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Protein Requirement in Granulated Microdiets for Olive Flounder (*Paralichthys oivaceus*) Larvae

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Abstract

The optimal protein requirement in granulated microdiets was determined for larval olive flounder (*Paralichthys olivaceus*). Five granulated microdiets (CP42, CP46, CP50, CP54 and CP58) containing 42 to 58 % protein levels at a constant energy level were prepared. As the protein levels increased in the granulated microdiets, all essential and nonessential amino acid (AA) contents increased. The weight gain, average growth rate and relative growth rate (RGR) of the flounder larvae fed the CP54 and CP58 diets were greater than of larvae fed the other diets. The total length of larval flounder fed the CP54 and CP58 diets was greater than of the flounder fed the CP42 and CP46 diets. The crude lipid content of the larval flounder fed the CP58 diet was higher than of the flounder fed the CP42, CP46 and CP50 diets. None of the whole body AA profiles of the founder larvae was affected by protein levels in the granulated microdiets. In conclusion, dietary protein requirement was estimated to be 55.4 % based on the RGR of the larval olive flounder.

*Keywords***:** Olive flounder (*Paralichthys olivaceus*), Granulated microdiet, Protein requirement, Essential amino acid (AA), Relative growth rate (RGR)

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Introduction

For the past three decades, the olive flounder (*Paralichthys olivaceus*) has been the most commercially important marine finfish for aquaculture in Korea due to its fast growth, high tolerance to fluctuation of water temperature and resistance against disease. The annual aquaculture production of this fish reached 45,737 tons in Korea in 2015 (MFAFF 2016).

As healthy larval and juvenile fish production should be secured to increase the aquaculture production of marine fish, studies focusing on larval marine fish production receive many attentions. The larvae of olive flounder are commonly supplied with live foods, such as rotifer (*Brachionus* sp.) and *Artemia* nauplii, microdiets and/or their combination after the newly hatched larval fish have consumed their yolk-sac. The development of a weaning diet to replace live food is critical for lowering production cost and for sustaining a constantly high quantity and quality of larval marine fish production. Kanazawa (2003) emphasized that the development of microparticulate diets as a substitute for live foods is necessary to further increase the productivity of larval marine fish.

The successful production of larval and juvenile marine fish has been reported by several research groups. The development of microdiets for larval marine fish, such as the Pacific bluefin tuna (*Thunnus orientalis*) (Haga et al., 2010, 2011; Takeuchi & Haga, 2015), red sea bream (*Pagrus major*) (López-Alvarado & Kanazawa, 1994a; Teshima, Koshio, Ishikawa, Alam & Hernadez, 2004), olive flounder (Bai, Cha & Wang, 2001; Takeuchi et al., 2003; Wang et al., 2004; Ji et al., 2013), gilthead seabream (*Sparus aurata*) (Saleh et al., 2013), European sea bass (*Dicentrachus labrax*) (Person-Le Ruyet, Alexandre, Thebaud & Mugnier, 1993) and Atlantic cod (*Gadus morhua*) (Baskerville-Bridges & Kling, 2000; Johnson, Cook, Nicklason & Rust, 2009) have been successfully made. Especially, Takeuchi et al. (2003) reported that a mixture of microparticle diets containing two different molecular weight peptides (1000-2000 and 30,000 Da) was a good source of protein and this type of diet can be given to olive flounder from the larval to juvenile stage. The apparent digestibility coefficients of the protein in the microparticulate diet in 8-week old Atlantic cod larvae were higher (ranging from 76 to 86%) than of those coefficients in *Artemia* (ranging from 47 to 58%) (Johnson et al., 2009). Cahu and Infante (2001) reviewed the

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substitution of live food with formulated diets in marine fish larvae emphasizing the physical aspects of the diet, which must be considered, and the digestibility of the diets in the larval fish and demonstrated that the nutritional requirements are not similar between larvae and juvenile fish. Kolkovski (2013) demonstrated the techniques and methods for manufacturing the microdiet particles and described the chemical and physical properties of the microdiet particles. Still, more studies on the development of microdiets are needed for the stable production of larval marine fish.

