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THE EFFECTS OF DIFFERENT DIETARY PROTEIN SOURCES ON THE GROWTH PERFORMANCES AND BODY COMPOSITION OF THE ROCK PORGY, *OPLEGNATHUS FASCIATUS*

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Key words: protein sources, rock porgy, growth performance, fishmeal.

ABSTRACT

This study investigated the effects of different dietary protein sources on the growth performance and muscle composition of the rock porgy, *Oplegnathus fasciatus*. Four isonitrogenous (48% crude protein) and isolipidic (10% crude lipid) diets were formulated with Peruvian fishmeal (PFM), Taiwanese fishmeal (TFM), poultry by-product meal (PBM) and meat and bone meal (MBM), respectively. The rock porgy (mean initial weight 10.58 g) were reared in 12 tanks and fed with one of the four treatment diets in triplicate for six weeks. The rock porgy fed with diet TFM had significantly higher weight gain than those fed with the other treatment diets. However, the rock porgy fed with diet MBM had significantly lower weight gain than those fed with the other treatment diets. Survival for all treatment groups ranged from 90 to 100%. Feed conversion ratio (FCR) of the rock porgy fed with diet TFM was lower than that of the fish fed with the other diets. The hepatosomatic index (HSI) of the rock porgy fed with diet PFM was lower than that of the fish fed with the other diets. The specific growth rate (SGR) of the rock porgy fed with diets TFM, PFM and PBM was significantly higher than that of fish fed with diet BMB. Therefore, it is suggested that TFM is the optimum protein source for the rock porgy.

I. INTRODUCTION

Protein is not only one of the most important elements in aquatic nutrition but also the most expensive nutrient in aquatic feed industry. Protein sources for aquatic feed are classified into animal protein and plant protein. Animal protein comes from aquatic products (white fishmeal, red fishmeal, squid meal, shrimp meal, etc.), poultry by-products (chicken meal, feather meal, etc.) and dairy products (casein and milk meal) (Lim and Dominy, 1990; Pelissero and Sumpter, 1992). Fishmeal is considered to be the major protein source for most aquatic species because of its high protein content, excellent digestibility, balanced amino acids, attractant properties and the essential fatty acids, namely eicosapentaenoic acid and docosahexaenoic acid (Bendiksen et al., 2011; Olsen and Hasan, 2012).

The production of aquaculture, cultured aquatic animal and plant, has been rapidly developed in mainland China, Japan, Philippines, Thailand, Taiwan and Vietnam (Tacon and Metian, 2008). In 2009, fish and shellfish supplied approximately 56 million tonnes globally. A total of 33 million tonnes of production from aquaculture were produced by mainland China (FAO, 2010; 2011). The fishmeal used in aquafeed is almost from wild capture. In 2009, around thirty percent of captured wild fish were converted into fishmeal and oil for fish and pet feed (FAO, 2011). In 1988, eighty percent of global fishmeal production was used in feed for land animal, and 10 percent of that was for aquaculture feed. However, in 2010, global fishmeal used for aquaculture feed was increased to 56 percent (Olsen and Hasan, 2012). In the 1990s, the average price of fishmeal varied from 400 to 600 USD/tonne. From 2000 to 2009, the price of fishmeal continued to increase from 400 to 1200 USD/tonne (Olsen and Hasan, 2012). The cost of fishmeal made from wild caught fish is getting higher.

Alternative protein sources, seafood by-products and soybean proteins can be used to replace fishmeal from the red sea bream, *Pagrus major* diet (Kader and Koshio, 2012). Fishmeal can be replaced with many good protein sources in aquaculture feed, such as plant proteins, PBM, fishery by-product, MBM, etc. The ingredients PBM and MBM are obtained from wastes of poultry and processing plants. The levels of leucine, lysine, methionine and tryptophan of PBM as well as MBM are lower than those of fishmeal (Yu, 2004). Bureau et al. (2000) indicated that the protein from MBM could partially replace dietary

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fishmeal protein without affecting the growth and feed efficiency on the rainbow trout (*Oncorhynchus mykiss*). The growth performance of the gilthead seabream, *Sparus aurata* fed with the diet containing high level of PBM was not significantly different from that of the fish fed with the diet containing fishmeal (Nengas et al., 1999).

