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Apparent digestibility coefficients of animal feed ingredients for olive flounder (*Paralichthys olivaceus*)

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Abstract
Apparent digestibility coefficients (ADCs) of dry matter, crude protein, crude lipid, nitrogen-free extract, energy and essential amino acids in animal-based feed ingredients were determined for olive flounder (*Paralichthys olivaceus*). A reference diet (RF) was formulated to contain 1.0% chromic oxide (Cr_2O_3) as an inert indicator. Nine test diets were formulated to contain RF and one of the feed ingredients (pollock meal [PM], jack mackerel meal [JMM], anchovy meal [AM], cod meal [CM], sardine meal [SM], sand eel meal [SEM], tuna meal [TM], meat meal [MM] and squid liver meal [SLM]) at a 7:3 ratio in each diet designated as PM, JMM, AM, CM, SM, SEM, TM, MM and SLM, respectively. Olive flounder, averaging 150 ± 8.0 g, were stocked at a density of 25 fish per tank in 400-L fiberglass tanks attached with fecal collection columns. Feces were collected from triplicate groups of fish one time a day for four weeks. Dry matter and crude protein ADCs of CM and SEM were significantly higher than the other tested ingredients. Lipid ADCs of JMM, CM and SEM were significantly higher than the other test ingredients. Energy ADCs of CM and SEM were significantly higher than that of the other tested ingredients. The availability of amino acids in CM was generally higher than the other animal protein sources. PM exhibited the lowest amino acid availability among the treatments. Interestingly, MM exhibited significantly higher nutrient digestibility than several marine-based ingredients. However, CM and SEM are seeming to be highly digestible and effective to use in olive flounder diet compared to the other tested ingredients. Overall, the results of this study provide information about the bioavailability of nutrients and energy in animal feedstuffs to apply when formulating cost-effective practical feeds for olive flounder.

Keywords: Olive flounder, Nutrient digestibility, Fish meal, Amino acid availability, Ingredient utilization

Introduction

The nutritive value of a feedstuff depends on several factors including chemical composition (Kokou & Fountoulaki, 2018), nutrient quality (Radhakrishnan et al., 2020) and digestibility which represent the digestion and absorption of nutrients and energy by animals (Riche et al., 2001; Tram et al., 2011; Turchini et al., 2019). Therefore, information on nutrient digestibility

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of feed ingredients is important for accurate diet formulations for fish. Chemical analysis and digestibility results of ingredients are also important to limit the waste produced by fish (Borghesi et al., 2008; Hien et al., 2010; Köprücü & Özdemir, 2005). Amino acid digestibility of ingredients is pivotal because the nutrient bioavailability can be affected by processing and manufacturing conditions of ingredients (Terrazas-Fierro et al., 2010). Moreover, the utilization of feedstuffs is influenced by several factors, such as the raw material (Kokou & Fountoulaki, 2018), recipient species (Refstie et al., 2000), freshness (Aksnes & Mundheim, 1997), processing (Drew et al., 2007; Opstvedt et al., 2003) and storage conditions (Camacho-Rodríguez et al., 2018) of the meal. Therefore, the determination of apparent digestibility coefficient (ADC) values of feedstuffs is one of the most important aspects of determining suitable feed ingredients to formulate nutritionally efficient diets (Irvin & Tabrett, 2005).

Fish meal (FM) is the most important ingredient in aquafeeds. It is widely used in many formulated aquaculture diets as a protein source because of its high protein content, essential amino acid content and excessive nutrient digestibility and palatability (Thompson et al., 2008; Zhou et al., 2004). However, FM is a limiting factor in the aquafeed sector because of the increasing demand, unstable supply and high price of quality FMs in the market (Galkanda-Arachchige et al., 2020; Gasco et al., 2018). Therefore, new FM sources and alternative feedstuffs were used to reduce and/or replace traditional FM from aquafeed (Cho et al., 2005a; Ha et al., 2021; Panase et al., 2018).

