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High Potential of Poultry by-product Meal as a Main Protein Source in the Formulated Feeds for a Commonly Cultured Grouper in Malaysia (*Epinephelus fuscoguttatus*)

(Potensi Tinggi Tepung Hasil Sampingan Ayam sebagai Sumber Utama Protein dalam Makanan Rumusan untuk Ikan Kerapu yang Biasa Diternak di Malaysia (*Epinephelus fuscoguttatus*))

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ABSTRACT

A trial was carried out to evaluate the possibility of replacing fish meal with poultry by-product meal (PBM) at high inclusion levels (50-100%) in the formulated feeds of tiger grouper, Epinephelus fuscoguttatus. *Fish meal-based feed (PBM0) served as the control feed and three PBM-based feeds with inclusion levels of 50 (PBM50), 75 (PBM75), and 100% (PBM100) were fed to triplicate groups of fish with mean body weight of 26.2±0.2 g. All formulated feeds were isoproteic (50%) and isolipidic (13%). Weight gain of juveniles ranged from 233 to 338% at the end of feeding trial. Final weight (g), weight gain (%) and specific growth rate (% day^{-1}) of fish fed PBM0 were lower than other fish groups. The feed conversion ratio ranged from 1.1 (PBM50) to 2.0 (PBM0) with no significant difference detected in all treatments. Apparent digestibility coefficients (ADCs) were influenced by the inclusion of PBM in the feeds, with PBM50 recording better values in all measured ADCs. Meanwhile, replacement of fish meal with PBM has little influence on the whole body proximate compositions and body indices. The present study shows that PBM is an excellent alternative protein source for farming the tiger grouper juveniles with fish meal protein replacement level of 50% resulted in the best overall performances.*

Keywords: Alternative ingredients; Epinephelus fuscoguttatus*; fish meal replacement; marine fish farming; poultry by-product meal; tiger grouper*

ABSTRAK

Satu kajian telah dijalankan untuk menilai kemungkinan mengganti tepung ikan dengan tepung hasil sampingan ayam (PBM) pada tahap penggantian yang tinggi (50-100%) di dalam makanan kerapu, Epinephelus fuscoguttatus. *Makanan berasaskan tepung ikan (PBM0) bertindak sebagai makanan kawalan dan tiga makanan berasaskan PBM dengan kadar penambahan 50 (PBM50), 75 (PBM75) dan 100% (PBM100) diberi makan kepada kumpulan triplikat ikan yang mempunyai berat badan purata 26.2±0.2 g. Kesemua diet berformulasi mengandungi protein (50%) dan lipid (13%) yang sama. Pertambahan berat juvenil menjulat antara 233 to 338% pada akhir percubaan. Berat akhir (g), pertambahan berat (%) dan kadar pertumbuhan spesifik (% hari^{-1}) ikan yang diberi makan PBM0 adalah lebih rendah daripada kumpulan lain. Kadar penukaran makanan menjulat dari 1.1 (PBM50) hingga 2.0 (PBM0) dengan tiada perbezaan bererti di antara semua rawatan. Koefisyen penghadaman ketara (ADCs) dipengaruhi oleh penambahan PBM dalam diet, dengan PBM50 memberikan nilai yang lebih baik bagi semua ADC yang diukur. Manakala penggantian tepung ikan dengan PBM tidak memberikan kesan yang besar terhadap kandungan proksimat dan indeks badan. Kajian ini menunjukkan PBM adalah sumber protein alternatif yang cemerlang untuk penternakan juvenil kerapu harimau dengan penggantian protein tepung ikan pada kadar 50% memberikan hasil keseluruhan yang terbaik.*

Kata kunci: Epinephelus fuscoguttatus; *kerapu harimau; penggantian protein; penternakan ikan marin; ramuan alternatif; tepung hasil sampingan ayam*

