

Replacing fish meal with a blend of poultry by-product meal and feather meal in diets for giant croaker (*Nibea japonica*)

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Abstract

An 8-week feeding trial was conducted to evaluate the potential of replacing fish meal with poultry by-product meal (PBM) and feather meal (FEM) in giant croaker (*Nibea japonica*) diet. The control diet (C) contained 400 g/kg fish meal, and 20%, 40%, 60% and 80% of the fish meal in diet C was replaced by a blend of PBM and FEM (PBM:FEM = 7:3) in diets B20, B40, B60 and B80, respectively. The weight gain and feed intake of fish fed diet C did not differ from those of fish fed diets B20 and B40 ($p > .05$), but were higher than those of fish fed diets B60 and B80 ($p < .05$). Phosphorus retention efficiency was lower in fish fed diets C, B20 and B40 than in fish fed diets B60 and B80 ($p < .05$). No significant differences were found in feed conversion ratio, nitrogen retention efficiency, condition factor, hepatosomatic index, body composition and nitrogen waste among the treatments ($p > .05$). Ratio of fish meal consumption to fish production linearly declined with the decrease in dietary fish meal level. This study indicates that dietary fish meal for giant croaker could be reduced to 240 g/kg by inclusion of the blend of PBM and FEM.

KEYWORDS

feather meal, feed utilization, giant croaker, poultry by-product meal, waste output, weight gain

1 | INTRODUCTION

The amount of fish meal used in aqua-feed has been growing due to the rapid expansion of global aquaculture industry. In 2010, 73% of fish meal production was used for aquaculture, which was 7.3 times higher than that used in 1980 (Shepherd & Jackson, 2013). Fish meal is a limited and expensive resource (Olsen & Hasan, 2012), and the prices of fish meal have been kept above US\$1100 per ton since 2006 (Hardy, 2010). Replacing fish meal with alternative protein ingredients in aqua-feed has been recognized as a way to establish sustainable aquaculture industry (Naylor et al., 2009).

Rendered animal protein ingredients, such as poultry by-product meal (PBM), meat and bone meal, feather meal (FEM) and blood meal, are excellent alternative ingredients of fish meal due to their high protein content, excellent amino acid profile, plenty in supplementation and competitive price (Allan et al., 2000; Bishop, Angus,

& Watts, 1995; Boger et al., 2001; Bureau et al., 2000; Metts et al., 2011; Papadopoulos, Boushy, Roodbeen, & Ketelaars, 1986). Among the rendered animal protein ingredients, PBM has been demonstrated a quality dietary fish meal substitute for carnivorous fishes, such as largemouth bass *Micropterus salmoides* (Tidwell, Coyle, Bright, & Yasharian, 2005), cuneate drum *Nibea miichthioides* (Wang, Guo, Bureau, & Cui, 2006), humpback grouper *Cromileptes altivelis* (Shapawi, Ng, & Mustafa, 2007), malabar grouper *Epinephelus malabaricus* (Li et al., 2009), cobia *Rachycentron canadum* (Zhou, Zhao, Li, Wang, & Wang, 2011), Florida pompano *Trachinotus carolinus* (Rossi & Davis, 2012) and Japanese sea bass *Lateolabrax japonicus* (Wang, Wang, Ji, Han, & Li, 2015). The potential of feather meal to replace dietary fish meal has been evaluated in chinook salmon *Oncorhynchus tshawytscha* (Fowler, 1990), Japanese flounder *Paralichthys olivaceus* (Kikuchi, Furuta, & Honda, 1994), African catfish *Clarias gariepinus* (Chor, Lim, & Shapawi, 2013) and tench *Tinca tinca* (González-Rodríguez, Celada, Carral, Sáez-Royuela, & Fuertes, 2014). Compared with PBM, FEM is a poor alternative ingredient of fish meal, and high feather meal

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inclusion results in growth decline of cuneate drum (Wang et al., 2006). Previous studies reported that more fish meal in diets for cuneate drum and malabar grouper could be replaced by a blend of PBM, meat and bone meal, FEM and blood meal than that replaced by these ingredients alone (Guo, Wang, & Bureau, 2007; Wang, Li, Han, Zheng, & Bureau, 2008). According to this viewpoint, using various rendered animal protein ingredients in combination might be a way to formulate highly nutritive and cost-effective fish feed because nutrient balance and economic performance of a blend of rendered animal protein ingredients are usually better than those of a single ingredient (Rawles et al., 2011).

