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EDITED BY
Pallab Sarker,
University of California, United States

REVIEWED BY
Md. Tawheed Hasan,
Sylhet Agricultural University, Bangladesh
Won Je Jang,
Pukyong National University, Republic of Korea

*CORRESPONDENCE
Sung Hwoan Cho
✉ chosunh@kmou.ac.kr

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Inclusion effect of jack mackerel meal in diets substituting fish meal with corn gluten meal on growth and feed utilization of olive flounder (*Paralichthys olivaceus*)

Md. Rabiul Islam[1,2], Sung Hwoan Cho[3]* and Taeho Kim[4]

[1]Division of Convergence Interdisciplinary Education of Maritime and Ocean Contents, Korea Maritime & Ocean University, Busan, Republic of Korea, [2]Department of Aquaculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh, [3]Division of Convergence on Marine Science, Korea Maritime & Ocean University, Busan, Republic of Korea, [4]Department of Marine Production Management, Chonnam National University, Yeosu, Republic of Korea

Inclusion effect of different amount of jack mackerel meal (JMM) in the diets substituting 50% fish meal (FM) with corn gluten meal (CGM) on growth, feed availability, biochemical composition, plasma, and serum chemistry of olive flounder (*Paralichthys olivaceus*) was elucidated. Seven experimental diets were formulated. The control (Con) diet contained 60% FM. Fifty percent of FM in the Con diet was substituted with CGM, and then 5, 10, 20, 30, 40, and 50% JMM were added at the expense of FM to formulate the CJ5, CJ10, CJ20, CJ30, CJ40, and CJ50 diets, respectively. All formulated feeds were assigned to triplicate groups of fish. A total of 420 juvenile fish averaging 18 ± 0.01 g (mean $\pm$ SEM) was uniformly divided into 21, 50-L tanks, with 20 fish per tank. Fish were carefully hand-fed to satiation for 8 weeks. The weight gain of olive flounder fed the Con diet was significantly ($p < 0.001$) higher than that of fish fed the CJ5, CJ10, and CJ20 diets, but not significantly ($p > 0.05$) different from that of fish fed the CJ30, CJ40, and CJ50 diets. Olive flounder fed the Con diet achieved significantly ($p < 0.001$ and $p < 0.04$) greater specific growth rates (SGR) and feed consumption than those of fish fed the CJ5 and CJ10 diets, but not significantly ($p > 0.05$) different from those of fish fed the CJ20, CJ30, CJ40, and CJ50 diets. However, there were no significant ($p > 0.05$) differences in feed utilization, proximate composition, amino acid profiles, and plasma and serum parameters of olive flounder. In conclusion, incorporation of 30, and 20% JMM are the most desirable treatments in the olive flounder diets replacing 50% FM with CGM based on weight gain, and SGR and feed consumption, respectively. This study will be very helpful to develop low-FM diets for sustainable olive flounder culture.

KEYWORDS

jack mackerel meal, fish meal substitution, olive flounder, corn gluten meal, feed stimulants, feed utilization

1 Introduction

Olive flounder (*P. olivaceus*) is a carnivorous demersal flatfish species (Hamidoghli et al., 2020). The Eastern Asian nations including Korea, China, and Japan are the major commercial producers of olive flounder because of its short grow-out time, good flavor, low susceptibility to disease, and high market value and demand (Kim et al., 2019; Stieglitz et al., 2021). Olive flounder has become the top species produced from aquaculture in Korea, with a production of 45,801 metric tons (MT) (50.6% of total marine aquaculture fin fish production) of fish in 2022 (KOSIS, 2023). Being a carnivorous fish species, in particular, olive flounder requires a high-protein (up to 60%) diet where FM is utilized as the primary protein source (Hamidoghli et al., 2020). However, the production of FM has reduced by 26.5%, and the price of FM has increased by 3.5 times in the last two decades due to increasing production costs and decreasing global marine production (Jannathulla et al., 2019). The increasing cost and unstable supply of FM will lower the inclusion level of FM in aquaculture diets in the long term (Tacon and Metian, 2008). Therefore, searching for a replacer for FM that is both inexpensive and supply-stable is a high priority for sustainable aquaculture production of fish.

Plant protein sources as a replacer for FM have been emphasized by fish nutritionists. Some plant protein sources including microalgae (Rahimnejad et al., 2017), macroalgae (Zeynali et al., 2020), rice distillers dried grain (Bae et al., 2015), and soybean meal and cotton seed meal (Pham et al., 2007; Lim and Lee, 2008) have been examined as the replacers to FM in the olive flounder diets because of their sufficient protein content and low market price. However, higher fiber content, amino acid (AA) imbalance, and the presence of anti-nutritional factors (ANF) limit the extensive use of plant proteins, which may lower the palatability and growth of fish (Jannathulla et al., 2019; Li et al., 2022). Corn gluten meal (CGM) have a high potential to be used as a replacer to FM in fish feeds due to its low fiber level, high protein content (60–70% of dry matter), and almost devoid of ANF (Peter et al., 2000; Oliva-Teles et al., 2015). CGM could replace FM up to 40.4% in turbot (*Psetta maxima*) (Regost et al., 1999) and 60% in both spotted rose snapper (*Lutjanus guttatus*) (Hernández et al., 2021) and gilthead sea bream (*Sparus aurata*) (Pereira and Oliva-Teles, 2003) feeds, respectively. In particular, Kikuchi (1999a) showed that CGM could replace up to 40% FM in olive flounder diets without having negative impacts on growth and feed utilization when juvenile olive flounder were fed with a 75% FM-based feed or one of the feeds replacing 20, 40, and 60% FM with CGM supplemented with limiting amino acid (AA) (arginine, lysine, and tryptophan) for 8 weeks. In his study, however, a feed substituting 40% FM with CGM without supplementation of limiting AA produced inferior growth and feed utilization compared to fish fed a 75% FM-basal feed or a feed substituting 40% FM with CGM supplemented with limiting AA.

Furthermore, CGM has been tested in combination with other alternatives, such as animal by-products (Ye et al., 2011), soybean meal (Sevgili et al., 2015), soy protein isolates (Gamboa-Delgado et al., 2013), and pea protein isolates (Wang et al., 2020) for FM in different aquaculture species. Nevertheless, the imbalanced AA profiles (deficiency in lysine and arginine) and the presence of non-soluble carbohydrates limit the application of CGM in fish diets because of its poor feed consumption (palatability) and feed utilization (Peter et al., 2000; Hernández et al., 2021). Therefore, inclusion of protein rich feed ingredient having feed attractants and stimulants effect into low-FM diets is one of the best methods to resolve those undesirable problems.

Feed attractants and/or stimulants are compounds that stimulate the feeding response and feed consumption of fish (Kubitza and Lovshin, 1999). A formulated diet must contain modest quantities of chemostimulants to be chemically appealing. Along with the proper nutritional balance, palatability is also considered an essential factor in the development of artificial diets (Takakuwa et al., 2019). The addition of attractants and/or stimulants to the diet allows fish to quickly access the feed, improve the palatability, and acceptability of the diet, in other words, minimize the leaching-out ratio of nutrients and deterioration of water quality (Kim et al., 2019; Hancz, 2020; Jeong et al., 2022). The effectiveness of the synthetic chemicals stimulants in practical feeding is still controversial (Morais, 2017). However, natural feed stimulants were found to increase feed consumption with a significant improvement in growth of fish (Kader et al., 2010; Nagel et al., 2014; Khosravi et al., 2018).

Jack mackerel (*Trachurus japonicus*) meal (JMM) was found to be an effective protein source having feed attractants and stimulants effect to olive flounder (Jeong et al., 2020, 2022; Jeong and Cho, 2023), rockfish (*Sebastes schlegeli*) (Kim and Cho, 2019; Baek et al., 2021), and yellowtail (*Seriola quinqueradiata*) (Hosokawa et al., 2001). Especially, Kim et al. (2019) and Jeong et al. (2020) emphasized that both rockfish and olive flounder exhibited the maximum attraction behavior (movement) to JMM among 15–16 various feed ingredients. Furthermore, Ikeda et al. (2012) proved that AA in the muscle extracts of jack mackerel, especially histidine showed outstanding feeding stimulant activity for olive flounder. The muscle extracts of jack mackerel are rich in AA and nucleotides, particularly inosine monophosphate (IMP) exhibiting a strong feed stimulatory effect on greater amberjack (*Seriola dumerili*) (Takakuwa et al., 2019).

