Aquaculture xxx (2008) xxx-xxx

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

# Potential of using a blend of rendered animal protein ingredients to replace fish meal in practical diets for malabar grouper (*Epinephelus malabricus*)

Yan Wang [a,*], Kai Li [b], Hua Han [c], Zhou-Xin Zheng [b], Dominique P. Bureau [d]

[a] College of Animal Sciences, Zhejiang University, Hangzhou 310029, Zhejiang, PR China
[b] College of Life Science, Shanghai Fisheries University, Shanghai 200090, PR China
[c] College of Food Science, Shanghai Fisheries University, Shanghai 200090, PR China
[d] Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada

ARTICLE INFO

*Article history:*
Received 1 November 2007
Received in revised form 20 March 2008
Accepted 21 March 2008
Available online xxxx

*Keywords:*
Malabar grouper
Fish meal
Rendered animal protein ingredients
Growth
Nitrogen retention efficiency

ABSTRACT

Two net pen experiments were carried out to assess the potential of using a blend of poultry by-products meal (PBM), meat and bone meal (MBM), feather meal (FM) and blood meal (BM) to replace fish meal in practical diets for malabar grouper *Epinephelus malabaricus*. The blend comprised of 50% PBM, 20% MBM, 20% BM and 10% FM. In the experiment I, triplicate groups of fish (initial body weight 51.0 g) were fed five isonitrogenous and isocaloric diets. The control diet (C) contained 50% herring meal, whereas in the diets M1, M2, M3 and M4, fish meal level was reduced to 37.5, 25, 12.5 and 0% by incorporating the blend as alternate protein source. No significant differences were found in feed intake, final body weight (FBW), weight gain (WG), feed conversion ratio (FCR), nitrogen retention efficiency (NRE), energy retention efficiency (ERE) and total nitrogen waste outputs (TNW) among fish fed the diet C, M1 and M2. Fish fed the diet M3 and M4 had higher feed intake, FCR and TNW, but lower FBW, WG, NRE and ERE, than that of fish fed the diet C. At the end of experiment I, there were no significant differences in condition factor (CF), hepatosomatic index (HSI) and contents of moisture, crude protein, crude lipid and ash in whole fish body among the diet treatments. In the experiment II, squid viscera meal was added at 1% as feeding stimulant in three diets used in the experiment I (diet SC vs. diet C, diet SM2 vs. diet M2, and diet SM3 vs. diet M3). No significant differences were found in feed intake, FBW, WG, FCR, NRE, ERE and TNW between fish fed the diet SC and C, and fish fed the diet SM2 and M2, and fish fed the diet SM3 and M3. At the end of experiment II, there were no significant differences in CF, HSI and whole fish body composition among the diet treatments. Results of the present study indicate the least dietary fish meal level should be 25% for malabar grouper reared in net pens, and adding squid viscera meal at 1% in diets cannot stimulate feeding and growth of the fish.



## 1. Introduction

Quality of dietary protein ingredients plays an important role in regulating growth, feed utilization and nitrogen waste outputs of the fish commercially farmed. Fish meal, excellent but expensive protein ingredients, are usually used at excessive 50% in carnivorous fish diets (Hertrampf and Piedad-Pascual, 2000; Glencross et al., 2007). Using economic terrestrial plant or animal protein ingredients to replace fish meal in fish diets can reduce feed cost of fish farming. In the past four decades, a great amount of studies focused on replacing fish meal with economic protein ingredients in fish diets, however, formulating fish meal free diets for carnivorous species is still a problem to fish nutritionist and culturist. The potential of using rendered animal protein ingredients, including poultry by-products meal (PBM), meat and bone meal (MBM) and feather meal (FM), as dietary protein sources have been examined in a wide range of fish species (Fowler, 1990, 1991; Steffens, 1994; Robaina, 1997; Quartararo et al., 1998; Nengas et al., 1999; Bureau et al., 2000; Kureshy et al., 2000; Webster et al., 2000; Milliamena, 2002; Wang et al., 2006b). Feeding gibel carp the diets in which high level of fish meal was replaced with MBM resulted in reduced feed intake (Xue and Cui, 2001). Palatability of the low fish meal diets could be improved by using feeding stimulants (Papatryphon and Sorares, 2000).