Giant croaker (*Nibea japonica*) is a carnivorous fish for marine aquaculture in South Korea and China (Chai, Ji, Han, Dai, & Wang, 2013; Lee, Cho, Lee, & Yang, 2001). The appropriate dietary protein, lipid and carbohydrate levels for juvenile giant croaker are 480 g/kg, 90 g/kg and 122–127 g/kg, respectively (Chai et al., 2013; Han et al., 2014; Li, Wang, Han, Hu, & Jiang, 2015). In commercial farming, the cost-effective feed for giant croaker has not been available, and the fish is fed with raw fish. The capacity of giant croaker in utilizing rendered animal protein ingredients as dietary protein source remains unknown. In this study, we examined the effect of replacing dietary fish meal with a blend of PBM and FEM on feed intake, growth, feed utilization, body composition and waste output of giant croaker. The objective aimed at evaluating the optimal inclusion level of fish meal, PBM and FEM in diet formulation for giant croaker.

2 | MATERIALS AND METHODS

2.1 | Feed ingredients and test diets

Fish meal used in this study was steam-dried red fish meal imported from New Zealand. Poultry by-product meal and feather meal were supplied by the Asian regional office, National Renderers Association (NRA). Menhaden fish oil (USP) was imported from America. The other feed ingredients were purchased from Kesheng Feed Company (Shaoxing, China). Proximate composition (g/kg) of the feed ingredients is shown in Table 1.

A single-factor experiment was designed. The control diet (C) was formulated to contain 400 g/kg fish meal. In the other four diets, 20%, 40%, 60% and 80% of the fish meal in diet C were replaced by a blend of poultry by-product meal (PBM) and feather meal (FEM) at an equal protein basis (the diets were abbreviated as B20, B40, B60 and B80).

The ratio of PBM to FEM in the blend was 7:3. The test diets were formulated to contain 480 g/kg crude protein and 90 g/kg crude lipid (Chai et al., 2013). Crystalline DL-methionine and L-lysine were added in diets B20, B40, B60 and B80 to prevent from the deficiency in methionine and lysine.

The feed ingredients were ground with a hammer grinder to pass through a 0.5-mm sieve. The feed ingredients were weighed, mixed by hand and then mixed in a kitchen mixer with quantitatively added water for 10 min. The pellets (3 mm diameter, 5 mm length) were extruded with a single-screw laboratory scale extruder (SLP-45; Fishery Machinery and Instrument Research Institute, Chinese Academy of Fishery Sciences). The test diets were dried at 24°C in an air-conditioned room and stored in plastic bags at -20°C until use. Formulation and proximate composition of the test diets are shown in Table 2, and amino acid profile is shown in Table 3.

2.2 | Fish and feeding trial

The feeding trial was carried out in the field station of the Marine Fisheries Institute of Zhejiang Province (Zhoushan, China). Giant croaker were hatched by the field station. The fish were reared in indoor concrete tanks (5 × 3 × 1.2 m) and fed with a commercial feed (New Love-Larva, Japan Forestry and Industry Co., LTD). Prior to the feeding trial, 375 fish with similar body size were selected and sorted into 15 flow-through polyethylene tanks (volume 200 L) at 25 fish per tank. The fish were acclimated in the tanks for 4 weeks during which the fish were fed with diet C twice daily.

At the start of the feeding trial, the acclimated fish were deprived of feed for 24 hr. Fifteen groups 20 fish each were bulk weighed and then randomly sorted into 15 tanks. Each treatment had three replicates. Initial body weight of fish was 20.7 ± 0.5 g (mean ± SD, n = 15). Three groups 10 fish each were sampled from the remaining fish. After anesthetized with clove oil (60 mg/L), body length and body weight were measured for determination of condition factor, and then, liver weight was measured for determination of hepatosomatic index. The sampled fish were frozen at -20°C for the analysis of initial body composition.

Duration of the feeding trial was 8 weeks during which fish were fed to satiation at 08:00 and 16:00 hr daily. Sand-filtered seawater flowed through the tanks at 3 L/min continuously. Water temperature was measured in the morning daily and fluctuated from 24.5 to 27.9°C. Salinity was measured weekly and ranged from 25 to 31 ppt.