Therefore, we evaluated the inclusion effect of various levels of JMM in low-FM diets substituting 50% FM by CGM on growth, feed availability, biochemical composition, and plasma and serum parameters of olive flounder in the current study.

2 Materials and methods

2.1 Experimental diet preparation

Seven experimental diets were formulated (Table 1). The main protein sources in the control (Con) diet were 60% FM and 15% fermented soybean meal. In the Con diet, 18.3% wheat flour, and 2.1% each of fish oil and soybean oil were included as the carbohydrate and lipid sources, respectively. Fifty percent of FM in the Con diet were replaced by CGM, and then various levels (5, 10, 20, 30, 40, and 50%) of JMM at the expense of FM were included to formulate CJ5, CJ10, CJ20, CJ30, CJ40, and CJ50 diets, respectively. However, a diet replacing 50% FM with CGM without JMM inclusion was not included in this experiment because the substitutability of FM with CGM was estimated to be 40% in the olive flounder feeds without AA supplementation (Kikuchi, 1999a). After properly combining the ingredients, a 3:1 ratio of water was added to the mixture to create a dough. Depending on the size of the fish's mouth, the dough was then pelletized by running it through a laboratory-style pellet extruder (4–6 mm in diameter) (Dongsung Mechanics, Korea). Finally, after being dried at 25°C in a forced air oven (SI-2400, Sin II Drying Machine Co. Ltd., Korea)

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TABLE 1 Ingredients and chemical composition of the experimental diets (%, dry matter basis).

	Experimental diets						
	Con	CJ5	CJ10	CJ20	CJ30	CJ40	CJ50
Ingredients (%, DM)							
Fish meal[1]	60.0	27.0	24.0	18.0	12.0	6.0	0.0
Corn gluten meal (CGM)[2]	0.0	33.1	33.1	33.1	33.1	33.1	33.1
Jack mackerel meal (JMM)[3]	0.0	3.0	6.0	12.0	18.0	24.0	30.0
Fermented soybean meal	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Wheat flour	18.3	12.5	12.5	12.5	12.5	12.5	12.5
Fish oil	2.1	4.8	4.8	4.8	4.8	4.8	4.8
Soybean oil	2.1	2.1	2.1	2.1	2.1	2.1	2.1
Vitamin premix[4]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[5]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Choline	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Nutrients (%, DM)							
Dry matter	96.4	96.6	96.6	96.5	96.6	96.5	96.7
Crude protein	55.4	55.1	55.3	55.7	55.4	56.0	55.9
Crude lipid	10.0	10.4	10.2	10.0	10.2	10.3	10.2
Ash	10.7	6.9	6.5	6.3	6.2	6.3	6.4

[1]Fish meal (crude protein: 73.3%, crude lipid: 8.6%, ash: 15.0%) composed of sardine meal and anchovy meal at the ratio of 1:1 was imported from Chile [USD 2.26/kg FM, USD 1 = 1,232 KRW (Korean currency)]. [2]Corn gluten meal (CGM) (crude protein: 69.4%, crude lipid: 1.0%, ash: 2.5%) was purchased from Hyunjin Farm, Busan, Korea (USD 0.92/kg CGM). [3]Jack mackerel meal (JMM) (crude protein: 73.8%, crude lipid: 9.1%, ash: 13.3%) was imported from Chile (USD 2.67/kg JMM). [4]Vitamin premix contained the following amounts, which were diluted in cellulose (g/kg mix): L-ascorbic acid, 121.2; 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; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003. [5]Mineral Premix contained the following ingredients (g/kg mix): $MgSO_4{\cdot}7H_2O$, 80.0; $NaH_2PO_4{\cdot}2H_2O$, 370.0; KCl, 130.0; ferric citrate, 40.0; $ZnSO_4{\cdot}7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3{\cdot}6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4{\cdot}H_2O$, 2.0; $CoCl_2{\cdot}6H_2O$, 1.0.

for 48 h, all sinking type pellets were kept at −20°C until further use.

2.2 Experimental condition and feeding trial

Healthy juvenile olive flounder were purchased from a commercial fish farm (Taean-gun, Chungcheongnam-do, Korea) and transported to the laboratory. Prior to initiation of the feeding experiment, fish were acclimatized to the rearing environments for 2 weeks by feeding with a commercial extruded pellet (55% crude protein and 8% crude lipid) twice a day at a ratio of 2–3% biomass of fish. A number of 420 juvenile fish averaging 18.0 ± 0.01 g (mean ± SEM) was uniformly assigned into twenty-one 50-L rectangular flow-through tanks (20 fish/tank). Each tank received sand-filtered seawater with a flow rate of 4.2 L/min. A multifunctional water quality meter (AZ-8603, AZ Instrument, Taichung, Taiwan) was used daily to monitor the water quality. Temperature, salinity, dissolved oxygen, and pH ranged 17.7–24.0°C (21.3°C ± 2.02°C; mean ± SD), 31.6–33.6 g/L (32.6 ± 0.47 g/L), 7.0–7.9 mg/L (7.3 ± 0.13 mg/L), and 7.1–7.5 (7.3 ± 0.12), respectively.

All experimental diets were assigned to triplicate groups of fish. Throughout the 8-week feeding experiment, fish were carefully hand-fed to apparent satiation twice a day (08:00 and 17:00). However, unfed feeds were not collected. Daily siphoning was used to clean the fish tanks' bottom, and the photoperiod followed the natural cycle (14 L: 10 D, h of light: dark cycle).

2.3 Determination of the biological indices of fish

At the termination of the 8-week experiment, all live fish were anesthetized with MS-222 at a concentration of 100 ppm after the 24-h starvation. The number of live fish from each tank was counted for calculating the survival and collectively weighed to evaluate weight gain. Ten anesthetized fish randomly taken from each tank were dissected to collect liver and viscera. Growth, feed utilization, and biological indices of fish were calculated by using the following formula: specific growth rate (SGR, %/day) = (Ln final weight of fish – Ln initial weight of fish) × 100/days of feeding (56 days), feed efficiency (FE) = weight gain of fish/feed consumption, protein efficiency ratio (PER) = weight gain of fish/protein consumption, protein retention (PR, %) = protein gain of fish × 100/protein consumption, condition factor (K, g/cm^3) = body weight of fish (g) × 100/total length of fish (cm)^3, viscerosomatic index (VSI, %) = viscera weight of fish × 100/body weight of fish, and hepatosomatic index (HSI, %) = liver weight of fish × 100/body weight of fish.

2.4 Analysis of the biochemical composition of the experimental diets and fish

Ten fish after measuring biological indices of fish and 4 remaining fish from each tank were homogenized and utilized for proximate

composition analysis after the 8-week feeding study. According to Association of Official Analytical Chemists (1990) standard method, the proximate composition for the experimental diets and fish were determined. Crude protein content was determined by Kjeldahl apparatus (Kjeltec 2,100 Distillation Unit, Foss Tecator, Hoganas, Sweden), and crude lipid content was determined by ether-extraction method (Soxtec TM 2043 Fat Extraction System, Foss Tecator, Hoganas, Sweden). Moisture content was determined by oven drying at 105°C for 24 h, and ash content was determined by using a muffle furnace at 550°C for 4 h. In order to analyze AA (apart from methionine and cysteine, and tryptophan), the experimental diets and the whole-body of olive flounder were hydrolyzed with 6 N HCl for 24 h at 110°C, followed by ion exchange chromatography with an AA analyzer (L-8800 Auto-analyzer: Hitachi, Tokyo, Japan). To measure methionine and cysteine content, the samples were oxidized with performic acid at below 5°C for 24 h to obtain methionine sulfone and cysteic acid, and they were then freeze-dried twice with deionized water. Then the freeze-dried samples were hydro-lysed and analyzed following similar process used for the other amino acids. Tryptophan analysis was conducted using high-performance liquid chromatography (S1125 HPLC pump system; Sykam GmbH, Eresing, Germany). Lipids for FA analyses in the experimental diets and the whole-body fish were extracted by a mixture of chloroform and methanol (2:1 v/v) according to the method of Folch et al. (1957). FA methyl esters were prepared by transesterification with 14% BF_3-MeOH (Sigma, St. Louis, MO, United States) and analyzed by using gas chromatography (Trace GC, Thermo, Waltham, MA, United States).