Fishmeal is brown powder obtained by cooking, pressing, drying and grinding. Most of the water and oil from fish or fish waste are removed. Generally, fishmeal is sold as powder and is used mostly in compound foods for poultry, pigs and farmed fish. The countries with major industrial fisheries are Norway, Peru and South Africa. The ingredient TFM is made from offal, which is the heads, skeletons and left over when the edible portions are cut off for human consumption. Therefore, the ingredient TFM mainly made from filleting offal usually consists of slightly lower protein content and a higher mineral content than does the meal made from whole fish. Fishmeal freshness is important for feed manufacture. Enzymatic and bacteriologic activities of fishmeal can rapidly decrease the content and quality of the fishmeal protein. Protein can be decomposed into amines and ammonia, the process that reduces the protein quality. The ingredient TFM is made from local industry and shows high freshness. Aung and Sheen (2015) indicated that dietary TFM can partially replace PFM without affecting the growth of orange-spotted grouper, *Epinephelus coioides*. Therefore, fishmeal made from fish processing waste can be considered a good animal protein source.

The rock porgy, *Oplegnathus fasciatus*, are coral reef fish which inhabit in the Pacific Ocean particularly in the waters of Japan, mainland China, South Korea and the Hawaiian Islands (Mundy, 2005). The rock porgy have been cultivated in Japan since 1976; however, the source of the fish larvae relies on wild catch (Yoshikoshi and Inoue, 1990). Compared with the milkfish and grouper, the rock porgy is likely to become a new aquaculture species in Taiwan because of the high economic value, excellent meat quality, and strong resistance to low temperature. Ikeda et al. (1988) indicated that 45-55% crude protein is required for rock porgy. The rock porgy fed with diet containing 40 or 50% crude protein with 16% crude lipid showed the best growth performance and the optimum protein requirement was 46% (Kang et al., 1998). However, Kim et al. (2016) pointed out that the juvenile *O. fasciatus* required 48.5% dietary protein for maximum growth based on broken-line analysis of weight gain.

A partial or a complete replacement of dietary fishmeal with various alternative animal protein sources for different fish species has been carried out by numerous studies (Portz and Cyrino, 2004; Muzinic et al., 2006; Goda et al., 2007; Guo et al., 2007; Li et al., 2009; Hernandez et al., 2014). However, the rock porgy fed with diets containing different animal protein sources have not been studied. Therefore, this study aimed to investigate the effects of different dietary protein sources, PFM, TFM, PBM and MBM on the growth performance and muscle composition of the juvenile rock porgy.

Table 1. Proximate composition (%) and acid value (mg KOH/g) of ingredients, PFM, TFM, PBM and MBM.

Compositions	Ingredients			
	PFM[a]	TFM[b]	PBM[c]	MBM[d]
Moisture	10.7	12.9	6.8	8.7
Ash[1]	15.7	15.7	10.7	10.2
Crude protein[1]	65.2	54.4	64.7	58.2
Crude lipid[1]	9.9	17.3	16.8	17.2
Acid value	19.0	16.9	10.9	34.9

[1] Expressed as dry weight
[a] purchased from Copeinca Ltd., Peru.
[b] purchased from Hanaqua Tech Inc., Taiwan.
[c] purchased from Agricom Sales Corporation, Australia.
[d] purchased from NH Foods, Ltd., Australia.

II. MATERIAL AND METHODS

1. Experimental Diets

Four isonitrogenous (48% crude protein) and isolipidic (10% crude lipid) diets were formulated with, respectively. The proximate analysis and acid value of dietary ingredients, PFM, TFM, PBM and MBM are shown in Table 1. The ingredients, PFM, TFM, PBM and MBM contained respectively 65.2, 54.4, 64.7 and 58.2% crude protein and 9.9, 17.3, 16.8 and 17.2% crude lipid. The acid values of the ingredients, PFM, TFM, PBM and MBM were 19.0, 16.9, 10.9 and 34.9 mgKOH/g, respectively. The ingredient compositions of experimental diets are shown in Table 2. The ingredients, PFM, TFM, PBM and MBM were served as main protein sources and isolated soy protein and shrimp meal were added to make diets to contain 48% crude protein. A mixture of 2:1 of fish oil and corn oil (w/w) was used as a lipid source. Corn starch and α-starch were served as carbohydrate sources and binders whereas α-cellulose was included to balance dietary compositions. To prepare the experimental diets, all dietary ingredients were ground into small particles using a grinder and then followed by passing through a 250 μm sieve. All ingredients were mixed manually into homogeneity and then oil was added. The water of approximately 20% of mash dry weight was added to form a moist dough. The dough was passed through a cold-extruder (2.5mm die diameter) and then dried in a dry-air oven at 60°C until reaching approximately 10% moisture content. After cooling, the experimental diets were stored at -20°C in a refrigerator.