The possibility of the complete replacement live food with microdiet alone for some marine fish larvae seems to be limited (Baskerville-Bridges & Kling, 2000; Takeuchi et al., 2003; Wang et al., 2004; Faulk & Holt, 2009; Tang, Chen & Wu, 2010; Alam et al., 2013; Li et al., 2013), but the combination of microdiet with live food effectively improved the survival and growth of the larval stages of marine fish (Kolkovski, Koven & Tandler, 1997; Yúfera et al., 2000; Teshima et al., 2004; Ji et al., 2013). Teshima et al. (2004), in particular, showed that live food (Rotifer and *Artemia*) could be completely replaced with a zein-microbound diets containing a molecular weight of 1000 and 3000 Da soybean peptides or with the combined half amount of zein-microbound diet containing a molecular weight of 1000 Da soybean peptides and live food for 30 days after hatching (DAH) in red sea bream larvae; however, for 15 DAH olive flounder larvae, the combined half amount of zein-microbound diet containing a molecular weight of 1000 Da soybean peptide and live food only.

Protein is one of the most important and expensive components in feed formulations for fish. The optimal dietary protein level for the maximum growth and survival of olive flounder larvae were suggested to be 60% or higher when microparticulate diets containing 40, 50 and 60% crude protein levels were supplied for 75 days (Bai et al., 2001). However, crude protein levels with 10% increments in the diets are too wide to determine accurate optimal protein levels for larval olive flounder, and the microparticulate diets used in their study was lab-pelletized. The outcome of their study has, therefore, little applicability to the formulation of practical microdiets for olive flounder larvae. The optimal protein requirement of the commercially granulated microdiet for larval olive flounder was determined in further detail in this study.

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Materials and methods

Spawning and Larval Rearing Conditions

The 1-day old fertilized eggs were purchased from Borame hatchery (Jeju Special Self-Governing Province, Korea) and transported to Garolim flounder hatchery (Seosan-si, Chungcheongnam-do, Korea). The 1 day old fertilized eggs were incubated in a 5 ton round-shaped flow-through tanks (water volume: 4 ton) at 18 °C (4 million eggs per tank). Water source was the sand-filtered and ultraviolet irradiated seawater at 33 ppt. Continuous aeration was provided throughout the incubation. The flow rate of the incubating 5 ton flow-through tank was 0.63 L min^{-1} $tank^{-1}$ since 3 DAH. The newly hatched larvae were started to be fed on rotifers at 3 DAH, with this food ending on 15 DAH, and the larvae were then fed *Artemia* nauplii (Great Salt Lake, Utah, USA) beginning on 14 DAH and ending on 25 DAH, with the microdiet feeding experiment beginning on 19 DAH. Rotifers and *Artemia* nauplii were enriched with AlgaMac-3050 Plus according to the manufacturer's recommendation to improve highly unsaturated fatty acids (HUFA) before fed to larvae. Rotifers and *Artemia* nauplii were daily supplied based on the feeding schedule and ration (Table 1). Larvae were reared in green water conditions until 18 DAH, provided by adding *Chlorella* sp. to the rearing tanks every morning. The designated amount of the granulated microdiet was handfed to larval fish 6-12 times a day between 06:00 and 18:00 hours.

Preparation of the Experimental Diets

The ingredients and feed formulation of the experimental diets are given in Table 2. Pollack and krill meals, wheat gluten and taurine are the protein sources in the experimental diets. Alpha-starch and dextrin were used as the carbohydrate sources, and fish oil was used as the lipid source in the experimental diets. Five granulated microdiets (CP42, CP46, CP50, CP54 and CP58), containing different levels of crude protein ranging from 42 to 58% with 4% increments at the expense of dextrin and at a constant estimated energy level (18.6 kJ g^{-1} diet), in triplicate were prepared. In the CP58 diet to increase protein content, however, 0.8% taurine and 2% α-starch content decreased due to shortage of dextrin content. Taurine requirement in the all experimental diets for olive

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flounder was met (> 1.5 % of 100 g diet) (Park, Takeuchi, Seikai & Yokoyama, 2001; Kim et al., 2005; Han et al., 2014).