2.5 Plasma parameters of fish

Three anesthetized fish from each tank had blood drawn from their caudal veins using heparinized syringes. Plasma was extracted and kept for subsequent analysis in separate aliquots in a freezer at −70°C after centrifugation (2,716 × g at 4°C) for 10 min. An automated chemistry system (Fuji Dri-Chem NX500i, Fujifilm, Tokyo, Japan) was used to analyze the following enzymes: aminotransferase (AST; 15,809,542, measurement range: 10–1,000 U/L), alanine aminotransferase (ALT; 16,654,035, measurement range: 10–1,000 U/L), alkaline phosphatase (ALP; 16,653,964, measurement range: 14–1,183 U/L), total bilirubin (T-BIL; 16,654,061, measurement range: 0.2–30.0 mg/dL), total cholesterol (T-CHO; 16,654,073, measurement range: 50–450 mg/dL), triglyceride (TG; 16,654,085, measurement range: 10–500 mg/dL), total protein (TP; 16,654,097, measurement range: 2.0–11.0 g/dL), and albumin (ALB; 16,653,952, measurement range: 1.0–6.0 g/dL).

2.6 Serum lysozyme activity and superoxide dismutase (SOD) of olive flounder

Three anesthetized fish from each tank had blood drawn from their caudal veins using syringes. Serum was extracted and kept for subsequent analysis in separate aliquots in a freezer at −70°C after centrifugation (2,716 × g at 4°C) for 10 min. Lysozyme activity was determined by the turbidimetric assay according to Lange et al. (2001). In brief, 100 µL test serum was added to a suspension (1.9 mL) of Micrococcus lysodeikticus (0.2 mg/mL; Sigma, St. Louis, MO, United States) in a 0.05 M sodium phosphate buffer, pH 6.2. The reaction was carried out at 25°C, and absorbance was measured at 530 nm on a spectrophotometer after 0 and 60 min. The quantity of enzyme needed to cause a 0.001/min reduction in absorbance was referred to as a lysozyme activity unit. SOD was determined by using a 19,160 SOD Determination Kit (Sigma-Aldrich Inc., St. Louis, MO, United States) following the manufacturer's direction. The same methods and procedures for measurements of lysozyme activity and SOD of fish were adopted in Jeong and Cho (2023)'s study.

2.7 Statistical analysis

Prior to statistical analysis, all data were tested for normality and homogeneity. Significant differences in means were analyzed by one-way ANOVA and Tukey's *post hoc* test with SPSS version 24.0 (SPSS Inc., Chicago, IL, United States). Percentage data were arcsine-transformed prior to statistical analysis. Furthermore, regression analysis was applied between weight gain, SGR, and feed consumption of fish and inclusion levels of JMM in diets substituting 50% FM by CGM.

3 Results

3.1 AA and FA profiles of the experimental feeds

The JMM is relatively high in essential AA (EAA) over FM; however, CGM is relatively rich in leucine and phenylalanine over FM (Table 2). Arginine, leucine, and lysine, as well as aspartic acid and glutamic acid are the abundant EAA and non-essential AA (NEAA), respectively, in all formulated diets. All EAA, except for phenylalanine tended to increase with dietary inclusion levels of JMM. The arginine and lysine content in all the experimental diets fulfilled the requirements of olive flounder, but were comparatively low for the dietary methionine requirement.

The FM is rich in total content of saturated FA (∑SFA), eicosapentaenoic acid (EPA, C20:5n-3), and total content of n-3 highly unsaturated FA (∑n-3 HUFA), while JMM is rich in total content of monounsaturated FA (∑MUFA), and docosahexaenoic acid (DHA, C22:6n-3) content (Table 3). The ∑MUFA and DHA content tended to increase with the inclusion of JMM in diets replacing 50% FM by CGM, but tended to decrease for the ∑SFA, except for the CJ50 diet, EPA, and ∑n-3 HUFA content.

3.2 Growth performance, feed availability, and biological indices of fish

All olive flounder survived at the completion of the experiment (Table 4). Weight gain of olive flounder fed the Con (69.0 ± 0.16 g) diet was significantly ($p < 0.001$) higher than that of the fish fed the CJ5 (64.7 ± 0.14 g), CJ10 (64.9 ± 1.14 g), and CJ20 (66.5 ± 0.54 g) diets, but not significantly ($p > 0.05$) different from that of the fish fed the CJ30 (66.9 ± 0.20 g), CJ40 (67.3 ± 0.16 g), and CJ50 (67.4 ± 0.10 g) diets (Figure 1). The SGR of olive flounder fed the

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TABLE 2 Amino acid (AA) profiles (% of diet) for feed ingredients and experimental diets.

					Experimental diets						
	FM	CGM	JMM	Requirement	Con	CJ5	CJ10	CJ20	CJ30	CJ40	CJ50
Essential amino acids (EAA, %)											
Arginine	4.08	2.15	4.19	2.04–2.10[a]	3.37	2.69	2.70	2.76	2.77	2.73	2.76
Histidine	1.69	1.32	3.03		1.24	1.32	1.30	1.30	1.40	1.51	1.58
Isoleucine	2.69	2.44	2.90		2.07	2.06	2.08	2.12	2.13	2.15	2.20
Leucine	5.01	10.95	5.29		4.97	6.08	6.09	6.12	6.24	6.22	6.23
Lysine	5.56	1.22	5.96	1.55–2.16[b]	3.86	2.67	2.67	2.68	2.70	2.73	2.73
Methionine	1.13	1.75	2.10	1.44–1.49[c]	1.25	1.25	1.26	1.28	1.32	1.33	1.37
Phenylalanine	2.78	4.02	2.86		2.35	2.76	2.72	2.74	2.70	2.70	2.71
Threonine	3.10	2.28	3.28		2.30	2.03	2.05	2.08	2.15	2.17	2.18
Tryptophan	1.14	0.28	1.22		0.49	0.39	0.42	0.42	0.45	0.46	0.46
Valine	3.37	2.92	3.54		2.60	2.20	2.24	2.24	2.24	2.24	2.29
∑EAA	30.55	29.33	34.37		24.50	23.45	23.53	23.74	24.1	24.24	24.51
Non-essential amino acids (NEAA, %)											
Alanine	4.37	4.67	4.57		3.50	4.08	4.06	4.14	4.15	4.17	4.21
Aspartic acid	6.36	3.22	6.69		4.92	4.26	4.28	4.32	4.37	4.38	4.43
Cysteine	1.24	1.30	0.90	0.06[c]	1.02	1.00	0.95	0.93	0.95	0.92	0.85
Glutamic acid	9.05	11.36	9.28		7.83	9.48	9.45	9.47	9.46	9.50	9.53
Glycine	4.26	1.43	4.39		3.14	2.42	2.42	2.42	2.41	2.46	2.53
Proline	3.14	5.14	3.05		2.46	3.46	3.42	3.42	3.41	3.50	3.48
Serine	2.95	2.72	3.05		2.38	2.53	2.50	2.53	2.55	2.54	2.56
Tyrosine	1.84	2.31	2.04		1.50	1.82	1.83	1.86	1.87	1.89	1.87
∑NEAA	33.21	32.15	33.97		26.75	29.05	28.91	29.09	29.17	29.36	29.46

[a]Arginine, [b]Lysine, and [c]Methionine requirements were obtained from Forster and Ogata (1998) and Alam et al. (2000, 2002) studies, respectively. ∑EAA: sum of essential amino acids. ∑NEAA: sum of non-essential amino acids.

Con (2.81 ± 0.004%/day), CJ40 (2.78 ± 0.004%/day), and CJ50 (2.78 ± 0.003%/day) diets were significantly ($p < 0.001$) higher than that of fish fed the CJ5 (2.72 ± 0.003%/day) and CJ10 (2.72 ± 0.024%/day) diets, but not significantly ($p > 0.05$) different from that of olive flounder fed the CJ20 (2.76 ± 0.012%/day) and CJ30 (2.77 ± 0.004%/day) diets (Figure 2). In regression analysis, the linear models were found to be the best fit model between inclusion levels of JMM in the olive flounder diets replacing 50% FM with CGM and weight gain (linear; $p = 0.0001$, adjusted $R^2 = 0.577$), and SGR (linear; $p = 0.0001$, adjusted $R^2 = 0.583$), respectively (Figures 1 and 2, respectively).