Olive flounder (*Paralichthys olivaceus*) is considered an important marine fish species which has been successfully cultured in East Asian countries including Korea, Japan and China. It offers many desirable characteristics for culture such as rapid growth rate, acceptance of dry pellet, high stocking density, consumer preference, ease of mass production and good market price. Their production is dependent on marine-originated protein sources as they are carnivores (Kim et al., 2021). Therefore, different types and levels of FMs, mainly produced using bycatch and byproducts, have been used in practical diets for olive flounder (Kim et al., 2014; Park et al., 2021). Pollock meal (PM) and cod meal (CM) are often used as white FM in diets for olive flounder (Choi et al., 2004; Khosravi et al., 2015; Kim et al., 2002). Anchovy meal (AM) and sardine meal (SM) are also supplemented in olive flounder diets as main protein sources (Back et al., 2020; Bae et al., 2020; Park et al., 2021). It was recently reported that jack mackerel meal (JMM) enhances feed consumption of olive flounder due to its high attractiveness (Jeong et al., 2020). Tuna meal (TM), produced by tuna byproducts, is incorporated in commercial olive flounder feed to reduce production costs as reported by Kim et al. (2014). TM is also an efficient FM replacer in olive flounder diet (Kim et al., 2021). Sand eels are bycatch species found in the Mediterranean Sea and the Atlantic Ocean and are widely used for FM production (Maynou et al., 2021; Rehbein, 2008). There is a lack of information about sand eel meal (SEM) in olive flounder diet. Meat meal (MM) is produced using inedible parts discarded from animal slaughterhouses and meat processing plants (Ha et al., 2021; Millamena, 2002). Ha et al. (2021) reported that MM replaces 60% of FM from olive flounder diet containing 650 g/kg FM without sacrificing growth, immunity and disease resistance. Squid liver meal (SLM), produced by squid processing byproducts, is used as feed attractant in aquafeed industry (Jang et al., 2021). Cho et al. (2005b) replaced 10% FM with SLM from olive flounder diet without deterioration of performance. Jang et al. (2021) also reported that SLM improves growth performance and feed utilization of olive flounder. Therefore, determination of digestibility is important to formulate efficient feed containing MM and SLM as FM replacers for olive flounder.

The nutrient digestibility might be changed with the type and level of FM in olive flounder diet (Kim et al., 2019; Yoo et al., 2006). Therefore, information about the nutrient digestibility of FMs used in olive flounder diet is important to formulate cost-effective practical diets because the nutrient digestibility of ingredients can also be specific to fish species (Sugiura et al., 1998). Kim et al. (2010) examined nutrient ADCs of several protein sources and reported that the digestibility of animal protein sources was higher in olive flounder compared to that of plant protein sources. In a previous study, we observed high protein and dry matter digestibility of extruded pellets containing different types of FM including AM, SM, TM and PM (Rahman et al., 2016). However, there is limited information available on the digestibility of major nutrients and energy from various animal protein feedstuffs for olive flounder. Therefore, the current study was designed to assess the ADCs of dry matter, crude protein, crude lipid, nitrogen free-extract (NFE), energy and essential amino acids in a range of FMs, MM and SLM for olive flounder.

Materials and Methods

Diet preparation

A reference diet (RF) was formulated using mackerel and anchovy FM (imported from Chile), and squid liver oil (E-Wha

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Oil & Fat, Busan, Korea) to meet the nutrient requirements of olive flounder (Lee et al., 2002) (Table 1). The RF was mixed with 1.0% chromic oxide (Cr_2O_3) as an inert indicator. Nine experimental diets were formulated using 70% RF and 30% of each of the test ingredients on an air-dry basis according to Cho & Slinger (1978). Test ingredients for ADCs were PM, JMM, AM, CM, SM, SEM, TM, MM and SLM. The experimental diets were designated using same abbreviations to respective ingredients as PM, JMM, AM, CM, SM, SEM, TM, MM and SLM respectively. FMs used in this study were produced by steam drying. Proximate and amino acid compositions of the test ingredients and diets are presented in Tables 2 and 3. All dry ingredients were thoroughly mixed and pelleted through a meat chopper machine after adding squid liver oil and distilled water (40%), air-dried and stored at –25℃.

Fish and experimental condition

A fecal collection system containing thirty fiberglass tanks of 400 L capacity, designed according to Lee (2002) was used for the experiment. Olive flounder were obtained from a hatchery (Namhae, Korea) and kept at Marine Biology Center for Research and Education at Gangneung-Wonju National University. Fish (initial mean weight, 150 ± 8.0 g) were randomly captured and distributed into each experimental tank at a density of 25 fish per tank. Sand-filtered seawater was supplied to rearing tanks at a 3 L/min flow rate. The water temperature was 20.2 ± 0.4 ℃ and the photoperiod was maintained by natural conditions. Prior to starting the experiment, fish were acclimated while feeding the RF to apparent satiation once daily for 2 weeks.

