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Key words: Asian sea bass(*Lates calcarifer*), poultry by-product meal, fish silage, fish protein hydrolysate, fishmeal replacement.

ABSTRACT

This feeding trial was conducted to investigate the effects of replacement of dietary fishmeal (FM) with a mixture of poultry by-product meal (PBM), fish silage (FS) and fish protein hydrolysate (FPH) on the growth performances of the Asian sea bass *Lates calcarifer*. Five isonitrogenous (46% crude protein) and isolipidic (12% crude lipid) diets were formulated to replace 0 (CON), 25 (FM 25), 50 (FM 50), 75 (FM 75) and 100% (FM 100) of fishmeal protein with the protein mixture (78% PBM, 11% FS, and 11% FPH). One additional diet, namely POM was designed to completely replace FM protein with PBM. The growth trial was conducted with ten fish (average initial weight, 1.72 g) stocked in triplicate in each of 60 L glass tanks and fed with the treatment diets to satiation by hand three times per day for 4 weeks. At the end of the feeding trial, decreasing final weight, weight gain percentage and SGR were noted with an increasing dietary PBM proportion. The final weight and weight gain percentage of fish fed with diets FM 25 and FM 50 were not significantly different from those of fish fed with the CON diet. Meanwhile, fish fed with diets FM 75, FM 100 and POM had significantly lower final weights and weight gain percentage than fish fed with the CON diet. Fish fed with the diets CON, FM 25, FM 50 and FM 75 had no significant difference in SGR. However, fish fed with the diets FM 100 and POM had a significantly lower SGR than fish fed with the CON diet. Therefore, the mixture of PBM, FS and FPH would seem to be able to replace dietary fishmeal protein for *L. calcarifer* up to 25 or 50% with no adverse effects.

I. INTRODUCTION

Fish meal (FM) has traditionally been a major dietary protein in commercial fish feeds and 70% of global FM was formulated in aquaculture diets (Hardy, 2010; Tacon and Metian, 2015). It provides an excellent protein and lipid source for marine carnivorous fish (Tacon and Metian, 2015). However, due to increasing demands on FM which decreased in supply annually (Olsen and Hasan, 2012), finding the suitable alternative protein sources to replace FM is urgent. The reliance of FM has been considered to be a risk to the aquaculture industry for a long time (Tacon and Metian, 2008).

An ideal candidate to replace FM in aquafeeds is the poultry by-product meal (PBM) that consists of leftovers from slaughtered poultry such as necks, heads, feet, undeveloped eggs, gizzards and intestines (Gümüş and Aydin, 2013; AAFCO, 2016). Moreover, the PBM contains high protein level and its amino acid profiles are relatively similar to FM (NRC, 2011). However, the substitution of PBM for dietary FM varies among fish species (Wang et al., 2015). The complete replacement of FM with PBM was reported in rainbow trout *Oncorhynchus mykiss* (Amirkolaie et al., 2014; Parés-Sierra et al., 2014; Barreto-Curiel et al., 2016), Japanese sea bass *Lateolabrax japonicas* (Wang et al., 2015), gilthead seabream *Sparus aurata* (Nengas et al., 1999), *Totoaba macdonaldi* (Zapata et al., 2016), Golden Pompano *Trachinotus ovatus* (Ma et al., 2014) and humpback grouper *Cromileptes altivelis* (Shapawi et al., 2007). Even though the PBM from the poultry industry is likely to be a potential pro

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Table 1. Proximate analysis of FM, PBM, FS and FPH.

Compositions	Ingredients			
	FM	PBM	FS[a]	FPH
Crude Protein*	68.0	65.0	68.5	77.5
Crude Lipid*	9.5	16.5	15.0	7.0

* Expressed as percent of dry matter.

[a] Fish silage: moisture 47.5%, ash 4.5%

tein supplement in aquaculture feeds, it lacks some essential amino acids and attraction for aquatic animals. Therefore, the lack of essential amino acids and attraction might be complemented by adding other protein rich products such as silage and hydrolysates.