Feed consumption of olive flounder fed the Con (65.4 ± 0.44 g) and CJ50 (65.1 ± 0.46 g) diets was significantly ($p < 0.04$) higher than that of fish fed the CJ5 (63.5 ± 0.22 g) and CJ10 (63.8 ± 0.20 g) diets, but not significantly ($p > 0.05$) different from that of olive flounder fed the CJ20 (64.6 ± 0.18 g), CJ30 (64.8 ± 0.11 g), and CJ40 (65.0 ± 0.72 g) diets (Figure 3). In regression analysis, the linear model was found to be the best fit model between inclusion levels of JMM in olive the flounder diet replacing 50% FM with CGM and feed consumption (linear; $p = 0.001$, adjusted $R^2 = 0.482$). The FE, PER, and PR of fish ranged from 1.02–1.06, 1.84–1.90, and 34.3–37.2%, respectively. These values were not significantly ($p > 0.1$, $p > 0.2$, and $p > 0.8$, respectively) affected by dietary treatments.

The K of olive flounder fed the Con diet was significantly ($p > 0.02$) higher than that of the olive flounder fed the CJ5, CJ10, and CJ20 diets, but not significantly ($p > 0.05$) different from that of the olive flounder fed the CJ30, CJ40, and CJ50 diets. However, VSI and HSI of olive flounder ranged 3.58–3.87% and 1.35–1.47%, respectively, and they were not significantly ($p > 0.05$) altered by dietary treatments.

3.3 Whole-body proximate composition of olive flounder

The moisture, crude protein, crude lipid, and ash content of the whole-body fish ranged 67.6–72.3%, 18.6–19.7%, 3.1–3.7%, and 3.5–4.3%, respectively (Table 5). Dietary interventions had no significant ($p > 0.05$) impact on these values.

3.4 Plasma parameters of olive flounder

AST, ALT, ALP, TB, T-CHO, TG, TP, and ALB level of fish ranged 12.9–13.2 U/L, 6.3–6.4 U/L, 110.6–112.8 U/L, 0.7–0.8 mg/dL, 357.7–370.8 mg/dL, 440.3–452.9 mg/dL, 5.3–5.7 g/dL, and 1.0–1.1 g/dL, respectively (Table 6). The experimental diets did not significantly ($p > 0.05$) alter any hematological parameter of olive flounder.

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TABLE 3 Fatty acid (FA) profiles (% of total FA) of the main feed ingredients and experimental diets.

					Experimental diets						
Fatty acid (%)	FM	CGM	JMM	Requirement	Con	CJ5	CJ10	CJ20	CJ30	CJ40	CJ50
12:0	0.42	0.03	0.53		0.11	0.06	0.07	0.06	0.06	0.06	0.06
14:0	5.83	0.07	3.88		3.05	1.19	1.24	1.32	1.41	1.46	1.53
16:0	22.34	12.91	19.88		17.09	14.17	14.15	14.26	14.28	14.22	14.16
18:0	3.72	2.12	7.07		3.71	4.26	4.18	3.95	3.82	3.74	3.76
20:0	0.29	0.49	0.29		0.25	0.32	0.33	0.32	0.32	0.31	0.32
22:0	2.37	0.33	1.45		0.31	0.33	0.32	0.32	0.33	0.32	0.32
∑SFA[a]	34.97	15.95	33.10		24.79	20.33	20.29	20.24	20.22	20.11	20.15
14:1n-5	0.21	0.02	0.26		0.47	0.20	0.19	0.19	0.21	0.23	0.24
15:1n-7	0.11	0.02	0.17		0.26	0.07	0.07	0.07	0.08	0.08	0.09
16:1n-7	6.53	0.23	5.43		3.87	2.33	2.24	2.24	2.20	2.20	2.19
17:1n-7	1.08	0.05	1.07		0.47	0.22	0.22	0.20	0.19	0.17	0.16
18:1n-9	15.47	25.88	21.41		21.30	25.00	25.22	25.70	26.03	26.23	26.40
20:1n-9	0.48	0.01	0.61		1.53	1.21	1.13	1.12	1.05	0.96	0.92
22:1n-9	0.07	0.00	0.15		0.55	0.35	0.31	0.27	0.28	0.25	0.23
∑MUFA[b]	23.84	26.19	28.93		28.19	29.31	29.31	29.72	29.96	30.04	30.14
18:2n-6	4.54	53.66	2.66		25.97	36.41	37.02	36.49	36.38	35.90	35.75
18:3n-6	0.08	0.01	0.08		0.29	0.32	0.31	0.31	0.30	0.29	0.28
18:3n-3	2.31	2.51	1.21		2.91	3.81	3.81	3.76	3.75	3.70	3.68
20:2n-6	0.25	0.05	0.39		1.31	0.74	0.70	0.67	0.63	0.58	0.56
20:3n-6	0.00	0.00	0.00		0.12	0.10	0.10	0.09	0.10	0.10	0.10
20:3n-3	1.62	0.03	1.87		0.07	0.06	0.05	0.06	0.06	0.06	0.07
20:4n-6	0.50	0.00	0.62		0.43	0.34	0.35	0.37	0.41	0.48	0.46
20:5n-3	13.40	0.00	10.09		6.48	3.17	3.10	2.98	2.85	2.75	2.65
22:5n-3	2.63	0.00	2.74		1.32	0.69	0.66	0.68	0.69	0.72	0.76
22:6n-3	12.15	0.00	13.99		5.87	3.16	3.20	3.23	3.30	3.36	3.37
∑n-3 HUFA[c]	29.80	0.03	28.69	7.84–9.80[d]	13.74	7.08	7.01	6.95	6.90	6.89	6.85
Unknown	3.71	1.60	4.32		2.25	1.56	1.10	1.40	1.35	1.91	2.03

[a]∑SFA: total content of saturated fatty acids. [b]∑MUFA: total content of monounsaturated fatty acids. [c]∑n-3 HUFA: total content of n-3 highly unsaturated fatty acids. [d]∑n-3 HUFA was obtained from Kim and Lee's (2004) study.

3.5 Serum lysozyme activity and SOD of olive flounder

Serum lysozyme activity of olive flounder ranged 538.4–583.3 U/mL and SOD ranged 71.5–73.5% (Table 7). The experimental diets did not significantly ($p > 0.9$ and $p > 0.8$, respectively) affect serum lysozyme activity and SOD of olive flounder.

3.6 AA and FA profiles of the whole-body olive flounder

AA profiles of the whole-body of olive flounder were not significantly ($p > 0.05$) altered by dietary treatments (Table 8).

The ∑SFA of the whole-body olive flounder fed the Con diet was significantly ($p < 0.0001$) higher than that of the fish fed all other diets (Table 9). EPA and DHA content of the whole-body of olive flounder fed the Con diet were significantly ($p < 0.0001$ for both) higher than those of fish fed all other diets. In particular, EPA content of olive flounder tended to decrease with inclusion levels of JMM in the CJ diets. The ∑n-3 HUFA content of the whole-body of olive flounder fed the Con diet was significantly ($p < 0.0001$) higher than that of olive flounder fed all other feeds.

4 Discussion

Comparable weight gain and SGR of olive flounder fed the CJ30, CJ40, and CJ50 diets or CJ20, CJ30, CJ40, and CJ50 diets to fish fed the Con diet in this study implied that 50% FM could be replaceable with CGM without deteriorating growth and SGR of olive flounder as long as 30–50% and 20–50% JMM were included at the expense of FM in a

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TABLE 4 Survival (%), feed efficiency (FE), protein efficiency ratio (PER), protein retention (PR), condition factor (K), viscerosomatic index (VSI), and hepatosomatic index (HSI) of olive flounder fed the experimental diets for 56 days.

