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# Apparent digestibility coefficients of the extruded pellet diets containing various fish meals for olive flounder, *Paralichthys olivaceus*

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## Abstract

Apparent digestibility coefficients (ADCs) of dry matter, crude protein, crude lipid, energy, essential amino acids, and fatty acids in extruded pellets containing various fish meals were determined for olive flounder (*Paralichthys olivaceus*). Eight extruded pellet diets were prepared to contain different fish meals (herring fish meal, anchovy fish meal, mackerel fish meal, sardine fish meal-A, sardine fish meal-B, tuna fish meal, pollock fish meal-A, and pollock fish meal-B) designated as HM, AM, MM, SM-A, SM-B, TM, PM-A, and PM-B, respectively. Chromic oxide (Cr<sub>2</sub>O<sub>3</sub>) was used as an inert indicator at a concentration of 0.5 % in the diet. Feces were collected from triplicate groups of fish (151 ± 4.0 g) using a fecal collection column attached to the fish rearing tank for 4 weeks. Dry matter ADCs of the MM, SM-A, SM-B, and PM-A diets were higher than those of all the other dietary groups, and the lowest digestibility of dry matter was observed in the PM-B diet. Fish fed the MM, SM-A, and PM-A diets showed significantly higher ADC of protein than those fed the AM, SM-B, TM, and PM-B diets. Lipid ADC of PM-B was significantly lower than that of the other diets. Energy ADCs of fish fed the MM, SM-A, and PM-A diets were significantly higher than those of the other diets. The availability of essential amino acids in the MM, SM-A, and PM-A diets were generally higher than that of the other fish meal diets, while TM showed the lowest values among all the experimental diets. ADCs of fatty acids in the AM, MM, SM-A, and PM-A diets were generally higher than those of fatty acids in the other diets, and the lowest values were recorded for the PM-B diet. These results provide information on the bioavailability of nutrients and energy in various fish meals which can be used to properly formulate practical extruded feeds for olive flounder.

**Keywords:** *Paralichthys olivaceus*, Apparent digestibility coefficient, Fish meals

## Background

Determination of the digestibility of nutrients in diets provides the first indication of their nutritional value and is considered as the first step of their quality evaluation (Allan et al. 2000; Glencross et al. 2007; Luo et al. 2009; Liu et al. 2009). Fish meal is certainly the best dietary protein source because it is quite palatable and provides an excellent balance of essential amino acids and fatty acids and

some other substances (Hardy 2010). Fish meal is the preferred animal protein supplement in the diets of aquatic animals. It carries huge quantities of energy and is rich in protein, lipids, minerals, and vitamins. It also serves as the benchmark ingredient in aquaculture diets because of its high nutrient content and digestibility (Udo et al. 2012). Fish meal in animal diets increases feed consumption, feed efficiency, and growth through better feed palatability and also improves nutrient uptake, digestion, and absorption among other ingredients (Yisa et al. 2013). Some studies have investigated apparent digestibility coefficients of various fish meals in several fish species such as grower

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rockfish, *Sebastes schlegeli* (Lee 2002); juvenile snakehead, *Ophiocephalus argus* (Yu et al. 2013); juvenile cobia, *Rachycentron canadum* (Zhou et al. 2004); Nile tilapia, *Oreochromis niloticus* (Köprücü and Özdemir 2005); Atlantic cod, *Gadus morhua* (Tibbetts et al. 2006); and juvenile haddock, *Melanogrammus aeglefinus* L. (Tibbetts et al. 2004). The raw materials of fish meal are processed by heating, pressing, separation, evaporation, and drying. Heating condenses the protein, breaks the fat depots, and also releases oil and water. Pressing improves the meal quality and decreases the moisture content of the press cake as much as possible. Drying process removes sufficient water from the wet and unstable mixture of press cake to form a stable fish meal.

Extrusion process can cause physical and chemical changes, such as ingredient particle size reduction and inactivation of enzymes. In addition, the heat associated with the extrusion process may also cause deactivation of anti-nutritional factors (Allan and Booth 2004) and improve the utilization of nitrogen-free extracts or other elements (Burel et al. 2000). Extrusion may also confer important benefits to the physical attributes of pellets including nutrient digestibility, palatability, pellet durability, water stability, and pellet storage life (Barrows and Hardy 2000). Extruded pellets are highly recommended for fish culture because of easy observation of feeding activity, easy management, and minimal water pollution. Cho et al. (2006) reported that extruded pellets can improve the digestibility of ingredients and they are generally well accepted by olive flounder, *Paralichthys olivaceus*.