Table 1. Reference and test diets formulation for the determination of nutrient digestibility coefficients of ingredients in olive flounder

Ingredients (%)	Reference diet	Test diet
Fish meal (mackerel+anchovy, 1:1)[1]	60.0	
Wheat flour	19.0	
α-potato starch	10.0	
Squid liver oil[2]	5.0	
Vitamin premix[3]	2.0	
Mineral premix[4]	2.0	
Vitamin C (50%)	0.5	
Vitamin E (25%)	0.2	
Choline salt[5]	0.3	
Cr_2O_3	1.0	
Reference diet		70.0
Test ingredients		30.0

[1] Imported from Chile.
[2] Produced by E-wha Oil & Fat, Busan, Korea.
[3] Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): 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; folicacid, 0.68; p-aminobezoicacid, 18.2; menadione, 1.8; retinylacetate, 0.73; cholecalficerol, 0.003; cyanocobalamin, 0.003.
[4] Mineral premix contained the following ingredients (g/kg mix): $MgSO_4.7H_2O$, 80.0; $NaH_2PO_4.2H_2O$, 370.0; KCl, 130.0; ferriccitrate, 40.0; $ZnSO_4.7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3.6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4.H_2O$, 2.0; $CoCl_2.6H_2O$, 1.0.
[5] Sigma-Aldrich, St. Louis, MO, USA.

Feces collection

After acclimation, triplicate groups of fish were hand-fed one of the test diets to apparent satiation (once a day, 15:00 h) for 4 weeks. Fecal collection was started 4 days after feeding the fish with the experimental diets to evacuate all previously ingested material from the gut. About two hours after feeding, the rearing tanks and collection columns were cleaned to remove any residual particulate matter including feces and uneaten feed. Feces were then allowed to settle overnight. Fecal samples were collected from the fecal collection columns at 09:00 h (approximately 16 h) each morning before next feeding. Collected feces were then filtered with filter paper (Whatman # 1) for 60 min at 4℃ and stored at –75℃ for further analyses.

Analytical methods

Proximate composition of both diet and fecal samples were analyzed in triplicate (AOAC, 1995). Chromic oxide levels were analyzed by a wet-acid digestion method according to Furukawa & Tsukahara (1966). Crude protein level was determined according to the Kjeldahl method with an Auto Kjeldahl System (Buchi, Flawil, Switzerland). Moisture content was determined after drying in an oven at 105℃ for 6 h. Crude lipid level was measured by the ether-extraction method. Crude fiber content was measured with an automatic analyzer (Fibertec, Tecator, Sweden). Ash content of samples was determined by burning in a muffle furnace at 600℃ for 4 h. NFE was calculated by the difference. Amino acid levels in the diets and fecal materials were analyzed using an automatic analyzer (Hitachi Model 835-50, Tokyo, Japan) consisting of an ion-exchange column.

The ADCs for the dry matter, crude protein, crude lipid, NFE, and energy, and the availability of amino acids for the test ingredients and diets were determined using the following equations:

Table 2. Proximate and amino acid compositions of the ingredients used in test diets

	Test ingredients								
	PM[1)]	JMM[2)]	AM[3)]	CM[4)]	SM[5)]	SEM[6)]	TM[7)]	MM[8)]	SLM[9)]
Proximate analysis (% of dry matter)									
Moisture	7.8	7.5	7.9	8.2	4.4	6.9	7.0	3.3	7.8
Crude protein	63.4	68.1	66.5	69.5	74.3	68.7	59.8	59.4	46.6
Crude lipid	2.7	6.6	8.2	9.2	9.7	10.1	6.7	14.6	15.6
Crude fiber	1.8	1.6	1.1	0.5	1.3	0.9	1.3	1.5	1.7
Ash	21.2	14.9	14.8	12.6	5.4	13.1	21.1	19.5	7.0
NFE[10)]	10.9	8.8	9.4	8.2	9.3	7.2	11.1	5.0	29.1
Gross energy (MJ/kg)[11)]	17.8	20.0	20.4	21.3	22.8	21.3	18.5	20.5	22.0
Amino acids (% of protein)									
Arg	7.0	6.9	7.1	7.0	7.0	6.9	5.4	4.9	4.9
His	1.4	2.6	2.2	1.4	1.4	1.5	1.2	1.2	1.1
Ile	1.9	2.0	2.2	2.3	1.9	2.1	1.7	1.3	1.5
Leu	3.4	3.4	3.6	3.6	3.1	3.5	2.6	2.4	2.3
Lys	6.8	7.2	7.8	7.6	7.1	7.6	4.4	4.5	3.7
Met + Cys	2.5	1.8	2.0	2.3	2.2	2.0	1.9	1.1	1.3
Phe + Tyr	3.1	3.4	3.8	3.7	3.2	3.6	2.7	2.3	2.7
Thr	4.1	4.0	3.8	4.2	4.0	4.1	3.2	2.6	2.5
Val	4.9	5.0	5.3	5.6	4.7	5.4	4.2	3.7	3.4

