Thesis for the Degree of Master of Science

Response of dietary substitution of fishmeal with various protein sources on growth, body composition and blood chemistry of olive flounder (*Paralichthys olivaceus*)

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by

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요 약

어분의 높은 가격으로 인하여 사료 내에서 어분을 대체 할 수 있는 동물성 또는 식물성 단백질원은 지속적으로 개발 되고 있는 실정이다. 본 연구의 목적은 잠용박, 프로메이트밀®, 육골분과 이들 혼합물을 사료 내 어분대체 물질로 사용하여 유어기 넙치 (*Paralichthys olivaceus*) 에게 미치는 영향을 규명 하는 것이다.

주요 단백질원으로서 60%의 어분이 사용된 실험구를 대조구(Con)로 표시 하였다. 어분대체 물질로서 잠용박과 육골분을 10%, 20% 대체한 실험구를 각각 SPM10, SPM20, MBM10 그리고 MBM20으로 표시 하였다. 또한 프로메이트밀®을 10%, 20%, 40%의 양으로 어분 대체한 실험구를 각각 PM10, PM20 그리고 PM40으로 표시 하였다. 마지막으로 잠용박과 프로메이트밀®의 혼합물을 10%, 20% 양으로 어분 대체한 실험구를 각각 SPM+PM20, SPM+PM20으로 표시 하였다.

실험어의 증체량과 성장률은 PM10실험구가 대조구, SPM20, PM20 그리고 SPM+PM20 실험구에 비해 높게 측정 되었다. 사료전환효율은 SPM10, MBM10, MBM20, PM10 그리고 SPM+PM10 실험구가 SPM+PM20, PM40실험구에 비해 높게 측정 되었다. 단백질전환효율은 MBM10, MBM20 실험구가 SPM20, PM20, PM40 그리고 SPM+PM20 실험구에 비해 높게 측정 되었다.

결론적으로, 10%의 잠용박, 20%까지의 육골분, 10%의 프로메이트밀® 그리고 10%의 잠용박과 프로메이트밀®의 혼합분이 사료내 어분대체 단백질원으로 그 사용이 유용 할 것으로 보인다.

Response of dietary substitution of fishmeal with various protein sources on growth, body composition and blood chemistry of olive flounder (*Paralichthys olivaceus*)

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Abstract

Animal and/or plant protein sources substituting fishmeal in the diets keep being developed due to its high price. The purpose of this study is to determine response of dietary substitution of fishmeal with silkworm pupae meal, promate meal®, meat and bone meal and/or their combination on performance of juvenile olive flounder (*Paralichthys olivaceus*).

A 60% fish meal was used as the main protein source, used as the control (Con) diet. The 10 and 20% fishmeal were substituted with silkworm pupae meal and meat and bone meal, referred to as the SPM10, SPM20, MBM10 and MBM20 diets, respectively. And the 10, 20 and 40% fishmeal

were substituted with promate meal®, referred to as the PM10, PM20 and PM40 diets, respectively. Finally the 10 and 20% fishmeal were substituted with combined silkworm pupae meal and promate meal®, refereed to as the SPM+PM10 and SPM+PM20 diets, respectively.

Weight gain and specific growth rate of fish fed the MBM10 diet were higher than those of fish fed the Con, SPM20, PM20, PM40 and SPM+PM20 diets. Feed efficiency ratio of fish fed the SPM10, MBM10, MBM20, PM10 and SPM+PM10 diets was higher than that of fish fed the SPM+PM20 and PM40 diets. Protein efficiency ratio of fish fed the MBM10 and MBM20 diets was higher than that of fish fed the SPM20, PM20, PM40 and SPM+PM20 diets.

In conclusion, dietary substitution of fishmeal with 10% SPM, 20% MBM, 10% PM and 10% SPM+PM could be made.

Keywords Olive flounder (*Paralichthys olivaceus*) . Fishmeal . Weight gain . Specific growth rate . Protein meal.

