Thesis for the Degree of Master of Science

Substitution effects of fishmeal with tuna byproduct meal in the diet on growth, body composition, plasma chemistry and amino acid profiles of juvenile olive flounder (*Paralichthys olivaceus*)

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A dissertation

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요 약

본 연구에서는 넙치용 배합사료내 어분대체원으로서 참치 가공부산물 대체에 따른 넙치의 성장, 체조성, 혈액성상 및 아미노산 조성에 미치는 영향을 조사하였다. 마리당 평균 12.6 g의 조피볼락 900마리를 무작위로 선별하여 30개의 180 L 유수식 수조에 수용하였다. 총 10종류의 실험사료를 준비하였다. 어분을 60% 첨가하여 제조한 대조구 사료(Con), 어분을 참치 가공부산물로 대체한 어분대체 5% (TBM5), 어분대체 10% (TBM10), 어분대체 20% (TBM20), 어분대체 30% (TBM30), 어분대체 40% (TBM40), 어분대체 60% (TBM60), 어분대체 80% (TBM80) 및 어분대체 100% (TBM100) 사료를 준비하였다. 또한 참치 가공부산물의 인 대체효과를 검증하기 위하여 TBM100 사료의 미네랄 premix내 0.37% sodium phosphate monobasic를 cellulose로 대체하였다(TBM100-NP). 모든 실험사료는 동일한 단백질(isonitronic) 함량과 지질(isolipidic) 함량을 가지게끔 제조하였다. 배합사료내 어분이 참치 가공부산물로 대체됨에 따라 사료내 필수아미노산인 lysine 함량이 감소하는 경향을 나타내었다. 증체량(weight gain)과 일일성장률(specific growth rate, SGR)은 대조구, TBM5와 TBM10 사료를 공급한 실험

구에서 TBM40, TBM60, TBM80, TBM100과 TBM 100-NP 사료를 공급한 실험구에서보다 높게 나타났다. 사료전환효율(feed efficiency ratio, FER)은 대조구 사료를 공급한 실험구에서 TBM30, TBM40, TBM60, TBM80, TBM100과 TBM100-NP 사료를 공급한 실험구에서보다 높게 나타났다. 단백질축적율(protein retention, PR)은 대조구 사료를 공급한 실험구에서 TBM30, TBM40, TBM60, TBM80, TBM100과 TBM100-NP 사료를 공급한 실험구에서보다 높게 나타났다. 8주간의 사육 실험 종료시 어체의 일반성분조성은 실험사료에 영향을 받았으며, 아미노산 조성은 실험구간에 유의적인 차이를 보이지 않았다. 또한 총단백질함량을 제외한 혈액성상분석 결과 실험구간에 유의적인 차이를 보이지 않았다. 본 연구 결과 넙치용 배합사료내 어분을 참치가공부산물로 대체할 경우 성장(SGR)에 있어서는 30%까지, 사료 이용성(FER과 PR)에 있어서는 20%까지 역효과 없이 대체 가능하다.

Keywords: 넙치(*Paralichthys olivaceus*); 참치 가공부산물(TBM); 어분; 성장; 사료 이용성

I. Experiment

Substitution effects of fishmeal with tuna byproduct meal in the diet on growth, body composition, plasma chemistry and amino acid profiles of juvenile olive flounder (*Paralichthys olivaceus*)

Abstract

Substitution effects of fishmeal with tuna byproduct meal (TBM) in the diet on growth, body composition, plasma chemistry and amino acid profiles of juvenile olive flounder were determined. Nine hundred fish averaging 12.6 g were randomly distributed into 30 of 180 l flow-through tanks. Ten experimental diets were prepared. A 60% fishmeal was included into the control (Con) diet. The 5, 10, 20, 30, 40, 60, 80 and 100% fishmeal were substituted with TBM at the expense of wheat flour and cellulose, referred to as the TBM5, TBM10, TBM20, TBM30, TBM40, TBM60, TBM80 and TBM100 diets, respectively. Finally, 0.37% sodium phosphate monobasic in mineral premix was replaced with cellulose in the TBM100 diet to clarify phosphorous substitution effect of TBM, referred to as the TBM100-NP diet. All experimental diets were prepared at isonitronic and isolipidic. An essential amino acid, lysine tended to decrease with an increased TBM substitution with fishmeal in the experimental diets. Weight gain and specific growth rate (SGR) of fish fed the Con, TBM5 and TBM10 diets were higher than those of fish fed the TBM40, TBM60, TBM80, TBM100 and TBM100-NP diets. Feed efficiency ratio (FER) of fish fed the Con diet was higher than that of fish fed the TBM30, TBM40, TBM60, TBM80, TBM100 and TBM100-NP diets. Protein retention (PR) of fish fed the Con diet was higher than that of fish fed the TBM30, TBM40, TBM60, TBM80, TBM100 and TBM100-NP diets. Proximate composition of fish was affected by the experimental diets. Amino acid profiles of fish were not different among the experimental diets. Plasma analysis was not different among the experimental diets. Based on these results, dietary substitution of fishmeal with up to 30 and 20% TBM could be made without adverse effect on growth (SGR) and feed utilization (FER and PR) of juvenile olive flounder, respectively.

Keywords: Olive flounder (*Paralichthys olivaceus*), Tuna byproduct meal (TBM), Fishmeal, Growth, Feed utilization

1. Introduction

Olive flounder (*Paralichthys olivaceus*) is one of the most commercially important marine fish species for aquaculture in Eastern Asia such as Korea, Japan and China and its annual aquaculture production in 2011 reached 40,805 metric tones, which was the highest in Korea (MFAFF, 2013). Therefore, many feeding trials to determine dietary nutrient requirements (Forster & Ogata, 1998; Lee et al., 2000a; Alam et al., 2002a; Lee et al., 2002; Kim & Lee, 2004), optimum feed allowance (Lee et al., 2000b; Kim et al., 2005; Cho et al., 2006a; Cho et al., 2007a) and feeding regime (Kim et al., 2002; Cho, 2005; Cho et al., 2006b; Abolfathi et al., 2012), and to develop dietary additive to improve performance (Lee et al., 1998; Cho et al., 2007b; Yoo, et al., 2007; Cho, 2011; Kim et al., 2011) for olive flounder have been reported.

