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Original Article

Fish by-product meal served as a good protein source in the formulated diets for red tilapia fry

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Abstract

The present study was conducted to determine the possibility of replacing the commercial fish meal (FM) with the locally produced fish by-product meal (FBPM) in the diets for red tilapia (phenotypically *Oreochromis niloticus*) fry (body weight ~0.54 g). Five isoproteic and isolipidic experimental diets with 0, 25, 50, 75, and 100% inclusion levels of FBPM were formulated and hand-fed to triplicate groups of fish (20 fish/ group) twice a day. After 6 weeks, fish fed FBPM 50 attained the highest final body weight (4.7±0.3 g; mean ±SD), weight gain (764.8±47.2%), specific growth rate (5.1±0.1%/day), feed intake (7.2±0.4 g/fish), protein efficient ratio (1.7±0.2), and the best feed conversion ratio (0.9±0.1). All fish fed FBPM-based diets also attained the better survival (93-95%) than those fed control diet (83%). Since FBPM is a by-product of marine fisheries industry, it should be considered as a major protein source in the diets for red tilapia fry.

Keywords: fish by-products, fish meal replacement, red tilapia, fry, feeding

1. Introduction

Red tilapia (phenotypically *Oreochromis niloticus*) is the most widely cultured freshwater fish species in Sabah, Malaysia. In 2014, nearly 50% (1,944 metric tonnes) of the total aquaculture production for freshwater fish in Sabah (3,908 metric tonnes) was dominated by the red tilapia with the wholesale value worth about RM 23 million (Fisheries Department of Malaysia, 2014). According to the surveillance on the operations of tilapia farms in Malaysia reported by Ng, Teh, Chowdhury, and Bureau (2013), feed cost represented the largest portion (range from 50 to 72%) in the total production cost of red tilapia. In Sabah scenario, feed cost comprised about 56% of the total production cost. The cost of feeds is high because fish meal (FM) has been conventionally used as the major dietary protein source, and the price of FM is hiking (Tacon & Metian, 2008). Therefore, in order to reduce the feed cost, many studies have been conducted to identify the suitable alternative protein source for replacing the expensive FM (Ng & Romano, 2013).

Sabah is popular for its high volume of marine fisheries landing. In 2013, the amount of marine fisheries landing in Sabah was the highest (196,522 metric tonnes) in Malaysia, after the Perak state (Fisheries Department of Malaysia, 2013). For this reason, fish by-products are easily available in most fish markets and fish processing factories in Sabah. Fish by-product is referred to the remaining parts of the fish which has no commercial value, such as the viscera, head, and skin (Rustad, Storrø, & Slizyte, 2011). In fact, it is proven that fish by-products meal (FBPM) can be used to replace FM in the feeds for several fish species such as African catfish *Clarias gariepinus* (Obasa, Akinyemi, Ogundijo, & Alade, 2011; Sotolu, 2009) and blue gourami *Trichogaster trichopterus* (Mohanta, Subramanian, & Korikanthimath, 2013), and other terrestrial animals including poultry (Lengkey, Bagau, Adriani, & Ludong, 2011; Ojewola, Okoye, & Ukoha, 2005). In Sabah, the head, viscera and skin of mackerels can be easily obtained but no study was conducted to evaluate their possibility as

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an alternative protein source in the diets for red tilapia. Although several studies have reported that the FBPM produced from tuna and sardine waste can be used to replace FM in the diets for different life stages of Nile tilapia, *Oreochromis niloticus* (Goddard, Al-Shagaa, & Ali, 2008; Gümüş, Erdogan, Kaya, & Erdogan, 2011; Bekibele *et al.*, 2013; Hernández *et al.*, 2013), the nutritional composition of FBPM produced from different fish species can be varied (see Goddard *et al.*, 2008). Therefore, the present study was conducted to evaluate the possibility of replacing FM in the diets for red tilapia with the FBPM produced from the local mackerel wastes. It would be more economically feasible if the dietary FM substitution can be started in younger stage of fish (Chor, Lim, & Shapawi, 2013). For that reason, red tilapia fry was used as the experimental fish in the present study.