Abbreviation

SPM	Silkworm pupae meal
MBM	Meat and bone meal
PM	Promate meal®
SPM+PM	Combined silkworm pupae meal and promate meal®
GOT	Glutamic oxaloacetic transaminase
GPT	Glutamic pyruvic transaminase
SGR	Specific growth rate
FER	Feed efficiency ratio
PER	Protein efficiency ratio
PR	Protein retention
CF	Condition factor
HSI	Hepatosomatic index
ARG	Arginine
HIS	Histidine
ILE	Isoluecine
LEU	Leucine
PHE	Phenylalanine
THR	Threonine
VAL	Valine
LYS	Lysine

I. 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. Therefore, many feeding trials to determine dietary nutrients requirements (Forster and Ogata 1998; Lee et al. 2000a; Lee et al. 2002), optimum feed allowance (Lee et al. 2000b; Cho et al. 2006a, 2007a) and optimum feeding strategy (Cho 2005; Cho et al. 2006b), and to develop dietary additive to improve immune response and/or quality (Lee et al. 1998; Cho et al. 2007b) for olive flounder have been performed.

In addition, some studies on development of alternative animal and/or plant protein sources for fishmeal in the diets for olive flounder have been performed. Substitution of fishmeal with 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 and Kikuchi 1997) and feather meal up to 40% (Kikuchi et al. 1994a) in the diets for olive flounder had been successfully made without retardation of growth. The alternative plant protein sources, such as soybean meal up to 44% (Kikuchi et al. 1994b) and corn gluten meal up to 40% (Kikuchi 1999a), and their combination, such as combined blood meal, corn gluten meal and blue mussel meat up to 45% (Kikuchi 1999b) for fishmeal in the diets were successfully made.

Furthermore Deng et al. (2006) showed that performance of olive flounder fed the diets substituting soy protein concentrate for 25-100% fishmeal was poorer than that of fish fed the fishmeal-based diet, but the diets with supplementation of amino acids improved.

Recently, the price of fishmeal has sharply increased due to its high demand for expansion of fish farm, overcatching of fish species commonly used as fishmeal resource and execution of international ocean regulation (FAO 2009). Thus development of alternative protein sources for fishmeal in the diet for olive flounder is highly needed more than ever.

Silkworm pupae (*Bombyx mori*) meal and promate meal®, which is a by-product of amino acids synthesized by microorganisms have been recently developed as the alternative protein sources for fishmeal in the aquafeed. In addition, Begum et al. (1994) reported that growth and utilization of the diets for Indian major carp (*Labeo rohita*) fingerling improved when fishmeal up to 50% was substituted with combined silkworm pupae and clam (*Lamellidens marginalis*) meat in the diets.

In this study, response of dietary substitution of fishmeal with silkworm pupae meal, promate meal®, meat and bone meal and/or their combination in the diets on growth, body composition and blood chemistry of juvenile olive flounder was determined.

II. 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 the initiation of the feeding trial. One thousand two hundred juvenile (an initial body weight of 14.5 g) fish were randomly chosen and distributed into 30 of 180-l flow-through tanks (water volume: 150-l) (forty fish per tank). 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 12.0 to 24.5°C (mean ± SD: 18.2 ± 3.80°C) and photoperiod followed natural conditions.

2.2. Design of the Feeding Trial and Preparation of the Experimental Diets

Ten experimental diets were prepared in triplicate (Table 1). A 60% fish meal, 5% soybean meal and 6% corn gluten meal were used as the protein source, which was used as the control (Con) diet. Wheat flour, and fish and soybean oils were used as the carbohydrate and lipid sources, respectively. The 10 and 20% fishmeal were substituted with silkworm pupae meal

Table 1 Ingredient and nutrient composition of the experimental diets used in the experiment