Although fishmeal have been used as one of the most popular protein sources in the diets for fish culture, but its international market price would have kept increasing sharply. Therefore, development of a new feed ingredient to replace for fishmeal in the diet is highly needed. Substitution of animal protein sources, such as meat and bone meal up to 18% (Kikuchi et al., 1997), meat meal up to 30% without supplementation of amino acids (Cho et al., 2005) and 60% with supplementation of amino acids (Sato & Kikuchi, 1997) and feather meal up to 40% (Kikuchi et al., 1994a) for fishmeal in the diets had been successfully made without retardation of growth of olive flounder. Lee et al. (2012a) recently reported that dietary substitution of animal protein sources (10% silkworm pupae meal, 20% meat and bone meal, 10% Promate meal® and 10% combined silkworm pupae meal and Promate meal®) for fishmeal could be made without retardation of growth of fish. However, animal protein sources, such as meat meal, meat and bone meal, blood meal and feather meal can not be safely used as an alternative protein source for fishmeal in the diet for fish culture anymore because of high risk of disease transfer or infection of land-animals originated, such as foot-and-mouth disease, mad cow disease and bird-flu to human being when used for feed ingredient (Greger, 2004).

Another alternative protein source for fishmeal in aquafeeds can be a plant protein source. The alternative plant protein sources for fishmeal have been successfully made for olive flounder: defatted soybean meal up to 45% (Kikuchi, 1999a) and corn gluten meal up to 40% (Kikuchi, 1999b). Hardy (2010) had proposed that the alternative ingredients from plant protein source whose global production should be sufficient to supply the needs of aquafeeds for future. However, plant protein sources, such as soybean and corn gluten meals can not be economically used as the alternative protein source for fishmeal anymore because of their high price resulted from an expansion of biofueling industry to develop soybean and corn as a seed stock for ethanol production over the world.

Therefore, a new feed ingredient that is both cheap and safe from disease transfer is highly needed to replace fishmeal in aquafeeds. Fishery byproduct could be the one. Uyan et al. (2006) reported that tuna muscle byproduct powder is a promising feed ingredient to replace 50% fishmeal protein without reduction in growth performance of olive flounder. However, availability of tuna muscle byproduct powder is quite limited to be used as feed ingredient of aquafeeds. And the 30 and 36% of fishmeal could be replaced with the blend of fermented soybean meal and squid byproduct at the ratio of 3:2 and fermented soybean meal and scallop byproduct at the ratio of 1:1 for red sea bream and olive flounder, respectively (Kader et al., 2011; Kader et al., 2012).

In this study, therefore, substitution effects of fishmeal with tuna byproduct meal (TBM) in the diet on growth, body composition, plasma chemistry and amino acid profiles of juvenile olive flounder were determined.

2. Materials and Methods

2.1. Fish and the experimental conditions

Juvenile olive flounder were purchased from a private hatchery and acclimated to the experimental conditions for 2 weeks before an initiation of the feeding trial. Nine hundred juvenile (an initial body weight of 12.6 g) fish were randomly chosen and distributed into indoor 30 of 180 l flow-through tanks (water volume: 150 l). The flow rate of water into each tank was 7.4 l/min/tank. The water source was sand-filtered natural seawater and aeration was supplied into each tank. Water temperature monitored daily at 15 h from 13.5 to 23.4°C (mean ± SD: 18.5 ± 2.76°C) and photoperiod followed natural conditions.

2.2. Design of the feeding trial and preparation of the experimental diets

A commercially available TBM (Ottogi SF Co. Ltd., Goseong-gun, Gyeongsangnam-do, Korea) has a high potential as the alternative protein source for fishmeal in aquafeeds because of its high nutrient content with crude protein of above 55% and crude lipid of above 10% and stable supply (100 tones daily available in Korea). TBM is a fermented mixture of tuna (yellowfin tuna, *Thunnus albacares* and skipjack tuna, *Katsuwonus pelamis*) byproducts, primary consisted of tuna head, bone, fin, blood and skin resulting from tuna canning, and soybean meal at a ratio of 6:4 by *Bacillus* sp. for 48 hours and is then dried. Ten experimental diets were prepared (Table 1). Fishmeal, TBM, soybean meal and casein were used as the protein sources in the experimental diets. Wheat flour, and fish and soybean oils were used as the carbohydrate and lipid sources, respectively. All experimental diets were prepared at isonitronic (50%) and isolipidic (11%). A 60% fishmeal was included into the control (Con) diet, which satisfied dietary nutrient (protein and energy) requirements for olive flounder (Lee et al. 2000a, 2002). The 5, 10, 20,

Table 1. Ingredient and nutrient composition of the experimental diets

	Experimental diets									
	Con	TBM5	TBM10	TBM20	TBM30	TBM40	TBM60	TBM80	TBM100	TBM100-NP
Ingredients (%, DM)										
Fishmeal[a]	60	57	54	48	42	36	24	12	0	0
Soybean meal[b]	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Tuna byproduct meal (TBM)[c]	0	3.5	7	14	21	28	42	56	70	70
Casein	0	0.25	0.6	1.3	1.9	3	4.5	6	7.8	7.8
Wheat flour	24	23.5	22.9	21.8	20.8	20.3	18	15.6	12	12
Fish oil	2	2	2	2	2	2	2	2	2	2
Soybean oil	2	2	2	1.9	1.8	1.7	1.5	1.4	1.2	1.2
Cellulose	5	4.75	4.5	4	3.5	2	1	0	0	0
Carboxymethy cellulose	1	1	1	1	1	1	1	1	1	1
Vitamin premix[d]	1	1	1	1	1	1	1	1	1	1
Mineral premix[e]	1	1	1	1	1	1	1	1	1	1
Choline	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Nutrients (%, DM)										
Dry matter	93.1	92.3	92.7	93.0	92.7	91.9	92.9	93.1	93.0	93.1
Crude protein	49.5	50.0	50.7	50.0	49.7	50.4	49.8	50.0	50.2	50.3
Crude lipid	11.0	11.1	10.8	10.8	10.8	11.1	10.8	11.0	10.9	10.9
Ash	11.0	11.1	10.8	11.1	10.8	11.1	11.3	11.0	11.3	10.9

[a]Fishmeal (crude protein: 75.0%, crude lipid: 8.2%, ash: 16.1%) and [b]Soybean meal (crude protein: 55.1%, crude lipid: 3.6%, ash: 6.4%) were by Jeilfeed Co Ltd., Haman-gun, Gyeongsangnam-do, Korea.

