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

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**Abstract**
This study was designed to determine the apparent digestibility coefficients of soybean meal, soy protein concentrate (SPC), soy protein isolate (SPI), rapeseed meal (RSM), pea protein concentrate (PPC), wheat gluten meal (WGM) and wheat flour (WF) for olive flounder, *Paralichthys olivaceus*. A reference diet (RF) was formulated to meet the nutrient requirements of olive flounder with 1% chromic oxide ($Cr_2O_3$) as an inert indicator. Test diets were prepared to contain 70% RF and 30% of the test ingredient. Olive flounder, averaging 150 ± 8.0 g, was cultured in 400-L fiberglass tanks at a density of 25 fish per tank. Fecal collection columns were attached to each tank. Fecal samples were obtained from triplicate groups of fish for 4 weeks. Dry matter digestibility of SPC (75%) and WGM (76%) were significantly higher than the other test ingredients. Protein digestibility of SPC (85%), PPC (88%) and WGM (89%) were significantly higher than the other test ingredients, and protein digestibility of RSM (77%) and WF (76%) was lower than the other ingredients tested. Lipid digestibility of SPC (72%) and SPI (69%) were significantly higher than the other test ingredients. Energy digestibility of SPC (85%) and WGM (82%) were significantly higher than that of others tested ingredients. The availability of amino acids in WGM was generally higher than in other plant-feed ingredients. Therefore, SPC and WGM were seems to be efficient as potential protein sources for olive flounder compared to other tested ingredients. Overall, findings of the current study may assist in more efficient and economical formulation of diets using plant feed ingredients for olive flounder.

**Keywords:** Olive flounder, Nutrient digestibility, Plant ingredients, Amino acid availability

## Introduction

Feed comprises the main operating cost in fish production. Accurate manipulation of feed ingredients is important to

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supply nutritional requirements for efficient production of fish (Sklan et al., 2004). Information on nutrient digestibility of feed ingredients is needed to improve the accuracy of diet for fish species in the case of formulating cost-effective diets by appropriate substitution of feedstuffs. Moreover, this information is also useful for regulating and preventing aquaculture waste (Lee, 2002; Liu et al., 2009; Zhou et al., 2004). Therefore, the determination of nutrient digestibility is considered one of the initial steps for evaluating potential ingredients in diets for target fish species (Chu et al., 2015; Li et al., 2013; Mo et al., 2019; Yu et al., 2013).

Olive flounder, *Paralichthys olivaceus,* is an important aquaculture species in Asian countries, especially Korea, Japan and China. Its production is steadily increasing since the late 1980s (Cho et al., 2006; Rahman et al., 2015). Olive flounder requires high levels of animal-based protein as they are carnivorous (Hamidoghli et al., 2020; Kim et al., 2002). Therefore, farmers use fish meal as the best protein source usually used in their diets (Jung et al., 2020). However, fish meal is a limiting factor for olive flounder culture because of increasing price and uncertain reliability of supply (Bai & Lee, 2010). Therefore, it was an important research priority to finding alternative protein ingredients for olive flounder (Kim et al., 2019; Lim et al., 2020). Several studies have been performed on nutrient requirements (Cho & Heo, 2011; Kim & Lee, 2013; Kim et al., 2005; Won et al., 2019) and the utilization of plant protein ingredients (Lim & Lee, 2008; Kim et al., 2019; Seong et al., 2018; Tharaka et al., 2020) for efficient production of olive flounder.

High level of plant protein sources was reported for reduced nutrient digestibility in olive flounder diets (Gunathilaka et al., 2020; Khosravi et al., 2018; Tharaka et al., 2020). Therefore, a number of studies were conducted to optimize the usage of plant protein sources in different feed formulations by reducing total fish meal levels in diets while improving performance of olive flounder in different growth stages (Hamidoghli et al., 2020). Many studies reported successful combinations of fish meal and plant protein sources. Moreover, functional ingredients and developed protein sources i.e., marine protein hydrolysates were used to optimize performance of olive flounder fed diets containing a high level of plant protein (Gunathilaka et al., 2020; Khosravi et al., 2018). Therefore, knowledge about nutrient digestibility of ingredients is obviously advantageous in the case of further developing low fish meal diets containing high levels of plant protein. Several studies were conducted to identify digestibility of feed ingredients in olive flounder. Rahman et al. (2016a) reported digestibility of various fish meal sources in extruded pellets for olive flounder. Nutrient digestibility of feed containing different carbohydrate sources was evaluated by Rahman et al. (2016b) and reported that dextrin and potato starch were efficient carbohydrate sources for olive flounder. Kim et al. (2010) observed lower nutrient digestibility in plant protein sources compared to animal-based protein sources. However, limited reports are available about the apparent digestibility coefficients (ADCs) of various plant feed ingredients for olive flounder to the best of our knowledge.