	Experimental diets									
	Con	SPM10	SPM20	MBM10	MBM20	PM10	PM20	PM40	SPM+PM10	SPM+PM20
Ingredients (%, DM)										
Fishmeal[1]	60	54	48	54	48	54	48	36	54	48
Dehulled soybean meal[1]	5	5	5	5	5	5	5	5	5	5
Corn gluten meal[1]	6	6	6	6	6	6	6	6	6	6
Wheat flour	22	22	22	22.1	22.2	22	22	20.4	22	22
Cellulose	2	1.2	0.5	1.5	1	1.1	0.2		1.2	0.3
Mixture of lysine and methionine[2]		0.5	1	0.5	1	0.5	1	2	0.5	1
Silkworm pupae meal dehydrated[1]		6	12						3	6
Meat and bone meal[1]				6	12					
Promate meal®[1]						6	12	24	3	6
Fish oil	1.5	1.6	1.7	1.6	1.7	1.6	1.7	1.8	1.6	1.7
Soybean oil	1.4	1.6	1.7	1.2	1.0	1.7	2.0	2.7	1.6	1.9
Vitamin premix[3]	1	1	1	1	1	1	1	1	1	1
Mineral premix[4]	1	1	1	1	1	1	1	1	1	1
Choline	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Nutrients (%, DM)										
Crude protein	55.4	57.2	57.7	53.7	52.6	55.6	56.4	56.0	55.5	57.3

Table 1 Continued

Crude lipid	9.3	9.4	9.5	8.8	8.5	9.9	9.6	8.7	9.7	9.9
Carbohydrate	24.9	23.6	23.3	25.9	25.6	24.7	24.9	27.5	25.3	23.7
Ash	10.4	9.8	9.5	11.6	13.3	9.8	9.1	7.8	9.5	9.1
Estimated energy (kJ/g diet)[5]	16.9	17.1	17.1	16.6	16.3	17.2	17.2	17.3	17.2	17.3

[1]Fishmeal(CP:63.6%, CL: 9.9%, ash: 14.6%),[1]Dehulledsoybeanmeal,[1]Cornglutenmeal,[1]Silkwormpupaemealdehydrated(CP: 79.2%, CL: 5.4%, ash: 6.3%), [1]Meatandbonemeal(CP: 42.6%, CL: 11.7%, ash: 29.0%) and [1]Promatemeal® (CP: 58.6%, CL: 3.1%, ash: 4.6%) were supplied by Jeilfeed Co. Ltd., Haman, Korea.

[2]Mixture of lysine and DL-methionine: purity of lysine and DL-methionine was over 98% and 99% (Sumitomo Chemical, Japan), respectively and they were mixed at the ratio of 1:1.

[3]Vitamin premix and [4]Mineralpremix were same as Lee et al. (2000a).

[5]Estimated energy calculated based on Garling and Wilson (1976)'s study.

MBM10 and MBM20 diets. In addition, the 10, 20 and 40% fishmeal were substituted with promate meal®, referred to as the PM10, PM20 and PM40 diets, respectively. Finally, the 10 and 20% fishmeal were substituted with combined silkworm pupae meal and promate meal®, refereed to as the SPM+PM10 and SPM+PM20 diets. Besides the mixture of lysine and methionine at the ratio of 1:1, which were likely to be deficient amino acids in alternative protein sources used was included into all experimental diets in proportion to percentage of fishmeal substitution except for the Con diet.

The ingredients of the experimental diets were well mixed with water at the ratio of 3:1 and pelletized by pellet-extruder. The experiment diets dried at room temperature overnight and stored into -20°C until use. All fish were hand-fed to apparent satiation twice a day (08:00 and 17:00 h) for 6 days a week throughout the 6-week feeding trial.

2.3. Analytical Procedures of the Experimental Diets and Fish

Five fish at the initiation and five fish from each tank at the 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 was 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 3 fish from each tank by using syringes after they were starved for 24 h and anesthetized with MS-222 at the concentration of 100 mg/l at the end of the feeding trial. Serum 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. Statistical Analysis

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 9.1 (SAS Institute, Cary, NC, USA).

