



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

Md Mostafizur Rahman¹, Buddhi E. Gunathilaka², Sang-Guan You³, Kang-Woong Kim⁴, Sang-Min Lee^{2,*}

¹ Aquacultural Engineering R&D Center, Dalian Ocean University, Dalian 116023, China

² Department of Aquatic Life Medicine, Gangneung-Wonju National University, Gangneung 25457, Korea

³ Department of Marine Food Science and Technology, Gangneung-Wonju National University, Gangneung 25457, Korea

⁴ Aquafeed Research Center, National Institute of Fisheries Science, Pohang 37517, Korea

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 (Cr₂O₃) 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

Received: Jun 3, 2022 Revised: Oct 4, 2022 Accepted: Nov 22, 2022

*Corresponding author: Sang-Min Lee

Department of Aquatic Life Medicine, Gangneung-Wonju National University, Gangneung 25457, Korea

Tel: +82-33-640-2414, Fax: +82-33-640-2955, E-mail: smlee@gwnu.ac.kr

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright © 2023 The Korean Society of Fisheries and Aquatic Science

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

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 ₂ O 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; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinylacetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.

⁴⁾Mineral premix contained the following ingredients (g /kg mix): MgSO₄·7H₂O, 80.0; NaH₂PO₄·2H₂O, 370.0; KCl, 130.0; Ferric citrate, 40.0; ZnSO₄·7H₂O, 20.0; Ca-lactate, 356.5; CuCl₂·2H₂O, 0.15; Na₂SeO₃·0.01; MnSO₄·H₂O, 2.0; CoCl₂·6H₂O, 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 °C.

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 °C 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 °C and stored at –75 °C 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). Cr₂O₃ 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 °C 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 °C 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:

$$\text{ADC of dry matter (\%)} = \frac{100 - (\text{dietary Cr}_2\text{O}_3 / \text{feces Cr}_2\text{O}_3) \times 100}{100}$$

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 (%) =

$$100 \times \left(1 - \frac{\text{dietary Cr}_2\text{O}_3}{\text{feces Cr}_2\text{O}_3} \times \frac{\text{feces nutrient or energy}}{\text{dietary nutrient or energy}} \right)$$

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

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

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.

Funding sources

This study was supported by a grant from the National Institute of Fisheries Science, Republic of Korea (No. R2023036) and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. 2018R1A6A1A03023584).

Acknowledgements

Not applicable.

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.

ORCID

Buddhi E. Gunathilaka <https://orcid.org/0000-0002-0654-9980>
 Sang-Guan You <https://orcid.org/0000-0002-8785-7167>
 Kang-Woong Kim <https://orcid.org/0000-0002-9891-4884>
 Sang-Min Lee <https://orcid.org/0000-0001-5746-391X>