2. Feeding Trial

The juvenile rock porgy (*Oplegnathus fasciatus*) were obtained from Aquatic Animal Center, National Taiwan Ocean University and were acclimatized in a 2,000 L fiberglass tank for two weeks. Fish were fed with a commercial diet (Shye-Yih Feeding Co., Ltd. Marine fish larvae feed NO. 3) three times per day prior to the feeding trial. At the beginning of the experiment, fish with the average weight of 10.58 g were starved for 24 h and randomly distributed into 12 glass aquaria (60 × 30 × 45 cm), each of which

Table 2. Composition (%) of the experimental diets for the rock porgy, *Oplegnathus fasciatus*.

Ingredients (%)	Diets			
	PFM	TFM	PBM	MBM
Peru fishmeal	41.4			
Taiwanese fishmeal		49.5		
Poultry by product meal			41.7	
Meat and bone meal				46.3
Isolated soy protein	15	15	15	15
Shrimp meal	10	10	10	10
Yeast	5	5	5	5
Lecithin	0.5	0.5	0.5	0.5
α-starch	10	10	10	10
Corn starch	4	4	4	4
Lipid[1]	4.5	0	1.6	0.6
Mineral mix[2]	2	2	2	2
Vitamin mix[3]	2	2	2	2
α-cellulose	5.6	2	8.2	4.6

[1] Fish oil: Corn oil = (V/V, 2:1)
[2] Bernhart-Tomarell, modified (Bernhart and Tomarell, 1966)
[3] Thiamin HCl 0.5%, riboflavin 0.8%, niacinamide 2.6%, D-biotin 0.1%, Ca-pantothenate 1.5%, pyridoxine HCl 0.3%, folic acid 0.5%, inositol 18.1%, ascorbic acid 12.1%, cyanobalamine 0.1%, para-aminobenzoic acid 3%, BHT 0.1%, cellulose 60.3%.

with 10 fish in triplicate. Fish were fed to apparent satiation three times (09:00, 15:00 and 21:00 h) per day by hand. The duration of this experiment was accordingly extended to six weeks. The experiment was conducted in recirculating seawater system and each aquarium was provided with continuous aeration through an air stone. The seawater recirculation system was assembled with a common filter, a biofilter, and a UV light to maintain the water quality. A photoperiod was maintained 12 h light: 12 h dark. The remaining feed and feces were removed before each feeding time. The amount of feed consumed by the fish in each tank was recorded weekly. At the end of the feeding trial, the fish in each tank were fasted for 24 h prior to being weighed. Each individual was weighed at both the beginning and the end of feeding trial.

The weight gain percentage (WG%), specific growth rate (SGR), feed conversion ratio (FCR) and survival were calculated according to the following equations: WG (%) = $100 \times (W_t - W_0)/W_0$, SGR (% day^{-1}) = $100 \times [(\ln W_t - \ln W_0)/t]$, FCR = feed intake (g)/weight gain (g) and survival (%) = 100 × (final number of fish/initial number of fish), where W_0 is the initial mean body weight (g), W_t is the final mean body weight (g) and t (day) is the feeding period. Fish were sacrificed by immersing in iced water. The liver tissues of nine samples from each treatment group were collected to determine Hepatosomatic Index (HSI). Fish muscle was carefully dissected, dried, homogenized and frozen for subsequent proximate analysis.

3. Proximate Analysis

Table 3. Proximate composition (%) and gross energy of diets for the rock porgy, *Oplegnathus fasciatus*.

Compositions	Diets			
	PFM	TFM	PBM	MBM
Moisture	4.82	5.03	3.52	2.93
Crude protein[1]	48.31	49.06	48.71	48.85
Crude lipid[1]	9.73	10.30	10.27	9.32
Ash[1]	12.56	14.12	10.61	10.9
Crude fiber[1]	10.56	4.21	12.90	10.36
NFE[2]	18.84	22.31	17.51	20.57
Gross energy (Kcal/100g)	448.0	443.4	467.7	468.9

[1] Expressed as percent of dry weight.
[2] NFE (nitrogen free extract): [100 − (crude protein + crude lipid + crude fiber + ash)]%.