Olive flounder is a commercially important carnivorous fish widely cultured in Eastern Asia including Korea, Japan, and China (Kim et al. 2014). Previous studies were conducted to investigate apparent digestibility coefficients of various fish meals for flounder (Deng et al. 2010; Kim et al. 2010). However, only limited information is available on the digestibility of different fish meals in flounder-extruded pellets. Therefore, the present study was conducted to determine the apparent digestibility coefficients of dry matter, crude protein, crude lipid, energy, essential amino acids, and selected fatty acids from different fish meals used in extruded diets for olive flounder.

## Methods

### Diet preparation

The proximate, essential amino acid and fatty acid (% of total fatty acids) compositions of the test ingredients (fish meals) are shown in Tables 1 and 2, respectively. Eight experimental diets were formulated using steam-dried herring fish meal, anchovy fish meal, mackerel fish meal, sardine fish meal-A, sardine fish meal-B, tuna fish meal, pollock fish meal-A, and pollock fish meal-B (designated as HM, AM, MM, SM-A, SM-B, TM, PM-A, and PM-B,

respectively) (Table 3). Chromic oxide ( $\text{Cr}_2\text{O}_3$ ) served as the inert indicator at a concentration of 0.5 % in the diet. All dry ingredients were thoroughly mixed, and the experimental diets were manufactured using a twin-screw extruder (Model ATX-2, Fesco Precision Co., Daegu, Korea). Extrusion conditions were as follows: feeder speed, 16 to 18 rpm; conditioner temperature, 75 °C; main screw speed, 640 rpm; and barrel temperature, 100 to 115 °C. Extruder pellets were oven-dried at 60 °C for 6 h to maintain the moderate moisture content of 5 to 8 % and stored at -25 °C until use.

### Fish and experimental condition

Juvenile olive flounder were obtained from a hatchery (Namhae, Korea) and acclimated to the laboratory conditions for 10 months. The experimental fish ( $151 \pm 4.0$  g) were then randomly distributed into 400-l cylindrical fiberglass tanks filled with 200 l of water at a density of 25 fish per tank. Filtered seawater was supplied at a flow rate of 3 l/min to each rearing tank. Fish rearing tanks had a sloping bottom leading to a centrally located drainage slot, and the effluent water was first directed over a fecal collection column before going to waste (Lee 2002). The water temperature was

**Table 1** Proximate and amino acid compositions of the fish meals used to test diets

	Fish meals							
	HM	AM	MM	SM-A	SM-B	TM	PM-A	PM-B
Proximate analysis (% in dry matter)								
Dry matter	93.3	92.2	92.4	91.3	94.0	92.0	93.7	93.1
Crude protein	73.4	67.3	76.6	71.5	71.0	62.7	74.7	63.3
Crude lipid	10.4	8.6	6.8	10.0	10.2	10.6	5.9	5.4
Ash	16.6	19.7	16.7	16.0	14.6	20.1	15.7	26.4
Gross energy (kcal/g)	4.9	4.5	4.6	4.7	4.8	4.3	4.7	3.9
Essential amino acids (% in protein)								
Arg	6.4	6.0	6.5	7.1	6.4	6.4	7.1	7.0
His	2.8	2.0	4.5	2.5	3.0	3.3	2.5	2.5
Ile	4.4	4.0	4.5	4.1	4.7	4.2	3.9	4.2
Leu	8.0	6.6	7.9	7.8	8.3	7.6	8.0	8.0
Lys	8.4	7.2	8.6	5.8	8.9	9.3	5.7	5.3
Met + Cys	4.2	3.9	4.3	2.7	4.3	4.0	2.8	2.9
Phe + Tyr	7.6	6.3	7.5	8.0	8.0	7.2	8.3	8.3
Thr	4.8	4.8	4.7	4.2	4.9	4.8	4.9	4.3
Val	5.9	4.9	5.0	4.4	5.3	5.6	4.3	4.7

HM herring fish meal, AM anchovy fish meal, MM mackerel fish meal, SM-A sardine fish meal-A, SM-B sardine fish meal-B, TM tuna meal, PM-A pollock fish meal-A, PM-B pollock fish meal-B

