4.0% and 3.8%, respectively (i.e., 10% and 15% replacements by PBM). Rice bran was used as a bulking filler. Feeds were manufactured as a floating pellet type at the Tigbauan main station of the Southeast Asian Fisheries Development Center/Aquaculture Department SEAFDEC/AQD, Tigbauan, Iloilo, Philippines.

Table 1. Composition of the experimental feeds

Feeds	CTF[1]	LPF[1]	HPF[1]
Ingredients (%)			
Fish meal[2, 3]	20.00	10.00	5.00
Soybean meal[2, 4]	20.00	20.00	20.00
Poultry by-product meal[5, 6]		8.00	12.00
Cod liver oil[7]	4.45	4.00	3.78
Wheat flour[8]	19.40	19.40	19.40
Mineral Mix[9]	2.00	2.00	2.00
Dicalcium phosphate	2.00	2.00	2.00
Vitamin Mix[9]	1.00	1.00	1.00
Vitamin C	0.05	0.05	0.05
Choline cloride	0.10	0.10	0.10
Rice bran[10]	31.00	33.45	34.67

[1] CTF: Control feed, LPF: low poultry by-product meal feed, HPF: high poultry by-product meal feed
[2] MCR Agri-Venture Corp., Iloilo, Philippines
[3] Dry matter, 92.0%; crude protein, 53.9%; crude fat, 3.3%
[4] Dry matter, 89.6%; crude protein, 48.6%; crude fat, 0.7%
[5] United Pharmachem Agrivet, Inc., Manila, Philippines
[6] Dry matter, 95.3%; crude protein, 62.4%; crude fat, 9.2%
[7] Alysons' Chemical Enterprises Inc., Metro Manila, Philippines
[8] Pilmico Foods Corp., Iligan City, Philippines
[9] Progressive Laboratories, Quezon City, Philippines
[10] Tamisen Rice Retailer, Iloilo, Philippines

Table 2 lists the nutrient contents of the experimental feeds. The contents of crude protein, crude fat, crude starch, and ash in the CTF were 27%, 10%, 31%, and 12%, respectively, and roughly equivalent to those of conventional commercial feeds for milkfish. The crude protein, crude fat, and crude starch contents in the LPF and HPF were similar to those of the CTF. Ash content was the highest in the CTF.

Table 2. Proximate analysis of the experimental feeds

Feeds	CTF[1]	LPF[1]	HPF[1]
Analysis (dry weight basis)			
Crude protein (N × 6.25%)	27.4	27.4	26.9
Crude fat (%)	10.3	9.5	9.8
Crude starch (%)	30.7	31.0	30.6
Ash (%)	12.4	11.2	10.7

[1] See the footnote of Table 1.

2. Stocked juveniles and feeding procedures

Juvenile milkfish were obtained from a private fish farmer in Igang, Nueva Valencia, Guimaras, Philippines and then transferred to the Igang Marine Station of the SEAFDEC/AQD. Ten thousand juveniles were initially stocked in a 10 m × 10 m × 4 m net cage and fed a commercial feed (Santeh Feeds Corp., Quezon City, Philippines) for 22 days to acclimate them before the experimental culture trials.

From the above juveniles, some 9,000 (mean ± standard deviation: 48.0 ± 0.8 g) were transferred and stocked in six net cages (5 m × 5 m × 4 m; 1,500 juveniles/cage) with duplication for each dietary treatment. The stocking density per cage was 15 fish /m^3. Immediately before the culture trials, 40 juveniles (49.8 ± 17.3 g) from the above stock were randomly sampled and stored in a –80°C freezer (= initial juveniles). Of these 40 juveniles, 20 were used for proximate composition analysis of the whole body, and the rest were used for biochemical composition analysis of the blood, liver, and dorsal muscle.