INTRODUCTION

The sustainable growth of tropical marine fish farming in Southeast Asia is hindered by several problems including the dependency of the industry on fish-based feeds. Apart from the use of problematic trash fish, the industry is also relying on formulated feed which is mostly based on fisheries resources as major ingredients. Search of suitable and sustainable feed ingredients is critical in order to support this rapidly growing industry. The tiger grouper, *Epinephelus fascoguttatus* is one of the most widely cultured grouper species due to its relatively faster growth and high adaptability in captivity compared with other grouper species. However, being a strict carnivorous fish, grouper requires high protein in the diets which results in higher feed cost. In addition, the decreasing retail price for cultured tiger grouper in recent years has caused farmers to find ways to reduce the production costs. Using a cheaper alternative protein source will help reduce the feeding cost

and support the sustainable growth of the grouper farming industry. Limited information is available on the full potential of PBM-based feeds in grouper. In a study using humpback grouper, *Cromileptes altivelis* (Valenciennes) (Shapawi et al. 2007) and malabar grouper, *Epinephelus malabaricus* (Li et al. 2009) as target species, poultry by-product meal (PBM) showed promising results when used to replace fish meal. Since fish response to their feed item is suggested to be species-specific (NRC 1993), the possibility of replacing fish meal with PBM in other grouper species deserves an investigation. Successful attempts to include PBM at high inclusion levels were reported by other authors using different fish species (Gaylord & Rawles 2005; Nengas et al. 1999; Takagi et al. 2000). PBM is seen as one of the most promising alternative ingredients for carnivorous fish species due to its good nutritional profile, high digestibility and supply which is available throughout the year. In the present study, significant levels of FM (50 -100%) were replaced with PBM in the diets of tiger grouper juveniles for the effects on growth, feed utilization, survival, body proximate composition, body indices and feed apparent digestibility coefficients (ADC).

Materials and Methods

FEEDING TRIAL

Four experimental feeds were formulated to contain equal amounts of crude protein (50%) and crude lipid (13%). Fishmeal was replaced with pet-food grade PBM (National Renderers Association (NRA), USA) at 50, 75 and 100% (PBM50, PBM75, PBM100) inclusion levels. The control diet (PBM0) contained 100% FM which was obtained from a local fish meal manufacturer (QL Marine Products Sdn. Bhd., Malaysia). Tiger grouper juveniles with the mean initial body weight of 26.2±2 g were obtained from a local fish farm in Sabah, Malaysia. A total of 15 cylindrical cages (42.5 cm in diameter and 43.5 cm in height) were randomly stocked with 30 fish in each cage. Cage arrangement was in a completely randomized design placed in a 150 ton FRP (fibreglass reinforced plastics) tank equipped with recirculation and aeration system. Water recirculation in the tank was 2080 L/ min, which allowed filtered water to be supplied continuously. Water parameters (dissolved oxygen, temperature and pH) were monitored daily throughout the experimental period. Fish were individually weighed at the beginning and the end of the feeding trial and bulk-weighed every fortnightly. Experimental feeds were given twice daily (0800 and 1600 h) to apparent satiation level to all triplicate treatments. The experiment was conducted for 16 weeks.

DIGESTIBILITY STUDY

At the completion of the feeding trial, remaining fish from the same treatments were pooled and distributed randomly into duplicate sets of fibreglass tanks (300 L) for faeces collection. Fresh and intact faeces were collected by slowly siphoning the tank bottom 2 h after each feeding (Shapawi et al. 2007). The collected faeces were then rinsed with distilled water, dried on filter paper and immediately frozen (Lin et al. 2004). Faeces collection was done daily from each tank until sufficient amount was obtained. Acid digestion method (Furukawa & Tsukahara 1966) was used to determine chromic oxide concentration in the experimental feeds and faeces.

SAMPLE COLLECTION AND ANALYSIS

A representative sample of five fish was sacrificed before commencing the feeding trial and kept frozen in -80°C prior to whole body proximate analysis. Samples of liver and viscera from fish in each treatment were removed and weighed to determine the hepatosomatic index (HSI) and viscerosomatic index (VSI). Dry matter was determined after dyring samples in an oven at 105°C until constant weight. Ash was analysed by incineration in a muffle furnace at 550°C. Crude protein was determined by Kjeldahl method and crude lipid analysis was conducted using the ether extraction method. Experimental feed samples were hydrolysed using 6N HCl at 110°C for 24 h and then derivatized with AccQ reagent (6-aminoquinolyl-N-hydroxysuccinimdyl carbamite) before chromatographic separation using an AccQ Tag™ reversed phase (3.9 × 150 mm) analytical column (Waters®).