III. Results and Discussion

Amino acid profiles of the experimental diets were given in Table 2. The essential amino acids, such as arginine (ARG), histidine (HIS), isoluecine (ILE), leucine (LEU), phenylalanine (PHE), threonine (THR) and valine (VAL) were relatively low in the PM20, PM40 and SPM+PM20 diets. ARG requirement of olive flounder was estimated to be 2.04 and 2.10% of the diets according to broken-line analyses of percent weight gain and feed conversion efficiency, respectively (Alam et al. 2002). Forster and Ogata (1998) reported that lysine (LYS) requirement of olive flounder were 1.6, 1.9 and 2.1% of the diets based on specific growth rate, feed efficiency and nitrogen retention of fish, respectively and proposed LYS requirement of 1.9-2.1% of the diets. LYS content of all experimental diets ranged from 2.43 to 2.69% in this study satisfied requirement of olive flounder.

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

	Experimental diets									
	Con	SPM10	SPM20	MBM10	MBM20	PM10	PM20	PM40	SPM+PM10	SPM+PM20
Alanine	3.04	2.86	2.77	2.97	2.75	2.99	2.72	2.64	2.76	2.63
Arginine	2.44	2.62	2.64	2.16	2.30	2.38	2.02	1.78	2.31	2.30
Aspartic	3.85	5.00	3.92	3.92	3.50	3.49	3.43	3.20	3.73	3.47
Glutamic	7.24	7.00	6.88	6.44	6.26	6.90	6.69	7.14	6.43	6.78
Glycine	3.93	3.75	3.46	3.84	3.87	3.55	3.08	2.64	3.43	3.32
Histidine	1.50	1.28	1.27	1.27	1.05	1.33	1.19	1.07	1.18	1.03
Isoleucine	1.90	1.79	1.76	1.75	1.67	1.78	1.68	1.48	1.68	1.63
Leucine	3.74	3.47	3.59	3.33	3.15	3.44	3.07	3.00	3.19	3.17
Lysine	2.58	2.69	2.64	2.63	2.59	2.65	2.43	2.49	2.68	2.60
Phenylalanine	2.06	1.90	1.84	1.86	1.76	1.86	1.52	1.58	1.76	1.75
Proline	3.30	4.05	2.86	2.75	2.67	2.80	2.83	2.70	2.76	2.55
Serine	1.88	1.84	1.99	1.69	1.73	1.72	1.46	1.38	1.76	1.78
Threonine	2.13	1.92	1.95	1.75	1.83	1.79	1.43	1.42	1.74	1.86
Tyrosine	1.18	1.35	1.24	1.10	1.23	1.13	0.96	0.93	1.14	1.36
Valine	2.29	2.18	2.22	2.18	1.93	2.13	1.88	1.83	2.08	1.95

Survival (%), weight gain (g/fish) and specific growth rate (SGR) of olive flounder fed the experimental diets substituting fishmeal with the various protein sources were presented in Table 3. Survival of fish ranged from 85.8 to 100% was not significantly ($P > 0.05$) different among the experimental diets. However, weight gain and SGR of fish fed the MBM10 diet were significantly ($P < 0.05$) higher than those of fish fed the Con, SPM20, PM20, PM40 and SPM+PM20 diets, but not significantly ($P > 0.05$) different from those of fish fed the SPM10, PM10, MBM20 and SPM+PM10 diets. In addition, weight gain and SGR of fish fed the PM20, PM40, which was the lowest and SPM+PM20 diets were significantly ($P < 0.05$) lower than those of fish fed the Con diet, probably resulted from the poor essential amino acids in the former (Table 2). No difference in weight gain and SGR of fish fed SPM10, SPM20 and PM10 diets compared to those of fish fed the Con diet in this study indicated that dietary substitution of 20% and 10% fishmeal with SPM and PM, respectively with supplementation of amino acids could be made without detrimental effect on growth of fish. Similarly, Begum et al. (1994) showed that fingerling Indian major carp fed the diets substituting combined silkworm pupae and clam meat for 50% fishmeal overgrew compared to fish fed the fishmeal-based diet.