The experimental feeds were given to the juveniles at four times a day (08:00, 10:00, 14:00, and 16:00) for 12 weeks (= 84 days from 17 February to 11 May 2016). The feeding amount was calculated based on fish weight using the feed guide suggested by commercial feed manufacturer. The initial feeding rate (= day 0) was 7.7% of the total body weight of stocked fish, and thereafter recalculated and decreased as the fish grew using total fish body weight that was monitored every four weeks (cage total body weight was estimated using the weight of the sampled fish which is 10% of total fish per cage). The final feed amount was 5.0% of total fish weight.

At the end of the culture period (= day 84), fish were weighed individually, and 30 juveniles per cage were sampled after one day of starvation prior to sampling. Of these 30 juveniles, 10 were used for proximate composition analysis of the whole body, and 15 were used for biochemical composition analysis of the blood, liver, and dorsal muscle. The remaining 5 juveniles were used for organoleptic examinations that included tests for smell, flavor, and texture. Water temperature during the culture trial was 28.3 ± 1.2°C.

3. Blood component analysis

Fish were anaesthetized in 0.01% 2-phenoxyethanol, and blood was taken using a syringe rinsed with 545 mM sodium citrate dihydrate solution and centrifuged at 3,500 × *g* for 5 min to separate the plasma. The concentrations of plasma glucose, triglyceride, total cholesterol, and phospholipid were measured using commercial kits (Glucose CII Test Wako, Triglyceride E Test Wako, Cholesterol E Test Wako and Phospholipid C Test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma total protein concentration was assayed using a phenol method (Lowry et al. 1951).

4. Chemical component analysis

Moisture, crude protein, crude fat, and ash of the feed, and that of the fish whole body, liver, and dorsal muscle were determined by drying for 8 h at 110°C, using the semi-micro Kjeldahl method (N × 6.25), using diethyl ether extraction through Soxhlet extractor, and heating at 600°C for 5 h, respectively. Crude starch content in the feeds was measured from the digestible simple sugar that was liberated in boiling 30% potassium hydroxide for 2 h, based on the phenol-sulfuric acid method (Dubois et al. 1956). Hepatic glycogen content was measured using a spectrophotometer (UV2400-PC, Shimadzu, Kyoto, Japan) after boiling the samples with anthrone and sulfuric acid (Carrol et al. 1956).

5. Organoleptic examinations

The taste of harvested fish was evaluated using a blind test. Ten fish per feed group (i.e., five fish per net cage) were steamed for 13 minutes, deboned and were served to each participant. A total of 48 people at SEAFDEC/AQD tasted the flesh, and evaluated the smell, flavor, and texture with a 5-grade evaluation. The total organoleptic evaluation of each fish was judged from the total score of each parameter.

6. Statistical analysis

The effects of experimental feeds on growth, feed utilization, and biochemical composition were evaluated using one-way ANOVA and Fisher's Protected Least Significant Difference test. A probability level of less than 0.05 was considered significant. Statistical analysis was conducted using Stat View software (SAS Institute, Cary, NC, USA).

Results

1. Growth performance of fish in the feeding trial

Table 3 shows the growth performance of the juveniles in each dietary group. Weight gain (WG) and specific growth rate (SGR) showed no difference among the dietary groups. The feed conversion ratio (FCR), protein efficiency ratio (PER), feed consumption rate, condition factor, and survival rate were also not significantly influenced by the dietary treatment.

2. Hematological characteristics

Table 4 lists the results of hematological parameter analyses of the juveniles. Plasma glucose, triglyceride, total cholesterol, phospholipid, and total protein concentrations were not significantly affected by PBM levels in the feeds.

3. Whole body, liver and dorsal muscle proximate composition

Table 5 lists the whole body proximate composition of the initial (day 0) and final (day 84) fish samples from each dietary group. Crude fat contents were higher in the final fish samples than the initial samples, whereas moisture content was lower in the final fish samples than in the initial fish samples in each dietary group. Moisture, crude protein, crude fat, and ash contents of the fish samples were not significantly different among the dietary groups.