Different types of plant protein sources are used in olive flounder diet. Soybean meal (SBM) is a widely used plant protein source in olive flounder diets (Pham et al., 2008; Ye et al., 2011; Lim et al., 2020). Soy protein concentrate (SPC) and soy protein isolate (SPI) were also used in olive flounder diet as developed soy byproducts (Gunathilaka et al., 2020; Hamidoghli et al., 2020; Khosravi et al., 2018). Wheat gluten is included in olive flounder diet in case of fish meal replacement and wheat flour (WF) is incorporated even in high fish meal diets as a carbohydrate source (Bae et al., 2015; Kim et al., 2019; Lim et al., 2020). However, rapeseed meal (RSM) and pea protein concentrate (PPC) were not studied as dietary supplements in high plant protein diets for olive flounder. Nutrient digestibility of these plant protein sources was not evaluated although SBM was compared with animal protein sources (Kim et al., 2010) and WF was tested as a carbohydrate source (Rahman et al., 2016b) in olive flounder diets. Therefore, this study was conducted to determine and compare ADCs of dry matter, crude protein, crude lipid, nitrogen-free extract (NFE), energy and essential amino acid availability of SBM, SPC, SPI, RSM, PPC, wheat gluten meal (WGM) and WF for olive flounder.

## Materials and Methods

### Diet preparation

A reference diet (RF) was formulated using mackerel and anchovy fish meal (imported from Chile), and squid liver oil (E-Wha Oil & Fat Industries, 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 by mixing 70% RF and 30% of each of the test ingredients on an air-dry basis according to Cho & Slinger (1979). Test ingredients


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**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[6)] | | 30.0 |

[1)]Imported from Chile.
[2)]Produced by E-wha Oil & Fat Industry, 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, St. Louis, MO, USA.
[6)]Test ingredients were soybean meal, soy protein concentrate, soy protein isolate, rapeseed meal, pea protein concentrate, wheat gluten meal and wheat flour.

for ADCs were SBM, SPC, SPI, RSM, PPC, WGM and WF. The experimental diets were also designated as SBM, SPC, SPI, RSM, PPC, WGM and WF to represent the test ingredients. Proximate and amino acid compositions of ingredients and diets are presented in Tables 2 and 3. All dry ingredients were thoroughly mixed and pelleted through a meat chopper machine, fitted with 5 mm die, after addition of squid liver oil and distilled water (40%). Then, moist strands were crushed into 8–12 mm long pellets, air-dried and stored at –25℃.

### Fish and experimental condition

Olive flounder were obtained from a hatchery (Namhae, Korea) and transported to Marine Biology Center for Research and Education at Gangneung-Wonju National University.

Prior to starting the experiment, fish were acclimated while feeding the RF to apparent satiation once daily for 2 weeks. A fecal collection system containing thirty fiberglass tanks of 400 L capacity, designed according to Lee (2002) was used for the experiment. The 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 3 L/min flow rate. The water temperature was 20.2 ± 0.4℃ and the photoperiod was maintained by natural conditions. Salinity, dissolved oxygen and pH levels of the water were 32.9 ± 0.51 ppt, 7.34 ± 0.46 mg/L and 7.45 ± 0.39 respectively.

### 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 on the 4th day after setting. About two hours after feeding, the rearing tanks and collection columns were cleaned to remove any residual particulate matter (feces and uneaten feed). Feces were then allowed to settle overnight. Fecal samples were collected 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. All feces collected from each tank during the digestibility trial were pooled.