Malabar grouper is a candidate for marine fish culture in Southeast Asia because of fast growth, hardiness to environment, high market value, and success in seed production of the fish (Li et al., in press). In commercial farming, malabar grouper are widely fed trash fish. Using trash fish as feed results in high feed cost and serious eutrophication problems, because nitrogen retention efficiency (NRE) is lower in the fish fed trash fish than the fish fed quality formulated diets (Wang et al., 2006a). Research on high nutritive, low polluting, and cost-effective diet formulation is essential to improve economical and environmental sustainability of malabar grouper farming. Early studies indicated optimal dietary protein and lipid level was 49%

* Corresponding author. Tel./fax: +86 571 86971891.
*E-mail address:* ywang@zju.edu.cn (Y. Wang).

0044-8486/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.aquaculture.2008.03.033



Please cite this article as: Wang, Y., et al., Potential of using a blend of rendered animal protein ingredients to replace fish meal in practical diets for malabar grouper (*Epinephelus malabricus*), Aquaculture (2008), doi:10.1016/j.aquaculture.2008.03.033

**Table 1**
Formulation (%), proximate composition (%) and energy content (MJ $kg^{-1}$) of the test diets used in the experiments

| | C | M1 | M2 | M3 | M4 | SC | SM2 | SM3 |
|---|---|---|---|---|---|---|---|---|
| Herring meal | 50 | 37.5 | 25 | 12.5 | | 50 | 25 | 12.5 |
| Squid viscera meal | | | | | | 1.0 | 1.0 | 1.0 |
| Poultry by-products meal | | 5.7 | 11.5 | 17.2 | 22.9 | | 11.5 | 17.2 |
| Meat and bone meal | | 2.3 | 4.6 | 6.9 | 9.2 | | 4.6 | 6.9 |
| Feather meal | | 1.2 | 2.3 | 3.4 | 4.6 | | 2.3 | 3.4 |
| Blood meal | 5.7 | 7.8 | 9.8 | 9.0 | 10.5 | 5.3 | 9.4 | 8.6 |
| Starch, gel | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Soybean meal | 15 | 15 | 15 | 22.6 | 24.1 | 15 | 15 | 22.6 |
| Rapeseed meal | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Wheat flour | 14.9 | 16.0 | 17.3 | 13.6 | 13.8 | 14.4 | 16.8 | 13.2 |
| $CaHPO_4$ | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| DL-Met | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Vitamin premix[a] | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Mineral premix[b] | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Fish oil | 3.4 | 3.5 | 3.5 | 3.8 | 3.9 | 3.3 | 3.4 | 3.6 |
| *Proximate composition and energy content* | | | | | | | | |
| Dry matter | 90.6 | 90.5 | 89.4 | 90.0 | 90.2 | 90.3 | 90.9 | 91.2 |
| Crude protein | 53.5 | 52.6 | 53.2 | 52.3 | 52.2 | 52.3 | 53.9 | 52.4 |
| Crude lipid | 9.3 | 9.2 | 8.9 | 10.3 | 9.4 | 10.0 | 7.5 | 9.9 |
| Ash | 12.4 | 11.9 | 11.2 | 10.8 | 10.1 | 12.5 | 11.1 | 10.5 |
| Gross energy | 20.93 | 21.02 | 21.29 | 21.38 | 21.58 | 20.86 | 20.86 | 21.36 |

Crude protein, crude lipid, ash and gross energy are expressed on a dry matter basis and given as means ($n=2$).

[a] Vitamin premix provides per kg of feed: vitamin A, 16,000 IU; vitamin $D_3$, 3000 IU; vitamin E, 200 mg; vitamin $K_3$, 20 mg; vitamin $B_1$, 13.4 mg; vitamin $B_2$, 20 mg; vitamin $B_6$, 30 mg; vitamin $B_{12}$, 27 mg; pantothenate, 80 mg; niacinamide, 130 mg; folic acid, 6.6 mg; biotin, 1000 mg; inositol, 270 mg; vitamin C, 200 mg; choline chloride, 1200 mg.