References

- Association of Official Analytical Chemists [AOAC] International. Official methods of analysis of AOAC International. 18th ed. Gaithersburg, MD: AOAC International; 2005.
- Bae KM, Kim KW, Lee SM. Evaluation of rice distillers dried grain as a partial replacement for fish meal in the practical diet of the juvenile olive flounder *Paralichthys olivaceus*. *Fish Aquat Sci*. 2015;18:151-8.
- Bai SC, Lee S. Olive flounder (*Paralichthys olivaceus*) (Korean perspective). In: Daniels HV, Watanabe WO, editors. Practical flatfish culture and stock enhancement. Hoboken, NJ: John Wiley & Sons; 2010. p. 156-68.
- Balmir F, Staack R, Jeffrey E, Berber Jimenez MD, Wang L, Potter SM. An extract of soy flour influences serum cholesterol and thyroid hormones in rats and hamsters. *J Nutr*. 1996;126:3046-53.
- Borghesi R, Dairiki JK, Cyrino JEP. Apparent digestibility coefficients of selected feed ingredients for dourado *Salminus brasiliensis*. *Aquac Nutr*. 2009;15:453-8.
- Cerri R, Niccolai A, Cardinaletti G, Tulli F, Mina F, Daniso E, et al. Chemical composition and apparent digestibility of a panel of dried microalgae and cyanobacteria biomasses in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 2021;544:737075.
- Cheng ZJ, Hardy RW. Effect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middlings, measured *in vivo* using rainbow trout (*Oncorhynchus mykiss*). *Aquac Nutr*. 2002;8:271-7.
- Cho CY, Slinger SJ. Apparent digestibility measurement in feedstuffs for rainbow trout. In: Halver J, Tiews K, editors. Finfish nutrition and fish feed technology: proceedings of a world symposium, vol. II. Berlin: Heenemann; 1979. p. 239-47.
- Cho SH, Heo TY. Effect of dietary nutrient composition on compensatory growth of juvenile olive flounder *Paralichthys olivaceus* using different feeding regimes. *Aquac Nutr*. 2011;17:90-7.
- Cho SH, Lee SM, Park BH, Lee SM. Effect of feeding ratio on growth and body composition of juvenile olive flounder *Paralichthys olivaceus* fed extruded pellets during the summer season. *Aquaculture*. 2006;251:78-84.
- Chu ZJ, Yu DH, Yuan YC, Qiao Y, Cai WJ, Shu H, et al. Apparent digestibility coefficients of selected protein feed ingredients for loach *Misgurnus anguillicaudatus*. *Aquac Nutr*. 2015;21:425-32.
- Dias J, Alvarez MJ, Arzel J, Corraze G, Diez A, Bautista JM, et al. Dietary protein source affects lipid metabolism in the European seabass (*Dicentrarchus labrax*). *Comp Biochem Physiol A Mol Integr Physiol*. 2005;142:19-31.
- Duncan DB. Multiple range and multiple F tests. *Biometrics*. 1955;11:1-42.
- El-Saidy DMSD, Gaber MMA. Complete replacement of fish meal by soybean meal with dietary L-lysine supplementation for Nile tilapia *Oreochromis niloticus* (L.) fingerlings. *J World Aquac Soc*. 2002;33:297-306.
- Francis G, Makkar HPS, Becker K. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*. 2001;199:197-227.
- Furukawa A, Tsukahara H. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. *Bull Jpn Soc Sci Fish*. 1966;32:502-8.
- Gatlin DM, Barrows FT, Brown P, Dabrowski K, Gibson Gaylord T, Hardy RW, et al. Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquac Res*. 2007;38:551-79.
- Gaylord TG, Barrows FT, Rawles SD. Apparent digestibility of gross nutrients from feedstuffs in extruded feeds for rainbow trout, *Oncorhynchus mykiss*. *J World Aquac Soc*. 2008;39:827-34.
- Gunathilaka BE, Khosravi S, Herault M, Fournier V, Lee C, Jeong JB, et al. Evaluation of shrimp or tilapia protein hydrolysate at graded dosages in low fish meal diet for olive flounder (*Paralichthys olivaceus*). *Aquac Nutr*.