[c]Tuna byproduct meal (TBM) (crude protein: 57.3%, crude lipid: 8.0%, ash: 11.7%) composed of tuna byproduct to soybean meal at the ratio of 6:4 was supplied by Ottogi SF Co Ltd., Goseong-gun, Gyeongsangnam-do, Korea.

[d]Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 121.2; DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.

[e]Mineral premix contained the following ingredients (g/kg mix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.

30, 40, 60, 80 and 100% fishmeal were substituted with TBM at the expense of wheat flour and cellulose, referred to as the TBM5, TBM10, TBM20, TBM30, TBM40, TBM60, TBM80 and TBM100 diets, respectively. Finally, 0.37% sodium phosphate monobasic ($Na_2P0_4{\cdot}2H_2O$) in mineral premix was replaced with cellulose in the TBM100 diet to clarify phosphorous substitution effect of TBM, referred to as the TBM100-NP diet. Each diet was fed to triplicate groups of fish.

The ingredients of the experimental diets were well mixed with water at the ratio of 3:1 and pelletized by a pellet-extruder. The experiment diets were dried at room temperature overnight and stored at -20°C until use. All fish were hand-fed to apparent satiation twice a day (09:00 and 17:00 h) for 7 days a week throughout the 7-week feeding trial.

2.3. Analytical procedures of the experimental diets and fish

Ten fish at an initiation and five fish from each tank at a termination of the feeding trial were sampled and sacrificed for proximate analysis. Crude protein was determined by the Kjeldahl method (Kjeltec 2100 Distillation Unit, Foss Tecator, Hoganas, Sweden), crude lipid was determined using an ether-extraction method (Soxtec TM 2043 Fat Extraction System, Foss Tecator, Sweden), moisture was determined by oven drying at 105°C for 24 h, fiber was determined using an automatic analyzer (Fibertec, Tecator, Sweden) and ash was determined using a muffle furnace at 550°C for 4 h, all methods were according to standard AOAC (1990). Amino acid composition of the experimental diets and the whole body of fish were determined by using an automatic amino acid analyzer (Sykam S4330, Eresing, Germany) after which the samples were hydrolyzed in 6 N HCl for 22 h at 110°C.

Blood samples were obtained from the caudal vein of randomly chosen five fish from each tank by using syringes after they were starved for 24 h at the end of the feeding trial. Plasma was collected after centrifugation (3,000 rpm for 10 min), stored freezer at -70°C as separate aliquots for analysis of total protein, glucose, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and triglyceride, and analyzed by using automatic chemistry system (Vitros DT60 II, Vitros DTE II, DTSC II Chemistry System, Johnson and Johnson Clinical Diagnostics Inc., New York, USA).

2.4. Calculations and statistical analysis

The following variables were calculated: specific growth rate (SGR, %/d) = 100´[(Ln final weight of fish-Ln initial weight of fish)/days of feeding], feed efficiency ratio (FER) = weight gain of fish/dry feed intake, protein efficiency ratio (PER) = weight gain of fish/protein consumed, protein retention (PR) = protein gain of fish/protein consumed, condition factor (CF) = body weight (g)/total length $(cm)^3$, hepatosomatic index (HSI) = liver weight (g)/body weight (g).

One-way ANOVA and Duncan`s multiple range test (Duncan, 1955) were used to analyze the significance of the difference among the means of treatments through SAS version 9.3 (SAS Institute, Cary, NC, USA). In addition, regression analysis was conducted between weight gain, SGR, FER, PER and PR of fish as the dependent variable and amount of TBN substitution in the experimental diets as the independent variable.

3. Results

Amino acid profiles of the experimental diets are presented in Table 2. An essential amino acid, lysine tended to decrease with an increased TBM substitution with fishmeal in the experimental diets. Lysine and arginine content ranged from 2.55 to 3.55% and 2.60 to 2.91% of the experimental diets, but 0.48 to 0.60% for methionine content, respectively.

Survival (%), weight gain (g/fish) and specific growth rate (SGR) of olive flounder fed the experimental diets substituting fishmeal with TBM for 7 weeks are given in Table 3. Survival of fish ranging from 95.6 to 98.9% was not significantly (P > 0.05) different among the experimental diets. The following correlation between weight gain of fish (Y) and TBM (X) was observed: $Y = -0.0005\ X^2 - 0.0906X + 33.012$ ($R^2 = 0.8515$, $P < 0.0001$), and $Y = -0.00005\ X^2 - 0.0034X + 2.6153$ ($R^2 = 0.8760$, $P < 0.0001$) for correlation between SGR of fish (Y) and TBM (X), respectively. However, weight gain and SGR of fish fed the Con, TBM5 and TBM10 diets were significantly (P < 0.05) higher than those of fish fed the TBM40, TBM60, TBM80, TBM100 and TBM100-NP diets, but not significantly (P > 0.05) different from those of fish fed the TBM20 and TBM30 diets. Weight gain and SGR of fish fed the TBM20 and TBM30 diets were significantly (P < 0.05) higher than those of fish fed the TBM60, TBM80, TBM100 and TBM100-NP diets, but not significantly (P > 0.05) different from those of fish fed the TBM40 diet. The poorest weight gain and SGR of fish was observed in fish fed the TBM100 diet.