1) PM was obtained from America.
2) JMM was obtained from Chile.
3) AM was obtained from Chile.
4) CM was obtained from Denmark.
5) SM was obtained from France.
6) SEM was obtained from Denmark.
7) TM was obtained from Korea.
8) MM was obtained from Korea.
9) SLM was obtained from Korea.
10) NFE was calculated by difference.
11) Based on 23.4 MJ/kg protein, 39.2 MJ/kg lipid and 17.2 MJ/kg NFE.
PM, pollock meal; JMM, jack mackerel meal; AM, anchovy meal; CM, cod meal; SM, sardine meal; SEM, sand eel meal; TM, tuna meal; MM, meat meal; SLM, squid liver meal; NFE, nitrogen-free extract.

$$\text{ADC of dry matter (\%)} = \left[100 - \left(\frac{\text{dietary } Cr_2O_3}{\text{feces } Cr_2O_3}\right) \times 100\right]$$

ADC of nutrients or energy (%)

$$= 100 \times \left(1 - \frac{\text{dietary } Cr_2O_3}{\text{feces } Cr_2O_3} \times \frac{\text{feces nutrient or energy}}{\text{dietary nutrient or energy}}\right)$$

The ADCs were calculated from the respective digestibility coefficients of the 70% RF and 30% of each of the test ingredients (Cho & Slinger, 1978).

ADC of test ingredient (%)
= [ADC in test diet – (0.7 × ADC in reference diet)] / 0.3

Statistical analysis

All data were subjected to one-way analysis of variance, followed by Duncan's multiple range test (Duncan, 1955) at the significance level of $p < 0.05$. Data are presented as mean ± standard error of mean of triplicate groups. Statistical analyses were performed with SPSS version 21.0 (IBM, Armonk, NY, USA).

Results

The ADCs of dry matter, crude protein, crude lipid, NFE and energy in the test ingredients consumed by olive flounder are presented in Table 4. ADCs of dry matter ranged from 68% to 95%. Dry matter ADC of CM was the highest among the treatments. In contrast, the dry matter digestibility of PM and AM

Table 3. Proximate and amino acid compositions of the reference and test diets fed to olive flounder

	Reference diet	Test diets (70% reference + 30% ingredient)								
		PM	JMM	AM	CM	SM	SEM	TM	MM	SLM
Proximate analysis (% of dry matter)										
Crude protein	53.1	56.2	57.6	57.1	58.0	59.5	57.8	55.1	55.0	51.2
Crude lipid	10.4	8.1	9.3	9.7	10.0	10.2	10.3	9.3	11.7	12.0
Ash (% DM)	15.2	17.0	15.1	15.1	14.4	12.3	14.6	17.0	16.5	12.7
NFE[1)]	21.3	18.2	17.6	17.7	17.4	17.7	17.1	18.2	16.4	23.6
Gross energy (MJ/kg)[2)]	20.2	19.5	20.2	20.2	20.5	21.0	20.5	19.7	20.3	20.7
Amino acids (% of protein)										
Arg	6.2	6.4	6.4	6.5	6.4	6.4	6.4	6.0	5.8	5.8
His	3.7	3.0	3.4	3.3	3.0	3.0	3.0	3.0	3.0	2.9
Ile	4.5	3.7	3.8	3.8	3.8	3.7	3.8	3.7	3.5	3.6
Leu	8.2	6.8	6.8	6.8	6.8	6.7	6.8	6.5	6.5	6.4
Lys	8.0	7.6	7.8	7.9	7.9	7.7	7.9	6.9	7.0	6.7
Met + Cys	4.8	4.1	3.9	4.0	4.1	4.0	4.0	3.9	3.7	3.8
Phe + Tyr	7.5	6.2	6.3	6.4	6.4	6.2	6.3	6.1	5.9	6.1
Thr	5.0	4.7	4.7	4.6	4.8	4.7	4.7	4.5	4.3	4.3
Val	5.6	5.4	5.4	5.5	5.6	5.3	5.5	5.2	5.0	4.9

1) NFE was calculated by difference.
2) Based on 23.4 MJ/kg protein, 39.2 MJ/kg lipid and 17.2 MJ/kg NFE.
PM, pollock meal; JMM, jack mackerel meal; AM, anchovy meal; CM, cod meal; SM, sardine meal; SEM, sand eel meal; TM, tuna meal; MM, meat meal; SLM, squid liver meal; NFE, nitrogen-free extract.