- 2020;26:1592-603.
- Hamidoghli A, Won S, Lee S, Lee S, Farris NW, Bai SC. Nutrition and feeding of olive flounder *Paralichthys olivaceus*: a review. *Rev Fish Sci Aquacult.* 2020;28:340-57.
- Irvin SJ, Williams KC. Apparent digestibility of selected marine and terrestrial feed ingredients for tropical spiny lobster *Panulirus ornatus*. *Aquaculture.* 2007;269:456-63.
- Jung JY, Kim S, Kim K, Lee BJ, Kim KW, Han HS. Feed and disease at olive flounder (*Paralichthys olivaceus*) farms in Korea. *Fishes.* 2020;5:21.
- Khosravi S, Bui HTD, Herault M, Fournier V, Kim KD, Lee BJ, et al. Supplementation of protein hydrolysates to a low-fishmeal diet improves growth and health status of juvenile olive flounder, *Paralichthys olivaceus*. *J World Aquac Soc.* 2018;49:897-911.
- Kim KD, Kim DG, Kim SK, Kim KW, Son MH, Lee SM. Apparent digestibility coefficients of various feed ingredients for olive flounder, *Paralichthys olivaceus*. *Korean J Fish Aquat Sci.* 2010;43:325-30.
- Kim KW, Kang YJ, Choi SM, Wang X, Choi YH, Bai SC, et al. Optimum dietary protein levels and protein to energy ratios in olive flounder *Paralichthys olivaceus*. *J World Aquac Soc.* 2005;36:165-78.
- Kim KW, Wang XJ, Bai SC. Optimum dietary protein level for maximum growth of juvenile olive flounder *Paralichthys olivaceus* (Temminck et Schlegel). *Aquac Res.* 2002;33:673-9.
- Kim MG, Shin J, Lee C, Lee BJ, Hur SW, Lim SG, et al. Evaluation of a mixture of plant protein source as a partial fish meal replacement in diets for juvenile olive flounder *Paralichthys olivaceus*. *Korean J Fish Aquat Sci.* 2019;52:374-81.
- Kim SS, Lee KJ. Comparison of leucine requirement in olive flounder (*Paralichthys olivaceus*) by free or synthetic dipeptide forms of leucine. *Anim Feed Sci Technol.* 2013;183:195-201.
- Köprücü K, Özdemir Y. Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). *Aquaculture.* 2005;250:308-16.
- Lee SM. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*). *Aquaculture.* 2002;207:79-95.
- Lee SM, Kim KD. Effect of various levels of lipid exchanged with dextrin at different protein level in diet on growth and body composition of juvenile flounder *Paralichthys olivaceus*. *Aquac Nutr.* 2005;11:435-42.
- Lee SM, Park CS, Bang IC. Dietary protein requirement of young Japanese flounder *Paralichthys olivaceus*. *Fish Sci.* 2002;68:158-64.
- Li MH, Oberle DF, Lucas PM. Apparent digestibility of alternative plant-protein feedstuffs for channel catfish, *Ictalurus punctatus* (Rafinesque). *Aquac Res.* 2013;44:282-8.
- Lim H, Kim MG, Shin J, Shin J, Hur SW, Lee BJ, et al. Evaluation of three plant proteins for fish meal replacement in diet for growing olive flounder *Paralichthys olivaceus*. *Korean J Fish Aquat Sci.* 2020;53:464-70.
- Lim SJ, Lee KJ. Supplemental iron and phosphorus increase dietary inclusion of cottonseed and soybean meal in olive flounder (*Paralichthys olivaceus*). *Aquac Nutr.* 2008;14:423-30.
- Liu H, Wu X, Zhao W, Xue M, Guo L, Zheng Y, et al. Nutrients apparent digestibility coefficients of selected protein sources for juvenile Siberian sturgeon (*Acipenser baerii* Brandt), compared by two chromic oxide analyses methods. *Aquac Nutr.* 2009;15:650-6.
- Luo Z, Li X, Gong S, Xi WQ. Apparent digestibility coefficients of four feed ingredients for *Synechogobius hasta*. *Aquac Res.* 2009;40:558-65.
- Luo Z, Tan X, Chen Y, Wang W, Zhou G. Apparent digestibility coefficients of selected feed ingredients for Chinese mitten crab *Eriocheir sinensis*. *Aquaculture.* 2008;285:141-5.
- Lusas EW, Riaz MN. Soy protein products: processing and use. *J Nutr.* 1995;125:573S-80S.
- Ma R, Liu X, Meng Y, Wu J, Zhang L, Han B, et al. Protein nutrition on sub-adult triploid rainbow trout (1): dietary requirement and effect on anti-oxidative capacity, protein digestion and absorption. *Aquaculture.* 2019;507:428-34.
- Medagoda N, Kim MG, Gunathilaka BE, Park SH, Lee KJ. Effect of total replacement of fish oil with tallow and emulsifier in diet on growth, feed utilization, and immunity of olive flounder (*Paralichthys olivaceus*). *J World Aquac Soc.* 2022;53:558-71.
- Mo AJ, Sun JX, Wang YH, Yang K, Yang HS, Yuan YC. Apparent digestibility of protein, energy and amino acids in nine protein sources at two content levels for mandarin fish, *Siniperca chuatsi*. *Aquaculture.* 2019;499:42-50.
- Mu YY, Lam TJ, Guo JY, Shim KF. Protein digestibility and amino acid availability of several protein sources for juvenile Chinese hairy crab *Eriocheir sinensis* H. Milne-Edwards (Decapoda, Grapsidae). *Aquac Res.* 2000;31:757-65.
- National Research Council [NRC]. Nutrient requirements of fish and shrimp. Washington, DC: National Research