Feed intake (g/fish), feed efficiency ratio (FER), protein efficiency ratio (PER), protein retention (PR), condition factor (CF) and hepatosomatic index (HSI) of juvenile olive flounder fed the experimental diets substituting fishmeal with TBM are given in Table 4.

Feed intake of fish was not significantly (P > 0.05) different among the experimental diets. The following correlation between FER (Y) and TBM (X) was observed: $Y = -0.00000007\ X^2 - 0.0032X + 0.9869$ ($R^2 = 0.8617$, $P < 0.0001$).

Table 2. Amino acid profiles of the experimental diets (DM % in the diet)

	Experimental diets									
	Con	TBM5	TBM10	TBM20	TBM30	TBM40	TBM60	TBM80	TBM100	TBM100-NP
Alanine	2.73	2.64	2.71	2.67	2.62	2.64	2.81	2.59	2.52	2.44
Arginine	2.70	2.57	2.66	2.64	2.60	2.65	2.91	2.70	2.73	2.65
Aspartic acid	6.43	6.44	6.50	6.53	6.57	6.70	7.51	6.99	6.74	6.93
Cystine	0.06	0.07	0.07	0.07	0.07	0.07	0.10	0.08	0.07	0.08
Glutamic acid	7.09	6.96	7.36	7.39	7.49	7.86	8.71	8.30	7.51	8.27
Glycine	2.84	2.80	2.90	2.85	2.82	2.89	3.05	2.78	2.87	2.71
Histidine	1.78	1.68	1.62	1.63	1.72	1.73	1.93	1.90	1.84	1.87
Isoleucine	1.91	1.86	1.90	1.90	1.88	2.02	2.21	2.11	1.89	1.97
Leucine	3.51	3.48	3.58	3.59	3.57	3.71	4.08	3.89	3.52	3.73
Lysine	3.55	3.38	3.39	3.31	3.21	3.25	3.16	3.06	2.55	2.59
Methionine	0.48	0.49	0.51	0.52	0.52	0.51	0.60	0.56	0.50	0.50
Phenylalanine	1.97	1.95	2.00	2.01	2.02	2.11	2.35	2.24	2.11	2.20
Proline	1.72	1.83	1.86	1.93	2.02	2.18	2.44	2.36	1.96	2.42
Serine	1.70	1.64	1.75	1.79	1.83	1.89	2.12	2.03	1.87	2.06
Threonine	2.19	2.16	2.26	2.22	2.21	2.28	2.45	2.39	2.23	2.24
Tyrosine	1.19	1.37	1.46	1.46	1.34	1.40	1.56	1.39	1.34	1.46
Valine	2.27	2.20	2.24	2.24	2.21	2.35	2.55	2.37	2.09	2.13

Table 3. Survival (%), weight gain (g/fish) and specific growth rate (SGR) of olive flounder fed the experimental diets substituting fishmeal with tuna byproduct meal (TBM) for 7 weeks

Experimental diets	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR[1] (%/day)
Con	12.6 ± 0.03	45.3 ± 1.14[a]	96.7 ± 1.92	32.6 ± 1.13[a]	2.60 ± 0.050[a]
TBM5	12.6 ± 0.01	45.2 ± 0.31[a]	97.8 ± 1.11	32.6 ± 0.32[a]	2.61 ± 0.016[a]
TBM10	12.6 ± 0.02	45.7 ± 2.57[a]	95.6 ± 2.94	33.0 ± 2.58[a]	2.62 ± 0.114[a]
TBM20	12.6 ± 0.01	43.4 ± 1.41[ab]	97.8 ± 2.22	30.8 ± 1.41[ab]	2.52 ± 0.066[ab]
TBM30	12.7 ± 0.01	42.1 ± 1.41[ab]	98.9 ± 1.11	29.4 ± 1.40[ab]	2.45 ± 0.066[ab]
TBM40	12.6 ± 0.01	40.9 ± 0.67[bc]	98.9 ± 1.11	28.3 ± 0.68[bc]	2.40 ± 0.035[bc]
TBM60	12.6 ± 0.01	38.3 ± 0.11[cd]	98.9 ± 1.11	25.7 ± 0.11[cd]	2.26 ± 0.006[cd]
TBM80	12.7 ± 0.03	36.6 ± 1.29[d]	97.8 ± 2.22	23.9 ± 1.30[d]	2.16 ± 0.073[d]
TBM100	12.6 ± 0.00	31.4 ± 0.29[e]	97.8 ± 1.11	18.8 ± 0.29[e]	1.86 ± 0.019[e]
TBM100-NP	12.6 ± 0.04	32.4 ± 0.62[e]	97.8 ± 2.22	19.8 ± 0.60[e]	1.92 ± 0.036[e]

Values (means of triplicate ± SE) in the same column sharing a common superscript are not significantly different ($P > 0.05$).

[1]SGR = (Ln final weight of fish - Ln initial weight of fish) × 100/days of feeding trial.

Table 4. Feed consumption (g/fish), feed efficiency ratio (FER), protein efficiency ratio (PER), protein retention (PR), condition factor (CF) and hepatosomatic index (HSI) of olive flounder fed the experimental diets substituting fishmeal with tuna byproduct meal (TBM) for 7 weeks