[b] Mineral premix composition is same to that described in Li et al. (in press).

and 9% for malabar grouper (Chen and Tsai, 1994; Shiau and Lan, 1996; Lin and Shiau, 2003), and the least dietary fish meal level was 25% when PBM or MBM was incorporated alone as fish meal substitutes (Li et al., in press).

Knowledge concerning the relationship between feeding behavior and dietary nutrient requirements in fish is still scarce. Guo et al. (2007) reported cuneate drum, a carnivorous species eating fast, grew normally when fed the diets containing 7% fish meal (using a blend of PBM, MBM, FM and BM as fish meal substitute). Potential of malabar grouper, a carnivorous species eating slowly, to utilize blended PBM, MBM, FM and BM as fish meal substitutes remains unknown. Squid meal and squid by-products meal were widely used in fish diets at 1–10% as feeding stimulants (Toften and Jobling, 1997; Eusebio and Coloso, 2000; Xue and Cui, 2001; Millamena, 2002; Catacutan and Pagador, 2004). Effect of adding squid viscera meal in diet formulation on feed intake and growth of malabar grouper has not been determined. Purpose of the present study is to assess the potential of using a blend of PBM, MBM, FM and BM as fish meal substitute in practical diets for malabar grouper, with or without adding squid viscera meal.

## 2. Materials and methods

### 2.1. Diet formulation and diet preparation

Poultry by-products meal, MBM, FM and BM were obtained from various suppliers in USA through National Rendered Association. Other ingredients were obtained from a local feed company (Tianbang Feed, Shanghai, China). Proximate composition, gross energy content and amino acid profile of the ingredients were described in Li et al. (in press).

The study consisted of two experiments (I and II). In the experiment I, five diet treatments were established. The control diet (C) contained 50% steam dried herring meal, whereas in the diets M1, M2, M3 and M4, fish meal level was reduced to 37.5, 25, 12.5 and 0% by incorporating a blend of PBM, MBM, BM and FM (50% PBM+20% MBM+20% BM+10% FM) as alternate protein source. In the experiment II, squid viscera meal was added at 1% as feeding stimulant in three diets used in the experiment I (diet SC vs. diet C, diet SM2 vs. diet M2, and diet SM3 vs. diet M3). Formulation, proximate composition and gross energy content of the test diets are shown in Table 1, and amino acid profile in Table 2. The test diets were pelleted following the procedure as described in Li et al. (in press).

### 2.2. Fish, husbandry and feeding

The experiments were carried out in net pens in Shenao Bay, Shantou, China. Malabar grouper *Epinephelus malabaricus* fingerlings were purchased from a marine fish hatchery at Zhaoan. After transportation, the fish were reared in commercial net pens (3 m×3 m×2 m), and weaned from minced trash fish to formulated diets with the procedure as described in Li et al. (in press) during a period of 10 weeks. Two weeks prior to the start of the experiments, 504 fish were selected and acclimated in 24 experimental pens (1 m×1 m×1.5 m) at 21 fish per pen, and fed the diet C twice daily. At the start of the experiment I, the acclimated fish were deprived of diet for 24 h, and pooled. Fifteen groups each of 20 fish weighing 51.0± 0.2 g (mean±S.E., $n=15$) were batch weighed, and randomly stocked into 15 experimental pens, with 3 replicates of each diet treatment. The experiment II was initiated at the same time following the procedure of the experiment I. Three sub-samples of 3 fish each were collected from the remaining acclimated fish for the determination of initial whole body composition. The sampled fish were frozen at −20 °C until analysis.

The experiments I and II lasted 10 weeks. During the experiments, fish were hand fed at 08:00 and 14:00 h daily as described in Li et al. (in press), except the days of strong waves or storm. Water temperature (ranged from 21 to 28 °C) and salinity (ranged from 32 to 35‰) were measured daily. Dead fish were recorded and weighed for calibrating the calculation of feed conversion ratio (FCR). At the end of the experiments I and II, fish in each pen were batch weighed, and three fish were sampled from each pen for the determination of condition factor (CF), hepatosomatic index (HSI) and final body composition. The sampled fish were frozen at −20 °C until analysis.