The amino acid analysis was performed on a HPLC system which consisted of Waters 1525 Binary HPLC Pump, 717 Plus auto-sampler (Waters®) and Waters 2475 Multi λ Fluorescence detector (wavelength excitation 250 nm, emission 395 nm). Chromatographic peaks were integrated, identified and quantified with Breeze™ software, version 3.20 by comparing to known standards (Amino acid standard H, Pierce, Rockford, Illinois, USA). Methionine and cysteine were determined from the same method of acid hydrolysis after treatment with performic acid oxidation. One-way ANOVA was used to compare the growth performance, feed utilization efficiency, whole-body proximate composition, body indices and ADC. Homogeneity of variances was tested with Levene's test and multiple comparisons among treatments were performed with a Tukey HSD post-hoc test. Significance level was set at 0.05. Statistical package SPSS v.11.0 for Windows was used for all statistical analyses.

Results

The dietary and proximate compositions of ingredients and experimental feeds are shown in Tables 1 and 2. Both FM and PBM contain high levels of protein (631-684 g kg^{-1}). The FM used in this experiment was low in fat and high in ash content, a typical composition of FM produced from fisheries by-catches. Analysed protein and lipid levels of all experimental feeds corresponded to the calculated levels. Amino acid composition of the experimental feeds indicated that the PBM-based feeds has numerically lower levels of the essential amino acid (EAA), lysine

and methionine (Table 3), with lysine as the first limiting EAA. Methionine level also decreased with increasing amounts of PBM. All EAA values were highest in PBM0 except for arginine and histidine. Average values of EAA in PBM diets showed that the higher the replacement, the lower the composition of EAA. Fish fed PBM50 performed significantly better than the control diet (PBM0) in terms of final weight, weight gain and SGR. The fish attained final weight of 119.5 g, percentage weight gain of 337.6% and specific growth rate of 1.2 % day^{-1} at the termination of the feeding trial (Table 4). There were no significant differences (p>0.05) detected in final weight, weight gain and SGR among fish fed PBM-based diets even though total replacement of fish meal with PBM (PBM100) resulted in slower growth of fish compared with partial replacement at 50 and 75%. Dry matter FCR values showed similar trend with growth values range between 1.1 (PBM50) and 2.0 (PBM0) and these values were not significantly different (p>0.05) among the formulated feeds. PER value was the lowest in PBM0 followed by PBM100, PBM75 and PBM50. No significant difference (p>0.05) was observed in NPUs among formulated feed treatments. Survival rates were all

TABLE 1. Dietary composition of experimental feeds

Ingredients (g kg^{-1})	Test Diets			
	PBM0	PBM50	PBM75	PBM100
Fish meal	793	396	198	0.0
Poultry by-product meal	0.0	365	548	731
Tapioca starch	40	43	45	46
Cod liver oil	22	41	51	43
Poultry fat	70	26	5	0.0
Vitamin premix	30	30	30	30
Mineral premix	20	20	20	20
Carboxymethylcellulose	10	10	10	10
Dicalcium phosphate	10	10	10	10
Chromic oxide	10	10	10	10
Alpha- cellulose	0.0	48	74	100

PBM0, Control diet; PBM50, PBM75 & PBM100 – Diets with poultry by-product meal replacing fish meal at 50, 75 and 100%, respectively

TABLE 2. Proximate composition of feed ingredients and experimental feeds (g kg^{-1}, dry matter basis)

Ingredients	Moisture	Ash	Crude lipid	Crude protein	*NFE
FM	101	268	48	631	53.4
PBM	40	147	119	684	49.5
Experimental feeds					
PBM0	128	117	128	491	136
PBM50	97	93	119	518	173
PBM75	109	83	124	516	168
PBM100	90	73	125	520	192

Refer to Table 1 for diet designations
*NFE, nitrogen-free extract= 1000 – (gkg^{-1} ash + gkg^{-1} lipid + gkg^{-1} protein)
FM, fish meal; PBM, poultry by-product meal

TABLE 3. Essential amino acid (EAA) composition in pelleted feeds (mean values, % dry matter)