Table 3. Growth performance of milkfish fed the experimental feeds[1]

Feeds	CTF[2]	LPF[2]	HPF[2]
Initial BW (g / fish)	48.9 ± 4.1	47.4 ± 8.5	47.8 ± 2.4
Final BW (g / fish)	350.7 ± 7.6	333.8 ± 8.9	327.8 ± 9.7
Initial number of fish	1,483 ± 1	1,483 ± 1	1,483 ± 1
Final number of fish	1,395 ± 64	1,437 ± 16	1,419 ± 18
WG (%)[3]	619 ± 45	614 ± 110	587 ± 54
SGR (%)[4]	2.35 ± 0.07	2.33 ± 0.18	2.29 ± 0.09
FCR[5]	2.06 ± 0.06	2.04 ± 0.10	2.17 ± 0.06
PER[6]	1.77 ± 0.05	1.79 ± 0.09	1.72 ± 0.05
Feed consumption rate (%BW / day)[7]	3.87 ± 0.04	3.68 ± 0.08	3.85 ± 0.09
Condition factor[8]	18.6 ± 0.1	18.5 ± 0.1	18.4 ± 0.0
Survival rate (%)[9]	94.07 ± 4.39	96.93 ± 1.00	95.68 ± 1.15

[1] Values are mean ± SD of duplicate cages.
[2] See the footnote of Table 1.
[3] Weight gain (WG) = 100 × (final BW − initial BW) / initial BW
[4] Specific growth rate (SGR) = 100 × {ln(final BW) − ln(initial BW)} / rearing period (days)
[5] Feed conversion ratio (FCR) = dry feed intake / (final BW − initial BW)
[6] Protein efficiency ratio (PER) = (final BW − initial BW) / dry feed protein intake
[7] Feed consumption rate = 100 × dry feed intake (g) / (0.5 × (initial BW + final BW) × days)
[8] Condition factor = 1,000 × BW / fork length^3
[9] Survival rate = 100 × final number of fish / initial number of fish

Table 4. Plasma components of milkfish fed the experimental feeds[1]

Feeds	Initial[2]	CTF[3]	LPF[3]	HPF[3]
Glucose (mg / 100mL)	147	203 ± 5	197 ± 15	192 ± 7
Triglyceride (mg / 100mL)	441	583 ± 180	594 ± 101	541 ± 14
Total cholesterol (mg / 100mL)	393	346 ± 56	364 ± 49	352 ± 22
Phospholipid (mg / 100mL)	1,462	1,542 ± 193	1,598 ± 157	1,533 ± 10
Total protein (g / 100mL)	4.90	4.97 ± 0.02	4.99 ± 0.11	4.85 ± 0.24

[1] Values are mean ± SD of duplicate cages.
[2] Average values of two pooled samples (10 fish / sample)
[3] See the footnote of Table 1.

Table 5. Proximate composition of whole body, liver and dorsal muscle of milkfish fed the experimental feeds

Feeds	Initial[1]	CTF[2]	LPF[2]	HPF[2]
Whole body[3]				
Moisture (%)	68.4	63.1 ± 0.4	63.1 ± 0.7	62.9 ± 0.2
Crude protein (N × 6.25%)	18.2	18.4 ± 0.1	18.6 ± 0.3	18.3 ± 0.2
Crude fat (%)	10.5	16.9 ± 0.3	16.0 ± 1.0	17.1 ± 0.3
Ash (%)	2.8	2.4 ± 0.1	2.5 ± 0.0	2.4 ± 0.0
Liver[4]				
Moisture (%)	62.8	62.4 ± 0.4	63.6 ± 0.7	63.9 ± 0.1
Crude protein (N × 6.25%)	15.6	15.1 ± 0.3	14.9 ± 0.5	14.7 ± 0.2
Crude fat (%)	16.2	18.3 ± 0.2	17.8 ± 0.3	17.6 ± 0.4
Glycogen (%)	1.22	1.16 ± 0.54	1.34 ± 1.06	1.54 ± 0.39
Ash (%)	1.4	1.9 ± 0.1	1.9 ± 0.5	1.4 ± 0.0
HSI (%)[5]	2.53	1.54 ± 0.01	1.42 ± 0.09	1.49 ± 0.05
Dorsal muscle[4]				
Moisture (%)	73.9	72.0 ± 0.4	72.6 ± 0.8	71.9 ± 0.2
Crude protein (N × 6.25%)	22.8	23.2 ± 0.4	23.3 ± 0.1	23.2 ± 0.4
Crude fat (%)	1.3	2.8 ± 0.4	2.2 ± 0.7	2.9 ± 0.4
Ash (%)	1.5	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0