Experimental diets	Feed consumption	FER[1]	PER[2]	PR[3]	CF[4]	HSI[5]
Con	33.5 ± 1.10	0.98 ± 0.012[ab]	1.97 ± 0.023[ab]	37.0 ± 0.61[a]	0.80 ± 0.009	0.87 ± 0.020[a]
TBM5	32.8 ± 0.08	1.00 ± 0.015[a]	1.99 ± 0.024[a]	37.0 ± 0.59[a]	0.79 ± 0.023	0.79 ± 0.003[bc]
TBM10	34.7 ± 2.27	0.95 ± 0.011[abc]	1.87 ± 0.025[bc]	35.2 ± 0.30[ab]	0.79 ± 0.010	0.85 ± 0.018[ab]
TBM20	34.4 ± 1.55	0.90 ± 0.025[bcd]	1.79 ± 0.048[cd]	37.3 ± 1.57[a]	0.79 ± 0.023	0.89 ± 0.012[a]
TBM30	33.6 ± 2.11	0.88 ± 0.027[cde]	1.77 ± 0.054[cd]	33.1 ± 0.30[bc]	0.79 ± 0.023	0.91 ± 0.035[a]
TBM40	33.0 ± 0.68	0.86 ± 0.020[de]	1.70 ± 0.032[de]	35.6 ± 0.80[ab]	0.79 ± 0.018	0.79 ± 0.029[bc]
TBM60	32.3 ± 0.91	0.81 ± 0.026[ef]	1.60 ± 0.042[e]	30.3 ± 1.07[d]	0.76 ± 0.022	0.80 ± 0.032[bc]
TBM80	32.4 ± 1.55	0.74 ± 0.017[f]	1.48 ± 0.023[f]	31.9 ± 0.37[cd]	0.77 ± 0.012	0.89 ± 0.026[a]
TBM100	29.6 ± 0.14	0.65 ± 0.019[g]	1.27 ± 0.025[g]	25.6 ± 0.76[e]	0.75 ± 0.025	0.75 ± 0.017[c]
TBM100-NP	29.7 ± 1.58	0.67 ± 0.021[g]	1.33 ± 0.041[g]	25.2 ± 0.94[e]	0.76 ± 0.025	0.76 ± 0.023[c]

Values (means of triplicate ± SE) in the same column sharing a common superscript are not significantly different ($P > 0.05$).

[1]Feed efficiency ratio (FER) = Weight gain of fish/feed consumed.

[2]Protein efficiency ratio (PER) = Weight gain of fish/protein consumed.

[3]Protein retention (PR) = Protein gain of fish/protein consume.

[4]CF = Body weight/total length^3.

[5]HSI = Liver weight/body weight.

However, FER of fish fed the Con diet was significantly ($P < 0.05$) higher than that of fish fed the TBM30, TBM40, TBM60, TBM80, TBM100 and TBM100-NP diets, but not significantly ($P > 0.05$) different from that of fish fed the TBM5, TBM10 and TBM20 diets. The following correlation between PER(Y) and TBM(X) was observed: $Y = -0.0000006\ X^2 - 0.0061X + 1.9627$ ($R^2 = 0.9338$, $P < 0.0001$). PER of fish fed the Con was significantly ($P < 0.05$) higher than that of fish fed the TBM20, TBM30, TBM40, TBM60, TBM80, TBM100 and TBM100-NP diets, but not significantly ($P > 0.05$) different from that of fish fed the TBM5 and TBM10 diets. The following correlation between PR (Y) and TBM (X) was observed: $Y = -0.0006\ X^2 - 0.0374X + 36.753$ ($R^2 = 0.7661$, $P < 0.0001$). PR of fish fed the Con diet was significantly ($P < 0.05$) higher than that of fish fed the TBM30, TBM60, TBM80, TBM100 and TBM100-NP diets, but not significantly ($P > 0.05$) different from that of fish fed the TBM5, TBM 10, TBM20 and TBM40 diets.

CF of fish was not significantly ($P > 0.05$) different among the experimental diets. However, HSI of fish fed the Con, TBM20, TBM30 and TBM80 diets was significantly ($P < 0.05$) higher than that of fish fed the TBM5, TBM40, TBM60, TBM100 and TBM100-NP diets, but not significantly ($P > 0.05$) different from that of fish fed the TBM10 diet.

Proximate composition of the whole body excluding liver and liver in olive flounder at the end of the 7-week feeding trial is presented in Table 5. Moisture content of the whole body excluding liver in fish fed the Con, TBM5, TBM10 and TBM30, TBM60 and TBM100-NP diets was significantly ($P < 0.05$) higher than that of fish fed the TBM20, TBM40, TBM80 and TBM100 diets. However, crude protein content of the whole body excluding liver in fish was not significantly ($P > 0.05$) different among the experimental diets. Crude lipid content of the whole body excluding liver in fish fed the TBM80 diet was significantly ($P < 0.05$) higher than that of fish fed the Con, TBM5, TBM10, TBM30, TBM60 and TBM100-NP diets, but not significantly ($P > 0.05$) different from that of fish fed the TBM20,

Table 5. Proximate composition (% of wet weight) of the whole body excluding liver and liver in olive flounder at the end of the 7-week feeding trial

Experimental diets	Whole body excluding liver			
	Moisture	Crude protein	Crude lipid	Ash
Con	75.9 ± 0.24[a]	18.2 ± 0.17	3.2 ± 0.05[bcde]	3.4±0.46[c]
TBM5	75.8 ± 0.37[a]	18.1 ± 0.27	3.1 ± 0.07[e]	3.4±0.44[c]
TBM10	75.6 ± 0.37[a]	18.2 ± 0.25	3.1 ± 0.07[de]	3.1±0.49[c]
TBM20	73.7 ± 0.30[bc]	18.4 ± 0.16	3.3 ± 0.04[ab]	3.6±0.13[bc]
TBM30	75.6 ± 0.36[a]	18.5 ± 0.08	3.1 ± 0.08[e]	3.3±0.23[c]
TBM40	73.6 ± 0.06[c]	18.6 ± 0.08	3.3 ± 0.03[abc]	3.9±0.51[bc]
TBM60	75.4 ± 0.16[a]	18.2 ± 0.13	3.1 ± 0.02[cde]	3.8±0.16[bc]
TBM80	73.0 ± 0.11[c]	17.9 ± 0.01	3.4 ± 0.06[a]	4.6±0.19[ab]
TBM100	74.5 ± 0.24[b]	18.0 ± 0.14	3.3 ± 0.03[abcd]	5.2±0.38[a]
TBM100-NP	75.6 ± 0.17[a]	18.0 ± 0.12	3.1 ± 0.01[de]	3.5±0.10[c]