2. Materials and Methods

2.1 Preparation of fish by-products meal (FBPM)

The fish by-products which comprised the heads, viscera, skin, and some flesh of mackerels were obtained from the local fish markets in Kota Kinabalu, Sabah. They were cleaned with tap water and boiled in hot water for about 20 minutes to prevent contamination by disease pathogens (Sotolu, 2009), dried in an oven, and finally grinded into fine powder. Subsequently, the FBPM was kept in a refrigerator until further used.

2.2 Formulation of experimental diets

The formulation and proximate composition of the experimental diets is shown in Table 1. Five experimental diets with different inclusion levels of the FBPM (0, 25, 50, 75 and 100 % replacement of FM protein) were formulated. All experimental diets were formulated to be isonitrogenous and isolipidic at 35% crude protein and 10% crude lipid levels, respectively (Ng *et al.*, 2013). The FM used in the present study was obtained from a local FM manufacturer.

2.3 Experimental fish and culture condition

A 6-week feeding trial was conducted to evaluate the growth performance and feed utilization of the red tilapia fry fed diets with different inclusion levels of FBPM. The

Table 1. Formulation and proximate composition of the experimental diets (dry weight g/ 100g).

Ingredients	FBPM 0	FBPM 25	FBPM 50	FBPM 75	FBPM 100
Fish meal[1]	42.0	31.5	21.0	10.5	0.0
Fish by-products meal[2]	0.00	8.6	17.2	25.8	34.3
Soybean meal[3]	21.3	21.3	21.3	21.3	21.3
Vegetable oil	6.3	6.4	6.5	6.6	6.7
Vitamin premix[4]	3.0	3.0	3.0	3.0	3.0
Mineral premix[5]	2.0	2.0	2.0	2.0	2.0
CMC[6]	1.5	1.5	1.5	1.5	1.5
Tapioca starch[7]	20.0	20.0	20.0	20.0	20.0
α–cellulose	2.9	4.7	6.5	8.3	10.2
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0
Proximate composition (%)					
Moisture	11.5±1.1	13.0±0.2	15.0±1.0	13.9±0.2	16.9±0.4
Ash	15.7±0.5	13.5±0.2	11.0±0.2	9.1±0.3	6.9±0.2
Crude protein	33.5±0.0	33.7±0.1	33.7±0.1	33.4±0.1	33.3±0.2
Crude lipid	8.6±0.1	8.1±0.1	8.5±0.2	8.6±0.3	8.5±0.4

[1] Proximate composition: crude protein, 57.0%; crude lipid, 8.1%. [2] Proximate composition: crude protein, 70.0%; crude lipid, 8.6%. [3] Proximate composition: crude protein, 47.0%; crude lipid, 1.5%. [4] Vitamin mixture (g/kg mixture): ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; niacin, 4.5; riboflavin, 1.0; pyridoxine HCl, 1.0; thiamine HCl,0.92; d-calcium panothenate, 3.0; retinyl acetate, 0.60; vitamin D3, 0.083; Menadione, 1.67; DL alpha tocopherol acetate, 8.0; d-biotin, 0.02; folic acid, 0.09;vitamin B12, 0.00135. All ingredients were diluted with alpha cellulose to 1 kg. [5] Mineral mixture (g/kg mixture): Calcium phosphate monobasic, 270.98; Calcium lactate, 327.0; Ferrous sulphate, 25.0; Magnesium sulphate, 132.0;Potassium chloride, 50.0; Sodium chloride, 60.0; Potassium iodide, 0.15; Copper sulphate, 0.785; Manganese oxide, 0.8; Cobalt carbonate, 1.0; Zinc oxide,3.0; Sodium salenite, 0.011; Calcium carbonate, 129.274. [6] Carboxymethyl cellulose (CMC), Sigma Brand. [7] Tapioca AAA brand. Bake with Me Sdn. Bhd.