**Table 2** Fatty acid compositions (% of fatty acids) of the fish meals

	Fish meals							
	HM	AM	MM	SM-A	SM-B	TM	PM-A	PM-B
C14:0	4.6	4.2	3.6	4.7	5.4	3.8	2.2	3.3
C14:1	0.4	0.3	0.6	0.6	0.5	1.0		0.4
C16:0	21.0	21.8	19.7	21.0	22.8	26.1	17.9	23.4
C16:1	3.5	5.6	3.6	5.1	6.0	4.7	3.8	5.7
C18:0	4.5	5.9	7.9	6.9	6.0	8.1	4.8	6.6
C18:1n-9	10.8	15.1	12.9	13.9	9.6	17.1	16.9	27.2
C18:2n-6	2.1	2.2	2.4	1.5	3.3	2.1	1.7	3.1
C20:0	0.3	0.4	1.8	0.8	2.6	0.5	1.5	0.3
C20:1n-9	3.3	1.7	0.9	1.0	0.8	1.2	3.5	3.3
C18:3n-3	0.8	0.4	2.6	0.5	3.1	0.8	1.8	0.3
C20:2n-6	2.6	2.0	1.4	1.5	1.4	1.2	1.3	0.6
C22:1n-9		0.5		0.9		0.9	2.5	0.9
C20:3n-3			1.6	0.7	2.0		1.1	
C20:4n-6	1.2	0.8	1.6	1.8	1.6	2.5	1.7	0.8
C22:2n-6	0.6		0.6	0.6	1.5		0.8	
C20:5n-3	12.4	16.4	9.4	11.1	13.0	6.2	14.1	7.9
C22:3n-3	0.4	0.7	0.6	0.5		0.3		
C22:5n-3	1.3	3.2	2.5	2.5	1.4	1.2	1.6	0.9
C22:6n-3	25.2	17.5	22.5	20.9	15.3	20.4	20.1	9.3
n-3HUFA	39.3	37.7	36.5	35.8	31.7	28.1	36.9	18.1

HM herring fish meal, AM anchovy fish meal, MM mackerel fish meal, SM-A sardine fish meal-A, SM-B sardine fish meal-B, TM tuna meal, PM-A pollock fish meal-A, PM-B pollock fish meal-B

21.4 ± 2.10 °C, and the photoperiod followed the natural conditions during the experimental period.

### Feces collection

Triplicate groups of fish were hand-fed with one of the experimental diets to apparent satiation once a day at 15.00 h. Two hours after feeding, the rearing tanks and collection column were brushed out in order to remove uneaten feed and fecal residues. The next day, feces were collected from the fecal collection columns at 9:00 h. Feces collected from the settling columns were immediately filtered with filter paper (Whatman # 1) for 60 min at 4 °C and stored at -75 °C for chemical analyses. Fecal samples from each tank were pooled at the end of the experiment.

### Analytical methods

Freeze-dried feed and feces samples were finely ground using a grinder. Fish scales were removed from the feces samples using a 300-µm sieve before analysis. Crude protein content was determined by the Kjeldahl method using an Auto Kjeldahl System (Buchi, Flawil, Switzerland). Crude lipid was determined by the ether-extraction method. Crude fiber was determined

**Table 3** Formulation and chemical composition of the experimental diets

	Diets							
	HM	AM	MM	SM-A	SM-B	TM	PM-A	PM-B
Ingredients (%)								
Herring fish meal	72							
Anchovy fish meal		72						
Mackerel fish meal			72					
Sardine fish meal-A				72				
Sardine fish meal-B					72			
Tuna meal						72		
Pollock fish meal-A							72	
Pollock fish meal-B								72
Wheat flour	14	14	14	14	14	14	14	14
α-potato-starch	5	5	5	5	5	5	5	5
Wheat gluten	2	2	2	2	2	2	2	2
Fish oil	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
Vitamin premix <sup>a</sup>	1	1	1	1	1	1	1	1
Mineral premix <sup>b</sup>	1	1	1	1	1	1	1	1
Stay-C (50 %)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin E (25 %)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Choline salt (50 %)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Cr <sub>2</sub> O <sub>3</sub>	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Nutrient content (dry matter basis)								
Crude protein (%)	53.4	51.7	54.9	52.9	51.8	47.6	54.0	47.5
Crude lipid (%)	9.3	8.5	7.8	9.6	8.6	9.7	8.0	7.5
Ash (%)	12.6	16.0	13.1	13.4	12.8	16.4	12.3	20.2
NFE (%) <sup>c</sup>	24.7	23.8	24.2	24.1	26.8	26.3	25.7	26.6

<sup>a</sup>Vitamin premix contained the following ingredients (g/kg premix), which were diluted in cellulose: 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; and cyanocobalamin, 0.003