The ingredients, experimental diets and fish muscle were analyzed for proximate composition based on the standard methods of AOAC (1984). Crude protein was determined using a Kjeldahl system (Kjeldahl system 1002, Tecator, Sweden) while crude lipid was determined by the chloroform and methanol (2:1, v/v) extraction method (Folch et al., 1957). Crude fiber was determined by acid and alkaline digestion using the Fibertec system M 1020 (FOSS Tecator, Sweden). Moisture and ash were determined by the conventional methods using an oven at 105°C and a muffle furnace at 540°C, respectively. Gross energy of diets was determined by using bomb calorimeter (IKA calorimeter system, C2000 basic, German). Acid value of four protein sources was measured according to AOCS method Da 14-48 (AOCS, 2006).

4. Statistical Analysis

A one-way analysis of variance (ANOVA) was used to test the different growth performance among fish fed with treatment diets. If significant differences were observed, the LSD multiple-comparison test was used to identify significant differences between treatment means. Probabilities of $P < 0.05$ were considered significant and statistical analyses were conducted using a Statistical Analysis System (SAS-PC) software program (V.9.4., SAS Institute, Cary, North Carolina, USA).

III. RESULTS

The proximate analysis of the experimental diets is shown in Table 3. Crude protein, crude lipid and ash of the experimental diets ranged from 48.31% to 49.06%, 9.32% to 10.30% and 10.61% to 14.12%, respectively. The fiber content of diet TFM (4.21%) was found to be the lowest of all treatment diets (10.36% to 12.90%).

Final weight, weight gain percentage, SGR, FCR, HSI and survival of the rock porgy fed with the experimental diets are shown in Table 4. The weight gain percentage of the rock porgy fed with diet TFM was significantly higher than that of fish fed with the other treatment diets ($P < 0.05$). However, the rock

Table 4. Growth performance and survival of the rock porgy, *Oplegnathus fasciatus* fed with the experimental diets for six weeks.

Diets	Initial weight (g)	Final weight (g)	Weight gain (%)	SGR (% day^{-1})	FCR	HSI (%)	Survival (%)
PFM	10.58 ± 0.09	37.30 ± 3.71^{b}	252.53 ± 35.88^{b}	2.99 ± 0.23^{a}	1.75	1.86	90
TFM	10.58 ± 0.05	43.89 ± 1.30^{a}	314.73 ± 12.79^{a}	3.39 ± 0.07^{a}	1.68	2.08	100
PBM	10.59 ± 0.05	37.44 ± 2.69^{b}	253.74 ± 25.59^{b}	3.00 ± 0.18^{a}	1.84	2.55	100
MBM	10.58 ± 0.02	22.94 ± 0.77^{c}	116.74 ± 7.03^{c}	1.84 ± 0.08^{b}	3.34	2.13	100

Values are mean ±SD, obtained from three replicates (n = 3) with 9 fish for each group.

[a,b,c] Means in the same column with the different letter are significantly different (P < 0.05).

Table 5. The proximate composition of muscle and liver of the rock porgy, *Oplegnathus fasciatus*, fed with the diets containing different dietary protein sources.

Compositions	Diets			
	PFM	TFM	PBM	MBM
Muscle				
Moisture	78.20	78.39	77.69	79.32
Crude protein*	84.58	84.44	83.31	85.46
Crude lipid*	9.24	9.65	10.76	8.52
Ash*	6.18	5.91	5.93	6.02
Liver				
Crude lipid*	42.13	38.26	51.47	27.16

* Expressed as percent of dry weight

porgy fed with diet MBM showed significantly lower weight gain percentage and SGR than those fed with diets TFM, PFM and PBM (P < 0.05). The weight gain percentages of the rock porgy fed with diets PFM and PBM were not significantly different (P >0.05). The FCR of the rock porgy fed with diet TFM was the lowest among fish fed with diets PFM, PBM and MBM. The HSI of the rock porgy fed with the treatment diets was in the range of 1.86 to 2.55%. Survival for all treatment groups ranged from 90 to 100%.

Moisture, crude protein, crude lipid and ash levels of muscle of the rock porgy at the end of the feeding trial are shown in Table 5. The moisture, crude protein and ash of fish muscle ranged from 77.69% to 78.39%, 83.31% to 85.46% and 5.91% to 6.18%, respectively. The crude lipid of muscle and liver of the rock porgy was in the range of 8.52 to 10.76% and 27.16 to 51.47%, respectively. The rock porgy fed with MBM had the lowest crude lipid levels in muscle and liver.