### 2.3. Chemical analysis, data calculation and statistical analysis

Contents of moisture, crude protein, crude lipid, ash and gross energy in the ingredients, test diets and fish, and contents of amino acids in the test diets were analyzed as described in Wang et al. (2006a). Feed intake, weight gain (WG), FCR, NRE, energy retention efficiency (ERE), total nitrogen waste output (TNW), CF and HSI were calculated as described in Li et al. (in press).

One-way ANOVA was performed to examine the differences in survival, feed intake, final body weight (FBW), WG, FCR, NRE, ERE, TNW, CF, HSI and whole body components (moisture, crude protein, crude lipid and ash) among the diet treatments in the experiment I. The differences in above variables among the diet treatments in the experiment II were examined using ANOVA for factorial layout. Mean

**Table 2**
Essential amino acid profile (%) of the test diets used in the experiment I

| Diets | Met | Lys | Thr | Ile | His | Val | Leu | Arg | Phe | Tyr |
|---|---|---|---|---|---|---|---|---|---|---|
| C | 0.89 | 3.52 | 2.12 | 1.95 | 1.91 | 2.35 | 3.90 | 3.02 | 2.26 | 1.53 |
| M1 | 1.08 | 3.53 | 2.16 | 1.96 | 1.95 | 2.53 | 4.09 | 3.09 | 2.39 | 1.64 |
| M2 | 1.00 | 3.39 | 2.19 | 1.95 | 1.97 | 2.56 | 4.20 | 3.19 | 2.31 | 1.46 |
| M3 | 0.88 | 3.22 | 2.15 | 1.98 | 1.89 | 2.58 | 4.17 | 3.51 | 2.44 | 1.57 |
| M4 | 0.76 | 3.09 | 2.17 | 1.95 | 1.83 | 2.60 | 4.17 | 3.42 | 2.38 | 1.46 |

Met, Lys, Thr, Ile, His, Val, Leu, Arg, Phe and Tyr are expressed on a dry matter basis and given as means ($n=2$).



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**Table 3**
Body weight (g fish$^{-1}$), weight gain (g fish$^{-1}$), feed intake (% d$^{-1}$), feed conversion ratio (feed gain$^{-1}$), nitrogen retention efficiency (%) and energy retention efficiency (%) of malabar grouper fed the test diets in the experiment I (Mean±S.E., $n$=3)

| Diets | Initial weight | Final weight | Weigh gain | Feed intake | Feed conversion ratio | Nitrogen retention efficiency | Energy retention efficiency |
|---|---|---|---|---|---|---|---|
| C | 50.9±0.5 | 137.1±2.5$^{a}$ | 86.2±2.4$^{a}$ | 1.32±0.02$^{a}$ | 1.00±0.01$^{a}$ | 32.0±0.8$^{a}$ | 31.1±0.7$^{a}$ |
| M1 | 50.4±0.5 | 133.0±2.7$^{ab}$ | 82.6±2.2$^{ab}$ | 1.32±0.02$^{a}$ | 1.02±0.02$^{a}$ | 32.2±0.8$^{a}$ | 30.5±0.2$^{a}$ |
| M2 | 50.9±0.2 | 130.1±3.6$^{ab}$ | 79.1±3.6$^{ab}$ | 1.35±0.01$^{a}$ | 1.09±0.03$^{a}$ | 30.0±0.6$^{a}$ | 28.8±1.0$^{a}$ |
| M3 | 51.1±0.5 | 124.7±4.2$^{b}$ | 73.6±4.5$^{b}$ | 1.48±0.02$^{b}$ | 1.21±0.05$^{b}$ | 27.3±1.0$^{b}$ | 24.8±1.1$^{b}$ |
| M4 | 51.0±0.9 | 110.2±3.6$^{c}$ | 59.2±3.1$^{c}$ | 1.44±0.11$^{b}$ | 1.38±0.03$^{c}$ | 23.4±1.0$^{c}$ | 21.3±0.3$^{c}$ |

Values within same column with different superscripts are statistically different at $P$<0.05.
Feed intake and feed conversion ratio are expressed on a dry diet basis.

values between the diet treatments in the experiment I or II were examined using Turkey HSD test. Survival, NRE, ERE and whole body components were arcsine transformed. Significance was accepted at $P$<0.05.