experimental fish fry (body weight 0.54±0.01g) was obtained from the Fish Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah, and 300 of them were evenly stocked into 15 cylindrical cages (cage dimension: 28.0 cm in diameter and 60.0 cm in height; stocking density: 20 fish/ cage) placed in a 10 tonnes fiberglass tank with de-chlorinated tap water. Each experimental diet was hand-fed to triplicate cages of fish twice daily (approximately at 08:00 and 15:00 hour) until apparent satiation. Fish mortality was observed, and about 20% of water in the tank was exchanged daily. All treatments of fish were bulk-weighted every two weeks, and individual fish total length and body weight were measured at the end of the feeding trial. Subsequently, the weight gain (WG), specific growth rate (SGR), survival (SR), total feed intake (TFI), feed conversion ratio (FCR), protein efficiency ratio (PER), and net protein utilization (NPU) for each dietary treatment of fish were calculated using the formula as follows:

WG (%) = (Final – Initial fish weight)/ Initial fish weight × 100
SGR (%/d) = [ln (Final weight) – ln (Initial weight)]/ days × 100
SR (%) = (Final fish number/ Initial fish number) × 100
TFI (g) = (Total given feed – Total uneaten feed)
FCR = Dry feed consumed (kg)/ Wet weight gain (kg)
PER = Wet weight gain/ Total protein intake
NPU = (Final – Initial fish body protein)/ Total protein intake × 100
CF = [Fish weight (g)/ Total length^3] × 100

2.4 Whole-body proximate analysis

Prior to the feeding trial, ten specimens from the same group of fish were sampled as the initial fish while another ten specimens were randomly sampled from each dietary treatment at the end of the trial for whole-body proximate analysis. All specimens were oven-dried, ground into powder, and kept in a refrigerator at 4°C until the analysis was carried out. The moisture, ash, crude protein and lipid of the fish whole-body were analyzed following the standard methods by AOAC (1997). In brief, the ash content was determined by incinerating the sample at 550°C in a muffle furnace for six hours and the remaining residue was weighted. The crude protein and lipid levels were determined following the Kjeldahl method using an automatic system machine (Kjeltec 2300) and the ether-extraction method using a soxhlet extraction unit (Soxtec 2043), respectively.

2.5 Statistical analysis

All calculated data was subjected to One-Way Analysis of Variance (ANOVA) with the Duncan's new multiple range tests using the SPSS software (version 21). Significant differences were assumed when $P<0.05$.

3. Results and Discussion

The growth performance and feed utilization of the red tilapia fry fed diets with different inclusion levels of FBPM are shown in Table 2. In the present study, all fish generally attained good growth performance with their weight gain (WG) and specific growth rate (SGR) in the range of 504.5-764.8% and 4.2-5.1%/ day, respectively. These results show that the freshly produced FBPM from the local FBP is indeed a good dietary protein source for the red tilapia fry, similar with the results reported by Goddard *et al.* (2008), Gümüş *et al*. (2011) and Hernández *et al*. (2013) that replaced FM with the tuna and sardine processing waste meal, tuna live meal, and tuna industry waste meal in the diets for Nile tilapia *O. niloticus* fry, respectively. In the present study, the fish fed FBPM 50 attained the highest final body weight (4.7 g), WG (764.8%), SGR (5.1%/ day) and total feed intake (7.2 g/ fish), although these results were not significantly higher ($P>0.05$) than those from the control – FBPM 0 group (3.8 g, 622.5%, 4.7%/ day, 6.6 g/ fish, respectively). Fish fed FBPM 50 also attained the best feed conversion ratio FCR (0.9) and

Table 2. Growth performance and feed utilization of the red tilapia fry fed diets with different inclusion levels of FBPM.