<sup>b</sup>Mineral premix contained the following ingredients (g/kg premix): MgSO<sub>4</sub>·7H<sub>2</sub>O, 80.0; NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 370.0; KCl, 130.0; ferric citrate, 40.0; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 20.0; Ca-lactate, 356.5; CuCl, 0.2; AlCl<sub>3</sub>·6H<sub>2</sub>O, 0.15; KI, 0.15; Na<sub>2</sub>Se<sub>2</sub>O<sub>3</sub>, 0.01; MnSO<sub>4</sub>·H<sub>2</sub>O, 2.0; and CoCl<sub>2</sub>·6H<sub>2</sub>O, 1.0

<sup>c</sup>Calculated = 100 - (crude protein + crude lipid + ash)

using an automatic analyzer (Fibertec, Tecator, Sweden), while ash content was determined by treatment in a muffle furnace at 600 °C for 4 h. Gross energy content was analyzed using an adiabatic bomb calorimeter (Parr, USA). For amino acid composition, samples were freeze-dried and then hydrolyzed with 6 N HCl at 110 °C for 24 h. Amino acid concentrations in the experimental diets and fecal samples were determined using an automatic analyzer (Hitachi Model 835-50, Japan) equipped with an ion exchange column (Hitachi Resin #

2619, 2.6 × 150 mm, Japan). Lipid for fatty acid analysis was extracted by a combination of chloroform and methanol (2:1, v/v) using the method of Folch et al. (1957). Fatty acid methyl esters were measured by transesterification with 14 % BF<sub>3</sub> methanol (Sigma, St Louis, MO, USA). The particular fatty acid composition was identified using a gas chromatography (PerkinElmer, Clarus 600, GC, USA) that has a flame ionization detector, equipped with SPTM-2560 capillary column (100 m × 0.25 mm i.d., film thickness 0.20 mm; Supelco, Bellefonte, PA, USA). Injector and detector temperatures were 260 °C. The column temperature was programmed from 140 to 240 °C at a rate of 5 °C/min. Helium was utilized by the carrier gas. Fatty acid composition from the samples was identified by comparison with retention times of the known standard fatty acid methyl esters (PUFA 37 component FAME Mix Supelco). Chromic oxide was determined by a wet-acid digestion method (Furukawa and Tsukahara 1966).

Apparent dry matter digestibility coefficients were calculated as 100 – (100 × (% Cr<sub>2</sub>O<sub>3</sub> in diet/% Cr<sub>2</sub>O<sub>3</sub> in feces)).

Apparent digestibility coefficients of nutrients, energy, essential amino acids, and selected fatty acids were calculated as 100 – (100 × (% feed marker/% feces marker) × (% nutrient, energy, amino acid, or fatty acid in feces/% nutrient, energy, amino acid, or fatty acid in feed)).

**Statistical analysis**

All data were subjected to one-way analysis of variance, followed by Duncan’s multiple range test (Duncan 1955) at a significance level of *P* < 0.05. Linear correlations were determined between nutrient digestibility and contents of the test ingredients (fish meals). All data are presented as mean ± SE (standard error) of three replicate groups. All statistical analyses were carried out using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

**Results**

The apparent digestibility coefficients (ADCs) of dry matter, crude protein, crude lipid, and energy of the extruded floating pellet diets containing various fish meals for olive flounder are shown in Table 4. The ADCs of dry matter ranged from 69 to 87 %. Dry matter ADCs of the MM, SM-A, SM-B, and PM-A diets were higher than those of the TM and PM-B diets. The dry matter ADC of PM-B was the lowest among the experimental groups.

Protein ADCs of diets ranged from 87 to 95 %. Protein ADCs of the MM, SM-A, and PM-A diets were significantly higher than those of the AM, SM-B, TM, and PM-B diets while the lowest values were observed in fish fed the TM and PM-B diets. Lipid ADCs ranged from 83

**Table 4** Apparent digestibility coefficients (%) of dry matter, crude protein, crude lipid, and energy in olive flounder fed the diets containing various fish meals