## 3. Results

Survival of the fish in the experiments I and II was 96.7±0.9% (mean±S.E., $n$=24). There was no significant difference in survival among the diet treatments in either the experiment I or II ($P$>0.05).

In the experiment I, there were no significant differences in feed intake, FBW, WG, FCR, NRE, ERE and TNW among fish fed the diet C, M1 and M2 ($P$>0.05, Table 3, Fig. 1). Fish fed the diet M3 and M4 exhibited higher feed intake, FCR and TNW, but lower FBW, WG, NRE and ERE, than that of fish fed the diet C ($P$<0.05). Weight gain linearly decreased with the decline of dietary fish meal level ($r$=0.849, $n$=15, $P$<0.05). At the end of the experiment I, no significant differences were found in CF, HSI and whole body components among the diet treatments ($P$>0.05, Tables 4 and 5).

In the experiment II, there was no significant difference in feed intake among fish fed the diet SC, SM2 and SM3 ($P$>0.05, Table 6). Fish fed the diet SM2 exhibited lower FBW and WG ($P$<0.05), but similar FCR, NRE, ERE and TNW ($P$>0.05), compared to fish fed the diet SC (Table 6, Fig. 2). Final body weight, WG, NRE and ERE were lower, while FCR and TNW higher, in fish fed the diet SM3 than fish fed the diet SC ($P$<0.05). There were no significant differences in FBW, WG, FCR, NRE, ERE and TNW between fish fed the diet C and SC, and fish fed the diet M2 and SM2, and fish fed the diet M3 and SM3 ($P$>0.05). At the end of the experiment II, no significant differences were found in CF, HSI and whole body components among the diet treatments ($P$>0.05, Tables 7 and 8).

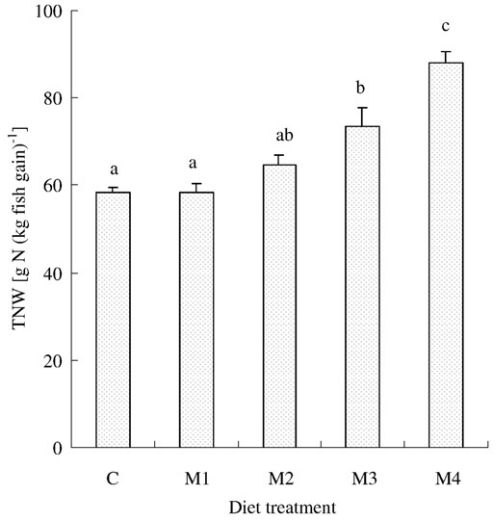


**Fig. 1.** Total nitrogen waste outputs (TNW) of malabar grouper fed the test diets in the experiment I (Mean±S.E., $n$=3). The letters present results of Tukey HSD test. The columns with different letters are significantly different at $P$<0.05.

## 4. Discussion

Feeding the least fish meal diets is practically important in commercial fish farming. In the present study, WG decreased with the decline of dietary fish meal level, and no significant differences were found in feed intake, FBW, WG, FCR, NRE, ERE and TNW among fish fed the diets containing 50, 37.5 or 25% fish meal. This suggests growth of malabar grouper is dependent on dietary fish meal level, and at least 25% fish meal should be used in diet formulation to maintain normal growth and feed utilization of the fish. Li et al. (in press) reported WG of malabar grouper decreased with the reduction of dietary fish meal level when PBM or MBM was used alone as fish meal substitutes, and recommended the least dietary fish meal level should be 25% for the fish reared in net pens. In contrast, orange spotted grouper grew normally when fed the diet containing 8% fish meal, with a blend of MBM and BM as fish meal substitute (Milliamena, 2002).