All ingredients, except for the fish oil, were ground by an air Z-mill (SK Z-mill 0405, Seishin Co. Ltd., Japan) and mixed well. The mixed ingredients were granulated with a granulator (Flow-Z granulator, Okawara Co. Ltd., Japan). The granulated microdiets were dried at 60 °C by a dryer (Horizontal Fluid Bed Dryer, Okawara Co. Ltd., Japan). The granulated microdiets were sieved and grouped into the two sizes (0.31-0.48 and 0.48-0.63 μm). The debris of the granulated microdiets was sent back to the granulator, but the oversized granular diets were sent to the roll mill, reground, and sieved again. The granulated microdiets were fish-oil coated and packed. The experimental microdiets were manufactured by Daehan Feed Ltd. (Incheon, Korea).

Experimental Conditions

9000 larvae were randomly distributed in 15 indoor 70 L square plastic tanks (600 larvae per tank) at 14 DAH and then 100 larvae from each tank were sampled for measurement of the initial weight of fish, dividing collected biomass of each tank by a number of fish (100 individual). Eventually, 7500 larvae were held in 15 tanks (500 larvae per tank) at 14 DAH for the feeding trial. Two size groups of granulated microdiets (0.31–0.48 and 0.48–0.63 μm) were supplied for 26 days as the fish grew (Table 1): the former for larval fish at 19 to 40 DAH and the latter for larval fish at 35 to 44 DAH. At 45 DAH, most of the fish larvae were bottom-settled after metamorphosis for all diet treatments. Larval fish were exposed to attenuated sun light.

Effective mircroorganisms (EM) (Boreong Agricultural Technology Center, Boryeong city, Chungcheongnam-do, Korea) were applied daily to each tank to purify the water at a concentration of 9.6 mL $tank^{-1}$ throughout the feeding trial. The bottom of each tank was siphon-cleaned twice a week. Water source was the sand-filtered and ultraviolet irradiated seawater (33 ppt). The water exchanged rate was 0.17 L $tank^{-1}$ min^{-1}. The water temperature ranged from 16.0 to 23.5°C (mean ± SE: 20.6 ± 0.39°C) throughout the feeding trial. At the end of the 26 days feeding trial, all surviving fish from each tank were counted, collectively weighed and sampled for growth and nutritional analysis.

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Calculation of Growth Rate of Fish Larvae

The following calculation were applied based on Lugert, Thaller, Tetens, Schulz & Krieter (2016)'s study: Absolute growth rate (AGR) = (Final weight of fish – initial weight of fish/days of feeding, and relative growth rate (RGR) = (Final weight of fish – initial weight of fish)×100/initial weight of fish.

Analytical Procedures for the Microdiets and Larval Fish

All surviving larval fish from each tank had been frozen at -20°C and later were thawed for chemical analysis. The fifty larval fish that had been randomly chosen among all frozen larval fish from each tank were individually measured for the final weight with an electronic analytical balance (ATX224, Shimadzu Corporation, Kyoto, Japan) and for total length by an eyepiece micrometer OM-500N (NaRiKa, Tokyo, Japan) while being viewed under a microscope (Eclipse E200, Nikon, Tokyo, Japan).

Prior to further examination, all samples were homogenized and used for proximate analysis. The crude protein content was determined by the Kjeldahl method (Auto Kjeldahl System, Buchi B-324/435/412, Switzerland), crude lipid was determined using an ether-extraction method, moisture was determined by oven drying at 105°C for 24 h, and ash was determined using a muffle furnace at 550°C for 4 h. All methods were in accordance with AOAC (1990) practices. The amino acid (AA) composition of the experimental microdiets and larval fish were determined by using a high speed AA analyzer (Hitachi L-8800, Tokyo, Japan) after sample hydrolysis in 6 N HCl for 24 h at 110 °C.

Statistical Analysis

A one-way ANOVA and Duncan`s multiple range test (Duncan, 1955) were used to determine the significance of the differences among the means of the treatments by using SAS version 9.3 program (SAS Institute, Cary, NC, USA). Broken-line analysis (Robbins, Norton & Baker, 1979) was used to determine dietary protein requirement of flounder larvae. Percentage data were arcsine-transformed prior to statistical analysis.

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Results

As the protein levels increased in the granulated microdiets, all essential and nonessential AA contents increased (Table 3). Glutamic acid, and leucine and lysine are the richest in the essential and nonessential AA in the experimental diets, respectively.