	Liver		
	Moisture	Crude protein	Crude lipid
Con	69.7 ± 0.16[c]	16.2 ± 0.22[c]	16.5 ± 0.07
TBM5	70.1 ± 0.51[c]	17.4 ± 0.17[ab]	16.4 ± 0.28
TBM10	72.7 ± 0.27[a]	16.1 ± 0.11[c]	15.4 ± 0.11
TBM20	71.9 ± 0.76[ab]	16.4 ± 0.41[bc]	15.8 ± 0.32
TBM30	72.2 ± 0.55[ab]	16.5 ± 0.27[bc]	15.6 ± 0.36
TBM40	70.0 ± 0.59[c]	17.9 ± 0.49[a]	16.5 ± 0.31
TBM60	71.4 ± 0.88[abc]	17.3 ± 0.62[ab]	16.0 ± 0.44
TBM80	70.9 ± 0.26[bc]	17.6 ± 0.21[a]	16.2 ± 0.04
TBM100	70.8 ± 0.18[bc]	18.0 ± 0.24[a]	16.2 ± 0.15
TBM100-NP	72.1 ± 0.41[ab]	17.3 ± 0.22[ab]	15.8 ± 0.19

Values (means of triplicate ± SE) in the same column sharing a common superscript are not significantly different ($P > 0.05$).

TBM40 and TBM100 diets. Ash content of the whole body excluding liver in fish fed the TBM100 diet was significantly ($P < 0.05$) higher than that of fish fed the all other diets, except for the TBM 80 diet. Moisture content of liver in fish fed the TBM10 diet was significantly ($P < 0.05$) higher than that of fish fed the Con, TBM5, TBM40, TBM80 and TBM100 diets, but not significantly ($P > 0.05$) different from that of fish fed the TBM20, TBM30, TBM60 and TBM100-NP diets. Crude protein content of liver in fish fed the TBM40, TBM80 and TBM100 diets was significantly ($P < 0.05$) higher than that of fish fed the Con, TBM10, TBM20 and TBM30 diets, but not significantly ($P > 0.05$) different from that of fish fed the TBM5, TBM60 and TBM100-NP diets. However, crude lipid content of liver in fish was not significantly ($P > 0.05$) different among the experimental diets.

Amino acid profiles of the whole body of olive flounder at the end of the 7-week feeding trial were not significantly ($P > 0.05$) different among the experimental diets (Table 6).

Plasma glucose, GOT, GPT, triglyceride and cholesterol in olive flounder was not significantly ($P > 0.05$) different among the experimental diets due to wide variation within the same diets (Table 7). However, plasma total protein in fish fed the TBM20, TBM30 and TBM40 diets was significantly ($P < 0.05$) higher than that of fish fed the TBM100 and TBM100-NP diets, but not significantly ($P > 0.05$) different from that of fish fed the Con, TBM5, TBM10, TBM60 and TBM80 diets.

Table 6. Amino acid profiles of olive flounder at the end of the 7-week feeding trial (% in the whole body)

	Experimental diets									
	Con	TBM5	TBM10	TBM20	TBM30	TBM40	TBM60	TBM80	TBM100	TBM100-NP
Alanine	1.30 ± 0.044	1.25 ± 0.068	1.26 ± 0.018	1.26 ± 0.038	1.24 ± 0.015	1.22 ± 0.023	1.21 ± 0.035	1.23 ± 0.030	1.20 ± 0.047	1.22 ± 0.052
Arginine	1.34 ± 0.035	1.30 ± 0.058	1.29 ± 0.015	1.25 ± 0.022	1.26 ± 0.010	1.25 ± 0.020	1.24 ± 0.032	1.25 ± 0.037	1.23 ± 0.038	1.22 ± 0.046
Aspartic	3.28 ± 0.121	3.22 ± 0.160	3.21 ± 0.003	3.13 ± 0.023	3.20 ± 0.075	3.16 ± 0.061	3.08 ± 0.052	3.13 ± 0.094	3.04 ± 0.095	3.01 ± 0.184
Cystine	0.08 ± 0.009	0.07 ± 0.009	0.07 ± 0.015	0.07 ± 0.012	0.07 ± 0.010	0.08 ± 0.009	0.08 ± 0.003	0.08 ± 0.003	0.08 ± 0.003	0.08 ± 0.006
Glutamic	3.53 ± 0.082	3.45 ± 0.142	3.47 ± 0.048	3.41 ± 0.033	3.41 ± 0.038	3.38 ± 0.047	3.39 ± 0.078	3.40 ± 0.101	3.31 ± 0.071	3.26 ± 0.143
Glycine	1.03 ± 0.025	1.02 ± 0.026	1.02 ± 0.012	0.98 ± 0.007	1.02 ± 0.015	0.99 ± 0.023	1.01 ± 0.030	0.99 ± 0.020	1.01 ± 0.025	0.96 ± 0.038
Histidine	0.56 ± 0.029	0.53 ± 0.030	0.54 ± 0.003	0.53 ± 0.009	0.53 ± 0.009	0.53 ± 0.007	0.50 ± 0.022	0.48 ± 0.044	0.50 ± 0.019	0.51 ± 0.020
Isoleucine	0.98 ± 0.041	0.96 ± 0.047	0.98 ± 0.020	0.94 ± 0.034	0.92 ± 0.029	0.95 ± 0.006	0.93 ± 0.019	0.94 ± 0.015	0.93 ± 0.031	0.92 ± 0.046
Leucine	1.80 ± 0.041	1.76 ± 0.080	1.77 ± 0.020	1.73 ± 0.023	1.72 ± 0.012	1.72 ± 0.020	1.71 ± 0.032	1.72 ± 0.051	1.66 ± 0.052	1.66 ± 0.069
Lysine	2.20 ± 0.075	2.14 ± 0.112	2.13 ± 0.019	2.08 ± 0.017	2.07 ± 0.015	2.08 ± 0.020	2.06 ± 0.047	2.08 ± 0.067	2.03 ± 0.066	2.00 ± 0.090
Methionine	0.24 ± 0.010	0.24 ± 0.007	0.24 ± 0.015	0.23 ± 0.013	0.25 ± 0.015	0.24 ± 0.013	0.24 ± 0.006	0.23 ± 0.006	0.23 ± 0.015	0.23 ± 0.019
Phenylalanine	0.91 ± 0.020	0.88 ± 0.040	0.89 ± 0.009	0.88 ± 0.017	0.87 ± 0.009	0.88 ± 0.007	0.85 ± 0.015	0.91 ± 0.043	0.83 ± 0.027	0.84 ± 0.038
Proline	0.69 ± 0.003	0.69 ± 0.012	0.68 ± 0.013	0.67 ± 0.000	0.70 ± 0.015	0.68 ± 0.006	0.71 ± 0.015	0.68 ± 0.010	0.67 ± 0.025	0.64 ± 0.035
Serine	0.88 ± 0.033	0.84 ± 0.059	0.86 ± 0.006	0.87 ± 0.019	0.85 ± 0.023	0.83 ± 0.023	0.82 ± 0.034	0.83 ± 0.045	0.80 ± 0.035	0.83 ± 0.036
Threonine	0.95 ± 0.025	0.94 ± 0.022	0.95 ± 0.038	0.92 ± 0.027	0.91 ± 0.087	0.93 ± 0.032	0.90 ± 0.038	0.92 ± 0.039	0.87 ± 0.013	0.85 ± 0.028
Tyrosine	0.72 ± 0.026	0.72 ± 0.033	0.72 ± 0.017	0.73 ± 0.007	0.71 ± 0.015	0.70 ± 0.015	0.69 ± 0.025	0.72 ± 0.026	0.67 ± 0.023	0.67 ± 0.037
Valine	1.05 ± 0.039	1.02 ± 0.042	1.04 ± 0.015	1.01 ± 0.034	0.99 ± 0.023	1.02 ± 0.010	1.00 ± 0.010	1.01 ± 0.006	0.98 ± 0.030	0.97 ± 0.047