### Analytical methods

Proximate composition of both diet and fecal samples were analyzed in triplicate (AOAC, 2005). $Cr_2O_3$ 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, Hoganas, 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) consist of an ion-exchange column.

ADC 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:

$$\text{ADC of dry matter (\%)} = (100 - (\text{dietary } Cr_2O_3 / \text{feces } Cr_2O_3) \times 100)$$

**Table 2. Nutrient and amino acid composition of the ingredients**

| | Ingredients | | | | | | |
|---|---|---|---|---|---|---|---|
| | SBM | SPC | SPI | RSM | PPC | WGM | WF |
| Proximate analysis (% of dry matter) | | | | | | | |
| Moisture | 10.8 | 6.9 | 6.4 | 9.1 | 7.0 | 4.5 | 10.9 |
| Crude protein | 47.6 | 74.1 | 68.9 | 36.1 | 48.7 | 83.7 | 16.4 |
| Crude lipid | 1.1 | 0.5 | 0.3 | 0.7 | 4.5 | 1.0 | 1.5 |
| Crude fiber | 5.0 | 3.1 | 3.5 | 4.6 | 3.5 | 2.8 | 3.6 |
| Ash | 6.2 | 6.6 | 8.1 | 9.5 | 3.8 | 0.5 | 2.2 |
| Carbohydrate[1)] | 40.1 | 15.7 | 19.2 | 49.1 | 39.5 | 12.0 | 76.3 |
| Gross energy (kJ/g) | 18.0 | 20.1 | 19.7 | 17.2 | 18.4 | 22.6 | 15.5 |
| Essential amino acids (% of protein) | | | | | | | |
| Arg | 6.7 | 6.6 | 6.7 | 6.4 | 7.0 | 4.8 | 6.3 |
| His | 3.3 | 3.4 | 3.2 | 3.6 | 3.2 | 3.1 | 3.7 |
| Ile | 4.4 | 3.9 | 4.2 | 3.8 | 4.2 | 4.2 | 3.9 |
| Leu | 7.7 | 7.8 | 8.0 | 7.7 | 8.2 | 7.1 | 7.7 |
| Lys | 7.4 | 7.3 | 7.5 | 7.6 | 7.9 | 5.5 | 7.6 |
| Met + Cys | 4.0 | 5.2 | 3.5 | 3.4 | 3.9 | 3.8 | 4.4 |
| Phe + Tyr | 7.7 | 7.4 | 7.5 | 7.0 | 7.8 | 7.2 | 7.2 |
| Thr | 4.8 | 5.0 | 4.7 | 5.2 | 4.7 | 3.8 | 5.0 |
| Val | 5.1 | 4.8 | 4.9 | 4.9 | 4.9 | 5.1 | 5.0 |

[1)]100 – (Moisture + Crude protein + Crude lipid + Ash).
SBM, soybean meal; SPC, soy protein concentrate; SPI, soy protein isolate; RSM, rapeseed meal; PPC, pea protein concentrate; WGM, wheat gluten meal; WF, wheat flour.

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 1979).

ADC of test ingredient (%) =

$$[\text{ADC in test diet} - (0.7 \times \text{ADC in RF})] / 0.3$$

### Statistical analyses

All experimental data were analyzed to one-way analysis of variance, followed by Duncan's multiple range test (Duncan, 1955) at the significance level of $p < 0.05$. Shapiro-Wilk's and Levene's tests were applied to verify whether the normality and homogeneity of variances are met. The data are presented as mean ± SE of triplicate groups. Relationship between nutrients and energy was examined using Pearson regression. Statistical analyses were evaluated using SPSS version 20.0 (IBM, Armonk, NY, USA).

## Results

The ADCs of dry matter, crude protein, crude lipid, NFE and energy of the test ingredients for olive flounder are presented in Table 4. ADCs of dry matter ranged from 62% to 76%. Dry matter ADCs of SPC and WGM were significantly higher than the other test ingredients ($p < 0.05$). In contrast, the dry matter digestibilities of SBM, RSM and WF were significantly lower than other ingredients tested ($p < 0.05$). SPI and PPC exhibited significantly higher dry matter ADC compared to SBM, RSM and WF although values were significantly lower than SPA and WGM groups.