	PBM0	PBM50	PBM75	PBM100
Arginine	3.75	4.01	4.30	4.25
Histidine	1.31	1.35	1.40	1.46
Isoleucine	2.17	2.12	2.00	1.97
Leucine	3.70	3.63	3.52	3.47
Lysine	4.25	3.42	3.22	3.45
Methionine	1.88	1.49	1.35	1.26
Phenylalanine	2.05	2.06	2.09	2.00
Threonine	2.16	2.20	2.27	2.14
Valine	2.42	2.40	2.31	2.31

Refer to Table 1 for diet designations

above 90% and did not influence by the dietary ingredients. The fish remained in good health throughout the feeding trial where neither deformity nor disease was observed. The whole-body moisture, ash, protein and lipid of fish fed formulated feeds ranged from 704.6 to 714.6 g kg^{-1}, 54.0 to 56.8 g kg^{-1}, 157.4 to 166.0 g kg^{-1} and 73.8 to 85.6 g kg^{-1}, respectively and were not affected by the dietary PBM inclusion. There were no significant differences (p> 0.05) recorded in the HSI and VSI in all treatments (Table 5). The highest value of dry matter ADC was recorded in PBM50 followed by PBM75, PBM0 and PBM100 and these values were not significantly different. The ADCs for protein were significantly higher (p<0.05) in PBM50 (87.06%) and PBM75 (85.85%) than those in PBM0 (73.37%) and PBM100 (74.47%). Apparent lipid digestibility (ALD) for PBM100 was significantly lower than other experimental feeds. Overall, PBM50 showed higher digestibility values in dry matter, protein and lipid than other groups (Table 6).

TABLE 4. Growth and feed utilization of tiger grouper fed experimental feeds and trash fish after 6 weeks

	Feeding treatments			
	PBM0	PBM50	PBM75	PBM100
Final weight (g)	90.5±8.3^{B}	119.5±7.9^{A}	110.7±5.5^{A}	106.5±1.3^{A}
*Weight gain (%)	240.0±27.5^{B}	337.6±26.6^{A}	305.0±31.8^{A}	291.6±10.8^{AB}
#SGR (% d^{-1})	1.0±0.06^{B}	1.2±0.05^{A}	1.2±0.06^{A}	1.1±0.02^{AB}
Survival (%)	93.0±1.5	97.0±0.5	97.0±0.5	97.0±0.0
Total feed intake (g/fish)	131.4±28.4^{B}	105.5±6.7^{B}	105.6±5.5^{B}	93.7±3.4^{B}
+FCR	2.0±0.20^{B}	1.1±0.03^{B}	1.3±0.04^{B}	1.2±0.07^{B}
^PER	1.0±0.1^{B}	1.7±0.0^{A}	1.5±0.0^{A}	1.6±0.1^{A}
~NPU	29.8±4.0^{A}	43.9±5.5^{A}	35.2±1.0^{A}	38.8±3.2^{A}

Refer to Table 1 for diet designations
Values are the mean triplicate groups of 30 fish.
Average weight of initial fish was 26.4±2 g
Values with different superscripts within rows are significantly different (P<0.05)
*Weight gain = (final weight – initial weight) x 100/initial weight
#Specific growth rate (SGR) = [(ln final weight – ln initial weight)/days] x 100
+Feed conversion ratio (FCR) = feed fed (g)/ weight gained (g)
^Protein efficiency ratio (PER) = wet weight gain (g)/ total protein intake (g)
~Net protein utilization (NPU) = 100 x (final – initial fish body protein)/ total protein intake

TABLE 5. Whole body proximate composition (g kg^{-1}) and body indices of experimental fish

Experimental feeds[1]	PBM0	PBM50	PBM75	PBM100
Moisture	714.6±1.6^{a}	708.4±1.6^{a}	709.2±3.0^{a}	704.6±1.7^{a}
Crude protein	163.6±0.5	166.0±0.7	158.6±0.5	157.4±0.4
Crude lipid	73.8±0.6^{a}	85.6±0.6^{a}	84.2±0.7^{a}	76.0±0.9^{a}
Ash	55.4±0.2^{ab}	56.8±0.2^{a}	55.4±0.2^{ab}	54.0±0.2^{ab}
HSI*	1.37±0.07^{ab}	1.12±0.09^{a}	0.99±0.46^{a}	1.53±0.32^{b}
VSI#	6.93±1.28	7.08±1.59	7.26±1.57	8.41±1.40