The survival (%), weight gain (g $fish^{-1}$), absolute growth rate (AGR, mg day^{-1}), relative growth rate (RGR, %) and total length (mm) of the larval flounder fed the granulated microdiets containing the various levels of crude protein are presented in Table 4. The survival, which ranged from 52.1 to 55.0%, was not significantly ($P > 0.05$) affected by the protein levels in the granulated microdiets. However, the weight gain, AGR and RGR of flounder larvae fed the CP54 and CP58 diets were significantly ($P < 0.003$ and $P < 0.006$, respectively) higher than those of the larvae fed the other (CP42, CP46 and CP50) diets. The total length of the larval flounder fed the CP54 and CP58 diets was also significantly ($P < 0.02$) longer than that of the flounder fed the CP42 and CP46 diets, but not significantly ($P > 0.05$) different from that of the flounder fed the CP50 diet.

The moisture, crude protein and ash content of the whole body of larval flounder were not significantly ($P > 0.05$) affected by the protein levels in the granulated microdiets (Table 5). However, the crude lipid content of the whole body of larval flounder fed the CP58 diet was significantly ($P < 0.02$) higher than that of the flounder fed the CP42, which was lowest, CP46 and CP50 diets, but was not significantly ($P > 0.05$) different from that of flounder fed the CP54 diet.

None of whole-body AA profiles of the founder larvae was significantly affected by the protein levels in the granulated microdiets (Table 6).

Broken-line model [$Y = 387.2 – 1.59 (R – X_{LR})$, $R = 55.4 \pm 2.93$ (SE)] showed that dietary protein requirement appeared to be 55.4% based on RGR of the larval olive flounder (Fig. 1).

Discussion

Bai et al. (2001) suggested that the dietary optimal protein level for maximum growth and survival of olive flounder larvae should be 60% or higher when the microparticulate diets containing fish muscle as main protein

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source at crude protein levels of 40, 50 and 60% were supplied to larval fish for 75 days from beginning on 8 DAH. However, the suggested values could have been overestimated because growth performance of larval flounder fed the experimental diets was poorer compared to fish larvae fed the commercial diet containing 55.8 to 61.2% protein levels. The poorer performance of the founder larvae fed on even the highest (60%) protein diet compared to the commercial diet probably resulted from the fact that larval fish might consume less protein content than the designated amount since nutrients in the all crumbled experimental diets could have more easily leached out than the commercial diet.

Our broken-line model (Fig. 1) indicated that 55.4% is the optimal protein level based on the growth rate of flounder larvae. Since the supply of protein content in excess in a diet may result in high feed costs and a deterioration of water quality, the optimum amount should be included in the microdiet. Similarly, the best growth and survival were observed in 15 DAH sea bass larvae fed the 50% protein diet when the 30, 40, 50, and 60% protein microencapsulated diets were fed for 21 days (Péres, Cahu, Zambonino Infante, Le Gall & Quazuguel, 1996).

Cahu and Infante (2001) demonstrated that the nutritional requirements are not similar between larval and juvenile fish. Juvenile olive flounder (an initial weight of 6 g) required 50% protein for best growth when eight experimental diets with four protein (41, 44, 47 and 50%) and two energy levels (20 and 19 kJ g^{-1}) were fed for 45 days (Yigit, Koshio, Teshima & Ishikawa, 2004). Kim, Wang, and Bai (2003) reported that the dietary protein requirements for the maximum growth of juvenile olive flounder (an initial weight of 13.3 g) were estimated to be between 40 and 44% when fish meal and casein-based diets containing protein levels from 30 to 60%, with 6% increments and at a constant energy level of 17 kJ g^{-1}, were fed for 8 weeks. These results indicated that the smaller or younger fish require higher protein content in their diets than is required for the larger or older fish. This is in agreement with other studies (Einen & Roem, 1997; Sweilum, Abdella & El-Din, 2005) showing that small fish required a high-protein and low-energy diet, whereas large fish required a low-protein and high-energy diet to achieve the best production in Nile tilapia (*Oreochromis niloticus*) and Atlantic salmon (*Salmo salar*).