[1] See the footnote of Table 3.
[2] See the footnote of Table 1.
[3] Values are mean ± SD of duplicate cages (two pooled samples, 5 fish / sample).
[4] Values are mean ± SD of duplicate cages (three pooled samples, 5 fish / sample).
[5] Hepatosomatic index (HSI) = 100 × liver weight / BW

Table 5 also shows the hepatic and dorsal muscular proximate compositions of the initial and final fish samples. Hepatic crude protein, crude fat, and glycogen were not influenced by the dietary groups. In addition, the hepatosomatic index (HSI) was not significantly affected by the inclusion levels of PBM in the feeds. Dietary PBM levels had no significant effect on the dorsal muscular moisture, crude protein, crude fat, or ash contents.

4. Organoleptic examinations of harvested fish

Table 6 lists the results of organoleptic examinations using the final fish. Although smell, flavor, and texture points were highest in the CTF group, the difference was very minimal at less than 3%.

Table 6. Organoleptic examination of milkfish fed the experimental feeds[1]

Feeds	CTF[2]	LPF[2]	HPF[2]
Parameter (Point / Share %)			
Smell	194 / 35	180 / 32	185 / 33
Flavor	194 / 35	182 / 33	176 / 32
Texture	187 / 35	173 / 32	180 / 33

[1] Taste test was assessed by 48 people using a 5-grade evaluation.
[2] See the footnote of Table 1.

Discussion

In this study, the main protein source of the CTF group was FM (20%) and SBM (20%) (Table 1), and both raw materials are very popular components of milkfish feed. In the LPF and HPF groups, 50% and 75% FM were replaced by PBM, but none of the growth performance parameters (i.e., WG, SGR, FCR, PER, feed consumption rate, condition factor, survival rate) were significantly different in these groups (Table 3).

The replacement efficiency of FM by PBM has been reported in various fish species. For carnivorous fish species such as Chinook salmon (Fowler 1991), Greater amberjack (Takakuwa et al. 2006), Cuneate drum (Wang et al. 2006), Japanese flounder (Wei et al. 2006), and Cobia (Saadiah et al. 2011), about 20% to

80% of FM can be replaced by PBM without negative impacts on growth. Similarly, the efficiencies of 50% and 66% replacement by PBM were verified in omnivorous fish species such as Gibel carp (Yang et al. 2004) and hybrid tilapia (Fasakin et al. 2005). These cited studies, as well as the results of the present study, indicate that PBM is considered an applicable alternative to FM, and its utilization was not affected by the feeding habits of the fish species. In addition, trials of the complete replacement of FM by PBM were successful in Nile tilapia (El-Sayed 1998), red sea bream (Takagi et al. 2000), and humpback grouper (Shapawi et al. 2007) with no negative effects on growth performance. However, in these past studies, PBM was the only alternative replacement to FM, and the PBM content was very high at 47%, 59%, and 74% in Nile tilapia, red sea bream, and humpback grouper, respectively, though such high PBM-containing feeds are not always practical. Conversely, an excess of PBM in the feed was reported to cause protein indigestion in gilthead seabream (Nengas et al. 1999) and gibel carp (Yang et al. 2004). Indigestible protein also causes environmental pollution of the aquaculture field via nitrogen deposition (Watanabe 2009). Moreover, the phosphorus in PBM is present as tri-calcium phosphate and the low absorption of this compound in fish also causes environmental pollution (Fowler 1991). And in general, the phosphorus content in animal-based protein is considerably higher than in plant-based protein (i.e., oil seed by-products, corn gluten meal) (Watanabe 2009, Gasco et al. 2018). Considering such environmental impacts, a feed with an excessive inclusion of PBM is not likely to be recommended, and a low FM feed containing PBM and plant-based protein is more recommended (Burr et al. 2013, Lu et al. 2015).