Diets	Dry matter	Crude protein	Crude lipid	Energy
HM	81.5 ± 1.47 <sup>bc</sup>	93.2 ± 0.31 <sup>cd</sup>	90.5 ± 1.24 <sup>b</sup>	90.7 ± 0.65 <sup>c</sup>
AM	80.7 ± 1.71 <sup>bc</sup>	91.6 ± 1.47 <sup>bc</sup>	94.6 ± 0.71 <sup>cd</sup>	90.3 ± 0.24 <sup>c</sup>
MM	83.6 ± 0.74 <sup>cd</sup>	95.3 ± 0.16 <sup>d</sup>	94.7 ± 0.91 <sup>cd</sup>	93.5 ± 0.49 <sup>d</sup>
SM-A	84.4 ± 0.51 <sup>cd</sup>	95.1 ± 0.18 <sup>d</sup>	95.9 ± 0.06 <sup>d</sup>	93.0 ± 0.07 <sup>d</sup>
SM-B	83.5 ± 0.06 <sup>cd</sup>	90.8 ± 0.08 <sup>b</sup>	93.1 ± 0.46 <sup>bcd</sup>	89.3 ± 0.11 <sup>c</sup>
TM	77.5 ± 1.04 <sup>b</sup>	87.2 ± 0.70 <sup>a</sup>	92.4 ± 1.59 <sup>bc</sup>	86.2 ± 0.40 <sup>b</sup>
PM-A	87.0 ± 0.45 <sup>d</sup>	95.4 ± 0.20 <sup>d</sup>	93.6 ± 1.24 <sup>bcd</sup>	93.9 ± 0.39 <sup>d</sup>
PM-B	69.2 ± 2.97 <sup>a</sup>	87.2 ± 1.29 <sup>a</sup>	83.0 ± 1.82 <sup>a</sup>	83.5 ± 0.98 <sup>a</sup>

Values (mean ± SE of triplicate groups) in the same column with different superscripts are significantly different (*P* < 0.05)

to 96 %. The lipid ADCs of the PM-B diet was significantly lower than those of the other diets, and the SM-A group showed the highest value. Energy ADCs ranged from 84 to 94 %. The energy ADCs of the MM, SM-A, and PM-A diets were significantly higher than those of the other groups while the PM-B diet showed the lowest value.

Essential amino acid ADCs of diets containing various fish meals for olive flounder are shown in Table 5. In general, essential amino acid availability reflected crude protein digestibility, with fish fed the MM, SM-A, and PM-A diets showing the highest values compared to the other experimental groups. Amino acid digestibility values, for most essential amino acids, in TM were the lowest for juvenile olive flounder among the fish meals tested. Fatty acid ADCs of diets containing various fish meals for olive flounder are shown in Table 6. Among all fish meals, the ADC of selected fatty acids in PM-B was significantly lower than that of fatty acids in other fish meals.

**Discussion**

Dry matter ADC of various protein feedstuffs offers an estimate of overall digestibility, and a low value generally indicates that a high level of indigestible material is present in the feedstuff (Li et al. 2013). Thus, dry matter ADCs have been considered to provide a better estimate of the amount of indigestible material present in feedstuffs in comparison with digestibility coefficients for individual nutrients (Luo et al. 2008). In this study, the MM, SM-A, SM-B, and PM-A diets were equally well digested and had higher dry matter ADCs than the TM and PM-B diets. These differences can be explained by the differences in origin, quality, and chemical composition of ingredients used in the diet. We found that the dry matter digestibility was positively correlated (*r* = 0.95) with ash content of fish meals tested in the current study.