Poultry by-products meal, MBM and FM have been demonstrated nutritionally adequate protein sources for many fish species (Fowler, 1991; Robaina, 1997; Wang et al., 2006b). Essential amino acid (EAA) deficiency is one of the factors limiting the utilization of economics protein sources as fish meal substitutes (Glencross et al., 2007). Compared to fish meal, PBM and MBM are lower in Met and Lys contents, and FM lower in Met, Lys and His contents (Hertrampf and

**Table 4**
Condition factor (g cm$^{-3}$) and hepatosomatic index (%) of malabar grouper fed the test diets in the experiment I (Mean±S.E., $n$=3)

| Diets | Condition factor | Hepatosomatic index |
|---|---|---|
| C | 2.78±0.06 | 4.20±0.43 |
| M1 | 2.73±0.13 | 4.28±0.28 |
| M2 | 2.72±0.05 | 4.82±0.39 |
| M3 | 2.76±0.16 | 4.84±0.22 |
| M4 | 2.74±0.09 | 4.93±0.28 |

Values within same column are not statistically different at $P$<0.05.

**Table 5**
Proximate composition (%) and energy content (MJ kg$^{-1}$) in whole body of malabar grouper fed the test diets in the experiment I (Mean±S.E., $n$=3)

| Diets | Moisture | Crude protein | Crude lipid | Ash | Gross energy |
|---|---|---|---|---|---|
| Initial | 71.8±0.1 | 16.6±0.1 | 5.8±0.2 | 5.1±0.0 | 6.2±0.0 |
| C | 71.4±0.4 | 17.0±0.1 | 5.7±0.3 | 4.8±0.1 | 6.4±0.1 |
| M1 | 71.3±0.0 | 17.1±0.1 | 6.2±0.2 | 4.7±0.1 | 6.4±0.1 |
| M2 | 71.2±0.1 | 17.1±0.1 | 6.2±0.1 | 4.8±0.1 | 6.5±0.1 |
| M3 | 71.6±0.2 | 17.0±0.2 | 5.9±0.2 | 4.8±0.1 | 6.3±0.1 |
| M4 | 71.9±0.2 | 16.7±0.3 | 5.9±0.1 | 4.8±0.1 | 6.3±0.0 |

Values within same column are not statistically different at $P$<0.05.
Crude protein, crude lipid, ash and gross energy are expressed on a wet weight basis.

**Table 6**
Body weight (g fish$^{-1}$), weight gain (g fish$^{-1}$), feed intake (% d$^{-1}$), feed conversion ratio (feed gain$^{-1}$), nitrogen retention efficiency (%) and energy retention efficiency (%) of malabar grouper fed the test diets in the experiment II (Mean±S.E., $n$=3)

| Diets | Initial weight | Final weight | Weigh gain | Feed intake | Feed conversion ratio | Nitrogen retention efficiency | Energy retention efficiency |
|---|---|---|---|---|---|---|---|
| C | 50.9±0.4 | 137.1±2.5$^{ab}$ | 86.2±2.4$^{a}$ | 1.32±0.02$^{a}$ | 1.00±0.01$^{a}$ | 32.0±0.8$^{a}$ | 31.1±0.7$^{a}$ |
| M2 | 50.9±0.2 | 130.1±3.6$^{abc}$ | 79.1±3.6$^{ab}$ | 1.35±0.01$^{a}$ | 1.09±0.03$^{a}$ | 30.0±0.6$^{abc}$ | 28.8±1.0$^{ab}$ |
| M3 | 51.1±0.5 | 124.7±4.2$^{cd}$ | 73.6±4.5$^{bc}$ | 1.48±0.02$^{b}$ | 1.21±0.05$^{b}$ | 27.3±1.0$^{bc}$ | 24.8±1.1$^{b}$ |
| SC | 51.2±0.4 | 138.3±1.6$^{a}$ | 87.1±1.7$^{a}$ | 1.32±0.01$^{a}$ | 1.03±0.03$^{a}$ | 31.1±1.1$^{a}$ | 30.6±1.0$^{a}$ |
| SM2 | 51.4±0.5 | 126.1±2.8$^{bcd}$ | 74.7±2.3$^{b}$ | 1.29±0.01$^{a}$ | 1.09±0.02$^{a}$ | 30.3±0.6$^{ac}$ | 28.3±0.4$^{ab}$ |
| SM3 | 51.1±0.4 | 117.1±5.2$^{d}$ | 66.0±5.1$^{c}$ | 1.34±0.03$^{a}$ | 1.20±0.05$^{b}$ | 28.3±1.2$^{c}$ | 26.1±1.8$^{ab}$ |

Values within same column with different superscripts are statistically different at $P$<0.05.
Feed intake and feed conversion ratio are expressed on a dry diet basis.