Experimental diets	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	FE[a]	PER[b]	PR[c] (%)	K[d] (g/cm^3)	VSI[e] (%)	HSI[f] (%)
Con	18.0 ± 0.01	87.0 ± 0.15^{a}	100.0	1.06 ± 0.011	1.90 ± 0.016	35.8 ± 0.614	1.06 ± 0.007^{a}	3.63 ± 0.053	1.39 ± 0.024
CJ5	18.0 ± 0.01	82.8 ± 0.14^{d}	100.0	1.02 ± 0.004	1.85 ± 0.008	37.2 ± 0.430	0.98 ± 0.011^{b}	3.87 ± 0.037	1.39 ± 0.004
CJ10	18.0 ± 0.00	83.0 ± 1.15^{cd}	100.0	1.02 ± 0.020	1.84 ± 0.037	34.3 ± 1.013	0.99 ± 0.008^{b}	3.80 ± 0.057	1.35 ± 0.030
CJ20	18.0 ± 0.00	84.5 ± 0.53^{bcd}	100.0	1.03 ± 0.007	1.85 ± 0.013	35.9 ± 0.138	1.01 ± 0.010^{b}	3.71 ± 0.080	1.35 ± 0.040
CJ30	18.0 ± 0.01	84.9 ± 0.20^{abcd}	100.0	1.03 ± 0.005	1.86 ± 0.009	36.6 ± 0.554	1.01 ± 0.018^{ab}	3.60 ± 0.023	1.37 ± 0.013
CJ40	18.0 ± 0.01	85.3 ± 0.15^{abc}	100.0	1.04 ± 0.002	1.85 ± 0.017	36.0 ± 3.099	1.01 ± 0.016^{ab}	3.66 ± 0.087	1.43 ± 0.063
CJ50	18.0 ± 0.01	85.4 ± 0.09^{ab}	100.0	1.03 ± 0.003	1.85 ± 0.011	36.0 ± 0.211	1.02 ± 0.008^{ab}	3.58 ± 0.108	1.47 ± 0.013
p-value		$P < 0.001$		$P > 0.1$	$P > 0.2$	$p > 0.8$	$p < 0.02$	$p > 0.08$	$p > 0.2$

Values (mean of triplicate ± SE) in the same column sharing the common superscript letter are not significantly different ($p > 0.05$). [a]Feed efficiency (FE) = weight gain of fish/feed consumption. [b]Protein efficiency ratio (PER) = weight gain of fish/protein consumption. [c]Protein retention (PR, %) = protein gain × 100/protein consumption. [d]Condition factor (K, g/cm^3) = body weight of fish (g) × 100/total length of fish (cm)^3. [e]Viscerosomatic index (VSI, %) = viscera weight of fish × 100/body weight of fish. [f]Hepatosomatic index (HSI, %) = liver weight of fish × 100/body weight of fish.

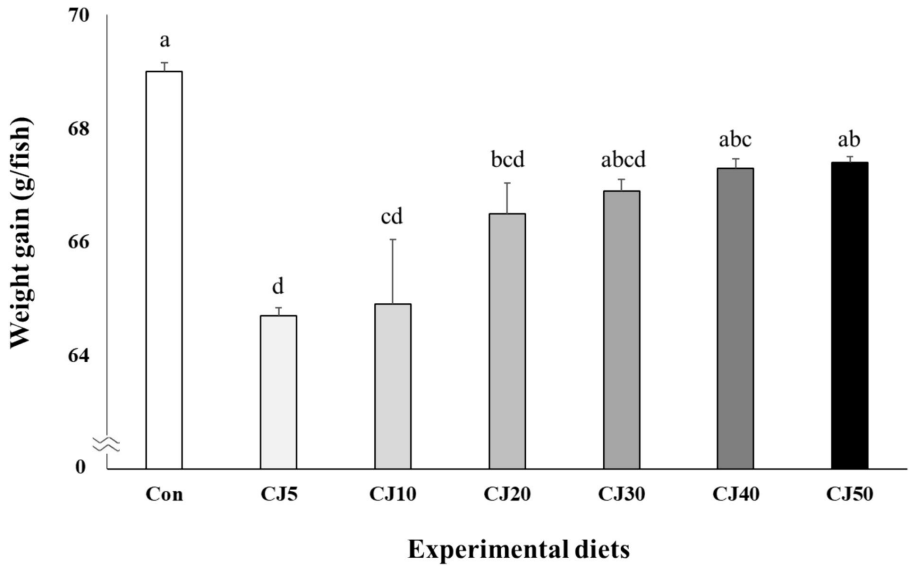

FIGURE 1
Weight gain (g/fish) of olive flounder (*Paralichthys olivaceus*) fed the experimental diets for 56 days (mean of triplicate ± SE) ($p < 0.001$). Regression analysis; $Y = 0.596143X + 64.2077$, $p = 0.0001$, adjusted $R^2 = 0.5773$. Different letters indicate statistically significant differences ($p < 0.05$).

60% FM-based diet, respectively. Linear improvement in weight gain and SGR of olive flounder versus JMM inclusion levels in low-FM diets replacing 50% FM with CGM might indicate that inclusion of JMM in low-FM diets could bring about improved growth performance of fish. Comparable feed consumption of olive flounder fed the CJ20, CJ30, CJ40, and CJ50 diets to fish fed the Con diet in this study indicated that 50% FM could be replaceable with CGM without deteriorating feed consumption of olive flounder as long as 20–50% JMM was included in a 60% FM-based diet. Improved weight gain and SGR of olive flounder were directly reflected from improved feed consumption of fish fed the low-FM diets substituting 50% FM with CGM. However, inferior weight gain, SGR and feed consumption of olive flounder fed the CJ5 and CJ10 diets to fish fed the Con diet indicated that inclusion of 5–10% JMM in low-FM diets could not compensate for the 60% FM-based (Con) diet in this study. Since JMM is one of the most expensive FM sources, 30 and 20% JMM are the most advised strategy in low-FM diets substituting 50% FM with CGM in terms of weight gain, and SGR and feed consumption, respectively. Unlike this study, Kikuchi (1999a) emphasized that FM up to 40% could be replaced with CGM in the olive flounder diets supplemented with limiting AA without compromising growth and feed utilization, however, a diet substituting 40% FM with CGM without supplementation of limiting AA led to inferior growth and feed utilization compared to fish fed a 75% FM-basal diet or diet substituting 40% FM with CGM supplemented with limiting AA. In considering results of Kikuchi's (1999a) study and this study, 50% FM could be replaceable by CGM without supplementation of limiting AA in low-FM diet as long as 20–50% JMM was included in olive flounder diet. Kim et al. (2019) and Jeong et al. (2020) also demonstrated that dietary inclusion of JMM led to improved growth performance and feed consumption of rockfish and olive flounder, respectively.

The CGM is a promising alternative to FM, but its utilization as a substitute for FM at an excess level deteriorated feed consumption and

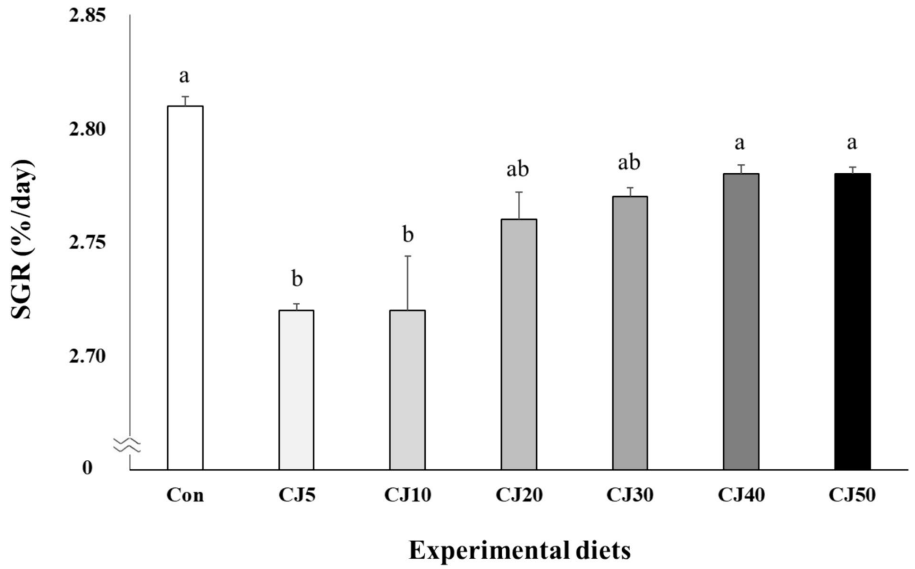

FIGURE 2
Specific growth rate (SGR, %/day) of olive flounder (*Paralichthys olivaceus*) fed the experimental diets for 56 days (mean of triplicate ± SE) ($p < 0.001$). Regression analysis; $Y = 0.012979X + 2.7094$, $p = 0.0001$, adjusted $R^2 = 0.5830$. Different letters indicate statistically significant differences ($p < 0.05$).