Table 4. Apparent digestibility coefficients (%) of dry matter, crude protein, lipid, NFE and energy in the test ingredients consumed by olive flounder

Ingredients	Dry matter	Crude protein	Crude lipid	NFE	Energy
PM	70.2 ± 0.28^{a}	68.1 ± 3.16^{a}	58.1 ± 1.27^{a}	72.0 ± 5.66^{bc}	56.5 ± 2.12^{a}
JMM	77.4 ± 1.46^{bc}	72.9 ± 0.48^{b}	90.6 ± 0.42^{d}	79.0 ± 2.50^{cd}	61.4 ± 1.45^{ab}
AM	68.0 ± 1.85^{a}	84.1 ± 1.82^{cd}	78.1 ± 2.34^{c}	59.0 ± 7.53^{a}	74.3 ± 4.38^{cd}
CM	94.7 ± 2.34^{e}	96.3 ± 0.87^{f}	86.5 ± 1.21^{d}	86.7 ± 2.28^{de}	95.0 ± 1.25^{e}
SM	78.2 ± 0.55^{bc}	86.3 ± 0.95^{d}	54.4 ± 1.37^{a}	62.3 ± 2.46^{ab}	73.0 ± 1.47^{cd}
SEM	88.4 ± 1.63^{d}	92.1 ± 1.57^{ef}	88.8 ± 2.12^{d}	94.1 ± 2.00^{e}	92.7 ± 2.07^{e}
TM	79.7 ± 0.83^{c}	87.8 ± 0.67^{de}	67.5 ± 0.94^{b}	73.2 ± 2.09^{bc}	77.6 ± 2.01^{d}
MM	75.1 ± 1.14^{b}	85.2 ± 0.95^{d}	76.1 ± 2.06^{c}	75.0 ± 2.96^{bcd}	79.2 ± 3.24^{d}
SLM	80.3 ± 1.77^{c}	80.1 ± 1.50^{c}	74.6 ± 1.44^{c}	78.4 ± 5.31^{cd}	68.7 ± 2.51^{bc}

a–f Values (mean ± standard error of mean) within the same column with different superscripts denote significant differences ($p < 0.05$).
PM, pollock meal; JMM, jack mackerel meal; AM, anchovy meal; CM, cod meal; SM, sardine meal; SEM, sand eel meal; TM, tuna meal; MM, meat meal; SLM, squid liver meal; NFE, nitrogen-free extract.

were significantly lower than the other ingredients tested ($p < 0.05$). Dry matter ADC of JMM was comparable with SM, TM, MM and SLM. MM exhibited significantly higher dry matter ADC than PM and AM while the result of SLM was significantly higher compared to MM ($p < 0.05$).

Protein digestibility of feedstuffs ranged from 68% to 96%. Digestibility of crude protein was higher for CM and SEM than those of the other ingredients. Protein digestibility of TM was comparable with SEM, SM, AM and MM. MM exhibited significantly higher protein digestibility than PM, JMM and SLM ($p < 0.05$). The values observed in SLM group were also significantly higher than PM and JMM ($p < 0.05$). PM showed the lowest protein digestibility value among all the tested ingredients.

Crude lipid digestibility ranged from 54% to 91%. The highest lipid digestibility coefficient was exhibited in JMM, CM and SEM and the lowest ADC for lipid was observed in PM and SM. MM and SLM showed comparable lipid digestibility which were significantly higher than PM, SM and TM.

The NFE digestibility of SEM was significantly highest among the test ingredients and the significantly lowest ADC for NFE was observed in AM ($p < 0.05$).

Energy digestibility of the animal protein feedstuffs tested in the current study ranged from 57% to 95%. The highest energy digestibility coefficient was observed in CM, followed by SEM and the energy ADC of PM was significantly lowest among those tested ($p < 0.05$). Energy digestibility values observed in AM, SM, TM and MM were comparable while that of SLM was comparable with SM and AM.

The ADC of amino acids of the tested ingredients are presented in Table 5. Generally, amino acid availability exhibited a similar pattern to crude protein digestibility. The ADC of amino acids in CM was generally higher than those of the other ingredients tested. The availability of essential amino acids in PM was the lowest for olive flounder compared to that of other ingredients.