**Table 5** Apparent amino acid digestibility coefficients (%) of diets containing various fish meals for olive flounder

Essential amino acids	Diets							
	HM	AM	MM	SM-A	SM-B	TM	PM-A	PM-B
Arg	94.3 ± 0.39 <sup>cd</sup>	93.1 ± 1.46 <sup>bc</sup>	98.1 ± 0.03 <sup>f</sup>	96.6 ± 0.19 <sup>ef</sup>	92.9 ± 0.27 <sup>bc</sup>	89.8 ± 0.65 <sup>a</sup>	96.1 ± 0.14 <sup>de</sup>	92.0 ± 0.39 <sup>b</sup>
His	93.2 ± 0.41 <sup>bc</sup>	90.8 ± 1.77 <sup>a</sup>	98.1 ± 0.13 <sup>e</sup>	96.1 ± 0.06 <sup>de</sup>	92.1 ± 0.15 <sup>ab</sup>	90.3 ± 0.62 <sup>a</sup>	95.0 ± 0.28 <sup>cd</sup>	89.8 ± 0.75 <sup>a</sup>
Ile	92.7 ± 0.60 <sup>cd</sup>	90.5 ± 2.23 <sup>bc</sup>	97.0 ± 0.13 <sup>e</sup>	94.9 ± 0.10 <sup>de</sup>	90.6 ± 0.28 <sup>bc</sup>	87.6 ± 0.86 <sup>a</sup>	94.8 ± 0.32 <sup>de</sup>	89.0 ± 0.48 <sup>ab</sup>
Leu	93.1 ± 0.48 <sup>cd</sup>	91.3 ± 2.04 <sup>bc</sup>	97.3 ± 0.11 <sup>e</sup>	95.3 ± 0.14 <sup>de</sup>	91.0 ± 0.21 <sup>bc</sup>	88.0 ± 0.79 <sup>a</sup>	95.0 ± 0.19 <sup>de</sup>	89.3 ± 0.43 <sup>ab</sup>
Lys	84.5 ± 0.62 <sup>de</sup>	92.3 ± 2.08 <sup>cd</sup>	97.8 ± 0.11 <sup>f</sup>	96.4 ± 0.04 <sup>ef</sup>	91.3 ± 0.12 <sup>bc</sup>	88.6 ± 0.93 <sup>a</sup>	95.3 ± 0.21 <sup>ef</sup>	89.5 ± 0.50 <sup>ab</sup>
Met + Cys	96.1 ± 0.26 <sup>d</sup>	94.5 ± 1.23 <sup>c</sup>	98.5 ± 0.02 <sup>e</sup>	97.3 ± 0.01 <sup>de</sup>	94.0 ± 0.11 <sup>c</sup>	88.5 ± 0.36 <sup>a</sup>	97.1 ± 0.09 <sup>de</sup>	91.1 ± 0.37 <sup>b</sup>
Phe + Tyr	92.2 ± 0.67 <sup>bc</sup>	90.2 ± 1.89 <sup>ab</sup>	96.9 ± 0.10 <sup>d</sup>	94.8 ± 0.21 <sup>d</sup>	90.2 ± 0.22 <sup>ab</sup>	88.5 ± 0.71 <sup>a</sup>	94.6 ± 0.35 <sup>cd</sup>	88.7 ± 0.42 <sup>a</sup>
Thr	92.0 ± 0.42 <sup>b</sup>	90.1 ± 1.83 <sup>b</sup>	96.7 ± 0.12 <sup>c</sup>	94.4 ± 0.24 <sup>c</sup>	90.1 ± 0.12 <sup>b</sup>	86.6 ± 0.66 <sup>a</sup>	94.7 ± 0.34 <sup>c</sup>	87.7 ± 0.48 <sup>a</sup>
Val	89.9 ± 0.36 <sup>c</sup>	86.5 ± 1.94 <sup>b</sup>	95.7 ± 0.16 <sup>e</sup>	92.7 ± 0.24 <sup>d</sup>	88.6 ± 0.20 <sup>bc</sup>	83.9 ± 0.81 <sup>a</sup>	93.4 ± 0.76 <sup>de</sup>	86.3 ± 0.51 <sup>ab</sup>

Values (mean ± SE of triplicate groups) in the same row with different superscripts are significantly different ( $P < 0.05$ )

It has been suggested that a high level of ash generally affects digestibility of dry matter and results in high waste outputs and can also cause mineral imbalances. Therefore, the low dry matter digestibility of the TM and PM-B diets may be attributed to their high ash content (20.1 and 26.4 %, respectively). Kitagima and Fracalossi (2011) reported low dry matter digestibility for fish and shrimp offal meal with high ash contents. Similar results have also been observed in rainbow trout (*Oncorhynchus mykiss*) (Bureau et al. 1999) and hybrid tilapia (*O. niloticus* × *Oreochromis aureus*) (Zhou and Yue 2012).

The protein quality of the dietary ingredients is usually the leading factor affecting fish performance and protein digestibility and is the first measure of its availability for fish (Yu et al. 2013). The ADC of protein in this study revealed that the protein of HM, MM, SM-A, and PM-A must be highly digestible by olive flounder. This indicates that each of these fish meals can be utilized efficiently as protein sources for olive flounder. The ADC of protein for the SM-A diet (95 %) is higher than that previously reported for rainbow trout (Gaylord et al.

2008). The ADC of protein for the MM diet (95 %) is higher than that reported for juvenile Pacific white shrimp, *Litopenaeus vannamei* (Lemos et al. 2009). The ADC of protein for the HM diet (93 %) is similar to that reported for herring fish meal in the Atlantic cod, *G. morhua* (Tibbetts et al. 2006), and salmonids such as the Atlantic salmon, *Salmo salar* (Anderson et al. 1997); coho salmon, *O. kisutch* (Sugiura et al. 1998); and rainbow trout (Burel et al. 2000). The ADC of protein for the AM diet (91 %) is similar to that reported for anchovy fish meal in salmonid species (Anderson et al. 1995; Sugiura et al. 1998, 2000; Thiessen et al. 2004; Glencross et al. 2005). In the present study, the protein ADCs of the TM and PM-B diets were lower than those of the other ingredients tested. The ADC of protein appeared to have a positive relationship with dry matter of the test ingredients ( $r = 0.84$ ). The differences in ADC of protein among fish meals can be attributed to their different nutrient compositions, raw materials, species, locations, seasons of catch, and processing conditions used to produce the meal (Luo et al. 2008; Lemos et al. 2009; Terrazas-Fierro et al. 2010).