Protein ADCs of the tested feedstuffs ranged from 76% to 89%. The highest protein ADC was observed in WGM. Protein ADC of SPC, PPC and WGM were significantly comparable and higher than the other test ingredients ($p < 0.05$). The protein ADC of RSM and WF were lower than the other ingredients tested ($p < 0.05$). WF exhibited the lowest protein digestibility. However, protein digestibility of SBM and SPI was significantly higher than that of WF ($p < 0.05$).

Crude lipid digestibility in the present study ranged


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**Table 3. Nutrient and amino acid composition of the reference and test diets fed to olive flounder**

| | Reference diet | Test diets (70% reference + 30% ingredient) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | SBM | SPC | SPI | RSM | PPC | WGM | WF |
| Proximate analysis (% of dry matter) | | | | | | | | |
| Crude protein | 53.1 | 51.5 | 59.4 | 57.8 | 48.0 | 51.8 | 62.3 | 42.1 |
| Crude lipid | 10.4 | 7.6 | 7.4 | 7.4 | 7.5 | 8.6 | 7.6 | 7.7 |
| Crude fiber | 3.6 | 4.0 | 3.5 | 3.6 | 3.9 | 3.6 | 3.4 | 3.6 |
| Ash | 15.2 | 12.5 | 12.6 | 13.1 | 13.5 | 11.8 | 10.8 | 11.3 |
| Carbohydrate[1)] | 17.7 | 24.4 | 17.1 | 18.2 | 27.1 | 24.2 | 16.0 | 35.3 |
| Gross energy (kJ/g) | 20.5 | 19.8 | 20.4 | 20.3 | 19.5 | 19.9 | 21.1 | 19.0 |
| Essential amino acids (% of protein) | | | | | | | | |
| Arg | 6.2 | 6.4 | 6.3 | 6.4 | 6.3 | 6.4 | 5.8 | 6.2 |
| His | 3.7 | 3.6 | 3.6 | 3.6 | 3.7 | 3.6 | 3.5 | 3.7 |
| Ile | 4.5 | 4.5 | 4.3 | 4.4 | 4.3 | 4.4 | 4.4 | 4.3 |
| Leu | 8.2 | 8.1 | 8.1 | 8.1 | 8.1 | 8.2 | 7.9 | 8.1 |
| Lys | 8.0 | 7.8 | 7.8 | 7.9 | 7.9 | 8.0 | 7.3 | 7.9 |
| Met + Cys | 4.8 | 4.6 | 4.9 | 4.4 | 4.4 | 4.5 | 4.5 | 4.7 |
| Phe + Tyr | 7.5 | 7.6 | 7.5 | 7.5 | 7.4 | 7.6 | 7.4 | 7.4 |
| Thr | 5.0 | 4.9 | 5.0 | 4.9 | 5.1 | 4.9 | 4.6 | 5.0 |
| Val | 5.6 | 5.5 | 5.4 | 5.4 | 5.4 | 5.4 | 5.5 | 5.4 |

[1)]100 – (Moisture + Crude protein + Crude lipid + Ash).
SBM, soybean meal; SPC, soy protein concentrate; SPI, soy protein isolate; RSM, rapeseed meal; PPC, pea protein concentrate; WGM, wheat gluten meal; WF, wheat flour.