Unlike the study by Bai et al. (2001) in which larval flounder were fed with live food (*Artemia* nauplii) to 45

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DAH, *Artemia* was supplied to larval fish until 18 DAH in this study. The survival and body weight (total length) of the larval fish at 45 DAH, ranging from 52.1 and 58.6 (17.3) to 55.0% and 61.2 mg (18.8 mm), respectively, in this study, were higher and heavier (longer) than those of larval fish at 41 DAH, which ranged from 43.0 and 54.8 (17.2) to 46.0% and 55.3 mg (17.5 mm), respectively, in the study by Bai et al. (2001). This indicated that the initiation of weaning to feeding with granulated microdiets at 18 DAH in this study was appropriate. Survival (66.1%) and total length (28.1 mm) of larval flounder at 40 DAH after being fed the live food (enriched rotifer and *Artemia* nauplii) were superior to those (7.4-24.5% and 19.0-20.7 mm) of larval fish fed a the combination of microparticle diet and one-third the amount of live food or one-third the amount of live food alone (Wang et al., 2004). Takeuchi et al. (2003) reported that olive flounder larvae at 11 DAH fed a combination of peptides of two molecular weights and live food at one-third the amount of live food for 10 days showed improved survival and growth rate, which were as good observed in the larval fish fed the live food alone.

The growth of red sea bream larvae at 20 DAH was enhanced by increasing the arginine level up to 2.4% of the diet when larvae were fed on the zein microbound diets containing various levels of arginine from 2.3 to 3.1% for 28 days (López-Alvarado & Kanazawa, 1994a). Similarly, the arginine requirement for flounder larvae seemed to be slightly higher than 3.2% of the CP54 diet due to estimated protein requirement of 55.4% in this study. The nutritional requirements of marine fish larvae have focused primarily on the fatty acid requirements since a few decades ago (Izquierdo, Socorro, Arantzamendi & Hernadez-Cruz, 2000; Kanazawa, 2003), but the AA requirements in recent years (Hamre et al., 2013; Li et al., 2013; Saavedra et al., 2015; Canada et al., 2016). In particular, histidine appeared to be the limiting AA in live food (enriched rotifers and *Artemia* nauplii) and in dry feed tested for meagre (*Agryrosomus regius*) larvae when essential AA profiles of fish carcass and diet were compared (Saavedra et al., 2015) or in enriched rotifers when they were fed to *Diplodus puntazzo* larvae at 4 DAH (Saavedra et al., 2007).

Neither chemical composition nor AA profiles of the whole body of larval founder differed among the granulated microdiets containing different levels of crude protein, except for the crude lipid content, in this study. Crude lipid content of the whole larval body was relatively well reflected from that of the granulated microdiets.

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Similarly, Bai et al. (2001) demonstrated that protein content in microparticulate diets did not change either the chemical composition or the whole-body fatty acid composition of larval flounder. The different dietary levels of crystalline AA or dietary arginine content did not affect any whole-body AA contents of olive flounder (López-Alvarado & Kanazawa, 1994b) and red sea bream larvae (López-Alvarado & Kanazawa, 1994a). However, unlike these studies, the chemical composition of the larval olive flounder was affected by either the different feed or the feeding ration (Wang et al., 2004), or the whole-body crude protein and lipid contents of large yellow croaker (*Larimichthys crocea*) larvae were affected by the dietary AA patterns (Li et al., 2013).

In conclusion, granulated microdiet protein requirements were estimated to be approximately 55.4% for larval olive flounders based on the RGR.

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Table 1. Feeding schedule and ration for olive flounder larvae by days after hatching (DAH) in this study

DAH	Rotifer (Number mL^{-1})	*Artemia* (Number mL^{-1})	Amount of microdiet (#3) (g^{-1} time)	Amount of mixture of #3 and #4 microdiets at 1:1 (g^{-1} time)	Amount of microdiet (#4) (g^{-1} time)	Daily feeding frequency
0						
3-9	4-5					
10-13	9-10					
14-15	8-10	4-5				
16-18		10-12				
19-21		5-6	0.05			6
22-25		2-3	0.06			8
26-29			0.06			12
30-34			0.07			12
35-40				0.08		12
41-44					0.09	12

Size of #3 and #4 microdiets were 0.31–0.48 and 0.48–0.63 μm, respectively.