Discussion

The actual ADC of feed ingredients provides insight to identify ingredient alternatives much more precisely for fish species. Digestibility of nutrients can be different based on the chemical composition of a particular ingredient. ADC of dry matter is more useful than ADC of individual nutrients to estimate the amount of indigestible material contained in feedstuffs (Yuan et al., 2010). In our study, dry matter digestibility of CM exhibited the highest values among all the tested ingredients. Cruz-Suárez et al. (2009) reported that dry matter digestibility appeared to be related to the fiber and ash contents of the material. In the previous study, we observed that dry matter digestibility was lower in feed ingredients containing high ash levels when feeds were prepared as extruded pellets (Rahman et al., 2016). Stone et al. (2000) reported that high protein content resulted in high dry matter digestibility in MM. SM contained the highest protein level and the lowest ash level although the dry matter and protein digestibility were significantly lower than CM and SEM in present study. Supportively, Sugiura et al. (1998) reported that dietary protein level had no significant correlation with protein digestibility. The proximate and amino acid composition of CM was also not considerably different from other ingredients (Table 2). Therefore, the high soluble nutrient content in CM which matches the requirement of olive flounder might be the reason for high dry matter digestibility. Nutrient absorption through the gastrointestinal tract can be accelerated by several factors including pH and ion concentration (Bucking & Wood, 2009). The CM and SEM might provide favorable levels of pH and ions to improve digestion and absorption of nutrients in olive flounder intestine. Therefore, further studies should be conducted to evaluate the factors affecting digestion and absorption in olive flounder fed diets containing CM and SEM. Moreover, MM and SLM exhibited higher or comparable dry matter digestibility to conventional FMs such as PM, JMM, AM and SM in the present study. Both MM and SLM contained high lipid levels (Table

Table 5. Apparent availability coefficients (%) of amino acids in test ingredients for olive flounder

Ingredients	Essential amino acids								
	Arg	His	Ile	Leu	Lys	Met + Cys	Phe + Tyr	Thr	Val
PM	83.6 ± 1.14^{a}	86.0 ± 0.78^{a}	79.9 ± 0.95^{a}	81.8 ± 0.92^{a}	83.1 ± 0.69^{a}	80.0 ± 1.71^{ab}	79.4 ± 1.25^{a}	80.4 ± 1.13^{a}	80.2 ± 0.97^{a}
JMM	87.5 ± 0.60^{b}	89.8 ± 0.06^{bc}	84.9 ± 0.44^{abc}	86.0 ± 0.49^{b}	87.7 ± 0.42^{b}	82.6 ± 0.67^{abc}	83.9 ± 0.60^{b}	84.8 ± 0.27^{b}	84.2 ± 0.45^{b}
AM	90.7 ± 1.88^{d}	90.7 ± 0.40^{cd}	87.7 ± 0.22^{bcd}	89.0 ± 0.40^{cd}	91.1 ± 0.38^{cd}	79.6 ± 4.53^{a}	84.0 ± 1.91^{b}	86.6 ± 1.50^{bc}	87.4 ± 0.12^{c}
CM	93.4 ± 0.35^{e}	93.5 ± 0.27^{g}	91.0 ± 0.43^{d}	91.9 ± 0.27^{e}	93.5 ± 0.47^{e}	91.1 ± 0.12^{e}	90.4 ± 0.40^{d}	91.3 ± 0.45^{e}	91.0 ± 0.47^{d}
SM	91.6 ± 0.18^{de}	91.0 ± 0.15^{de}	86.9 ± 0.25^{bcd}	88.5 ± 0.38^{c}	91.0 ± 0.17^{cd}	85.5 ± 0.95^{bcd}	86.9 ± 0.35^{bc}	88.5 ± 0.29^{cd}	87.3 ± 0.32^{c}
SEM	90.0 ± 0.12^{cd}	91.5 ± 0.26^{de}	86.7 ± 0.61^{bcd}	88.5 ± 0.35^{c}	90.1 ± 0.27^{c}	87.1 ± 0.83^{cde}	86.7 ± 0.28^{bc}	87.8 ± 0.22^{cd}	87.0 ± 0.49^{c}
TM	91.6 ± 0.17^{de}	92.2 ± 0.19^{ef}	88.6 ± 0.15^{cd}	90.0 ± 0.15^{d}	92.1 ± 0.10^{d}	80.5 ± 1.11^{ab}	88.1 ± 0.22^{bc}	89.2 ± 0.43^{de}	88.2 ± 0.13^{c}
MM	91.2 ± 0.24^{de}	91.5 ± 0.23^{de}	82.6 ± 4.75^{ab}	88.9 ± 0.31^{cd}	90.8 ± 0.24^{c}	84.3 ± 1.01^{abc}	86.9 ± 0.47^{bc}	87.6 ± 0.31^{cd}	87.4 ± 0.37^{c}
SLM	87.8 ± 0.50^{bc}	88.7 ± 0.72^{b}	84.6 ± 0.79^{abc}	85.6 ± 0.09^{b}	88.1 ± 0.48^{b}	83.1 ± 1.35^{abc}	85.4 ± 1.40^{bc}	85.0 ± 0.23^{b}	84.5 ± 0.62^{b}