Refer to Table 1 for diet designations
Values with different superscripts within rows are significantly different (P<0.05)
Moisture, crude protein, crude lipid and ash of initial fish were 706±0.4, 166.8±0.2, 71.0± 0.5 and 55.4± 0.3 g kg^{-1}, respectively
HSI*, hepatosomatic index
VSI#, viscerosomatic index

TABLE 6. Apparent digestibility coefficient (ADCs) for dry matter, crude protein and lipid of the experimental feeds

Experimental feeds	ADCs (%)		
	ADMD*	APD$^{\alpha}$	ALD$^{¥}$
PBM0	58.97±1.46^{a}	73.37±5.93^{b}	89.23±2.38^{a}
PBM50	62.42±1.40^{a}	87.06±1.63^{a}	88.06±2.09^{a}
PBM75	60.06±4.72^{a}	85.85±0.22^{a}	88.14±1.71^{a}
PBM100	58.67±3.53^{a}	74.47±5.42^{b}	86.41±3.04^{b}

Refer to Table 1 for diet designations
Values with different superscripts within column are significantly different (p<0.05)
*ADMD (Apparent dry matter digestibility) = 100 X [1- (% dietary chromic oxide/ faeces chromic oxide)
$^{\alpha}$APD (Apparent protein digestibility)= 100 x [1- (%faeces protein / % dietary protein) x (% dietary chromic oxide / faeces chromic oxide)]
$^{¥}$ALD (Apparent lipid digestibility) = 100 x [1- (%faeces lipid / % dietary lipid) x (% dietary chromic oxide / faeces chromic oxide)]

Discussion

The tiger grouper with an average initial weight of 26.2 g attained up to 337% weight gain after 16-weeks of feeding trial. A significantly lower (p>0.05) growth of fish fed control diet compared to PBM-based diets suggests that PBM can be efficiently utilized by tiger grouper juveniles. Numerous attempts have been made to evaluate the effects of PBM on growth and feed utilization efficiency of fish and the findings were remarkably promising. PBM has been demonstrated nutritionally adequate protein source for various fish species. However, not many studies have been carried out to investigate the full potential of poultry by-product meal in the feeds of grouper species. In a similar study conducted on humpback grouper (*Cromileptes altivelis*), high quality PBM was able to replace FM at up to 100% replacement level without adverse effects on growth performance (Shapawi et al. 2007). High replacement level (>50%) of FM with PBM were also reported in different fish species such as salmonids (Steffens 1994), gilthead seabream (Nengas et al. 1999), red sea bream (Takagi et al. 2000) and largemouth bass (Subhadra et al. 2006). Meanwhile, negative effects on growth performance and nutrient utilization were observed when more than 50% of FM was replaced with PBM in black sea turbot (Yigit et al. 2006), European eel (Gallagher & Degani 1988) and chinook salmon (Fowler 1991). The use of local FM (PBM0) as a sole source of protein did not give superior results compared to PBM-based diets. The FM used in the present study is characterized by high ash content which can cause low digestibility in fish feed (Millamena 2002). Even though there was no significant difference detected in FCR of the pelleted feeds, it is noteworthy that FCR of the control diet was higher than in PBM-based diets. The lower quality of FM used in the present study might also have contributed to the poorer growth and FCR of the control diet. Fish meal manufacturers in Malaysia use fish from the fishery by-catches. It is well-documented that fish meal quality is dependent on the quality of the raw fish and processing technology used. Interestingly, combination of good quality PBM and local fish meal at 1:1 ratio apparently is able to support good growth, feed utilization and survival of tiger grouper in captivity. The experimental feeds were acceptable by fish at all levels of FM replacement.