IV. DISCUSSION

Fishmeal is a perfect protein source and is most widely used in aquatic feed because of the high value of protein and sufficient essential amino acid (Vechklang et al., 2011). However, there are risks associated with fishmeal used in aquafeed industry, such as availability, price and quality fluctuations (Borghesi et al., 2009). The quality of fishmeal is affected by many factors: the temperature and the methods when fish are caught, the temperature when fish are stored prior to processing, the length of storage prior to processing, the composition of fish catch, manufacture processing and transportation time (Hertramph and Piedad-Pascual, 2000). Macan et al. (2006) indicated that level of histamine increases when fishmeal is in long-term storage. Köse et al. (2003) demonstrated that the level of histamine in fishmeal increases with rising temperature of storage condition. However, the improper manufacture of fishmeal may also give rise to toxic histamine. In addition, the price of fishmeal depends on the transportation cost globally (Huntington, 2004). It is likely that a few months should be taken to ship the PFM from Peru to Taiwan. Therefore, the freshness of fishmeal may deteriorate. When the freshness of seafood by-products decreases, the palatability of diet supplemented with these by-products may be changed (Kader and Koshio, 2012). The rock porgy fed with diet TFM have the best growth performance in this study. It is likely that the ingredient TFM, a meal made from aquaculture or fishery by-product in Taiwan, has an advantage considering the transportation time and storage time. In addition, the acid value of ingredient TFM is lower than that of ingredient PFM in this study. Therefore, ingredient TFM is fresher than ingredient PFM.

The alternative protein sources such as MBM and PBM, included in diets need to be considered their suitable nutritional quality, anti-nutrition factor, sustainable and cost effective (Koumi et al., 2009; Khan et al., 2013). The growth performance of the rock porgy fed with diets PFM and PBM showed no significant differences in this study. The ingredient PBM is highly digestible in protein and energy, particularly, similar amino acid compositions with fishmeal (Yu, 2004). The growth performance

Table 6. Amino acid profile ($g/100g^{-1}$ dry weight) of Peruvian fishmeal, poultry by-product meal and meat and bone meal.

Composition	Ingredients		
	Peruvian fishmeal[a]	Poultry by-product meal[a]	Meat and bone meal[b]
Arginine	5.05	5.64	3.25
Histidine	1.62	1.57	0.84
Isoleucine	3.66	2.8	1.55
Leucine	6.11	4.99	2.99
Lysine	5.67	4.12	2.6
Methionine	2.07	1.76	0.63
Phenylalanine	3.48	2.84	1.63
Threonine	3.38	2.7	1.75
Tryptophan	0.69	0.63	0.28

[a] Source: Shapawi et al. (2007)
[b] Source: Yu (2004)

of sunshine bass fed with the diet containing the PBM as a protein source showed no adverse effect (Pine et al., 2008). Steffens (1994) showed that PBM is a suitable ingredient without any effect on growth parameters when supplemented with lysine and methionine for the rainbow trout, *Oncorhynchus mykiss*. The ingredient PBM can successfully provide nearly half of the dietary protein in a commercial diet for hybrid striped bass, *Morone chrysops* × *M. saxatilis* (Rawles et al., 2006). Therefore, ingredient PBM can be a good animal protein source in aquafeed.

The ingredient MBM is either able or unable to be a good protein source for aquatic animals. The weight gain and SGR of large yellow croaker (*Pseudosciaena crocea*) fed with the diets containing over 45% MBM were significantly decreased (Ai et al., 2006). Bharadwaj et al. (2002) indicated that weight gain and FCR of 55 g hybrid striped bass (*M. chrysops* × *M. saxatilis*) fed with the diet containing 45% MBM were not significantly different from those of fish fed with a fishmeal diet. The rock porgy fed with MBM as the sole protein source showed the lowest growth performance in this study. However, Yu (2004) indicated that lysine, isoleucine and methionine of the ingredient MBM are limited (Table 6). Some species cannot endure high levels of dietary MBM (Ai et al., 2006; Guo et al., 2007; Hu et al., 2008; Lee et al., 2012; Rossi and Davis, 2014), while others can accept high levels of dietary MBM (Robaina et al., 1997; Bharadwaj et al., 2002). Therefore, based on the above observations as well as the result in this study, MBM in aquatic feed is suggested to be designed for specific fish species.

In conclusion, the rock porgy (*O. fasciatus*) fed with the diet included TFM showed the best and significantly higher growth performance than those fed with the diets included PFM, PBM and MBM. Therefore, it is suggested that TFM is the optimum protein source for the rock porgy.

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