One of the protein rich products is fish protein hydrolysate (FPH), the fishery by-products digested by enzymes (Chalamaiah et al., 2012). It not only consists of small peptides ranging from 2 to 20 amino acids, but also consists of various free amino acids that are known to serve as attractants for fish (Jones, 1989; Chalamaiah et al., 2012). Importantly, the small molecules in FPH, indicated by Kotzamanis et al. (2007), seem to be ingested easily by animals. Furthermore, the FPHs that have been used in aquaculture feeds are able to enhance the growth and survival of Atlantis salmon *Salmo salar* parr (Burt, 1987), Atlantic salmon fry (Berge and Storebakken, 1996), rainbow trout *Oncorhynchus mykiss* (Aksnes et al., 2006b), tilapia *Oreochromis niloticus* (Fagbenro et al., 1994), carp larvae *Cyprinus carpio* (Carvalho et al., 1997) and Japanese sea bass *Lateolabrax japonicus* (Liang et al., 2006).

The other protein rich product is the fish silage (FS), a liquid product processed from fishery by-products either by the addition of formic acid or by the anaerobic microbial fermentation (Vidotti et al., 2002). During the extrusion process, Fagbenro and Jauncey (1995) found that the FS used in aqua-feeds is able to decrease the fish meal proportion, raise the moisture content and improve the firmness of pellets. In addition, Vidotti et al. (2002) showed that the fish silage contain high amount of histidine, threonine and serine, while the fishery by-products without hydrolysis contains high amount of valine, isoleucine and leucine. Since proteins are hydrolyzed into free amino acids, both the silage and hydrolysates should provide the most available amino acid resources for aquatic animals (Espe et al., 1989).

Asian sea bass（*Lates calcarifer*）is an important commercial carnivorous species in Australia and Southeast Asia (Tucker et al., 2002). This species is a fast growing euryhaline fish of good taste (Mohd-Yusof et al., 2010). Recently, the Asian sea bass aquaculture has been expanded widely in Asia in order to meet the demands in market places (Glencross, 2006). In 2014, global aquaculture production of Asian sea bass was estimated to be 71,581 tons (FAO, 2016). The replacement of fish meal with alternative ingredients in the Asian sea bass diet has been intensively studied (Boonyaratpalin et al, 1998; Williams et al., 2003; Glencross et al., 2011; Kek and Sheen, 2015; Glencross et al., 2016); however, studies related to diets with a mixture of alternative ingredients to replace fish meal for this fish species are limited. Therefore, the aim of the present study is to evaluate the effects of the replacement of dietary fish meal protein with a mixture of PBM, FS and FPH on the growth performance and tissue composition of the Asian sea bass.

II. MATERIALS AND METHODS

1. Experimental diets

The proximate analysis of different protein sources is shown in Table 1. The crude protein and crude lipid of FM, PBM, FS and FPH ranged from 65.0 to 77.5% and 7.0 to 16.5%, respectively. The formula of the experimental diets is shown in Table 2. Five isonitrogenous (46% crude protein) and isolipidic (12% crude lipid) diets were formulated to replace 0 (CON), 25 (FM25), 50 (FM50), 75 (FM75) and 100% (FM100) of fishmeal protein with the protein mixture (78% PBM (Shye Yih Feeding Co., Taiwan), 11% FS (ScanPro 35/4, ScanBio, Trondheim, Norway), and 11% FPH (Aquativ Co., France)). In addition, another diet was formulated to contain PBM as the sole protein source to replace FM protein, namely POM. The mixture of 2:1 of fish oil (Sigma) and corn oil (Sigma) was added to the CON diet and fish oil (Sigma) was added to FM25, FM50, FM75, FM100 and POM to maintain isolipidic diets. The fish oil contains 28.11% n-3 HUFA (highly unsaturated fatty acids, 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3). Dietary ingredients were first ground to small particle size with a hammer mill and then passed through a 250-μm mesh sieve. After all the dry ingredients were mixed until homogenous, corn oil, fish oil and FS were added. Finally, water was added (approximately 30% of mash dry weight) to form a moist dough which was then passed through a 3.0-mm diameter die using an extruder to produce pellets. The pellets were dried in an air-dry oven at 60°C to approximate 5% moisture content and stored frozen. The proximate analysis of experimental diets is shown in Table 2. The crude protein and crude lipid of experimental diets were in the range of 45.6 to 46.6% and 12.4 to 13.6%, respectively. Ash contents decreased from 13.2 to 10.0% with the increasing replacement levels of the mixture of PBM, FS and FPH, while, crude fiber contents increased from 6.3 to 9.5% with the increasing replacement levels of the mixture of PBM, FS and FPH.