In this study, the main source of fat in the CTF was FO (4.5%) (Table 1). Given the higher crude fat content of PBM than that of FM, 10% and 15% FO was replaced by PBM for the LPF and HPF groups. The FO replacement level by PBM was lower than the FM replacement level. However, the price of FO is considerably higher than that of FM; thus, partial replacement of FO by this fat source reduces feed costs (FAO 2016). According to Borlongan (1992a), (n-3) fatty acids, especially eicosapentaenoic acid (EPA, 20:5(n-3)) and docosahexaenoic acid (DHA, 22:6(n-3)), which are only found in FO, and are essential for milkfish, indicate that very low FO may have caused nutrient deficiency in the EPA and DHA groups. However, the growth of the fish examined in this study did not show any negative impacts; therefore, the replacement levels applied here (i.e., up to 15%) were considered acceptable for milkfish.

Plasma total cholesterol concentration decreased with increasing PBM content in the feed, as previously reported in red sea bream (Takagi et al. 2000) and greater amberjack (Takakuwa et al. 2006). Another study reported that FM increases blood cholesterol content (Goulding et al. 1983). Together, these studies suggest that the decrease in plasma total cholesterol concentration was caused not by an increase in the PBM content, but by a decrease in FM content in the feed. In this study, neither the concentration of plasma total cholesterol nor other components were affected by the levels of PBM included in the feed (Table 4). The cause of the difference in plasma total cholesterol concentration between the previous studies and this study is not clear, but a difference in feeding habits may provide some explanation. Red sea bream and greater amberjack are carnivorous, while milkfish is omnivorous and has a lower protein requirement (i.e., low FM requirement) in their feed.

The results of organoleptic examinations indicated that the smell, flavor, and texture of the fillet were almost the same among the feed groups (Table 6). In addition, the proximate composition of the dorsal muscle as well as the whole body and liver were not influenced by the feed groups (Table 5). These results illustrate that the meat quality was not significantly affected by the level of PBM included in the feed (i.e., up to 12%).

Various raw materials are used for aquaculture feed, but their prices depend on various factors such as climate, region, demand, and foreign exchange rate, as well as quality or grade. Therefore, simple comparisons of raw material prices would be difficult. The average import price is a good indicator used to compare the prices of feed ingredients. The main protein sources in this study were FM, SBM, and PBM, and the average import price of FM was more than three times higher than those of SBM and PBM in 2016 (Tridge 2019). Therefore, our results suggest that changing the level of PBM could greatly contribute to reducing milkfish feed costs.

Taking our observations and others into account, we consider that PBM is an acceptable alternative to FM as a good cost performance protein source in milkfish feed. Moreover, PBM did not adversely affect the taste of the harvest fish. However, the quality and nutritional contents of PBM largely vary depending on the raw materials and manufacturing process (Nengas et al. 1999). According to Dong et al. (1993), crude protein and crude fat varied from 55% to 75% and from 10% to 19%, respectively, among six different PBM manufacturers. Such variations in PBM quality can affect the growth performance and apparent protein

digestibility coefficient (Shapawi et al. 2007). Thus, the quality control of PBM is required for further application in fish feed.

It is possible that the FM content in milkfish feed could be completely replaced by PBM. It would be interesting to compare the performance between commercial feed and our original PBM feed. Therefore, we aim to conduct further demonstration trials using non-FM feed and commercial feed to further develop a suitable milkfish feed low in FM content.

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