Values (means of triplicate ± SE) in the same row sharing a common superscript are not significantly different ($P > 0.05$).

No significant difference was found among fish fed the experimental diets.

Table 7. Plasma chemistry of olive flounder at the end of the 7-week feeding trial

Experimental diets	Total protein (g/dL)	Glucose (mg/dL)	GOT (IU/L)	GPT (IU/L)	Triglyceride (mg/dL)	Cholesterol (mg/dL)
Con	2.67 ± 0.067[ab]	71.0 ± 15.13	33.3 ± 2.33	2.67 ± 0.333	126.3 ± 6.17	134.7 ± 2.85
TBM5	2.80 ± 0.153[ab]	76.7 ± 13.78	42.3 ± 9.87	4.00 ± 0.577	119.3 ± 20.58	146.3 ± 7.80
TBM10	2.83 ± 0.067[ab]	60.7 ± 11.14	34.0 ± 6.03	3.00 ± 0.000	127.0 ± 15.18	144.0 ± 3.51
TBM20	3.10 ± 0.153[a]	76.3 ± 10.99	33.0 ± 6.24	3.33 ± 0.333	137.3 ± 3.84	151.0 ± 4.36
TBM30	2.93 ± 0.088[a]	73.3 ± 19.34	42.7 ± 6.89	4.00 ± 0.577	142.7 ± 7.13	155.7 ± 5.24
TBM40	2.87 ± 0.219[a]	81.7 ± 39.82	39.0 ± 6.24	5.00 ± 1.000	144.3 ± 11.10	149.0 ± 7.64
TBM60	2.83 ± 0.067[ab]	55.0 ± 4.62	36.3 ± 2.33	4.00 ± 0.000	155.3 ± 6.69	156.3 ± 5.61
TBM80	2.70 ± 0.100[ab]	61.7 ± 2.60	35.0 ± 3.21	3.33 ± 0.333	147.7 ± 4.67	138.7 ± 8.82
TBM100	2.40 ± 0.058[b]	40.3 ± 2.33	31.7 ± 8.21	3.00 ± 0.000	113.0 ± 2.65	133.3 ± 0.88
TBM100-NP	2.40 ± 0.208[b]	41.3 ± 6.89	52.3 ± 25.04	3.33 ± 0.333	144.3 ± 9.02	126.3 ± 13.32

Values (means of triplicate ± SE) in the same column sharing a common superscript are not significantly different ($P > 0.05$).

4. Discussion

A few essential amino acid requirements for olive flounder have been known: lysine (1.9-2.1% of the diet), methionine (1.44% of the diet in the presence of 0.06% of cystine), arginine (2.04-2.10% of the diet) and (Forster & Ogata, 1998; Alam et al., 2000, 2002a). Lysine and arginine content (2.55-3.55% and 2.60-2.91% of the diets, respectively) in the experimental diets were all satisfied for dietary requirements for olive flounder, but methionine content (0.48-0.60% of the experimental diets in the presence of 0.06-0.10% cystine) was relatively low in this study. The low methionine content of the experimental diets did not deteriorate performance of olive flounder in this study because SGR obtained in fish fed the Con diet was comparable to that in juvenile fish reported in other studies (Kim et al., 2005; Deng et al., 2006; Yoo et al., 2007).

A single alternative animal or plant protein source for fishmeal in the diet is likely to be deficient in the essential amino acids, such as lysine and methionine. Therefore, substitution of fishmeal with the combined animal and plant protein sources in the diet was more effective than a single protein (either animal or plant protein) source for carp (*Cyprinus carpio*) (Hossain & Jauncey, 1989), largemouth bass (*Micropterus salmoides*) (Tidwell et al., 2005), cuneate drum (*Nibea miichthioides*) (Guo et al., 2007), red sea bream (*Pagrus major*) (Kader et al., 2010; Kader & Koshio, 2012) and olive flounder (Kader et al., 2012). Kikuchi (1999a) also reported that a 45% fishmeal could be replaced with the combined soybean meal, blood meal, corn gluten meal and blue mussel meat for olive flounder.