$^{a–g}$ Values (mean ± standard error of mean) within the same column with different superscripts denote significant differences ($p < 0.05$).
PM, pollock meal; JMM, jack mackerel meal; AM, anchovy meal; CM, cod meal; SM, sardine meal; SEM, sand eel meal; TM, tuna meal; MM, meat meal; SLM, squid liver meal.

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2) compared to FMs used in the study. Especially, MM contains high oleic acid level (Ha et al., 2021) which are easily absorbed and stored in olive flounder liver (Medagoda et al., 2022). Ha et al. (2021) observed significantly higher monoene fatty acids in whole-body samples of olive flounder fed MM. SLM also rich in oleic acid and DHA (MoonLee et al., 2012). Therefore, these fatty acids might be responsible for high dry matter digestibility of MM and SLM in olive flounder. Fatty acid digestibility of both ingredients should be tested in future studies to prove the assumption although the lipid digestibility was higher in MM and SLM compared to PM and SM. However, high level of dietary MM was reported to decrease the growth of fish while high dietary SLM resulted in significant cadmium accumulation in organs (Jang et al., 2021). Therefore, these phenomena should be considered when using MM and SLM in olive flounder diet.

The results of protein digestibility revealed that CM and SEM possess a protein content that is highly digestible in olive flounder indicating that each of these animal origin feed ingredients is suitable protein sources for olive flounder. The protein ADC for JMM (73%) is lower than that reported in white leg shrimp, *Litopanaeus vannamei* (89%) (Lemos et al., 2009). Protein ADC for AM (84%) is higher than that reported in sunshine bass, *Morone chrysops* × *Morone saxatilis* (79%) (Thompson et al., 2008) and it is slightly lower than those reported for AM in Atlantic cod, *Gadus morhua* (92%) (Tibbetts et al., 2006), coho salmon, *Oncorhynchus kisutch* (91%) and rainbow trout, *Oncorhynchus mykiss* (94%) (Sugiura et al., 1998) and Nile tilapia, *Oreochromis niloticus* (91%) (Köprücü & Özdemir, 2005). The protein ADC for SM (86%) is slightly lower than values reported for rainbow trout (90%) (Gaylord et al., 2008). The lower digestibility of protein in JMM, AM and SM reported in the present study might be a result of species and/or size differences (Thompson et al., 2008). The protein quality of FM depends both on the freshness of the raw material and the processing conditions in the manufacturing of the FM (Anderson et al., 1995; Sørensen, 2012). Some studies have reported that the effect of the processing condition of FM may lead to reduce in protein digestibility due to changes in the chemical composition or shape of proteins (Anderson et al., 1993; Opstvedt et al., 2003). Moreover, the nutritional compositions of FM were reported to be different with harvest location and season (Boran et al., 2008; Bragadóttir et al., 2004). In the current study, the protein digestibility of PM was the lowest among the ingredients tested. This result may also be attributed to origin of raw materials, location, species, catching season and processing conditions followed to manufacture. Interestingly, both MM and SLM resulted in higher protein digestibility compared to PM and JMM. Also, result of MM was comparable with AM, SM and TM while protein digestibility of SLM was comparable with AM. It has been previously reported that MM exhibited comparable protein digestibility to FM in *Labeo rohita* (Hussain et al., 2011). Stone et al. (2000) reported that digestibility of MM was improved in silver perch, *Bidyanus bidyanus*, when product contain high protein level. However, protein level of MM was comparatively lower than in other ingredients used in the present study (Table 2). However, the RF in this study contained FM making a mixture of FM, MM or SLM in MM and SLM diets. Therefore, total FM level was also reduced in both MM and SLM diets. It was well documented that efficiency of a diet is enhanced when contains a mixture of protein sources (Bae et al., 2020). Accordingly, we assumed that proteins in MM and SLM diets were efficiently digested by olive flounder in the present study.