Table 2. Ingredient composition of experimental diets for *L. calcarifer*.

Ingredients	Diets					
	CON	FM25	FM50	FM75	FM100	POM
Fish meal	66.2	49.6	33.1	16.5	0	0
Poultry by- product meal	0	13.1	26.3	39.4	52.6	69.2
Fish silage	0	1.9	3.7	5.6	7.4	0
Hydrolyzed fish protein	0	1.9	3.7	5.6	7.4	0
α-starch	10	10	10	10	10	10
Corn starch	8.5	8.5	8.5	8.5	8.5	8.5
Yeast	5	5	5	5	5	5
Mineral mix[a]	2	2	2	2	2	2
Vitamin mix[b]	2	2	2	2	2	2
Oil*	5.7	0	0	0	0	0
Fish oil	0	4.7	3.8	2.7	1.7	0.6
Cellulose	0.1	0.8	1.4	2.2	2.9	2.2
Lecithin	0.5	0.5	0.5	0.5	0.5	0.5
	Analyzed composition(dry-matter basis)					
Moisture	4.0	3.2	3.9	4.8	4.7	4.3
Ash	13.2	12.4	11.5	10.6	10.0	10.6
Crude Protein	46.0	46.6	46.6	46.1	46.0	45.6
Crude Lipid	12.4	13.2	12.9	13.1	12.4	13.6
Crude Fiber	6.3	6.3	7.0	7.8	9.5	6.7
NFE[c]	22.1	22.5	22.1	22.4	22.1	23.5
Dietary energy (Kcal/100g)[d]	366.5	366.5	366.5	366.5	366.5	366.5

[*] Fish oil : Corn oil = 1:1

[a] Calcium carbonate 2.1%, Calcium phosphate dibasic 73.5%, Citric acid 0.227%, Cupric acid 0.046%, Ferric acid (16~17% Fe) 0.558%, Magnesium oxide 2.5%, Magnesium citrate 0.835%, Potassium sulfate 6.8%, Sodium chloride 3.06%, Sodium phosphate 2.14%, Zinc citrate 0.133%, Potassium iodine 0.001% and Potassium phosphate dibasic 8.1% (Bernhart and Tomarelli, 1996)

[b] 0.5%Thiamin HCl; 0.8%Riboflavin; 2.6%Niacinaminde; 0.1%D-Biotin; 1.5%Ca-Pantothenate; 0.3%Pyridoxine HCl; 0.5%Folicacid; 18.1%Inositol; 12.1%Ascorbic acid; 3%Para-Aminobenzoic acid;0.1%Cyanocobalamin; 0.1%BHT; 60.3%α-Cellulose.