Table 3 Survival, weight gain (g/fish) and specific growth rate (SGR) of olive flounder fed substituting fishmeal with the various protein sources for 6weeks (±SE)[1]

	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR[2]
Con	14.5±0.02	53.6±1.49	85.8±7.12	39.1±1.47^{b}	3.11±0.063b
SPM10	14.6±0.04	54.3±0.56	98.3±1.67	39.8±0.55^{ab}	3.13±0.023ab
SPM20	14.5±0.01	51.9±0.13	97.5±1.44	37.4±0.14^{b}	3.03±0.008b
MBM10	14.5±0.04	57.7±1.57	98.3±0.83	43.2±1.53^{a}	3.28±0.059a
MBM20	14.6±0.05	54.9±0.67	93.3±5.46	40.3±0.64^{ab}	3.16±0.025ab
PM10	14.6±0.06	54.8±1.14	96.7±3.33	40.2±1.15^{ab}	3.14±0.053ab
PM20	14.5±0.03	48.0±1.30	96.7±3.33	33.5±1.30^{c}	2.85±0.065c
PM40	14.5±0.17	39.8±0.91	98.8±1.22	25.3±0.75^{d}	2.41±0.027d
SPM+PM10	14.5±0.04	54.7±0.31	100±0.00	40.1±0.31^{ab}	3.15±0.014ab
SPM+PM20	14.5±0.17	48.1±1.71	86.0±7.38	33.6±1.72^{c}	2.85±0.091c

[1]Values in the same column sharing a common superscript are not significantly different (P>0.05).

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

MBM is generally considered to be an inferior animal protein source to fishmeal in the diet for fish culture, however, improvement in weight gain and SGR of olive flounder fed the MBM10 and MBM20 diets in this study indicated that fish fed the properly formulated diets substituting MBM with supplementation of amino acids for 20% fishmeal could overgrow fish fed the fishmeal-based diet. Similarly, Kikuchi et al. (1997) showed that growth of olive flounder fed the diets substituting MBM with supplementation of amino acids for 18% fishmeal was comparable to that of fish fed the fishmeal-based diet. Growth of yellowtail (*Seriola quinqueradia*) and gibel carp (*Carassius auratus gibelio*) substituting MBM for 20% fishmeal was comparable to that of fish fed the fishmeal-based diet (Shimeno et al. 1993; Yang et al. 2004; Zhang et al. 2006). Dietary substitution of 30 and 24% fishmeal with MBM supplemented with methionine could be made without deterioration of performance of cuneate drum (*Nibea miichthioides*) and malabar grouper (*Epinephelus malabaricus)*, respectively (Guo et al. 2007; Li et al. 2009). In addition, weight gain of gilthead seabream (*Sparus aurata*) fed the diets substituting MBM for 40% fishmeal was comparable to that of fish fed the fishmeal-based diet although apparent digestibility of protein in the latter was higher than in the former (Robaina et al. 1997). Ai et al. (2006) also showed that 45% fishmeal could be replaced with MBM for yellow croaker (*Pseudosciaena crocea*) and reduced growth with higher MBM was due to lower digestibility and imbalance of essential amino acids.

Comparable weight gain of olive flounder fed the SPM+PM10 diet, but inferior weight gain of fish the PM20 and SPM+PM20 diets compared to fish fed the Con diet in this study indicated that dietary substitution of fishmeal with 10% SPM+PM could be made without reduction in weight gain of fish, but not with 20% PM or SPM+PM. No more than 5% inclusion of SPM is recommended for the commercial feed for olive flounder because of its high level of non-protein nitrogen (Personal communication; Dr. J. Y. Yoo, Jeilfeed Co. Ltd., Haman, Korea). Dietary substitution of fishmeal with combined animal and plant protein sources effectively improved performance of common carp (*Cyprinus carpio*), olive flounder and cuneate drum rather than the single protein source (Hossain and Jauncey 1989; Kikuchi 1999b; Guo et al. 2007).