**Table 6** Apparent fatty acid digestibility coefficients (%) of diets containing various fish meals for olive flounder

Fatty acids	Diets							
	HM	AM	MM	SM-A	SM-B	TM	PM-A	PM-B
C14:0	91.7 ± 0.12 <sup>b</sup>	94.9 ± 0.12 <sup>bc</sup>	96.1 ± 0.16 <sup>c</sup>	97.0 ± 1.51 <sup>c</sup>	93.0 ± 0.47 <sup>bc</sup>	92.9 ± 0.59 <sup>bc</sup>	93.2 ± 0.47 <sup>bc</sup>	80.6 ± 3.12 <sup>a</sup>
C16:0	90.0 ± 0.13 <sup>bc</sup>	92.2 ± 0.34 <sup>bc</sup>	94.6 ± 0.17 <sup>c</sup>	93.1 ± 0.40 <sup>c</sup>	89.4 ± 0.71 <sup>bc</sup>	87.4 ± 0.77 <sup>b</sup>	93.7 ± 0.26 <sup>c</sup>	69.7 ± 4.70 <sup>a</sup>
C18:0	86.8 ± 0.27 <sup>bcd</sup>	90.7 ± 0.66 <sup>de</sup>	91.9 ± 0.24 <sup>de</sup>	94.1 ± 0.37 <sup>e</sup>	84.3 ± 1.02 <sup>bc</sup>	83.7 ± 0.82 <sup>b</sup>	89.4 ± 0.38 <sup>cde</sup>	68.9 ± 4.69 <sup>a</sup>
C18:1n-9	92.5 ± 0.62 <sup>b</sup>	95.5 ± 0.17 <sup>b</sup>	95.8 ± 0.31 <sup>b</sup>	95.2 ± 0.25 <sup>b</sup>	93.8 ± 0.32 <sup>b</sup>	95.9 ± 2.16 <sup>b</sup>	95.2 ± 0.21 <sup>b</sup>	80.5 ± 2.53 <sup>a</sup>
C18:2n-6	88.9 ± 0.74 <sup>b</sup>	96.6 ± 0.27 <sup>c</sup>	97.1 ± 0.29 <sup>c</sup>	96.9 ± 0.22 <sup>c</sup>	93.3 ± 0.75 <sup>bc</sup>	95.5 ± 0.22 <sup>bc</sup>	93.9 ± 0.24 <sup>bc</sup>	72.1 ± 6.24 <sup>a</sup>
C18:3n-3	93.4 ± 0.11 <sup>cd</sup>	96.5 ± 0.27 <sup>cd</sup>	97.8 ± 0.80 <sup>d</sup>	96.4 ± 0.27 <sup>cd</sup>	90.1 ± 0.40 <sup>bc</sup>	85.8 ± 0.60 <sup>b</sup>	91.7 ± 0.44 <sup>bcd</sup>	61.3 ± 6.39 <sup>a</sup>
C20:4n-6	95.7 ± 0.19 <sup>c</sup>	97.4 ± 0.18 <sup>c</sup>	97.1 ± 0.12 <sup>c</sup>	94.3 ± 0.43 <sup>bc</sup>	92.7 ± 1.87 <sup>bc</sup>	94.4 ± 0.63 <sup>bc</sup>	89.9 ± 3.15 <sup>b</sup>	82.6 ± 2.59 <sup>a</sup>
C20:5n-3	97.2 ± 0.07 <sup>b</sup>	98.5 ± 0.08 <sup>b</sup>	98.7 ± 0.08 <sup>b</sup>	97.3 ± 0.48 <sup>b</sup>	97.5 ± 0.21 <sup>b</sup>	98.2 ± 0.34 <sup>b</sup>	97.9 ± 0.20 <sup>b</sup>	92.5 ± 1.52 <sup>a</sup>
C22:6n-3	96.7 ± 0.05 <sup>b</sup>	98.0 ± 0.14 <sup>b</sup>	98.0 ± 0.36 <sup>b</sup>	97.3 ± 0.20 <sup>b</sup>	96.8 ± 0.14 <sup>b</sup>	96.9 ± 0.57 <sup>b</sup>	97.4 ± 0.10 <sup>b</sup>	88.0 ± 1.89 <sup>a</sup>