[c] NFE(Nitrogen free extract): [100-(crude protein + crude lipid + crude fiber + ash)]%

[d] Calculated digestible energy = [4 (% protein) + 9 (% lipid) + 4 (% carbohydrate)]

2. Feeding trial

The juvenile Asian sea bass, *L. calcarifer* were obtained from a local farm. Prior to the feeding trail, the juvenile Asian sea bass was acclimated and fed with the control diet for two weeks in a 2,000 L fiberglass tank. At the beginning of the experiment, 1.72 g fish of similar size were individually weighed and randomly distributed to 18 glass aquaria (57 × 30 × 35 cm) containing 50 L freshwater with 10 fish in each aquarium. The feeding trial was carried out for four weeks. Six treatment diets were randomly assigned to three replicates for each experiment diet. The experimental fish were fed by hand three times a day (09:00, 15:00, and 21:00 h) to visual satiation. Uneaten feed and fecal matter were removed before each feeding by siphoning. Each aquarium was provided with continuous aeration through an air stone connected to a central air compressor. During the experiment period, 12 h light: 12 h dark was maintained and water temperature ranged from 28 to 31°C. Feeding was stopped 12 h prior to weighing, and fish were individually weighed at the beginning and the end of feeding trial.

At the end of feeding trial, all fish from each aquarium were sampled to determine the proximate composition of muscle and liver. Fish were sacrificed by immersing in ice water. Fish muscle and liver were carefully dissected, dried, homogenized and frozen for subsequent proximate analysis. The homogenates were collected into one sample for each dietary treatment and analyzed in duplicates.

3. Proximate analyses

Proximate analysis of feed ingredients, experimental diets, fish muscle and fish liver was followed by the procedures of AOAC (1984). Samples were dried to constant weight at 105°C to determine moisture. Crude protein was determined by a Kjeltec semi-autoanalyzer system 1002 (Tecator, Sweden). Crude lipid was determined by chloroform and methanol (2:1, v/v) extraction procedure according to Folch et al. (1957). Crude fiber was determined by acid and alkaline digestion using Fibertec system M 1020 (FOSS Tecator, Sweden). Ash was determined by combustion at 540°C in a muffle furnace.

Table 3. Initial weight, final weight, weight gain, SGR, FCR, feed intake, HSI and survival of *L. calcarifer*.

Diets	Initial weight(g)	Final weight(g)	Weight gain (%)	SGR(% day-1)	FCR	Feed intake(g)	HSI	Survival (%)
CON*	1.72 ± 0.07	17.55 ± 0.24 a	929.90 ± 68.55 a	8.04 ± 0.23 a	1.75	27.7	3.94 ± 0.41 a	100
FM25*	1.72 ± 0.06	17.02 ± 0.60 a	872.16 ± 50.05 ab	7.84 ± 0.18 a	1.75	26.7	3.94 ± 0.64 a	75
FM50	1.72 ± 0.02	14.70 ± 1.23 ab	755.88 ± 79.80 ab	7.39 ± 0.33 a	1.78	23.1	3.60 ± 0.50 ab	100
FM75	1.72 ± 0.02	13.31 ± 2.84 b	672.31 ± 170.57 b	7.00 ± 0.73 a	1.85	21.4	3.46 ± 0.33 bc	97
FM100	1.72 ± 0.01	9.65 ± 1.32 c	460.89 ± 75.64 c	5.92 ± 0.48 b	2.24	17.8	3.42 ± 0.55 bc	90
POM	1.72 ± 0.04	5.99 ± 1.19 d	249.48 ± 78.34 d	4.26 ± 0.74 c	3.00	12.8	3.19 ± 0.54 c	83

[*] Two replicates

[a,b,c,d] Means with different superscripts in each column are significantly different (p<0.05).

Weight gain (%) = 100 x [final weight (g) – initial weight (g)] / initial weight (g).

SGR (specific growth rate) = 100 x [ln final weight (g) – ln initial weight (g)] / day.

FCR (feed conversion ration) = feed intake (g) / weight gain (g)

HSI (Hepatosomatic index) = (liver weight / body weight) x 100

Survival (%) = 100 x (final number of fish / initial number of fish).

4. Data calculation and statistical analysis

Weight gain percentage (WG %), specific growth rate (SGR), feed conversion ratio (FCR), survival (SUR) and hepatosomatic index (HSI) were calculated according to the following equations:

WG (%) = 100 x [final weight (g) – initial weight (g)] / initial weight (g).