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 various protein sources were given in Table 4. Feed consumption of fish fed the Con and MBM10 diets was significantly ($P < 0.05$) higher than that of fish fed the PM20 and PM40 diets, but not significantly ($P > 0.05$) different from that of fish fed the SPM10, SPM20, MBM20, PM10, SPM+PM10 and SPM+PM20 diets. Feed consumption of fish decreased with the increased fishmeal-substitution over 10% PM in the diets and resulted to poor performance, indicating that dietary substitution of fishmeal with PM

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 the various protein sources for 6 weeks (means of triplicate ± SE)[1]

Experimental diets	Feed consumption	FER[2]	PER[3]	PR[4]	CF[5]	HSI[6]
Con	36.2±0.44[a]	1.08±0.049[ab]	1.95±0.088[ab]	42.4±0.39[ab]	1.00±0.019	2.00±0.104[abc]
SPM10	35.5±1.07[ab]	1.12±0.047[a]	1.96±0.082[ab]	38.7±4.49[ab]	0.95±0.011	1.66±0.074[d]
SPM20	34.5±0.61[ab]	1.08±0.017[ab]	1.88±0.029[bc]	35.0±0.50[abc]	0.98±0.009	1.74±0.050[cd]
MBM10	36.3±0.83[a]	1.19±0.020[a]	2.21±0.037[a]	44.6±1.11[a]	1.02±0.037	1.83±0.106[bcd]
MBM20	35.0±1.20[ab]	1.16±0.024[a]	2.20±0.047[a]	44.9±4.37[a]	1.03±0.017	1.98±0.054[abc]
PM10	35.2±1.70[ab]	1.14±0.025[a]	2.05±0.044[ab]	46.2±5.76[a]	0.95±0.020	2.10±0.096[ab]
PM20	31.8±1.19[bc]	1.06±0.064[ab]	1.88±0.113[bc]	35.3±2.72[abc]	0.95±0.010	2.20±0.028[a]
PM40	28.4±1.74[c]	0.89±0.010[c]	1.58±0.018[d]	24.7±2.03[c]	0.98±0.027	2.25±0.040[a]
SPM+PM10	35.0±0.21[ab]	1.15±0.010[a]	2.07±0.017[ab]	43.7±3.63[ab]	1.02±0.022	1.94±0.038[abc]
SPM+PM20	36.0±2.38[ab]	0.94±0.091[bc]	1.65±0.159[cd]	32.5±3.09[bc]	0.97±0.012	1.95±0.148[abc]

[1]Values in the same column sharing a common superscript are not significantly different (P>0.05).

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

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

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

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

[6]HSI = Liver weight/body weight.

must be limited and carefully considered for olive flounder.

FER of fish fed the SPM10, MBM10, MBM20, PM10 and SPM+PM10 diets was significantly ($P < 0.05$) higher than that of fish fed the SPM+PM20 and PM40 diets, which was the lowest, but not significantly ($P > 0.05$) different from that of fish fed the Con, SPM20 and PM20 diets. Improvement in FER of fish fed the SPM10, PM10, MBM10, MBM20 and SPM+PM10 diets in this study indicated that substitution of fishmeal up to 10% with SPM and PM, 20% with MBM, and 10% with SPM+PM in the diets could be made without deterioration of FER.

PER of fish fed the MBM10 and MBM20 diets was significantly ($P < 0.05$) higher than that of fish fed the SPM20, PM20, PM40 and SPM+PM20 diets, but not significantly ($P > 0.05$) different from that of fish fed the Con, SPM10, PM10 and SPM+PM10 diets. PR of fish fed the MBM10, MBM20 and PM10 diets was significantly ($P < 0.05$) higher than that of fish fed the PM40 and SPM+PM20 diets, but not significantly ($P > 0.05$) different from that of fish fed the Con, SPM10, SPM20, PM20 and SPM+PM10 diets. Improvement in PER and/or PR of fish fed the MBM10, MBM20 and PM10 diets in this study indicated that substitution of fishmeal up to 20% MBM and 10% PM in the diets could improve protein utilization of fish, resulted from an effective improvement in weight gain of fish. Similarly, protein utilization of fish commonly improved when fishmeal was replaced with the alternative animal and/or plant protein sources in the diets (Sato and Kikuchi 1997; Kikuchi 1999b; Yang et al. 2004; Zhang et al. 2006).