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

| Ingredients | Dry matter | Crude protein | Crude lipid | NFE | Energy |
|---|---|---|---|---|---|
| SBM | $63.1 \pm 0.75^{a}$ | $83.0 \pm 0.87^{c}$ | $63.3 \pm 0.44^{b}$ | $47.7 \pm 1.06^{a}$ | $71.3 \pm 0.92^{a}$ |
| SPC | $75.1 \pm 3.03^{c}$ | $85.1 \pm 3.77^{cd}$ | $72.0 \pm 1.23^{c}$ | $71.5 \pm 0.88^{e}$ | $85.2 \pm 2.15^{c}$ |
| SPI | $67.5 \pm 0.12^{b}$ | $82.5 \pm 1.03^{bc}$ | $69.3 \pm 0.56^{c}$ | $55.9 \pm 1.17^{bc}$ | $74.9 \pm 1.28^{ab}$ |
| RSM | $62.0 \pm 0.80^{a}$ | $77.1 \pm 2.05^{ab}$ | $64.0 \pm 1.23^{b}$ | $53.4 \pm 1.39^{b}$ | $71.1 \pm 0.94^{a}$ |
| PPC | $67.4 \pm 0.41^{b}$ | $88.2 \pm 1.27^{cd}$ | $63.8 \pm 2.00^{b}$ | $57.6 \pm 0.64^{cd}$ | $77.3 \pm 1.19^{b}$ |
| WGM | $76.1 \pm 0.32^{c}$ | $88.8 \pm 0.46^{d}$ | $63.6 \pm 1.93^{b}$ | $70.4 \pm 0.57^{e}$ | $82.0 \pm 1.71^{c}$ |
| WF | $62.2 \pm 0.53^{a}$ | $76.3 \pm 0.47^{a}$ | $58.7 \pm 0.84^{a}$ | $59.4 \pm 0.53^{d}$ | $70.2 \pm 1.87^{a}$ |

[a–e]Values (mean ± SE of triplicate groups) in the same column with different superscripts are significantly different ($p < 0.05$).
NFE, nitrogen-free extract; SBM, soybean meal; SPC, soy protein concentrate; SPI, soy protein isolate; RSM, rapeseed meal; PPC, pea protein concentrate; WGM, wheat gluten meal; WF, wheat flour.

from 59% to 72%. The significantly highest ADC of lipid was exhibited in SPC and SPI and the lowest ADC of lipid was observed in WF ($p < 0.05$). Other tested ingredients showed significantly higher lipid digestibility than the WF group.

SPC and WGM had the significantly highest ADC for NFE while SBM exhibited the lowest value. The significantly lowest ADC of NFE was observed in SBM group ($p < 0.05$).

The energy digestibility of the plant protein feedstuffs tested here ranged from 70% to 85%. The highest energy digestibility was observed in SPC, followed by WGM and the significantly lowest ADC for energy was obtained in SBM, RSM and WF ($p < 0.05$).

Apparent amino acid availability for olive flounder is shown in Table 5. The availability of amino acids in WGM was generally higher than those of the other ingredients tested, while WF showed the lowest value.

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

| Ingredients | Essential amino acids | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Arg | His | Ile | Leu | Lys | Met + Cys | Phe + Tyr | Thr | Val |
| SBM | 86.7 ± 0.38[b] | 88.4 ± 0.49[bc] | 81.3 ± 0.22[b] | 83.5 ± 0.33[b] | 85.5 ± 0.28[b] | 78.3 ± 2.11[ab] | 81.5 ± 0.44[b] | 83.0 ± 0.54[b] | 80.7 ± 0.84[b] |
| SPC | 88.0 ± 1.36[bc] | 88.4 ± 1.40[bc] | 81.6 ± 1.99[b] | 84.0 ± 1.68[bc] | 86.4 ± 1.43[bc] | 84.4 ±1.23[cd] | 83.0 ± 1.80[b] | 83.7 ± 1.82[b] | 81.3 ±1.93[bc] |
| SPI | 87.7 ± 0.12[bc] | 87.0 ± 0.58[b] | 82.3 ± 0.53[bc] | 83.2 ± 0.39[b] | 85.9 ± 0.74[b] | 76.2 ±1.78[ab] | 81.8 ± 0.74[b] | 81.9 ± 0.43[b] | 80.9 ± 0.98[b] |
| RSM | 87.5 ± 0.30[b] | 88.4 ± 0.14[bc] | 80.9 ± 0.73[ab] | 83.8 ± 0.32[b] | 86.5 ± 0.26[bc] | 74.2 ± 1.18[a] | 81.6 ± 0.07[b] | 83.7 ± 0.33[b] | 80.7 ± 0.73[b] |
| PPC | 90.0 ± 0.21[cd] | 89.2 ± 0.29[c] | 85.0 ± 0.47[c] | 86.9 ± 0.30[c] | 89.2 ± 0.26[cd] | 80.2 ± 2.01[bc] | 85.6 ± 0.32[c] | 84.8 ± 0.15[b] | 84.2 ± 0.57[c] |
| WGM | 91.5 ± 1.26[d] | 93.0 ± 0.58[d] | 90.2 ± 0.93[d] | 85.0 ± 1.64[bc] | 91.8 ± 1.76[d] | 87.3 ± 1.17[d] | 88.2 ± 0.06[d] | 88.1 ± 1.05[c] | 90.0 ± 0.95[d] |
| WF | 83.5 ± 0.48[a] | 83.7 ± 0.43[a] | 78.2 ± 0.55[a] | 78.7 ± 0.53[a] | 82.0 ± 0.44[a] | 73.3 ± 1.82[a] | 78.3 ± 0.70[a] | 77.5 ± 0.46[a] | 77.1 ± 0.59[a] |