SGR (% day^{-1}) = 100 x [ln final weight (g) – ln initial weight (g)] / day.

FCR = feed intake (g) / weight gain (g)

HSI (%) = (liver weight / body weight) x 100

SUR (%) = 100 x (final number of fish / initial number of fish).

A one-way analysis of variance (ANOVA) was used to test the differences in weight gain percentages, final weight, SGR, HSI and survival among treatment fish fed with the treatment diets. When a significant treatment effect was observed, a Duncan's multiple range test was used to compare differences among means. Probabilities of $p < 0.05$ were considered significant and all statistical analyses were conducted using the SAS software program for windows (V.9.4., SAS Institute, Cary, North Carolina, USA).

III. RESULTS

The growth performances, FCR, feed intake and survival of the Asian sea bass fed with the experimental diets are shown in Table 3. The death of fish fed with diets CON and FM25 in one tank of triplicate tanks was caused by the failure of aeration. There are insignificant differences in survival among treatments. After 4 weeks of feeding trial, a decreasing trend of the final weight, weight gain percentage and SGR was noted with an increasing level of the mixture of PBM, FS and FPH. The final weight and weight gain percentage of fish fed with the diets FM25 and FM 50 were not significantly different from those of fish fed with the CON diet ($p > 0.05$). Meanwhile, fish fed with the diets FM100 and POM had significantly lower final weight and weight gain percentage than those fed with the other treatment diets ($p < 0.05$). The FCR of fish fed with the diets CON, FM25, FM 50 and FM75 is lower than that of fish fed with the diets FM100 and POM. In addition, feed intake of fish decreased with increasing dietary mixture of PBM, FS and FPH. The relationship between weight gain percentage and dietary levels of FM replacement with mixture of PBM, FS and FPH is shown in Fig. 1. The weight gain percentage of Asian sea bass decreased with the increasing dietary mixture of PBM, FS and FPH linearly. The Asian sea bass fed with a POM diet containing PBM without FS and FPH showed the lowest final weight, weight gain percentage, SGR, and FCR among treatment groups. The feed intake of fish fed with diets CON, FM25, FM50 and FM75 was quite similar, while that of fish fed with POM was equal to

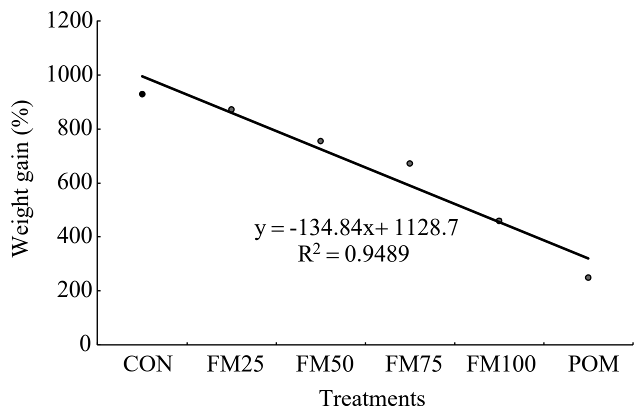

Fig. 1. The relationship between weight gain percentage and dietary levels of FM replacement with mixture of PBM, FS and FPH for *L. calcarifer*.

Table 4. Proximate analysis of *L. calcarifer* muscle and liver.

Compositions	Diets					
	CON	FM25	FM50	FM75	FM100	POM
Muscle						
Moisture	78.69	79.41	79.17	79.87	80.72	81.83
Ash*	1.55	1.10	1.19	1.31	1.00	1.09
Crude Protein*	84.14	82.98	82.89	82.79	82.19	83.48
Crude Lipid*	6.40	7.19	7.32	7.48	8.77	6.74
Liver						
Crude lipid*	38.03	43.47	43.86	44.35	49.01	40.76

* Expressed as percent of dry matter.