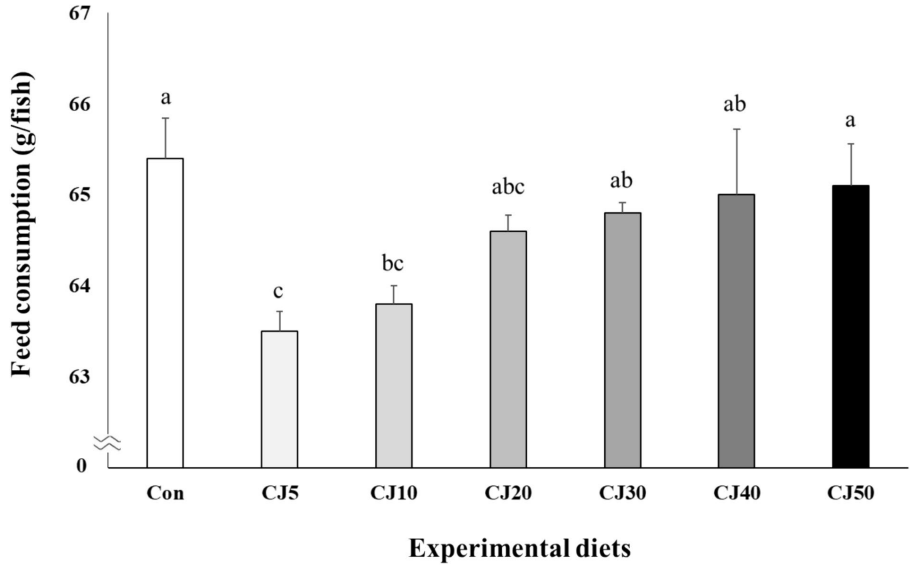

FIGURE 3
Feed consumption (g/fish) of olive flounder (*Paralichthys olivaceus*) fed the experimental diets for 56 days (mean of triplicate ± SE) ($p < 0.04$). Regression analysis; $Y = 0.338438X + 63.3041$, $p = 0.001$, adjusted $R^2 = 0.4819$. Different letters indicate statistically significant differences ($p < 0.05$).

feed utilization, and eventually deteriorated the growth of turbot (Regost et al., 1999) and spotted rose snapper (Hernández et al., 2021). The imbalanced AA (deficiency in lysine and arginine) profiles of CGM in diets led to deteriorated growth of fish (Peter et al., 2000). Incorporated feed stimulants were found to be an effective method to enhance growth and feed consumption of fish fed the low-FM diet or non-FM diet, where FM was partially replaced with plant proteins (Papatryphon and Soares, 2000; Kader et al., 2012).

There were no significant differences in FE, PER, and PR of olive flounder fed all diets in this study. Similarly, dietary inclusion of graded levels of JMM as feed stimulants did not influenced feed utilization of olive flounder (Jeong et al., 2022; Jeong and Cho, 2023). Unlike this study, however, Kikuchi (1999b) revealed the greatest weight gain, feed consumption, FER, and PER in olive flounder fed a defatted soybean meal-basal diet as a replacer for FM with supplementation of blue mussel meat as feed enhancer. According to Hancz (2020) this disparity in the impact are possible due to the differences in type and doses of feed attractants and/or stimulants.

Fish require EAA to support their somatic and reproductive growth; hence, EAA requirements have to be taken into consideration when formulating low-FM diet (Hamidoghli et al., 2020). The arginine and lysine content of all experimental diets fulfilled the requirements (2.04–2.10% and 1.55–2.16%, respectively) of olive flounder (Forster and Ogata, 1998; Alam et al., 2002), but the methionine content of the

experimental diets including the Con diet appeared to be comparatively lower than its requirement (1.44–1.49%) in the presence of 0.06% cysteine (Alam et al., 2000). The comparatively low methionine content in all experimental diets did not lower the growth of olive flounder because the relatively high cysteine content (0.85% in the CJ50 diet–1.02% in the Con diet) in all experimental diets could spare the methionine requirement (Finkelstein et al., 1988). Likewise, Harding et al. (1977) and Twibell et al. (2000) unveiled that cysteine could spare 50 and 60% of methionine in yellow perch (*Perca flavescens*) and channel catfish (*Ictalurus punctatus*) diets, respectively.

Marine fish including olive flounder need ∑n-3 HUFA for normal growth and development, which is mainly composed of EPA and DHA (Furuita et al., 2002). The EPA and DHA help to preserve the structural integrity of cell membranes, modulate carbohydrate and lipid metabolism, and regulate several inflammatory responses of fish (Lutfi et al., 2023). Farmed fish need to supply long-chain n-3 HUFA through diets because they have limited capacity to develop them in their bodies (Lee et al., 2020). The requirement (7.84–9.80% of total FA) of ∑n-3 HUFA for olive flounder (Kim and Lee, 2004) seemed to be met in the Con diet only in the current study. The ∑n-3 HUFA content in the experimental diets tended to decrease with increased JMM inclusion levels because FM contained a comparatively high ∑n-3 HUFA over JMM. Higher ∑SFA, EPA, DHA, and ∑n-3 HUFA content of whole-body of olive flounder fed the Con diet compared to those of fish fed all other diets were well responded from the FA profiles of the experimental diets. Likewise, the findings of this study are consistent with other studies, in which dietary FA profiles were well reflected in the whole-body FA profile of fish (Ha et al., 2021; Kim et al., 2021).

The biological parameters of fish including VSI and HSI in this study were not significantly impacted by dietary treatments. However, the K appeared to be well responded to weight gain of fish, which tended to increase with JMM inclusion levels in low-FM diets. The findings of this study were well proved by Baek et al. (2021)'s study, in which K of rockfish tended to increase with dietary inclusion levels of JMM. Likewise, Kader et al. (2010) explained that K of red sea bream (*Pagrus major*) tended to increase when crystalline AA, fish soluble, squid meal, and their combination were included as feed enhancer in low-FM diet.

Plasma parameters are useful indicators for the health status and stress level of fish (Satheeshkumar et al., 2012). In this study, no distinctive differences in plasma measurements of olive flounder were found among dietary treatments. Similarly, dietary inclusion of protein hydrolysates (shrimp, krill, and tilapia) showed no discernible differences in hematological measurements of olive flounder and red sea bream (Bui et al., 2014; Khosravi et al., 2015, 2018). Tusche et al. (2011) and Nagel et al. (2014) also reported that the inclusion of blood meal, mussel meal, and free AA mix as feed attractants in a plant protein concentrate-based diets did not affect the hematological parameters, except for the hematocrit level in rainbow trout (*Oncorhynchus mykiss*) and plasma protein level in turbot. No discernible difference in plasma chemistry of olive flounder in this study indicates that olive flounder fed the low-FM diets replacing 50% FM by CGM were in good health and nutritional condition.

The SOD protects cells from the toxic effects of superoxide radicals and lysozyme activity protects from pathogenic invasion (Sharf and Khan, 2020). However, lysozyme activity and SOD of olive flounder were not influenced by dietary treatments in this experiment. The findings of this study were supported by other studies, in which no remarkable changes in SOD and lysozyme activity were found in olive flounder fed diets substituting FM with fermented tuna by-product meal (Oncul et al., 2019) and meat meal (Ha et al., 2021). In contradiction to this study, inclusion of protein hydrolysate (shrimp, krill, and tilapia hydrolysate) tended to increase SOD and lysozyme activity of olive flounder fed a FM-basal feed (Khosravi et al., 2015) or low-FM feeds substituting 50% FM by soy

TABLE 5 Proximate composition (%, wet weight) of the whole-body olive flounder at the end of the 56-day feeding trial (mean of triplicate ± SE).

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
Con	71.1 ± 0.19	18.7 ± 0.16	3.7 ± 0.11	3.7 ± 0.14
CJ5	69.6 ± 0.20	19.7 ± 0.24	3.4 ± 0.17	4.2 ± 0.19
CJ10	72.3 ± 0.35	18.6 ± 0.23	3.3 ± 0.31	3.5 ± 0.16
CJ20	67.6 ± 1.87	19.2 ± 0.14	3.6 ± 0.57	4.0 ± 0.15
CJ30	70.4 ± 0.45	19.4 ± 0.18	3.1 ± 0.10	4.3 ± 0.21
CJ40	69.7 ± 3.21	19.2 ± 1.26	3.4 ± 0.23	4.0 ± 0.52
CJ50	69.9 ± 1.51	19.2 ± 0.16	3.3 ± 0.31	4.1 ± 0.29
P-value	$p > 0.5$	$p > 0.7$	$P > 0.8$	$p > 0.4$

TABLE 6 Plasma parameters of olive flounder at the end of the 56-day feeding trial (means of triplicate ± SE).