[a–d]Values (mean ± SE of triplicate groups) in the same column with different superscripts are significantly different ($p < 0.05$).
SBM, soybean meal; SPC, soy protein concentrate; SPI, soy protein isolate; RSM, rapeseed meal; PPC, pea protein concentrate; WGM, wheat gluten meal; WF, wheat flour.

## Discussion

Nutrient ADCs of ingredients are utilized in diet formulation programs to satisfy the nutrient requirements of a target fish species at the least cost. The nutrient digestibility varies based on the chemical composition of ingredients used (Cerri et al., 2021). In the current study, SBM, RSM and WF showed significantly lower apparent dry matter digestibility compared to all the other plant feedstuffs tested. Plant protein sources containing high levels of carbohydrate and fiber have relatively lower dry matter digestibility (Luo et al., 2008). The statement may be further supported by the current study where the plant protein ingredients with relatively lower fiber and carbohydrate contents (SPC and WGM) showed significantly higher ADC of dry matter compared to those with relatively higher fiber and carbohydrate contents (SBM, RSM and WF). Moreover, the results showed that dry matter ADCs were significantly correlated with fiber and carbohydrate content of ingredients in the present study. The phenomenon might be attributed to the chemical composition and structure of ingredients because higher level of fiber and carbohydrate contents interfere enzymes from accessing their substrates or directly interacting with enzymes decelerating digestive processes (Sklan et al., 2004). In addition to fiber and carbohydrates, Mo et al. (2019) indicated that high ash levels in feed ingredients can negatively affect dry matter digestibility. The trend was not clear in the present study although WGM contained lower fiber, carbohydrate and ash levels while exhibiting the highest dry matter digestibility among tested ingredients. Also, low or high dietary protein levels were reported to reduce nutrient digestion and utilization in fish (Kim et al., 2002; Ma et al., 2019). However, dry matter digestibility of SPI and PPC was comparable although the ingredient contained different protein levels. Therefore, we assumed that fiber, carbohydrates and ash levels in ingredients can be considered as indicators of dry matter digestibility in olive flounder.

It is well known that protein quality of feed ingredients is generally the major factor that affects fish performance and protein digestibility (Köprücü & Özdemir, 2005; Zhou & Yue, 2012). In the present study, the low ADC values for WF and RSM protein for olive flounder agree with several other studies. Protein ADC of RSM was low for omnivorous loach, *Misgurnus anguillicaudatus* (Chu et al., 2015) and several other carnivorous species such as javelin goby, *Synechogobius hasta* (Luo et al., 2009), juvenile cobia, *Rachycentron canadum* (Zhou et al., 2004) and rainbow trout, *Oncorhynchus mykiss* (Cheng & Hardy, 2002) than other plant protein sources. Sklan et al. (2004) found that protein from corn and wheat exhibited the lowest protein digestibility among all the plant protein ingredients fed to tilapia. However, the values found here for flounder were slightly lower than those reported for tilapia and higher than rainbow trout (Gaylord et al., 2008) because carnivorous species exhibit low efficiency in utilizing protein in plant protein sources compared to omnivorous species (Cheng & Hardy, 2002; Luo et al., 2009). Moreover, it has been suggested that the lower protein digestibility in plant protein ingredients could be a reason of; i) unbalance amino acid profile (NRC, 2011); ii) antinutritional factors (ANFs) (Francis et al., 2001); and iii) inadequate levels of energy in these feedstuffs (El-Saidy & Gaber, 2002). In this regard, it was reported that RSM contains ANFs and non-starch polysaccharides (cellulose and pectin), that may restrict growth performance and protein utilization (Yusuf et al., 2021; Zhang et al., 2020). This phenomenon may partly explain the high level of protein digestibility observed in SPC, PPC and WGM in the