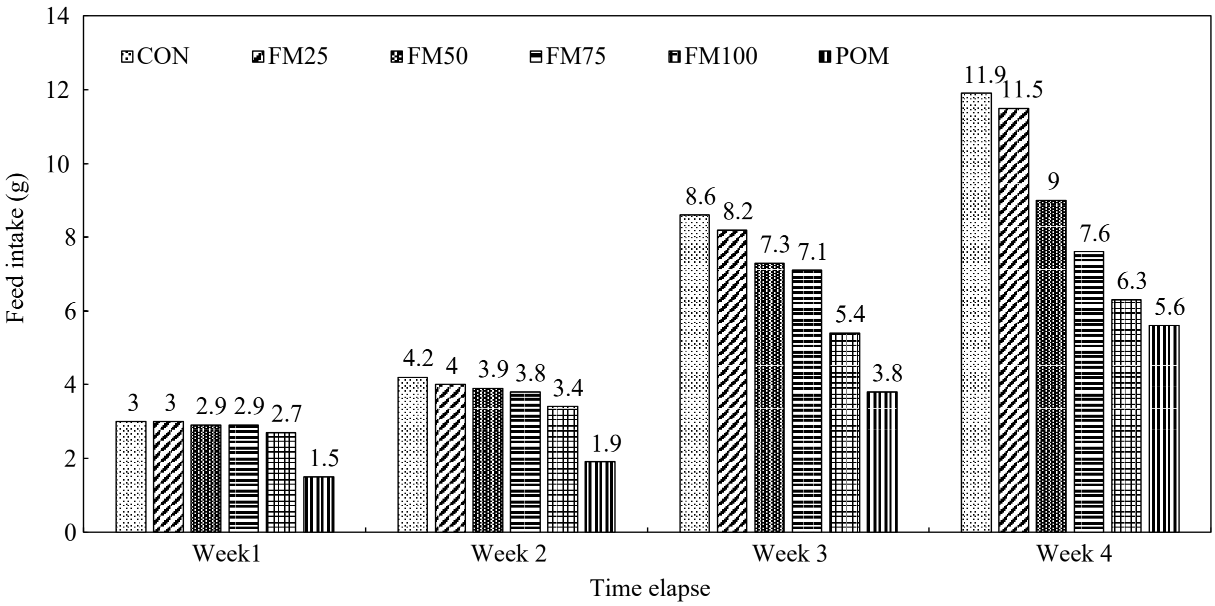

Fig. 2. Feed intake of each treatment diet from week 1 to week 4.

half feed intake of fish fed with the CON diet for the first and the later weeks (Fig 2). Moreover, the feed intake of fish gradually became more followed by time elapsed. Also, feed intake of fish became less when they were fed with the diets containing high level of the mixture of PBM, FS and FPH. HSI of fish fed with the diets FM75, FM100 and POM was significantly lower than that of fish fed with the diets CON and FM25 ($p < 0.05$). However, the HSI of fish fed with the CON, FM25 and FM50 was not significantly different ($P > 0.05$). The survival of fish ranged from 75 to 100% with no significant differences among treatment groups.

The proximate analysis of fish muscle and liver is shown in Table 4. The crude protein content of fish muscle tends to decrease with increasing dietary mixture of PBM, FS and FPH, ranging from 84.14 to 82.19%. The crude lipid of fish muscle and fish liver tends to increase with increasing dietary mixture of PBM, FS and FPH, ranging from 6.40 to 8.77% and 38.03 to 49.01%, respectively.