Experimental diets	AST (U/L)	ALT (U/L)	ALP (U/L)	T-BIL (mg/dL)	T-CHO (mg/dL)	TG (mg/dL)	TP (g/dL)	ALB (g/dL)
Con	13.2 ± 0.32	6.4 ± 0.25	111.2 ± 1.48	0.8 ± 0.04	368.3 ± 8.17	442.2 ± 4.29	5.5 ± 0.09	1.1 ± 0.04
CJ5	12.9 ± 0.23	6.3 ± 0.35	111.7 ± 1.91	0.8 ± 0.04	367.6 ± 8.61	441.2 ± 9.54	5.3 ± 0.09	1.1 ± 0.04
CJ10	13.0 ± 0.35	6.4 ± 0.25	110.6 ± 1.06	0.8 ± 0.06	365.8 ± 8.68	444.7 ± 7.16	5.7 ± 0.06	1.1 ± 0.04
CJ20	13.1 ± 0.47	6.4 ± 0.33	112.2 ± 1.42	0.8 ± 0.04	370.8 ± 9.77	440.3 ± 8.66	5.5 ± 0.07	1.1 ± 0.03
CJ30	13.1 ± 0.51	6.3 ± 0.20	112.8 ± 1.10	0.8 ± 0.04	370.8 ± 7.96	440.4 ± 6.25	5.6 ± 0.10	1.0 ± 0.03
CJ40	13.1 ± 0.49	6.4 ± 0.33	112.2 ± 1.41	0.8 ± 0.04	365.2 ± 7.58	452.9 ± 7.09	5.6 ± 0.11	1.0 ± 0.04
CJ50	13.1 ± 0.53	6.4 ± 0.29	111.0 ± 1.40	0.7 ± 0.03	357.7 ± 11.48	445.0 ± 7.67	5.6 ± 0.11	1.0 ± 0.03
p-value	$P > 0.9$	$P > 0.9$	$P > 0.8$	$P > 0.7$	$P > 0.9$	$P > 0.9$	$p > 0.5$	$p > 0.3$

AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; T-BIL, total bilirubin; T-CHO, total cholesterol; TG, triglyceride; TP, total protein; and ALB, albumin.

protein concentrate (Khosravi et al., 2018). Bui et al. (2014) and Gunathilaka et al. (2021) reported that the protein hydrolysates prepared from fishery by-products contain low-molecular-weight peptides, which may induce the immunomodulatory activity in fish by triggering the activity of fish macrophages.

No distinctive differences in the proximate composition and AA profiles of the whole-body olive flounder were found in this study. Likewise, the inclusion of fishery protein hydrolysate and freeze-dried blue muscle meal as feed attractants in low-FM diets substituting various levels of FM with soy and rapeseed protein concentrate did not influence the proximate composition of olive flounder and turbot, respectively (Nagel et al., 2014; Khosravi et al., 2018). Similarly, no significant alterations in AA profiles were observed in the whole-body olive flounder fed diets replacing FM by various protein sources (Choi et al., 2020; Ha et al., 2021; Kim et al., 2021). Alam et al. (2002) also did not show any difference in AA profile of the whole-body olive flounder fed diets containing different AA patterns. However, there were some contradictory results presenting remarkable changes in the proximate composition (Kader et al., 2012; Kim et al., 2014; Peng et al., 2022) and AA profiles (Alam et al., 2018; Chen et al., 2019; Mastoraki et al., 2020) of fish by dietary replacement of FM with other protein sources.

TABLE 7 Serum lysozyme activity and superoxide dismutase (SOD) of olive flounder at the end of the 56-day feeding trial (mean of triplicate ± SE).

Experimental diets	Lysozyme activity (U/mL)	SOD (%)
Con	534.6 ± 17.59	73.1 ± 0.51
CJ5	539.6 ± 20.93	71.6 ± 1.81
CJ10	539.2 ± 29.12	71.5 ± 0.64
CJ20	551.4 ± 25.77	73.2 ± 0.53
CJ30	540.9 ± 46.45	72.6 ± 1.04
CJ40	583.3 ± 44.10	73.4 ± 0.24
CJ50	538.4 ± 34.04	73.5 ± 0.30
p-value	$p > 0.9$	$p > 0.8$

5 Conclusion

Weight gain of olive flounder fed the CJ30, CJ40, and CJ50 diets was comparable to fish fed the Con diet. Furthermore, SGR and feed consumption of olive flounder fed the CJ20, CJ30, CJ40, and CJ50 diets were comparable to fish fed the Con diet. However, feed utilization, biological indices except for K, proximate composition, AA profiles, and hematological measurements of fish were not altered by dietary treatments. Inclusion of 30 and 20% JMM inclusion were the most recommendable in the olive flounder diets substituting 50% FM with CGM based on weight gain, and SGR and feed consumption, respectively.

TABLE 8 Amino acid (AA) profiles (%, wet weight) of the whole-body of olive flounder at the end of the 56-day feeding trial (means of triplicate ± SE).

	Experimental diets							
	Con	CJ5	CJ10	CJ20	CJ30	CJ40	CJ50	*P*-value
Essential AA (%)								
Arginine	0.38 ± 0.02	0.37 ± 0.01	0.34 ± 0.02	0.38 ± 0.03	0.35 ± 0.03	0.36 ± 0.02	0.39 ± 0.09	$P > 0.5$
Histidine	0.70 ± 0.02	0.74 ± 0.01	0.66 ± 0.03	0.72 ± 0.01	0.73 ± 0.01	0.70 ± 0.03	0.76 ± 0.03	$p > 0.6$
Isoleucine	0.80 ± 0.02	0.78 ± 0.01	0.73 ± 0.01	0.76 ± 0.01	0.70 ± 0.03	0.78 ± 0.03	0.85 ± 0.02	$p > 0.3$
Leucine	0.15 ± 0.04	0.12 ± 0.04	0.12 ± 0.04	0.13 ± 0.03	0.13 ± 0.05	0.14 ± 0.05	0.15 ± 0.05	$P > 0.3$
Lysine	0.91 ± 0.03	0.82 ± 0.03	0.87 ± 0.02	0.82 ± 0.05	0.79 ± 0.03	0.82 ± 0.03	0.84 ± 0.10	$P > 0.4$
Methionine	1.40 ± 0.04	1.41 ± 0.02	1.41 ± 0.02	1.42 ± 0.02	1.34 ± 0.01	1.42 ± 0.01	1.50 ± 0.03	$P > 0.8$
Phenylalanine	1.72 ± 0.03	1.74 ± 0.03	1.80 ± 0.03	1.84 ± 0.05	1.66 ± 0.05	1.79 ± 0.02	1.88 ± 0.03	$P > 0.6$
Threonine	1.52 ± 0.03	1.54 ± 0.02	1.52 ± 0.04	1.53 ± 0.04	1.43 ± 0.03	1.43 ± 0.02	1.57 ± 0.06	$p > 0.1$
Tryptophan	0.90 ± 0.01	0.90 ± 0.00	0.95 ± 0.01	0.96 ± 0.01	0.93 ± 0.01	0.96 ± 0.00	1.01 ± 0.01	$p > 0.06$
Valine	0.45 ± 0.02	0.48 ± 0.01	0.46 ± 0.02	0.48 ± 0.06	0.44 ± 0.01	0.48 ± 0.02	0.49 ± 0.08	$p > 0.4$
Non-essential AA (%)								
Alanine	1.21 ± 0.03	1.15 ± 0.04	1.14 ± 0.03	1.16 ± 0.02	1.10 ± 0.05	1.13 ± 0.06	1.19 ± 0.08	$p > 0.4$
Aspartic acid	0.71 ± 0.06	0.71 ± 0.07	0.69 ± 0.04	0.72 ± 0.05	0.71 ± 0.05	0.72 ± 0.07	0.76 ± 0.08	$p > 0.2$
Cysteine	1.35 ± 0.01	1.36 ± 0.02	1.39 ± 0.02	1.37 ± 0.02	1.30 ± 0.02	1.37 ± 0.02	1.46 ± 0.01	$P > 0.5$
Glutamic acid	1.56 ± 0.10	1.50 ± 0.06	1.53 ± 0.04	1.52 ± 0.04	1.44 ± 0.10	1.53 ± 0.06	1.59 ± 0.18	$P > 0.9$
Glycine	0.52 ± 0.09	0.51 ± 0.06	0.52 ± 0.04	0.49 ± 0.06	0.51 ± 0.02	0.53 ± 0.02	0.53 ± 0.14	$P > 0.7$
Proline	0.22 ± 0.04	0.23 ± 0.03	0.24 ± 0.02	0.23 ± 0.01	0.25 ± 0.05	0.21 ± 0.04	0.25 ± 0.05	$P > 0.4$
Serine	2.57 ± 0.03	2.63 ± 0.04	2.61 ± 0.03	2.63 ± 0.04	2.51 ± 0.05	2.58 ± 0.05	2.67 ± 0.05	$P > 0.7$
Tyrosine	0.85 ± 0.02	0.87 ± 0.02	0.86 ± 0.01	0.92 ± 0.02	0.83 ± 0.02	0.84 ± 0.02	0.89 ± 0.03	$P > 0.5$

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TABLE 9 Fatty acid (FA) profiles (% of total FA) of the whole body of olive flounder at the end of the 56-day feeding trial.