No significant differences in weight gain and SGR of fish fed the TBM30 and Con diets in this study indicated that fishmeal could be replaced with up to 30% TBM in the diet without adverse effect of growth and SGR of juvenile olive flounder. Therefore, TBM is a promising feed ingredient to substitute fishmeal in the diet for olive flounder. However, substitution of fishmeal with greater than 30%

TBM in the experimental diets resulted in poorer weight gain and SGR of fish. Similarly, the 30 and 36% fishmeal could be replaced with the blend of fermented soybean meal (by *Bacillus* spp.) and squid byproduct at the ratio of 3:2 and fermented soybean meal and scallop byproduct at the ratio of 1:1 for red sea bream and olive flounder, respectively (Kader et al., 2011, 2012). Uyan et al. (2006) reported that tuna muscle byproduct powder could replace 50% fishmeal without reduction in growth performance of olive flounder. However, it can not be used as a cost-effective ingredient for aquafeed due to limitation of its supply and its high price.

No difference in FER and PR of fish fed the TBM20 diet compared to those of fish fed the Con diet in this study indicated that fishmeal up to 20% could be replaced with TBM without deterioration of FER and PR for juvenile olive flounder, resulted from an effective improvement in weight gain of fish. However, PER of fish fed the TBM20 diet was poorer than that of fish fed the Con diet. Similarly, FER, PER and/or PR of olive flounder (Kikuchi, 1999a; Kader et al., 2012), gibel carp (*Carassius auratus gibelio*) (Yang et al., 2004; Zhang et al., 2006) and red sea bream (*Pagrus major*) (Kader et al., 2011; Kader & Koshio, 2012) improved when fishmeal was replaced with the alternative animal and/or plant protein sources in the diet.

No difference in weight gain (SGR) and feed utilization (FER, PER and PR) of fish fed the TBM100 and TBM100-NP diets in this study indicated that phosphorous probably originated from bone of tuna in the TBM100-NP diet could be equally effective as 0.37% sodium phosphate monobasic in the TBM100 diet for olive flounder, probably indicating that TBM could be used as a supplemental phosphorous source in the diet for olive flounder. Similarly, Uyan et al. (2006) reported that 50% fishmeal could be replaced with tuna muscle byproduct powder without reduction in growth performance of olive flounder and resulted in 50% lower phosphorous loading than the control diet (fishmeal-based diet) into the environment. Vielma et al. (1999) also reported that supplementation of the herring bone meal in the low phosphorous basal diet increased the growth rate of rainbow rout and suggested that particle size of bone meal could significantly affect performance of fish. However, unlike these studies, Lee et al. (2010) reported that fish bone meal derived from Alaskan seafood processing byproduct could be used in rainbow trout feed as a supplemental calcium source, but not as the primary source of phosphorous because of the low bioavailability of phosphorous by fish in the diets. A further study to determine substitution effect of TBM with minerals, especially, phosphorous source in the diet for fish is needed in detail.

Proximate composition of fish was affected by the experimental diets, except for crude protein of the whole body excluding liver and crude lipid of liver. Especially, ash content in fish body seemed to be relatively well reflected from that of the experimental diets. Similarly, the proximate composition of the whole body of fish was affected by the various alternative animal and/or plant protein sources for fishmeal in the diets (Kikuchi, 1999a; Deng et al., 2006; Uyan et al., 2006; Lee et al., 2010; Zhou et al., 2011; Lee et al., 2012a; Kader & Koshio, 2012). However, dietary substitution of the combined animal and plant protein sources for fishmeal did not change the proximate composition of fish (Cho et al., 2005; Tidwell et al., 2005; Lee et al., 2012b). Zeitler et al. (1984) demonstrated that the whole body composition of fish correlated with fish species, feeding and diet formulation.

No difference in amino acid profiles of the whole body of fish was observed in this study. This was probably explained by Yamamoto et al. (2000)'s study showing that the whole body amino acid composition of rainbow trout (*Oncorhynchus mykiss*) fed the various dietary compositions did not show marked differences because body proteins are synthesized based on the genetic information from DNA, so that amino acid composition of specific body proteins is the same irrespective of dietary. Similarly, the different amino acid patterns in the diet did not alter a marked difference in the amino acid composition of olive flounder (Alam et al., 2002b). Unlike this study, however, amino acid profiles of fish were affected by the dietary substitution of fishmeal with soy protein concentrate (Deng et al., 2006).

Alternative protein sources in the diets affected plasma constituents of fish (Kikuchi et al., 1994b; Kikuchi, 1999a; Lim & Lee, 2011). In addition, serum total protein, triglyceride and cholesterol concentration of olive flounder would be affected by dietary substitution of fishmeal with soybean meal although the whole body composition of fish was not affected (Ye et al., 2011). Kader & Koshio (2012) also reported that plasma glucose and total cholesterol was affected by dietary substitution of fishmeal with seafood byproduct and soybean protein for red sea bream. However, unlike this study, dietary substitution of fishmeal with the various protein sources did not affect serum chemistry of fish (Cho et al., 2005; Lee et al., 2012a, 2012b). Lim & Lee (2011) also reported that plasma chemistry of Nile tilapia (*Oreochromis niloticus*) was not affected by dietary substitution of fishmeal with the fermented plant protein sources.

Based on these results, it can be concluded that dietary substitution of fishmeal with up to 30 and 20% TBM could be made without adverse effect on growth (SGR) and feed utilization (FER and PR) of juvenile olive flounder, respectively.

II. Conclusion

Substitution effects of fishmeal with tuna byproduct meal (TBM) in the diet on growth, body composition, plasma chemistry and amino acid profiles of juvenile olive flounder (*Paralichthys olivaceus*) were determined. It can be concluded that dietary substitution of fishmeal with up to 30 and 20% TBM could be made without adverse effect on growth (SGR) and feed utilization (FER and PR) of juvenile olive flounder, respectively.

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