Ingredient	Dry matter	Crude protein	Crude lipid	Ash
Fish meal, steam-dried	908	662	84	162
Poultry by-product meal	943	671	126	124
Feather meal	931	822	100	23
Soybean meal	900	454	29	62
Rapeseed meal	881	372	5	20
Wheat middling	875	177	41	33

TABLE 1 Proximate composition (g/kg) of the feed ingredients

Crude protein, crude lipid and ash are expressed on basis of the ingredients stored in air (n = 2).

TABLE 2 Formulation (g/kg) and proximate composition (g/kg) of the test diets

Ingredients	C ^a	B20 ^a	B40 ^a	B60 ^a	B80 ^a
Fish meal, steam-dried	400	320	240	160	80
Poultry by-product meal	35	87	139	190	242
Feather meal	15	37	59	82	104
Soybean meal	240	240	240	240	240
Rapeseed meal	60	60	60	60	60
Wheat middling	123	128	133	139	134
Starch, gel.	20	20	20	20	20
Brewer's yeast	20	20	20	20	20
Ca(H ₂ PO ₄) ₂	15	15	15	15	15
Choline chloride	2	2	2	2	2
L-Lysine	0	2	4	6	8
DL-Methionine	0	1	2	3	4
Vitamin and mineral premix ^b	20	20	20	20	20
Menhaden fish oil	50	48	46	43	41
Dry matter	857	874	871	877	881
Crude protein ^c	457	465	464	472	476
Crude lipid ^c	101	103	103	96	99
Ash ^c	105	102	95	99	85
Phosphorus ^c	16	15	14	13	12

^aC: Control; B20: 20% of the fish meal was replaced; B40: 40% of the fish meal was replaced; B60: 60% of the fish meal was replaced; B80: 80% of the fish meal was replaced.

^bVitamin and mineral premix (per kg diet): retinyl acetate, 3,000 IU; cholecalciferol, 2,400 IU; all-rac- α -tocopheryl acetate, 60 IU; menadione sodium bisulphite, 1.2 mg; ascorbic acid monophosphate (49% ascorbic acid), 120 mg; cyanocobalamin, 0.024 mg; d-biotin, 0.168 mg; choline chloride, 1,200 mg; folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine.HCl, 6 mg; riboflavin, 7.2 mg; thiamine.HCl, 1.2 mg; sodium chloride (39% Na, 61% Cl), 3077 mg; ferrous sulphate (20% Fe), 65 mg; manganese sulphate (36% Mn), 89 mg; zinc sulphate (40% Zn), 150 mg; copper sulphate (25% Cu), 28 mg; potassium iodide (24% K, 76% I), 11 mg; Celite AW521 (acid-washed diatomaceous earth silica), 1,000 mg.

^cCrude protein, crude lipid, ash and phosphorus are expressed on basis of the diets stored in air.

TABLE 3 Amino acid content (g/kg) of the test diets

Diet	Met	Lys	Thr	Ile	His	Val	Leu	Arg	Phe	Tyr	Asp	Ser	Glu	Pro	Gly	Ala
C ^a	5.3	30.7	17.5	18.8	15.8	20.6	33.6	24.8	19.2	9.9	43.2	20.8	69.0	18.2	26.0	26.9
B20 ^a	6.8	31.8	17.7	19.2	15.4	21.2	34.1	26.1	19.6	10.2	39.6	22.5	70.0	20.3	28.2	27.2
B40 ^a	5.5	31.0	17.9	19.2	14.8	21.5	34.1	26.6	19.8	9.8	45.7	24.1	69.7	23.0	30.2	26.6
B60 ^a	6.8	32.4	18.8	20.3	15.1	22.8	35.8	29.0	21.2	11.0	40.4	26.8	72.1	24.6	33.2	27.8
B80 ^a	6.8	31.4	18.4	19.8	14.2	22.5	34.8	28.8	20.5	10.4	40.2	27.2	69.6	26.5	34.6	26.9

Amino acid contents are expressed on basis of the diets stored in air ($n = 2$).

^aC: Control; B20: 20% of the fish meal was replaced; B40: 40% of the fish meal was replaced; B60: 60% of the fish meal was replaced; B80: 80% of the fish meal was replaced.

Dissolved oxygen was measured occasionally and was always greater than 6.0 mg/L. Ammonia concentration was undetectable in the tanks. Photoperiod was 14 hr light: 10 hr dark.