Values (mean ± SE of triplicate groups) in the same row with different superscripts are significantly different ( $P < 0.05$ )



The quality of dietary protein depends on its amino acid composition and their digestibility and availability (Rollin et al. 2003). Lack of an essential amino acid leads to poor dietary protein utilization and therefore reduces growth and decreases feed efficiency. Although the data presented in this study suggest a reasonable agreement between protein and amino acid digestibilities, individual amino acid availabilities within a feed ingredient are variable. The amino acid availability coefficients of the MM, SM-A, and PM-A diets were significantly higher than those of the other experimental diets, suggesting that olive flounder can efficiently utilize these fish meals. In most of the cases, ADCs of essential amino acid in the TM diet were the lowest of all the fish meals that were tested, possibly due to lower quality of the starting raw material. Many researchers have reported that some amino acids of fish meal are inefficiently utilized or made unavailable due to differences in the processing conditions or the low quality of the raw material processed (Wilson et al. 1981; Anderson et al. 1992, Anderson et al. 1995; Yamamoto et al. 1998; Mu et al. 2000, Chu et al. 2015).

The ADC of dietary lipid usually ranges from 85 to 95 % in fish (NRC 1993). In the present study, lipid digestibilities were considered to be high (>90 %), except for PM-B (83 %). Previous studies reported ADC values of lipid in different fish meals including Peruvian fish meal (94 %) for juvenile snakehead, *O. argus* (Yu et al. 2013); white fish meal (78 %); and brown fish meal (76 %) for loach, *Misgurnus anguillicaudatus* (Chu et al. 2015). The digestibility of lipids is known to be influenced by a number of factors, including degree of unsaturation, dietary lipid level, and various other constituents (Yuan et al. 2010).

Digestibility of fatty acids is identified to be influenced by a number of factors including their chain length, degree of unsaturation, level of incorporation in dietary fat, and other constituent fatty acids and their melting points (Olsen et al. 2000; Martins et al. 2009; Oujifard et al. 2012). High specificity towards unsaturated fatty acids has commonly been found for fish digestive lipases (Caballero et al. 2002). In the present study, all diets showed high fatty acid digestibility except for PM-B. The low digestibility coefficient of fatty acids for the PM-B diet may be attributed to the poor quality of raw material processed. However, digestibility of individual fatty acids has been affected by other factors including emulsification, enzymatic hydrolysis, and micellar incorporation (Francis et al. 2007).

The ADCs of energy for the HM and SM-A diets, in the current study, are in the same range as reported in Atlantic cod (93 %) (Tibbetts et al. 2006) and rainbow trout (95 %) (Gaylord et al. 2008). It has been reported that carnivorous fish are capable of efficiently utilizing

energy from animal products (Sullivan and Reigh 1995; Gaylord and Gatlin 1996; McGoogan and Reigh 1996; Lee 2002; Zhou et al. 2004). It was found that a high ash content of fish meal might reduce energy digestibility (Gomes et al. 1995).

## Conclusions

The MM, SM-A, and PM-A diets showed higher dry matter, crude protein, crude lipid, and energy ADCs than the other diets. Due to variation within individual amino acid and fatty acid ADCs among diets, the use of specific amino acid and fatty acid ADCs may allow more accurate and economical formulation of the feed for olive flounder.

## Abbreviations

ADCs, apparent digestibility coefficients; AM, anchovy fish meal; Arg, arginine; His, histidine; HM, herring fish meal; Ile, isoleucine; Leu, leucine; Lys, lysine; Met + Cys, methionine + cysteine; MM, mackerel fish meal; Phe + Tyr, phenylalanine + tyrosine; PM-A, pollock fish meal-A; PM-B, pollock fish meal-B; SE, standard error; SM-A, sardine fish meal-A; SM-B, sardine fish meal-B; Thr, threonine; TM, tuna fish meal; Val, valine

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## Availability of data and materials

All datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

MMR conducted the feeding trial and drafted the manuscript. HSH, KWK, KDK, and BJL manufactured the experimental feed and performed the analyses. SML conceived and designed the study and experimental facility and also revised the manuscript. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

## Consent for publication

Not applicable

## Ethics approval and consent to participate

Experimental protocols followed the guidelines of the Animal Care and Use Committee of Gangneung-Wonju National University.

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