Piedad-Pascual, 2000). Blood meal are rich in Lys (Hertrampf and Piedad-Pascual, 2000), and can be used to balance dietary Lys content when PBM, MBM and FM are used, alone or in combination, as fish meal substitutes (Milliamena, 2002; Guo et al., 2007). Previous studies reported dietary fish meal level for cuneate drum could be reduced to 17.5% by incorporating PBM (Wang et al., 2006b), or 7% by incorporating a blend of PBM, MBM, BM and FM (Guo et al., 2007). In the present study, the test diets were formulated to be isonitrogenous and isocaloric, but the diets containing 50–25% fish meal (diet C, M1 and M2) had higher Met content than that of the diets containing 12.5–0% fish meal (diet M3 and M4). Contents of Ile, His, Val, Leu, Phe and Tyr were higher, while contents of Met and Lys lower, in the test diets than whole body of the malabar grouper fed trash fish (Li et al., in press). Lys content was higher, while dietary Met content lower, in the test diets than the dietary requirements for orange spotted grouper (Luo et al., 2005, 2006). These facts reveal the decreased WG and NRE of malabar grouper fed the diets containing 12.5 or 0% fish meal are mainly attributable to low dietary Met content. Robaina et al. (1997) reported gilthead seabream had low digestibility to the diets incorporated in MBM. Poor digestibility of MBM and FM might be another factor responsible to the decreased growth of malabar grouper fed the diets containing 12.5 or 0% fish meal.

Destroyed palatability has been demonstrated responsible to the reduced feed intake and growth of fish fed the diets in which high levels of dietary fish meal was replaced with economic plant or animal protein ingredients (Davis et al., 1995; Xue and Cui, 2001). Adding feeding stimulants was recommended to improve palatability of fish diets (Papatryphon and Sorares, 2000). The effect of adding feeding stimulants on feed intake and growth of fish varies among fish species and the stimulatory substances used. Gly could stimulate feeding of pinfish (Carr and Chaney, 1976), but rainbow trout did not respond positively to Gly (Adron and Mackie, 1978). Tilapia was sensitive to acidic amino acids and citric acid, but not sensitive to alkaline and neutral amino acids (Adams et al., 1988). Squid meal and squid by-products meal were generally used at 1–10% in diets for many fish species, such as sea bass (Eusebio and Coloso, 2000), gibel carp (Xue and Cui, 2001), grouper (Millamena, 2002) and red snapper (Catacutan and Pagador, 2004). Toften and Jobling (1997) indicated adding squid extract at 1% in Atlantic salmon diets did not result in improved feed intake and growth. Mai et al. (2006) reported adding squid viscera meal at 5–10% in diets significantly enhanced growth of Japanese sea bass. In the present study, fish fed the diets containing 0–12.5% fish meal had higher feed intake and FCR, but lower WG and NRE, than that of fish fed the diet containing 50% fish meal, and adding squid viscera meal at 1% in diet formulation did not enhance feed intake, WG and NRE of the fish. This suggests the reduced growth and feed utilization of malabar grouper fed the diets containing 0–12.5% fish meal did not result from the change in palatability of the diets, and adding squid viscera meal in diets failed to stimulate feeding and growth of the fish.