- Council of the National Academies; 2011.
- Peach RW. Protein and energy utilization by juvenile Atlantic halibut (*Hippoglossus hippoglossus*) [M.Sc. Thesis]. Truro, NS: Dalhousie University; 2005.
- Pham MA, Lee KJ, Dang TM, Lim SJ, Ko GY, Eo J, et al. Improved apparent digestibility coefficient of protein and phosphorus by supplementation of microbial phytase in diets containing cottonseed and soybean meal for juvenile olive flounder (*Paralichthys olivaceus*). *Asian-Australas J Anim Sci*. 2008;21:1367-75.
- Rahman MM, Choi J, Lee SM. Influences of dietary distillers dried grain level on growth performance, body composition and biochemical parameters of juvenile olive flounder (*Paralichthys olivaceus*). *Aquac Res*. 2015;46:39-48.
- Rahman MM, Han HS, Kim KW, Kim KD, Lee BJ, Lee SM. Apparent digestibility coefficients of the extruded pellet diets containing various fish meals for olive flounder, *Paralichthys olivaceus*. *Fish Aquat Sci*. 2016a;19:27.
- Rahman MM, Lee KJ, Lee SM. Effect of dietary carbohydrate sources on apparent nutrient digestibility of olive flounder (*Paralichthys olivaceus*) feed. *Fish Aquat Sci*. 2016b;19:15.
- Romarheim OH, Skrede A, Gao Y, Krogdahl Å, Denstadli V, Lilleeng E, et al. Comparison of white flakes and toasted soybean meal partly replacing fish meal as protein source in extruded feed for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 2006;256:354-64.
- Seong M, Lee S, Lee S, Song Y, Bae J, Chang K, et al. The effects of different levels of dietary fermented plant-based protein concentrate on growth, hematology and non-specific immune responses in juvenile olive flounder, *Paralichthys olivaceus*. *Aquaculture*. 2018;483:196-202.
- Sklan D, Prag T, Lupatsch I. Apparent digestibility coefficients of feed ingredients and their prediction in diets for tilapia *Oreochromis niloticus* × *Oreochromis aureus* (Teleostei, Cichlidae). *Aquac Res*. 2004;35:358-64.
- Tharaka K, Gunathilaka BE, Veille A, Kim MG, Shin J, Lim H, et al. Algae-clay powder (sea lettuce, *Ulva lactuca* and red algae, *Solieria chordalis* in exfoliated micronized montmorillonite) supplementation in a fish meal-reduced diet for olive flounder (*Paralichthys olivaceus*). *Aquac Rep*. 2020;18:100498.
- Tibbetts SM, Milley JE, Lall SP. Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758). *Aquaculture*. 2006;261:1314-27.
- Tibbetts SM, Scaife MA, Armenta RE. Apparent digestibility of proximate nutrients, energy and fatty acids in nutritionally-balanced diets with partial or complete replacement of dietary fish oil with microbial oil from a novel *Schizochytrium* sp. (T18) by juvenile Atlantic salmon (*Salmo salar* L.). *Aquaculture*. 2020;520:735003.
- Tomás-Vidal A, Monge-Ortiz R, Jover-Cerdá M, Martínez-Llorens S. Apparent digestibility and protein quality evaluation of selected feed ingredients in *Seriola dumerili*. *J World Aquac Soc*. 2019;50:842-55.
- Weththasinghe P, Hansen JØ, Nøklund D, Lagos L, Rawski M, Øverland M. Full-fat black soldier fly larvae (*Hermetia illucens*) meal and paste in extruded diets for Atlantic salmon (*Salmo salar*): effect on physical pellet quality, nutrient digestibility, nutrient utilization and growth performances. *Aquaculture*. 2021;530:735785.
- Won S, Lee S, Hamidoghli A, Lee S, Bai SC. Dietary choline requirement of juvenile olive flounder (*Paralichthys olivaceus*). *Aquac Nutr*. 2019;25:1281-8.
- Ye J, Liu X, Wang Z, Wang K. Effect of partial fish meal replacement by soybean meal on the growth performance and biochemical indices of juvenile Japanese flounder *Paralichthys olivaceus*. *Aquac Int*. 2011;19:143-53.
- Yu HR, Zhang Q, Cao H, Wang XZ, Huang GQ, Zhang BR, et al. Apparent digestibility coefficients of selected feed ingredients for juvenile snakehead, *Ophiocephalus argus*. *Aquac Nutr*. 2013;19:139-47.
- Yusuf HA, Piao M, Ma T, Huo R, Tu Y. Enhancing the quality of total mixed ration containing cottonseed or rapeseed meal by optimization of fermentation conditions. *Fermentation*. 2021;7:234.
- Zhang X, Wang H, Zhang J, Lin B, Chen L, Wang Q, et al. Assessment of rapeseed meal as fish meal alternative in diets for juvenile Asian red-tailed catfish (*Hemibagrus wyckioides*). *Aquac Rep*. 2020;18:100497.
- Zhou QC, Tan BP, Mai KS, Liu YJ. Apparent digestibility of selected feed ingredients for juvenile cobia *Rachycentron canadum*. *Aquaculture*. 2004;241:441-51.
- Zhou QC, Yue YR. Apparent digestibility coefficients of selected feed ingredients for juvenile hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus*. *Aquac Res*. 2012;43:806-14.