IV. DISCUSSION

No adverse effects on growth performance was observed from the Asian sea bass fed with the diets containing up to 50% of FM protein replaced with the mixture of PBM, FS and FPH in this study. The juvenile tench *Tinca tinca* fed with the diets containing up to 25% of FM protein replaced with PBM showed no adverse growth performance (Gonzalez-Rodriguez et al., 2016). Similarly, up to 60% of FM protein could be replaced with PBM for juvenile red drum *Sciaenops ocellatus* (Kureshy et al., 2000) and orange-spotted grouper *Epinephelus coioides* (Ridwanudin et al., 2013), while 100% of FM protein could be replaced with PBM for Japanese sea bass *Lateolabrax japonicas* (Wang et al., 2015), humpback grouper *Cromileptes altivelis* (Shapawi et al., 2007), gilthead seabream *Sparus aurata* (Nengas et al., 1999) and rainbow trout *Oncorhynchus mykiss* (Parés-Sierra et al., 2014). Thus, the adverse effects on growth performance seems to be dependent on fish species and quantity of PBM. The Asian sea bass would seem to have a limited capacity to utilize PBM (up to 50%) as a FM substitute.

In this study, the fish fed with the FM100 diet had a significantly higher growth performance than those fed with the POM diet. The rainbow trout (Barreto-Curie et al., 2016) and orange-spotted grouper (Ridwanudin and Sheen, 2014) fed with the diet containing FS also had a significantly higher growth performance than those fed with the diet without FS. Therefore, the growth performance of fish fed the PBM-based diets supplemented with fish hydrolysate products are better than that of fish fed those diets without fish hydrolysate products. The fish hydrolysate products, the attractants in fish diets, are conducive to fish growth and feed utilization (Liaset et al., 2000; Aksnes et al., 2006a, b; Zheng et al., 2012). In addition, the PBM-based diet supplemented with FS consists of a large quantity of taurine (Barreto-Curie et al., 2016), a nutrient that is able to promote fish growth, feed conversion and protein retention efficiency for most aquatic animals (Gaylord et al., 2006, 2007; Salze and Davis, 2015).The FS and FPH supplemented in the PBM diets in this study would improve the growth performance of Asian sea bass.

The crude protein content of fish muscle from treatment groups decreases as the amount of dietary mixture of PBM, FS and FPH increases, while the crude lipid content of fish muscle from treatment groups increases with the increasing dietary mixture of PBM, FS and FPH in this study. The decreasing amount of crude protein, together with the increasing amount of crude lipid, was also observed from cobia (Zhou et al., 2011) and tench *Tinca tinca* (Gonzalez-Rodriguez et al., 2016) after they were fed with diets containing increasing PBM. Due to the imbalance profile of amino acids in the PBM, the black sea turbot muscle protein, as well as the whole body protein of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) decreased with an increasing of dietary PBM (Turker et al., 2005; Rawles et al., 2006; Yigit et al., 2006). Furthermore, the whole body lipid content from Japanese sea bass *Lateolabrax japonicus* (Wang et al., 2015) and snakehead *Channa striata* (Abdul-Halim et al., 2014) increased as the fish were fed with increasing dietary PBM. Based on the above results, it seems that dietary mixture of PBM, FS and FPH would affect the crude protein and crude lipid contents of fish muscle.

The lipid content of fish liver increases with increasing amount of dietary mixture of PBM, FS and FPH in this study. The increasing lipid content of livers from rainbow trout (Amirkolaie et al., 2014), largemouth bass, *Micropterus salmoides* (Subhadra et al., 2006) and Japanese sea bass (Hu et al., 2013) is also observed when they were fed with diets containing increasing amount of PBM. Amirkolaie et al. (2014) indicated that fat content of liver of rainbow trout increased with increasing amount of PBM suggesting fish cannot handle poultry fat well. Japanese sea bass fed diets containing low level of dietary n-3 HUFA showed higher liver lipid level than those fed diets containing higher level of n-3 HUFA (Hu et al., 2013). Thus, it seems that these fish mentioned above fail to utilize poultry fat efficiently. It can be referred that the lack of n-3 HUFA in diets containing PBM may give rise to increasing lipid content in fish liver.

In conclusion, the present study demonstrated that no adverse effect was found on Asian sea bass fed with the diets containing 25 or 50% of FM protein replaced with the mixture of PBM, FS and FPH.

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