Results of the present study reveal that dietary fish meal level for malabar grouper can be reduced to 25% by incorporating a blend of PBM, MBM, FM and BM, either with or without adding squid viscera

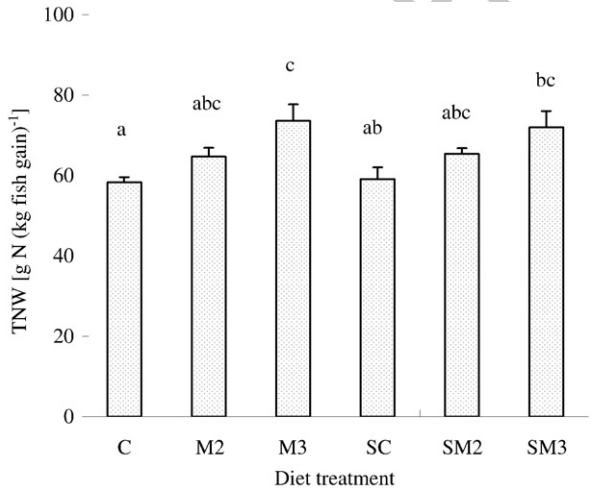


**Fig. 2.** Total nitrogen waste outputs (TNW) of malabar grouper fed the test diets in the experiment II (Mean±S.E., $n$=3). The letters present results of Tukey HSD test. The columns with different letters are significantly different at $P$<0.05.

**Table 7**
Condition factor (g cm$^{-3}$) and hepatosomatic index (%) of malabar grouper fed the test diets in the experiment II (Mean±S.E., $n$=3)

| Diets | Condition factor | Hepatosomatic index |
|---|---|---|
| C | 2.78±0.06 | 4.20±0.43 |
| M2 | 2.72±0.05 | 4.82±0.39 |
| M3 | 2.76±0.16 | 4.84±0.22 |
| SC | 2.92±0.10 | 4.31±0.27 |
| SM2 | 2.75±0.04 | 4.38±0.10 |
| SM3 | 2.73±0.05 | 3.89±0.34 |

Values within same column are not statistically different at $P$<0.05.

**Table 8**
Proximate composition (%) and energy content (MJ kg$^{-1}$) in whole body of malabar grouper fed the test diets in the experiment II (Mean±S.E., $n$=3)

| Diets | Moisture | Crude protein | Crude lipid | Ash | Gross energy |
|---|---|---|---|---|---|
| Initial | 71.8±0.1 | 16.6±0.1 | 5.8±0.2 | 5.1±0.0 | 6.2±0.0 |
| C | 71.4±0.4 | 17.0±0.1 | 5.7±0.3 | 4.8±0.1 | 6.4±0.1 |
| M2 | 71.2±0.1 | 17.1±0.1 | 6.2±0.1 | 4.8±0.1 | 6.5±0.1 |
| M3 | 71.6±0.2 | 17.0±0.2 | 5.9±0.2 | 4.8±0.1 | 6.3±0.1 |
| SC | 71.5±0.0 | 17.0±0.1 | 6.4±0.2 | 4.7±0.1 | 6.4±0.0 |
| SM2 | 71.6±0.2 | 17.3±0.1 | 5.6±0.1 | 4.9±0.1 | 6.3±0.0 |
| SM3 | 71.0±0.3 | 17.3±0.1 | 6.2±0.4 | 5.0±0.1 | 6.5±0.1 |

Values within same column are not statistically different at $P$<0.05.
Crude protein, crude lipid, ash and gross energy are expressed on a wet weight basis.



Please cite this article as: Wang, Y., et al., Potential of using a blend of rendered animal protein ingredients to replace fish meal in practical diets for malabar grouper (*Epinephelus malabricus*), Aquaculture (2008), doi:10.1016/j.aquaculture.2008.03.033

meal. The least dietary fish meal level for malabar grouper is higher than that for cuneat drum (Guo et al., 2007), suggesting the fish eating slowly, such as malabar grouper, has lower ability to utilize PBM, MBM, FM and BM as alternate protein of fish meal, relative to the fish eating fast, such as cuneate drum. Therefore, feeding behavior should be considered as a factor affecting the potential of fish to utilize rendered protein ingredients as fish meal substitutes.

## Acknowledgements

Fats and Protein Research Foundation (FPRF, Bloomington, IL) and National Natural Science Foundation of China (Grant No. 30771673) funded the research. We thank Dr. Yu Yu for the help in procurement of rendered animal ingredients. Sincere thanks are also extended to Miss Rui-Li Jiang, Wen-xiu Ji, Ning-xia Xie and Lei Bi, and Mr. Wei-zhou Chen, Ze-wei Sun and Yuan-xi Lin for their help in various aspects.

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