	Experimental diets							
	Con	CJ5	CJ10	CJ20	CJ30	CJ40	CJ50	*P*-value
12:0	0.41 ± 0.024	0.36 ± 0.040	0.40 ± 0.041	0.36 ± 0.028	0.36 ± 0.006	0.39 ± 0.062	0.32 ± 0.022	$P > 0.6$
14:0	3.67 ± 0.048^{a}	2.92 ± 0.159^{abc}	3.13 ± 0.144^{b}	2.78 ± 0.119^{bcd}	2.86 ± 0.125^{bcd}	2.54 ± 0.061^{cd}	2.30 ± 0.137^{d}	$P < 0.0001$
16:0	18.23 ± 0.139^{a}	17.30 ± 0.205^{ab}	17.59 ± 0.331^{ab}	17.39 ± 0.297^{ab}	17.57 ± 0.402^{ab}	16.94 ± 0.222^{ab}	16.52 ± 0.382^{b}	$p < 0.009$
18:0	4.10 ± 0.161	4.01 ± 0.067	4.04 ± 0.069	4.13 ± 0.162	4.14 ± 0.046	4.09 ± 0.013	4.26 ± 0.045	$p > 0.6$
20:0	0.36 ± 0.023^{a}	0.22 ± 0.031^{a}	0.24 ± 0.044^{a}	0.18 ± 0.018^{b}	0.18 ± 0.024^{b}	0.19 ± 0.043^{b}	0.20 ± 0.040^{b}	$P < 0.02$
22:0	1.99 ± 0.040^{a}	0.30 ± 0.061^{b}	0.27 ± 0.035^{b}	0.26 ± 0.063^{b}	0.28 ± 0.026^{b}	0.33 ± 0.015^{b}	0.34 ± 0.043^{b}	$p < 0.0001$
∑SFA^{a}	28.76 ± 0.248^{a}	25.11 ± 0.370^{b}	25.66 ± 0.562^{b}	25.10 ± 0.501^{b}	25.40 ± 0.509^{b}	24.48 ± 0.322^{b}	23.94 ± 0.534^{b}	$p < 0.0001$
14:1n-5	0.09 ± 0.006	0.05 ± 0.009	0.08 ± 0.015	0.06 ± 0.015	0.08 ± 0.007	0.06 ± 0.006	0.07 ± 0.012	$P > 0.2$
15:1n-7	0.26 ± 0.015	0.24 ± 0.046	0.23 ± 0.021	0.20 ± 0.019	0.25 ± 0.030	0.20 ± 0.033	0.19 ± 0.018	$P > 0.4$
16:1n-7	4.49 ± 0.018	3.99 ± 0.064	4.64 ± 0.701	3.90 ± 0.382	4.15 ± 0.103	3.92 ± 0.147	3.76 ± 0.148	$P > 0.4$
17:1n-7	0.68 ± 0.024	0.57 ± 0.038	0.70 ± 0.084	0.57 ± 0.047	0.68 ± 0.067	0.61 ± 0.070	0.59 ± 0.103	$P > 0.6$
18:1n-9	24.39 ± 0.266	24.90 ± 0.429	23.17 ± 0.910	24.11 ± 0.303	24.27 ± 0.930	24.44 ± 0.628	25.25 ± 0.255	$p > 0.3$
20:1n-9	1.79 ± 0.059	2.20 ± 0.272	1.87 ± 0.261	1.95 ± 0.218	1.99 ± 0.424	1.74 ± 0.335	1.97 ± 0.136	$P > 0.9$
22:1n-9	1.29 ± 0.069	1.32 ± 0.147	1.47 ± 0.223	1.59 ± 0.209	1.63 ± 0.101	1.52 ± 0.216	1.15 ± 0.091	$p > 0.3$
24:1n-9	0.24 ± 0.012	0.15 ± 0.029	0.17 ± 0.035	0.16 ± 0.025	0.21 ± 0.015	0.21 ± 0.026	0.16 ± 0.017	$p > 0.1$
∑MUFA^{b}	33.22 ± 0.318	33.42 ± 0.167	32.33 ± 0.316	32.54 ± 0.218	33.26 ± 1.014	32.70 ± 0.743	33.14 ± 0.168	$P > 0.6$
18:2n-6	18.16 ± 0.231^{b}	25.17 ± 0.773^{a}	25.19 ± 0.821^{a}	27.07 ± 0.678^{a}	25.34 ± 1.815^{a}	26.44 ± 0.862^{a}	25.74 ± 1.913^{a}	$p < 0.002$
18:3n-6	0.21 ± 0.012	0.25 ± 0.033	0.20 ± 0.030	0.23 ± 0.015	0.23 ± 0.038	0.24 ± 0.023	0.29 ± 0.048	$P > 0.4$
18:3n-3	1.68 ± 0.075^{b}	2.39 ± 0.027^{a}	2.28 ± 0.105^{a}	2.35 ± 1.01^{a}	2.26 ± 0.043^{a}	2.46 ± 0.044^{a}	2.39 ± 0.132^{a}	$P < 0.001$
20:2n-6	0.55 ± 0.015^{b}	0.78 ± 0.31^{a}	0.71 ± 0.094^{ab}	0.84 ± 0.044^{a}	0.81 ± 0.026^{a}	0.87 ± 0.69^{a}	0.83 ± 0.92^{a}	$p < 0.03$
20:3n-6	0.03 ± 0.006	0.02 ± 0.003	0.04 ± 0.009	0.03 ± 0.010	0.03 ± 0.015	0.04 ± 0.012	0.03 ± 0.010	$P > 0.9$
20:3n-3	0.75 ± 0.028	0.79 ± 0.062	0.72 ± 0.021	0.78 ± 0.071	0.75 ± 0.023	0.81 ± 0.026	0.79 ± 0.043	$p > 0.8$
20:4n-6	0.18 ± 0.009	0.22 ± 0.035	0.24 ± 0.026	0.22 ± 0.035	0.25 ± 0.018	0.25 ± 0.023	0.26 ± 0.022	$P > 0.4$
20:5n-3	6.35 ± 0.132^{a}	4.61 ± 0.113^{b}	4.55 ± 0.078^{bc}	4.34 ± 0.247^{bcd}	4.28 ± 0.178^{bcd}	3.82 ± 0.130^{cd}	3.60 ± 0.151^{d}	$P < 0.0001$
22:2n-6	0.31 ± 0.019	0.25 ± 0.025	0.23 ± 0.023	0.28 ± 0.012	0.26 ± 0.032	0.22 ± 0.008	0.27 ± 0.020	$P > 0.4$
22:6n-3	6.23 ± 0.119^{a}	4.65 ± 0.70^{b}	4.60 ± 0.039^{b}	4.67 ± 0.138^{b}	4.80 ± 0.075^{b}	4.80 ± 0.058^{b}	4.83 ± 0.052^{b}	$P < 0.0001$
∑n-3 HUFA^{c}	13.33 ± 0.140^{a}	10.04 ± 0.176^{b}	9.87 ± 0.128^{b}	9.79 ± 0.361^{b}	9.83 ± 0.224^{b}	9.43 ± 2.12^{b}	9.22 ± 0.216^{b}	$P < 0.0001$
Unknown	3.55 ± 0.345	2.40 ± 0.808	2.62 ± 0.686	1.62 ± 2.41	2.39 ± 0.662	2.95 ± 1.106	3.88 ± 1.378	

Values (means of triplicate ± SE) in the same row sharing the same superscript letter are not significantly different ($p > 0.05$). [a]∑SFA: total content of saturated fatty acids. [b]∑MUFA: total content of monounsaturated fatty acids. [c]∑n-3 HUFA: total content of n-3 highly unsaturated fatty acids.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Korea Maritime and Ocean University (KMOU IACUC 2021–05). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MI: Data curation, Investigation, Writing – original draft. SC: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. TK: Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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