CF of olive flounder at the end of the 6-week feeding trial was not significantly ($P > 0.05$) different among the experimental diets. However, HSI of fish fed the PM20 and PM40 diets was significantly ($P < 0.05$) higher than that of fish fed the SPM10, SPM20 and MBM10 diets, but not significantly ($P > 0.05$) different from that of fish fed the Con, PM10, MBM20, SPM+PM10 and SPM+PM20 diets. High (20 and 40%) substitution of fishmeal with PM in the diets resulted to an increased HSI of fish in this study, probably resulted from the high non-protein nitrogen content in PM, being agreement with Lee and Kim (2005)'s study showing that HSI of fish was affected by dietary energy content.

Proximate composition of olive flounder at the end of the feeding trial was presented in Table 5. Moisture, crude protein and ash content of the whole body of fish without liver was not significantly ($P > 0.05$) different among the experimental diets. However, crude lipid content of the whole body of fish without liver fed the Con diet was significantly ($P < 0.05$) higher than that of fish fed the SPM10, SPM20, PM20, PM40, MBM20, SPM+PM10 and SPM+PM20 diets, but not significantly ($P > 0.05$) different from that of fish fed the PM10 and MBM10 diets.

Moisture content of liver in olive flounder fed the PM10, MBM10, MBM20 and SPM+PM10 diets was significantly ($P < 0.05$) higher than that

Table 5 Proximate composition (% ofwet weight) of olive flounder at the end of the feeding trial (means of triplicate ± SE)[1]

Experimental diets	Whole body without liver			
	Moisture	Crude protein	Crude lipid	Ash
Con	72.9±0.66	20.2±0.53	4.4±0.06^{a}	3.8±0.09
SPM10	73.2±0.59	18.7±1.13	3.1±0.24^{bc}	3.3±0.03
SPM20	73.5±0.49	18.0±0.01	3.1±0.02^{bc}	3.5±0.16
MBM10	73.7±0.35	19.2±0.36	4.3±0.94^{ab}	3.5±0.16
MBM20	74.1±0.31	19.3±1.26	3.0±0.26^{c}	3.5±0.11
PM10	72.9±0.37	20.9±2.36	3.6±0.30^{abc}	3.5±0.09
PM20	73.1±0.95	18.0±0.48	3.2±0.23^{bc}	3.7±0.16
PM40	74.1±1.03	17.8±1.16	3.0±0.24^{c}	3.8±0.14
SPM+PM10	73.0±0.65	19.8±1.30	2.9±0.26^{c}	3.5±0.04
SPM+PM20	74.3±0.71	18.7±0.08	3.0±0.33^{c}	3.7±0.04

	Liver		
	Moisture	Crude protein	Crude lipid
Con	70.2±0.22^{a}	14.0±1.18^{a}	22.8±1.09^{bc}
SPM10	68.3±1.00^{ab}	14.3±0.64^{a}	24.8±1.68^{b}
SPM20	67.1±1.84^{ab}	13.6±0.74^{a}	31.1±0.07^{a}
MBM10	71.3±0.62^{a}	13.3±0.46^{ab}	25.0±0.74^{b}
MBM20	68.9±1.12^{a}	12.8±0.54^{ab}	16.2±0.21^{d}
PM10	70.2±0.58^{a}	11.7±0.52^{ab}	22.9±0.69^{bc}
PM20	67.4±1.83^{ab}	10.8±0.13^{b}	22.2±0.81^{c}
PM40	64.4±1.52^{b}	11.9±0.24^{ab}	21.7±0.68^{c}
SPM+PM10	69.3±1.17^{a}	13.2±0.33^{ab}	21.1±0.07^{c}
SPM+PM20	67.2±1.75^{ab}	14.2±1.52^{a}	21.1±0.29^{c}

[1]Values in the same column sharing a common superscript are not significantly different (P>0.05).