At the end of the feeding trial, fish were deprived of feed for 24 hr and then were captured from each tank and bulk weighed. Three fish were sampled from each tank. After measurement of condition factor and hepatosomatic index, the sampled fish were frozen at -20°C for analysis of final body composition.

2.3 | Chemical analysis

The sampled fish were autoclaved at 120°C for 20 min, homogenized with a laboratory grinder and dried at 105°C . Contents of moisture, crude protein, crude lipid, ash and phosphorus of the feed ingredients, test diets and fish were analysed with the method described in AOAC (1995). Content of amino acid of the test diets was analysed with a Sykam-433 amino acid analyser (Munich, Germany).

2.4 | Data calculation and statistical analysis

Feed intake, weight gain, feed conversion ratio (FCR), nitrogen retention efficiency (NRE), phosphorus retention efficiency (PRE), condition factor, hepatosomatic index, nitrogen waste, phosphorus waste and the ratio of fish meal consumption to fish production (RCP) were calculated as below (Wang et al., 2015):

$$\text{Feed intake}(\%/ \text{day}) = 100 \times I / [(W_0 + W_t) / 2 \times t]$$

$$\text{Weight gain}(\text{g}) = W_t / N_t - W_0 / N_0$$

$$\text{FCR} = I / (W_t - W_0)$$

$$\text{NRE}(\%) = 100 \times (W_t \times C_{N_t} - W_0 \times C_{N_0}) / (I \times C_{N_f})$$

$$\text{PRE}(\%) = 100 \times (W_t \times C_{P_t} - W_0 \times C_{P_0}) / (I \times C_{P_f})$$

$$\text{Condition factor}(\text{g}/\text{cm}^3) = 100 \times W_B / L^3$$

$$\text{Hepatosomatic index}(\%) = 100 \times W_L / W_B$$

$$\text{Nitrogen waste [g N / (kg fish gain)]} \\ = 1000 \times (I \times C_{N_t}) \times (1 - \text{NRE}) / (W_t - W_0)$$

$$\text{Phosphorus waste [g P / (kg fish gain)]} \\ = 1000 \times (I \times C_{P_t}) \times (1 - \text{PRE}) / (W_t - W_0)$$

$$\text{RCP (g g}^{-1}\text{)} = \text{WG} \times \text{FCR} \times \text{FL} / (W_t / N_t \times \text{DMF}_t - W_0 / N_0 \times \text{DMF}_0)$$

where I (g) is total amount of the dry feed consumed by fish; W_0 (g) is total initial body weight and W_t (g) is total final body weight; t (d) is the duration of the trial; N_t is number of fish at the end of the trial and N_0 is at the start; C_{N_t} (%) is nitrogen content of fish body at the end of the trial and C_{N_0} (%) is at the start; C_{N_f} (%) is nitrogen content of test diets; C_{P_t} (%) is phosphorus content of fish body at the end of the trial and C_{P_0} (%) is at the start; C_{P_f} (%) is phosphorus content of test diets; W_B (g) is body weight of the sampled fish, and L (cm) is body length; W_L (g) is liver weight of the sampled fish; FL (g/kg) is fish meal content of the test diets; DMF_t (g/kg) is dry matter content of the fish sampled at the end of the trial and DMF_0 (g/kg) at the start.

The differences in final body weight, weight gain, feed intake, FCR, NRE, PRE, condition factor, hepatosomatic index, body components (moisture, crude protein, crude lipid, ash and phosphorus), nitrogen waste, phosphorus waste and RCP among fish fed diets C, B20, B40, B60 and B80 were examined with one-way ANOVA. The further comparison between the treatments was performed with Tukey's honestly significant difference (HSD) test. The data expressed in percentage (feed intake, NRE, PRE, hepatosomatic index and body components) were arcsine-transformed prior to ANOVA. $p < .05$ was considered significant in difference. The statistical analysis was performed using IBM SPSS (version 21.0).