The lipid digestibility of fish diets was usually reported from 85% to 95% in previous studies (NRC, 1993). The ADC of lipid value for AM (78%) is lower than those previously observed in Nile tilapia (98%) (Köprücü & Özdemir, 2005) and sunshine bass, *M. chrysops* × *M. saxatilis* (82%) (Thompson et al., 2008). The lipid digestibility is influenced by lipid levels and different other factors including unsaturation level and chain length of fatty acids in particular ingredients (Yuan et al., 2010). Olive flounder has a low ability to utilize dietary lipid compared to other marine carnivorous fish (Lee et al., 2000). However, JMM, CM and SEM exhibited higher lipid digestibility compared to the other ingredients tested in the present study. MM and SLM contained high lipid levels although the lipid digestibility was lower compared to JMM, CM and SEM. Therefore, the lipid digestibility of ingredients tested in the current study might not be correlated with dietary lipid levels. Lipid digestibility is dependent on the lipid source (Caballero et al., 2002). Accordingly, it is assumed that JMM, CM and SEM contain highly digestible lipids for olive flounder. In the case of MM and SLM, we assumed that efficiently digestible fatty acid in both ingredients were responsible for the high lipid digestibility compared to PM, SM, and TM.

The ADC of energy value for AM (74%) is lower than values recorded in Atlantic cod (86%) (Tibbetts et al., 2006) and Nile tilapia (92%) (Köprücü & Özdemir, 2005). Several studies have revealed better energy utilization of animal products in fish species (Gaylord & Gatlin, 1996; Lee, 2002; McGoogan & Reigh, 1996; Zhou et al., 2004). Carnivorous species can efficiently utilize energy in FM and other animal protein sources than in plant protein sources (Kim et al., 2010; Lee et al., 2020; Sullivan & Reigh, 1995; Yu et al., 2013). According to Yu et al. (2013), digestibility of energy in selected ingredients is dependent on the chemical composition and quantities of each ingredient are responsible for the total energy digestibility of diet. Further, they mentioned that utilization of carbohydrate energy in snakehead, *Ophiocephalus argus*, can be maximized by supplementing the proper ratio of dietary carbohydrate and protein. In the present study, CM and SEM contained approximately similar levels of protein, lipid, ash and moisture indicating that both diets exhibited proper carbohydrate and protein ratio to increase the energy digestion in olive flounder. Energy digestibility of MM was significantly higher than PM and JMM indicating that MM was efficiently utilized by olive flounder. Similar trend was observed in dry matter, protein and lipid digestibility of MM. The increased nutrient digestibility might be associated with increased energy digestibility in MM. Moreover, differences in the energy digestibility of the feed ingredients tested for olive flounder may be due to differences in the source, freshness of the raw materials and processing conditions involved in the production of the final meal (Maina et al., 2002). High ash content of FM was also reported to decrease the digestibility of energy according to Gomes et al. (1995) although it was not obvious in the present study. Those phenomena should be examined in future studies.

The availability of amino acids reflected the protein digestibility of ingredients. The higher availability of amino acids represents high-quality protein sources for fish. On the contrary, low amino acid availability indicates poor utilization of dietary protein (Halver & Hardy, 2002; Lee, 2002). Therefore, Allan et al. (2000) reported the importance of amino acid availability data when formulating efficient diets for fish. In the present study, the availabilities of measured amino acids were variable although the present data suggest a fair agreement concerning protein and amino acid digestibilities. The amino acid availability coefficient for CM was significantly higher than the other tested ingredients, indicating that CM is a good quality feed ingredient that contains highly digestible protein for olive flounder. PM had a lower availability coefficient of essential amino acids than the other ingredients tested. However, ADCs of essential amino acids in FM are generally higher (> 90%) in some fishes, such as Atlantic salmon, *Salmo salar*, (Anderson et al., 1995), common carp, *Cyprinus carpio* (Yamamoto et al., 1998) and striped bass (Small et al., 1999). The low amino acid availability of PM in this study could be due to differences in the meal processing conditions or the differences in the quality of the raw material processed. Supportively, Mu et al. (2000) suggested that some amino acids of FM are inadequately utilized or made unavailable because of the manufacturing process of FM.

In conclusion, the results of this study provide information about the bioavailability of nutrients and energy in tested feedstuffs. The amino acid digestibility coefficients are useful for accurate and economical feed formulation for olive flounder because of variation of amino acid availabilities among tested ingredients. MM and SLM exhibit similar or higher nutrient digestibility than conventional FMs except CM and SEM. However, effects of both MM and SLM on growth performance of olive flounder should be considered to formulate efficient feed. Overall, CM and SEM are seeming to be highly digestible and effective to use in olive flounder diet compared to the other tested ingredients.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and materials

Upon a reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

This article does not require IRB/IACUC approval because there are no human and animal participants.

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