in fish fed the PM40 diet, but not significantly ($P > 0.05$) different from that in fish fed the SPM10, SPM20, PM20 and SPM+PM20 diets. Crude protein content of liver in fish fed the Con, SPM10, SPM20 and SPM+PM20 diets was significantly ($P < 0.05$) higher than that in fish fed the PM20 diet, but not significantly ($P > 0.05$) different from that in fish fed the MBM10, MBM20, PM10, PM40 and SPM+PM10 diets. Crude lipid content of liver in fish fed the SPM20 diet was significantly ($P < 0.05$) higher than that in fish fed the all other experimental diets. In addition, crude lipid content of liver in fish fed the SPM10 and MBM10 diets was significantly ($P < 0.05$) higher than that in fish fed the MBM20, which was the lowest, PM20, PM40, SPM+PM10 and SPM+PM20 diets. Lowest crude lipid content of liver in fish fed the MBM20 diet was well reflected from its lowest crude lipid content, partially agreeing with other studies showing that body composition of fish was well reflected from dietary nutrient content (Kikuchi et al. 1997; Lee and Kim 2005; Li et al. 2009).

Serum total protein level ranged from 2.9 to 3.5 g/dl, glucose level ranged from 11.7 to 33.7 mg/dl, GOT level ranged from 7.3 to 15.0 IU/l, GPT level ranged from 4.0 to 14.3 IU/l and triglyceride level ranged from 50.0 to 95.7 mg/dl of fish at the end of feeding trial was not significantly ($P > 0.05$) different among the experimental diets (Table 6). Unlike this study, however, dietary alternative protein sources affected plasma constituents; for instance, dietary substitution of fishmeal with soybean meal tended to increase serum triglycerides and/or glucose concentration (Kikuchi et al. 1994b; Kikuchi 1999b). Plasma total protein and triglyceride level was affected by both dietary protein and lipid levels (Lee and Kim 2005).

According to these results, it can be concluded that dietary substitution of fishmeal with 10% SPM, 20% MBM, 10% PM and 10% SPM+PM could be made without detrimental effect on growth and feed utilization of olive flounder.

Table 6 Serum chemistry of olive flounder at the end of the feeding trial (means of triplicate ± SE)[1]

Experimental diets	Total protein (g/dl)	Glucose (mg/dl)	GOT (IU/l)	GPT (IU/l)	Triglyceride (mg/dl)
Con	3.3±0.15	13.0±3.00	12.0±3.06	8.7±1.45	82.0±37.58
SPM10	3.2±0.06	12.7±3.18	15.0±6.51	14.3±6.84	95.7±31.67
SPM20	3.0±0.10	32.7±16.71	8.7±2.33	5.0±1.53	75.3±27.09
MBM10	3.2±0.19	33.7±19.17	13.3±3.18	13.3±4.70	73.0±29.84
MBM20	3.3±0.17	11.7±3.84	7.3±1.76	5.7±2.03	57.0±9.07
PM10	3.5±0.10	16.3±3.48	8.3±0.33	4.0±1.00	64.0±3.06
PM20	2.9±0.07	32.0±18.61	12.7±4.81	10.0±6.03	53.7±4.18
PM40	3.0±0.12	16.3±0.67	7.3±0.88	4.0±0.58	72.0±29.72
SPM+PM10	3.0±0.07	16.7±3.33	14.0±4.51	9.0±4.00	50.0±20.41
SPM+PM20	3.1±0.29	18.3±3.48	11.0±3.21	4.0±1.00	77.0±19.30

[1]Values in the same column sharing a common superscript are not significantly different (P>0.05).

IV. Conclusion

In this study, response of dietary substitution of fishmeal with silkworm pupae meal, promate meal®, meat and bone meal and/or their combination in the diets on growth, body composition and blood chemistry of juvenile olive flounder (*Paralichthys olivaceus*) was determined.

Weight gain and specific growth rate of fish fed the MBM10 diet were higher than those of fish fed the Con, SPM20, PM20, PM40 and SPM+PM20 diets. Feed efficiency ratio of fish fed the SPM10, MBM10, MBM20, PM10 and SPM+PM10 diets was higher than that of fish fed the SPM+PM20 and PM40 diets. Protein efficiency ratio of fish fed the MBM10 and MBM20 diets was higher than that of fish fed the SPM20, PM20, PM40 and SPM+PM20 diets.

According to these results, it can be concluded that dietary substitution of fishmeal with 10% SPM, 20% MBM, 10% PM and 10% SPM+PM could be made without detrimental effect on growth and feed utilization of olive flounder.

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