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Table 2. Feed ingredients of the experimental microdiets (%, dry matter basis)

	Experimental diets				
	CP42	CP46	CP50	CP54	CP58
Ingredients (%)					
Pollack meal[1]	28	32.5	37	41.5	46
Krill meal	30.5	32	33.5	35	36.5
Wheat gluten	4	4	4	4	4
Taurine	2.5	2.5	2.5	2.5	1.7
α-starch	2	2	2	2	0
Dextrin	20	14.3	8.6	2.9	0
Fish oil	6.5	6.2	5.9	5.6	5.3
Soybean lecithin	0.65	0.65	0.65	0.65	0.65
Vitamin premix[2]	4	4	4	4	4
Choline chloride (50 %)	0.85	0.85	0.85	0.85	0.85
Mineral premix[3]	1	1	1	1	1
Nutrients (%)					
Dry matter	94.0	94.0	94.2	94.1	94.2
Crude protein	42.6	46.2	50.4	54.3	58.0
Crude lipid	16.0	16.2	16.5	17.1	17.3
Ash	9.0	9.3	10.0	10.4	11.1
Estimated energy (kJ g^{-1})[4]	18.6	18.6	18.6	18.6	18.6

[1] Pollack meal imported from Russia.

[2] Vitamin premix contained the following amount which were diluted in brewer's yeast (mg kg^{-1} diet): L-ascorbic acid, 51.24; DL-α-tocopheryl acetate, 150.0; thiamin hydrochloride, 20.0; riboflavin, 40.0; pyridoxine hydrochloride, 20.0; nicotinic acid, 150.0; D-calcium-pantothenate, 70.0; inositol, 300.0; D-biotin, 0.2; folic acid, 10.0; p-aminobenzoic acid, 18.2; menadione sodium hydrogen sulfite, 10.0; retinyl acetate, 6.0; cyanocobalamin, 0.001.

[3] Mineral premix contained the following amount which were diluted in brewer's yeast (mg kg^{-1} diet): $MgSO_4 \cdot 7H_2O$, 496.92; $C_4H_2FeO_4$, 65.8; $FeSO_4$, 103.04; $CuSO_4$, 5.97; $CoSO_4.7H_2O$, 3.42; CaI_2, 3.91; $ZnSO_4$, 68.85; $Al(OH)_3$, 3.81; $MnSO_4 \cdot H_2O$, 65.8.

[4] Estimated energy calculated based on 16.8 kJ g^{-1} for protein and carbohydrate, and 37.8 kJ g^{-1} for lipid (Garling & Wilson, 1976).

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Table 3. Amino acid (AA) profiles of the main protein sources and experimental microdiets (% of the diet)

			Experimental diets				
	Pollack meal	Krill meal	CP42	CP46	CP50	CP54	CP58
Alanine	4.16	2.96	2.49	2.68	2.76	3.06	3.44
Arginine	4.27	3.34	2.61	2.78	2.90	3.20	3.53
Aspartic acid	7.17	5.77	4.25	4.50	4.71	5.13	5.70
Cystine	0.82	0.39	0.38	0.39	0.46	0.51	0.53
Glutamic acid	9.23	7.22	6.53	6.82	7.10	7.60	8.55
Glycine	3.49	2.46	2.48	2.68	2.82	3.09	3.53
Histidine	1.52	1.13	0.95	1.01	1.04	1.15	1.27
Isoleucine	3.29	2.92	2.08	2.21	2.36	2.58	2.79
Leucine	5.52	4.47	3.39	3.57	3.83	4.18	4.53
Lysine	5.89	4.06	3.38	3.62	3.74	4.13	4.63
Methionine	2.37	1.58	1.10	1.14	1.27	1.42	1.55
Phenylalanine	3.35	2.53	1.88	1.99	2.03	2.21	2.48
Proline	2.41	2.06	2.00	2.14	2.23	2.45	2.73
Serine	3.04	2.19	1.79	1.89	2.07	2.24	2.40
Threonine	3.11	2.41	1.83	1.95	2.11	2.31	2.46
Tyrosine	2.30	1.21	1.39	1.46	1.58	1.78	1.83
Valine	3.84	2.83	2.20	2.34	2.50	2.73	2.96