Parameters	FBM 0	FBM 25	FBM 50	FBM 75	FBM 100
Final body weight (g)	3.8±0.2^{ab}	3.6±0.3^{a}	4.7±0.3^{b}	3.3±0.5^{a}	3.4±1.0^{a}
Weight gain (%)	622.5±20.6^{ab}	561.1±43.6^{a}	764.8±47.2G	504.5±83.6^{a}	522.8±186.6^{a}
Specific growth rate (%/ day)	4.7±0.1^{ab}	4.5±0.2^{ab}	5.1±0.1G	4.3±0.3^{a}	4.2±0.7^{a}
Survival (%)	83.0±7.6^{a}	92.0±2.9^{ab}	93.0±5.8^{ab}	95.0±5.0G	93.0±2.9^{ab}
Total fish intake (g/fish)	6.6±0.6^{ab}	5.9±0.6^{ab}	7.2±0.4^{a}	5.9±0.2^{ab}	5.6±1.1G
Feed conversion ratio (FCR)	1.0±0.0^{ab}	1.0±0.1^{ab}	0.9±0.1^{a}	1.2±0.1^{b}	1.1±0.2^{ab}
Protein efficient ratio (PER)	1.5±0.1^{a}	1.6±0.1^{a}	1.7±0.2^{a}	1.4±0.2^{a}	1.4±0.3^{a}
Net protein utilization (NPU)	23.8±1.5^{a}	27.8±3.2^{a}	36.4±7.2^{a}	42.2±0.4^{ab}	58.2±24.4G
Condition factor (CF)	1.4±0.1^{a}	1.6±0.2^{a}	1.6±0.1^{a}	1.6±0.0^{a}	1.7±0.2^{a}

Different superscript indicates significant different ($P<0.05$).

Table 3. Whole-body proximate of *Oreochromis* sp. fed diets with different inclusion levels of FBPM.

Proximate (%)	Initial	FBPM 0	FBPM 25	FBPM 50	FBPM 75	FBPM 100
Moisture	75.3±0.2	76.5±1.4	78.0±2.2	76.8±0.4	77.2±2.2	77.7±1.4
Crude Protein	9.1±0.2	14.3±0.8	13.5±1.2	15.0±0.4	14.1±1.4	15.0±0.9
Crude Lipid	6.8±0.5	3.8±0.3	3.4±0.3	3.4±0.3	3.4±0.2	3.6±0.3
Ash	2.6±0.1	4.0±0.3^{a}	3.9±0.4^{ab}	3.7±0.1^{ab}	3.6±0.5^{ab}	3.4±0.2^{b}

Among the treatments, different superscript indicates significant difference ($P<0.05$).

protein efficiency ratio PER (1.7). No significant difference ($P<0.05$) was detected in the condition factor (CR) among all dietary treatments. In addition, survival of fish was generally higher in the FBPM-based diets group (92%-95%) than the control group (83%). Apparently, the optimum inclusion level of FBPM in the diets for the red tilapia fry was 50%, based on its growth performance and feed utilization.

In the present study, fish fed FBPM 100 attained the highest net protein utilization NPU value (58.2) among all groups, and this result was significantly higher ($P<0.05$) than those fed FBPM 0 (23.8), FBPM 25 (27.8), and FBPM 50 (36.4). Also, there was a trend of increasing in NPU when the FBPM inclusion level increased. This outcome can be due to differences in quality of protein in the FM and the FBPM used. According to Johnson and Parsons (1997) and Shirley and Parsons (2001), high ash content can cause amino acids imbalance and reduction of the protein quality in animals flesh. In the present study, ash content in the FBPM 0 (15.7%) was the highest among all diets, while the dietary ash content decreased (13.5%-6.9%) when the inclusion level of FBPM increased from 25 to 100% (Table 1). In fact, similar trend was also reflected in the whole-body ash content of the experimental fish. The fish fed FBPM 0 contained the highest whole-body ash content (4.0%) compared to those fed FBPM 25-100, and the fish whole-body ash content decreased (3.9-3.4%) when the dietary inclusion level of FBPM increased (25-100%) (Table 3). These results reflected the high ash content of the commercial FM, and confirmed for the better protein quality in the FBPM than the commercial FM used in the present study.

The fish fed with FBPM 100 attained the highest NPU; however, their weight gain was significantly lower than those fed FBPM 50. Such result could be due to the poor palatability of FBPM 100. The total feed intake for FBPM 100 (5.6 g/fish) was the lowest among all dietary treatments and was significantly lower than that of FBPM 50 (7.2 g/fish), similar with the results of Hernández *et al.* (2013) when they replaced FM with tuna silage hydrolysates in the diets for *O. niloticus* fry. Therefore, complete FM replacement with the FBPM was not recommended.

4. Conclusions

The locally-produced FBPM was a good protein source to replace FM in the diets for red tilapia fry, and its optimum inclusion level was determined at 50%, according to the fish growth performance and utilization of the diet.

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