The EAA composition of PBM-based feeds in the present study is also similar to the EAA composition of PBM-based feeds in the previous study using humpback grouper as a target species (Shapawi et al. 2007) and is strongly influenced by PBM level in the diets where methionine and lysine are often reported as the limiting amino acids (Gaylord & Rawles 2005; Shapawi et al. 2007). To the best of our knowledge, there is no information on the dietary EAA required of tiger grouper. The dietary methionine requirement was reported to be 1.18% in juveniles humpback grouper (Giri et al. 2005) and 1.31% in orange-spotted grouper (Lou et al. 2005). Except in PBM100, methionine content in all experimental feeds in the present study was above 1.3%. In a separate study by Luo et al. (2006), the optimum requirement of juveniles orange-spotted grouper for L-lysine was 2.83% of the diet. The optimum requirement of arginine was very similar to lysine which was reported to be around 2.73 – 2.8% of the diets in *E. coioides* and *E. awoara* (Luo et al. 2007; Zhou et al. 2012). In the present study, lysine and arginine contents in all formulated experimental feeds ranged between 3.22% (PBM75) and 4.25 % (PBM0) and 3.75% (PBM0) and 4.30% (PBM75), respectively. It was also observed that arginine and histidine values were higher in the replacement diets compared with the control treatment (PBM0). This trend was also observed in our previous study on humpback grouper (Shapawi et al. 2007). Unfortunately, the optimum requirement of tiger groupers for both arginine and histidine are not yet known. Apart from the origin of the PBM, the performance of PBM-based feeds in various fish species is also significantly influenced by the processing methods used (Dong et al. 1993).

Whole-body proximate composition was not affected by the replacement of FM with PBM. This is in agreement with Takagi et al. (2000) which reported no significant difference in whole-body composition of red sea bream fed different levels of dietary PBM. In the previous study using humpback grouper (Shapawi et al. 2007), replacement of fish meal with PBM did not influence the whole-body moisture and lipid content. However, whole-body ash tends to increase with the increase of PBM in the diets. Higher HSI of fish fed PBM100 than fish in other groups were observed. HSI of fish in PBM0, PBM50 and PBM75 were comparable to HSI values of other grouper species (Shapawi et al. 2007; Wang et al. 2008). The VSI values were not significantly different in all treatments. The results indicated that the different inclusion levels of PBM have little influence on the body indices of tiger grouper. The ADCs of experimental diets were influenced by the inclusion level of PBM. The lower dry matter and crude protein ADCs in PBM0 and PBM100 were probably one of the major factors influencing the poorer growth of fish in this group. The ash content of FM used in the present study is considered high and may relate to the reduced digestibility in the diets. Meals that contain high ash are generally considered to be less digestible for fish (Parsons 1997; Stone et al. 2000). Dry matter and crude protein ADCs for FM-based diets ranged between 54 and 89.2% and 71 and 98.5%, respectively, in humpback grouper and orange-spotted grouper (Eusebio et al. 2004; Laining et al. 2004; Shapawi et al. 2007). Protein ADCs (73 – 87%) in the present study were comparable to our previous study using *C. altivelis* (Shapawi et al. 2007) and in gibel carp (Yang et al. (2006) and slightly better than the values reported in rainbow trout (60%) (Nengas et al. 1999) and in sunshine bass (*Morone chrysops x M. saxatlis*) (55 – 61%) (Pine et al. 2008). The highest protein ADC in PBM50 might explain the better growth performance of tiger grouper fed PBM50. Different source and quality of PBM might also influence the digestibility of the feeds (Dong et al. 1993). The present lipid ADCs of PBM-based feeds (86.41 – 88.14%) were slightly lower than the values reported in humpback grouper (91.7 – 96.7%)

(Shapawi et al. 2007) and tiger grouper (91.2 to 95.4%) (Usman et al. 2007). In the present study, added poultry fat was slightly higher in PBM100 which probably resulted in slightly lower lipid digestibility. The effects of essential amino acid (Gaylord & Rawles 2005; Gropp et al. 1979), phosphorus and organics acids supplementation (Sarker et al. 2012) in feeds formulated with alternative ingredients deserves an investigation in grouper species to improve the performance of these non-fish meal-based feeds.

CONCLUSION

In general, high quality PBM showed high potential to be used as a main source of protein in the diets of juvenile tiger grouper which will help reduce the dependency of tropical marine fish farming on fish-based feeds.

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