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Table 4. Survival (%), weight gain (mg fish^{-1}), specific growth rate (SGR), absolute growth rate (AGR), relative growth rate (RGR) and total length (mm) of olive flounder larvae at the end of feeding trial

Experimental diets	Initial weight (mg fish^{-1})	Final weight (mg fish^{-1})	Survival (%)	Weight gain (mg fish^{-1})	AGR[1] (mg day^{-1})	RGR[2] (%)	Total length (mm)
CP42	12.5 ± 0.03	58.6 ± 0.84^{c}	52.1 ± 1.07^{a}	46.0 ± 0.69^{b}	1.77 ± 0.027^{b}	367.2 ± 6.04^{b}	17.3 ± 0.34^{c}
CP46	12.5 ± 0.01	59.1 ± 0.27^{c}	53.0 ± 1.31^{a}	46.5 ± 0.30^{b}	1.79 ± 0.012^{b}	371.7 ± 2.59^{b}	17.9 ± 0.22^{bc}
CP50	12.5 ± 0.01	59.6 ± 0.31^{bc}	53.2 ± 1.33^{a}	47.1 ± 0.33^{b}	1.81 ± 0.013^{b}	375.7 ± 2.64^{b}	18.1 ± 0.04^{ab}
CP54	12.5 ± 0.01	61.2 ± 0.35^{a}	54.6 ± 1.51^{a}	48.6 ± 0.35^{a}	1.86 ± 0.013^{a}	387.1 ± 2.78^{a}	18.8 ± 0.32^{a}
CP58	12.6 ± 0.01	61.1 ± 0.58^{ab}	55.0 ± 1.10^{a}	48.6 ± 0.15^{a}	1.87 ± 0.006^{a}	387.2 ± 1.05^{a}	18.8 ± 0.20^{a}

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).
[1]Absolute growth rate (AGR) = (Final weight of fish – initial weight of fish/days of feeding
[2]Relative growth rate (RGR) = (Final weight of fish – initial weight of fish)×100/initial weight of fish

Table 5. Proximate composition (% of wet weight) of the whole body of olive flounder larvae at the end of feeding trial

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
CP42	81.0 ± 0.38^{a}	11.4 ± 0.24^{a}	2.4 ± 0.06^{c}	1.8 ± 0.01^{a}
CP46	81.4 ± 0.42^{a}	11.5 ± 0.29^{a}	2.5 ± 0.05^{bc}	1.7 ± 0.03^{a}
CP50	82.1 ± 0.31^{a}	11.3 ± 0.18^{a}	2.5 ± 0.03^{bc}	1.7 ± 0.05^{a}
CP54	82.1 ± 0.22^{a}	11.5 ± 0.13^{a}	2.6 ± 0.02^{ab}	1.7 ± 0.04^{a}
CP58	81.9 ± 0.43^{a}	11.9 ± 0.27^{a}	2.7 ± 0.04^{a}	1.7 ± 0.04^{a}

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

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Table 6. Whole-body amino acid (AA) profiles of olive flounder larvae at the end of the feeding trial (% of wet weight)