3 | RESULTS

3.1 | Survival, feed intake, weight gain and feed utilization efficiency

Survival was 100% in all the tanks. The final body weight, weight gain, feed intake and PRE were significantly affected by fish meal replacement level (ANOVA, $p < .05$, Table 4), whereas FCR and NRE were independent on fish meal replacement (ANOVA, $p > .05$). The final body weight, weight gain and feed intake were higher in fish fed diets C,

B20 and B40 than those in fish fed diets B60 and B80 (HSD test, $p < .05$). No significant differences were found in final body weight, weight gain and feed intake either between fish fed diets C, B20 and B40 (HSD test, $p > .05$) or between fish fed diets B60 and B80 (HSD test, $p > .05$). No significant differences were found in FCR and NRE among fish fed diets C, B20, B40, B60 and B80 (ANOVA, $p > .05$), while the PRE was lower in fish fed diets C, B20 and B40 than in fish fed diets B60 and B80 (HSD test, $p < .05$).

3.2 | Condition factor, hepatosomatic index and body composition

At the end of the feeding trial, condition factor, hepatosomatic index and body contents of moisture, crude protein, crude lipid, ash and phosphorus were independent on fish meal replacement level (ANOVA, $p > .05$, Table 5). No significant differences were found in condition factor, hepatosomatic index and body composition between fish fed diets C, B20, B40, B60 and B80 (ANOVA, $p > .05$).

3.3 | Waste outputs and the ratio of fish meal consumption to fish production

Phosphorus waste and RCP were dependent on fish meal replacement level (ANOVA, $p < .05$, Table 6), while nitrogen waste was independent on fish meal replacement level (ANOVA, $p > .05$). No significant difference was found in nitrogen waste among fish fed diets C, B20, B40, B60 and B80 (ANOVA, $p > .05$). The phosphorus waste was higher in fish fed diets C, B20 and B40 than in fish fed diets B60 and B80 (HSD test, $p < .05$), while no significant difference was found in phosphorus waste either between fish fed diets C, B20 and B40 or between fish fed diets B60 and B80 (HSD test, $p > .05$). The RCP linearly declined with the decrease in dietary fish meal level (DL), and the regression equation could be described as: $\text{RCP} = 0.0037 \times \text{DL}$ ($R^2 = 0.963$, $p < .05$, $n = 15$). The RCP was lower in fish fed diets B40, B60 and B80 than in fish fed diet C (HSD test, $p < .05$).

4 | DISCUSSION

In the present study, no significant difference was found in weight gain between fish fed diet C, B20 and B40, suggesting that dietary

TABLE 4 Initial body weight (IBW, g/fish), final body weight (FBW, g/fish), weight gain (g/fish), feed intake (%/d), feed conversion ratio (FCR, feed fish/gain), nitrogen retention efficiency (NRE, %) and phosphorus retention efficiency (PRE, %) of giant croaker fed the test diets

Diet	IBW	FBW	Weight gain	Feed intake	FCR	NRE	PRE
C ¹	20.3 ± 0.2	123.3 ± 2.0 ^b	103.0 ± 2.2 ^b	2.52 ± 0.05 ^{ab}	0.97 ± 0.00	40.01 ± 0.64	33.17 ± 1.92 ^a
B20 ¹	20.7 ± 0.7	115.4 ± 5.7 ^{ab}	94.8 ± 5.2 ^{ab}	2.60 ± 0.04 ^b	1.05 ± 0.04	36.30 ± 1.99	34.03 ± 2.60 ^a
B40 ¹	20.8 ± 0.1	114.2 ± 7.3 ^{ab}	93.4 ± 7.2 ^{ab}	2.52 ± 0.02 ^{ab}	1.03 ± 0.03	37.29 ± 1.54	35.82 ± 2.43 ^a
B60 ¹	21.1 ± 0.3	111.0 ± 6.5 ^a	89.9 ± 6.3 ^a	2.32 ± 0.18 ^a	0.96 ± 0.09	39.82 ± 3.81	42.41 ± 1.79 ^b
B80 ¹	21.0 ± 0.6	106.1 ± 1.4 ^a	85.1 ± 1.9 ^a	2.36 ± 0.07 ^a	0.99 ± 0.03	37.57 ± 0.72	46.84 ± 2.50 ^b

Data are presented as mean ± SD ($n = 3$). The data with different superscripts in the same row mean significant difference at $p < .05$.

¹C: Control; B20: 20% of the fish meal was replaced; B40: 40% of the fish meal was replaced; B60: 60% of the fish meal was replaced; B80: 80% of the fish meal was replaced.