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current study as their ANFs were reduced through processing technologies, improving their functional and nutritional properties (Gatlin et al., 2007). Especially, PPC exhibited comparable protein digestibility although dry matter, NFE and energy digestibility were lower than SPC and WGM indicating that a low level of ANFs contains in PPC after processing.

In the current study, the ADCs of lipid were < 72% in plant protein ingredients tested. Similar results were observed by Lee & Kim (2005). Among the plant feedstuffs tested in the current study, SPC and SPI showed relatively higher values compared to the other ingredients. Researchers have suggested that physical properties of plant protein influence lipid digestibility (Dias et al., 2005; Romarheim et al., 2006). Amphiphilic globulins found in plant proteins can bind to fat in the chyme decreasing lipid digestibility (Lusas & Riaz, 1995). Balmir et al. (1996) also reported that the undigested high molecular fraction of plant protein can bind lipids, specifically conjugated bile salts and increase steroid excretion with feces. WGM exhibited lower lipid digestibility although dry matter and protein digestibility were higher. However, the trend was not observed in greater amberjack, *Seriola dumerili* (Tomás-Vidal et al., 2019). Source and quality are major factors that can affect the digestibility of lipid in fish (Medagoda et al., 2022; Tibbetts et al., 2020; Weththasinghe et al., 2021). Therefore, fatty acid digestibility of each ingredient in olive flounder should be measured in future studies.

The energy ADC values of SPC, PPC and WGM for olive flounder were slightly lower than those reported for other fish species (Peach, 2005; Tibbetts et al., 2006). The energy digestibility of a particular feed ingredient is dependent on the chemical composition (Yu et al., 2013). Plant feed ingredients had low energy digestibility due to high carbohydrate (Irvin & Williams, 2007) and fiber levels (Lee, 2002). Therefore, we also assumed that the energy ADCs was significantly correlated with fiber and carbohydrate content of ingredients in the present study. Supportively, SBM, RSM and WF which are having higher carbohydrate contents resulted in lower energy digestibility compared to the other plant protein ingredients. Differences in energy digestibility may be due to variations in the fiber and carbohydrate contents of the ingredients. Moreover, the high level of energy digestibility observed in SPC and WGM in the current study might be attributed to a reduction in ANFs because their functional and nutritional properties were improved through processing technologies (Tibbetts et al., 2006).

The availabilities of each amino acid in each ingredient were different in the present study. Methionine and cystine availability in SBM, SPI, RSM and WF were lowest for olive flounder. This indicates that the imbalance of amino acids may reduce their availability. The other factors responsible for low amino acid availability might be chemical composition and processing methods (Chu et al., 2015). Supportively, Borghesi et al. (2009) reported that both chemical composition and processing were the main reasons affecting amino acid digestibility in plant protein ingredients because special techniques such as thermal processing are useful to deactivate ANFs. The amino acid availability coefficient for WGM in this study was the highest among all the ingredients tested, indicating that WGM is good quality, highly digestible plant protein source for olive flounder. The amino acid availability of WF showed the lowest value among the ingredients indicating that high carbohydrate content decreases the amino acid digestibility. Mu et al. (2000) also observed large variations in the availability of essential amino acids in a protein source indicating the necessity of amino acid availability information.

In conclusion, the use of specific amino acid digestibility coefficients may allow a more accurate and economical formulation of the feed for olive flounder due to variation within individual amino acid availabilities among ingredients. However, the effects of these ingredients on growth performance of olive flounder should be considered to formulate efficient feed. Among plant protein ingredients tested, SPC and WGM were better digested than other plant protein ingredients and thus these ingredients can be used efficiently as alternative protein sources for olive flounder.

## 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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