	Experimental diets				
	CP42	CP46	CP50	CP54	CP58
Alanine	0.74 ± 0.015	0.73 ± 0.013	0.74 ± 0.049	0.77 ± 0.024	0.76 ± 0.020
Arginine	0.66 ± 0.018	0.71 ± 0.010	0.65 ± 0.049	0.70 ± 0.052	0.67 ± 0.038
Aspartic acid	1.10 ± 0.032	1.16 ± 0.026	1.09 ± 0.087	1.16 ± 0.080	1.10 ± 0.068
Cystine	0.13 ± 0.003	0.13 ± 0.003	0.13 ± 0.003	0.14 ± 0.009	0.12 ± 0.003
Glutamic acid	1.60 ± 0.047	1.68 ± 0.027	1.61 ± 0.098	1.72 ± 0.075	1.65 ± 0.064
Glycine	0.80 ± 0.015	0.80 ± 0.010	0.79 ± 0.038	0.81 ± 0.035	0.80 ± 0.025
Histidine	0.27 ± 0.003	0.28 ± 0.003	0.27 ± 0.006	0.27 ± 0.018	0.29 ± 0.033
Isoleucine	0.50 ± 0.003	0.52 ± 0.007	0.50 ± 0.023	0.53 ± 0.024	0.51 ± 0.024
Leucine	0.93 ± 0.003	0.95 ± 0.012	0.92 ± 0.031	0.95 ± 0.048	0.94 ± 0.055
Lysine	0.96 ± 0.013	0.95 ± 0.026	0.95 ± 0.039	1.01 ± 0.046	0.97 ± 0.034
Methionine	0.35 ± 0.009	0.35 ± 0.018	0.35 ± 0.015	0.37 ± 0.015	0.32 ± 0.012
Phenylalanine	0.49 ± 0.003	0.48 ± 0.009	0.47 ± 0.026	0.49 ± 0.023	0.45 ± 0.020
Proline	0.45 ± 0.015	0.46 ± 0.012	0.42 ± 0.046	0.44 ± 0.028	0.45 ± 0.017
Serine	0.52 ± 0.029	0.54 ± 0.017	0.51 ± 0.044	0.53 ± 0.038	0.53 ± 0.035
Threonine	0.58 ± 0.009	0.60 ± 0.003	0.57 ± 0.023	0.58 ± 0.034	0.56 ± 0.027
Tyrosine	0.34 ± 0.031	0.32 ± 0.020	0.33 ± 0.012	0.38 ± 0.012	0.37 ± 0.015
Valine	0.60 ± 0.003	0.62 ± 0.006	0.61 ± 0.023	0.63 ± 0.023	0.63 ± 0.038

None of AA profiles of the whole body of fish (means of triplicate ± SE) was significantly affected by crude protein levels in granulated microdiets ($P > 0.05$)

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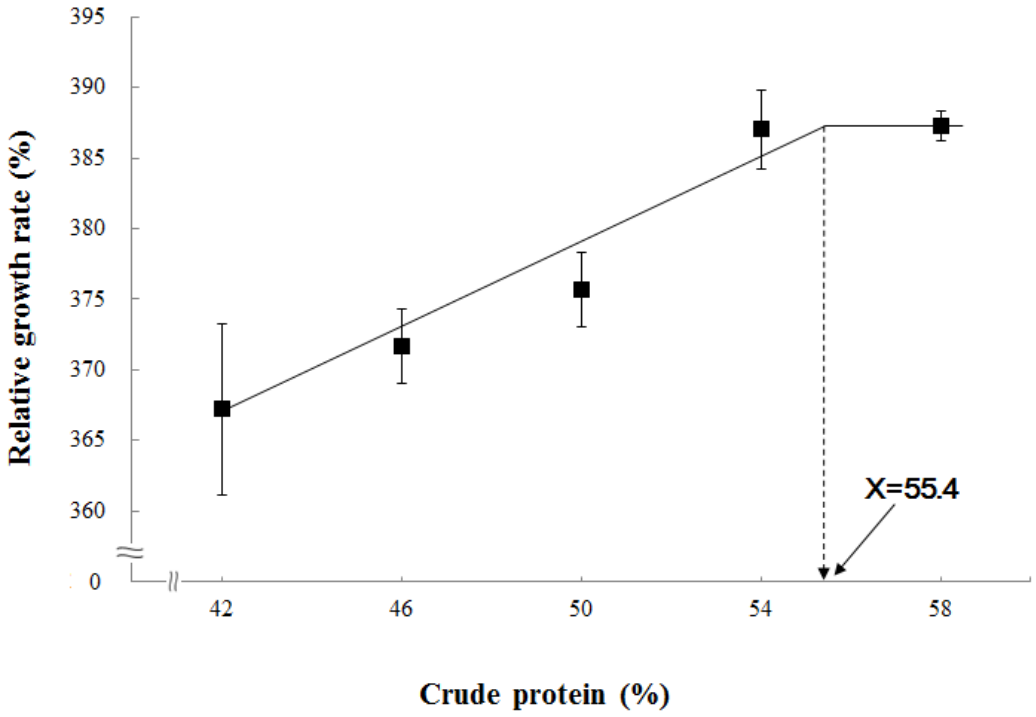

Figure 1. Effect of protein levels in granulated microdiets on relative growth rate of olive flounder larvae (means of triplicate ± SE). Y = 387.2 – 1.59 (R – X_{LR}), R = 55.4 ± 2.93 (SE).