TABLE 5 Condition factor (g/cm^3), hepatosomatic index (%) and whole body composition (g/kg) of giant croaker fed the test diets

Diet	Condition factor	Hepatosomatic index	Moisture	Crude protein	Crude lipid	Ash	Phosphorus
Initial	1.45 ± 0.11	1.70 ± 0.12	755 ± 0	177 ± 0	30 ± 0	42 ± 0	6.4 ± 0.2
C ^a	1.75 ± 0.02	2.86 ± 0.05	732 ± 5	176 ± 3	61 ± 3	33 ± 2	5.1 ± 0.4
B20 ^a	1.75 ± 0.13	2.75 ± 0.08	728 ± 6	178 ± 3	63 ± 5	34 ± 1	5.5 ± 0.2
B40 ^a	1.81 ± 0.08	2.86 ± 0.02	729 ± 0	178 ± 2	62 ± 3	33 ± 1	5.4 ± 0.1
B60 ^a	1.75 ± 0.11	2.70 ± 0.10	730 ± 2	178 ± 2	60 ± 3	35 ± 1	5.4 ± 0.2
B80 ^a	1.76 ± 0.15	2.71 ± 0.09	730 ± 3	176 ± 4	59 ± 2	35 ± 1	5.7 ± 0.1

Data are presented as mean ± SD ($n = 3$).

^aC: Control; B20: 20% of the fish meal was replaced; B40: 40% of the fish meal was replaced; B60: 60% of the fish meal was replaced; B80: 80% of the fish meal was replaced.

TABLE 6 Nitrogen waste ([g N/(kg fish gain)]), phosphorus waste ([g P/(kg fish gain)]) and ratio of fish meal consumption to fish production (RCP, kg/kg) of giant croaker fed the test diets

Diet	C ¹	B20 ¹	B40 ¹	B60 ¹	B80 ¹
Nitrogen waste	42.71 ± 0.48	49.99 ± 3.36	48.09 ± 2.61	43.63 ± 6.69	46.86 ± 1.76
Phosphorus waste	10.30 ± 0.29 ^b	10.22 ± 0.76 ^b	9.29 ± 0.63 ^b	7.05 ± 0.87 ^a	6.31 ± 0.48 ^a
RCP	1.38 ± 0.08 ^d	1.25 ± 0.12 ^d	0.92 ± 0.07 ^c	0.55 ± 0.05 ^b	0.29 ± 0.00 ^a

Data are presented as mean ± SD ($n = 3$). The data with different superscripts in the same row mean significant difference at $p < .05$.

¹C: Control; B20: 20% of the fish meal was replaced; B40: 40% of the fish meal was replaced; B60: 60% of the fish meal was replaced; B80: 80% of the fish meal was replaced.

fish meal for giant croaker could be reduced to 240 g/kg when the blend of PBM and FEM was used as a fish meal substitute. Nengas, Alexis, and Davies (1999) reported that weight gain of gilthead sea bream *Sparus aurata* declined when dietary fish meal level was reduced to 180 g/kg by inclusion of a blend of poultry meat meal and feather meal (PBM: FEM = 3:1). Milliamena (2002) reported that complete replacement of dietary fish meal with a blend of meat meal and blood meal (meat meal: blood meal = 4:1) did not negatively affect weight gain of grouper *Epinephelus coioides*. Guo et al. (2007) reported that dietary fish meal level for cuneate drum could be reduced to 70 g/kg by inclusion of a blend of PBM, meat and bone meal, FEM and blood meal (PBM: meat and bone meal: FEM: blood meal = 6:2:1:1). Wang et al. (2008) reported that reducing dietary fish meal level to 250 g/kg by inclusion of a blend of PBM, meat and bone meal, FEM and blood meal (PBM: meat and bone meal: FEM: blood meal = 5:2:1:1) did not negatively affect weight gain of malabar grouper. Zhu et al. (2011) reported that replacement of dietary fish meal with a blend of meat and bone meal, PBM and FEM (meat and bone meal: PBM: FEM = 2:2:1) did not result in growth decline of Siberian sturgeon *Acipenser baerii* until fish meal level was reduced to 240 g/kg. Compared with cuneate drum (Guo et al., 2007; Wang et al., 2006), giant croaker required more fish meal in diet formulation when PBM and FEM were used in combination as a fish meal substitute.

Deficiency of some essential amino acids, such as Met and Lys, has been recognized as one of the factors limiting dietary fish meal replacement with terrestrial animal and plant ingredients (Glencross, Booth, & Allan, 2007). In some studies, however, supplementation of crystalline Met and Lys could not increase the level of fish meal

replacement with soybean meal in diets for Japanese sea bass and golden pompano (Wu, Ren, Qin, Han, & Wang, 2016; Zhang et al., 2016). Wang et al. (2015) assumed that low dietary Met and Lys contents should not be the primary factor limiting fish meal replacement by PBM in Japanese sea bass diets. In the present study, crystalline DL-Met and L-Lys were added to improve amino acid profile of the diets with fish meal replaced by PBM and FEM. The weight gain was lower in fish fed diets B60 and B80 than that in fish fed diet C although no significant differences were found in contents of Met and Lys between diets C, B60 and B80. This result reveals that some factors except dietary amino acid profile might negatively affect growth of the fish fed diets B60 and B80. The present study is in agreement with the conclusion that deficiency of essential amino acids should not be the primary factor limiting fish meal replacement by alternative ingredients in fish diets (Wang et al., 2015).

In the present study, feed intake slightly decreased with the decrease in dietary fish meal level, while no significant differences were found in the FCR and NRE between fish fed diets C, B20, B40, B60 and B80. This finding is consistent with the reports on gilthead sea bream (Nengas et al., 1999), grouper (Milliamena, 2002), cuneate drum (Guo et al., 2007; Wang, Kong, Li, & Bureau, 2010) and Siberian sturgeon (Zhu et al., 2011) that indicated the lowered feed utilization efficiency should not be the mechanism responsible to growth decline of fish fed the diets with fish meal replaced by blended rendered animal protein ingredients. The reduced feed intake might be attributed to the negative effect of FEM inclusion on dietary palatability. Moreover, the NRE of fish fed diets C, B20, B40, B60 and B80 (<40%) was similar to that (32%–38%) of giant croaker fed formulated feed, but was much lower than that (55%) of giant croaker fed quality fish fillet (Chai et al.,

2013). This result reveals that there is a great space to improve NRE of warm carnivorous fishes fed formulated feed by optimization of diet formulation.

Previous studies reported that replacing dietary fish meal with rendered animal protein ingredients did not significantly affect morphology and body composition of grouper (Milliamena, 2002), cuneate drum (Guo et al., 2007) and malabar grouper (Wang et al., 2008). In the present study, no significant differences were found in condition factor, hepatosomatic index and body composition between fish fed diets C, B20, B40, B60 and B80. This result reveals that replacement of dietary fish meal with a blend of PBM and FEM could not apparently change morphology and body composition of giant croaker.

The impact of replacement of fish meal with alternative ingredients in fish diets on wild fishery resource and aquaculture environment could be evaluated with RCP and nutrient wastes derived from feeding (Wang et al., 2015). In the present study, the RCP was reduced from 1.38 (diet C) to 0.92 (diet B40) by replacing fish meal with the blend of PBM and FEM. This result reveals that fish meal consumption in giant croaker farming was 1.38 times higher than fish production when fed the diets containing fish meal at 400 g/kg and could be reduced to 92% of fish production by feeding diets containing fish meal at 240 g/kg. The RCP of fish fed B40 was much higher than that of Japanese seabass (RCP = 0.29, Wang et al., 2015) and Largemouth bass (RCP = 0.60, Ren et al., 2017), suggesting high dependence of giant croaker farming on fish meal. On the other hand, nitrogen and phosphorus wastes of fish fed diets B20 and B40 did not significantly differ from those of fish fed diet C, suggesting that partial replacement of dietary fish meal with the blend of PBM and FEM did not increase waste output of nitrogen and phosphorus in giant croaker farming. The nitrogen waste [48 g N/(kg fish gain)] and phosphorus waste [9 g P/(kg fish gain)] of fish fed diet B40 were lower than those of malabar grouper (Li et al., 2009; Wang et al., 2008), cuneate drum (Wang, Kong, Li, & Bureau, 2007) and golden pompano *Trachinotus ovatus* (Wu, Han, Qin, & Wang, 2015). This result reveals that environmental pollution was lower in giant croaker farming than those in the farming of malabar grouper, cuneate drum and golden pompano.

In conclusion, fish meal level could be reduced to 240 g/kg (B40) by inclusion of a blend of PBM and FEM in diets for giant croaker. Fed the diets containing 240 g/kg fish meal, fish meal consumption in commercial farming accounted for 92% of fish production.

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