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Optimum dietary protein level in granulated microdiet for larval rockfish *Sebastes schlegeli* Hilgendorf 1880

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The Graduate School

Korea Maritime and Ocean University

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by

Bok-Il Jang

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Optimum dietary protein level in granulated microdiet for larval rockfish *Sebastes schlegeli* Hilgendorf 1880

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Abstract

The object of this study is to determine protein requirement in granulated microdiet for larval rockfish. Rockfish broodstocks were stocked into a 20-ton fiberglass reinforced plastic (FRP) circular tank that was filled with seawater sterilized by ultraviolet radiation. Broodstocks were taken out after parturition from the tank and newborn larvae were held in the tank until 9-DAP. 7,200 larvae were randomly transferred to 24, 80-L square plastic tanks (300 larvae per tank) at 9-DAP for the feeding trial. Rotifer were fed for 1-DAP to 6-DAP larval rockfish, *Artemia* nauplii and granulated microdiets (#3 and #4) were fed for 6-DAP to 10-DAP and for 10-DAP to 29-DAP larval rockfish, respectively as larval fish grew. 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 fish oil at constant estimated energy level (4.42 kcal/g diet) were prepared in two size (0.31-0.48, 0.48-0.63 μm). Fish meal, soluble fish protein concentrate, krill meal, wheat gluten and taurine were used as the primary protein sources in the experimental diets. Alpha-starch and dextrin and fish oil were used as the carbohydrate and lipid sources in the experimental diets. Granulated microdiets were carefully hand-fed to larvae 8-12 times a day between 06:00 and 18:00 h.

In the granulated microdiets, as the protein levels increased, all essential and nonessential amino acids (EAA and NEAA) contents increased. The survival of larval rockfish was not significant ($P > 0.05$) different among the experimental diets. Weight gain and growth rate of larval rockfish fed the CP54 diet were significantly ($P < 0.05$) higher than that of larval fish fed the all other diets (CP42, CP46, CP50 and CP58 diets). Broken-line model [Y = 198.4 − 2.18 (R − X_{LR}), R=54.0 ± 1.77 (SE)] showed that dietary protein requirement was estimated to be 55.4% based on weight gain of larval rockfish. Amino acid profiles of the whole-body of larval rockfish fed the experimental diets at the end of the feeding trial were not significantly ($P > 0.05$) affected by protein levels in the granulated microdiets, except for histidine. Moisture, crude protein and crude lipid content of the whole-body of larval rockfish were not significantly ($P > 0.05$) different among the experimental diets except ash. according to these results, protein requirement for larval rockfish was estimated to be 54.0% based on the broken-line model.

KEY WORDS: Rockfish (*Sebastes schlegeli*) larvae; Granulated microdiet; Protein requirement

자어기 조피볼락용 과립형 미립자 사료내 적정 단백질 요구량

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요 약

본 연구는 산출 후 자어기 조피볼락용 과립형 미립자 사료내 적정 단백질 요구량을 규명하였다. 20톤의 유수식 Fiber glass reinforced plastic (FRP) 수조에 어미 조피볼락을 수용한 뒤 산출 후 9일령까지 자어기 조피볼락을 20톤 FRP 수조에서 사육하였다. 총 7,200 마리의 자어기 조피볼락을 무작위로 24개의 유수식 80-L 사각수조에 수용하였다. 산출 후 1일령부터 6일령까지 로티퍼를 먹이로 공급하였고, 산출 후 6일령부터 10일령까지 알테미아를 공급하였고, 산출 후 10일령부터 실험의 종료시(산출 후 29일)까지 제조한 조피볼락용 과립형 미립자 사료를 공급하여 주었다. 단백질 함량을 달리한 총 5종류(조단백질 함량: 42%, 46%, 50%, 54%, 58%)의 실험사료(CP42, CP46, CP50, CP54, CP58)를 2종류 크기(0.31-0.48, 0.48-0.63 μm)로 준비하였다. 실험 사료내 정어리, 가수분해 농축분, 크릴분, wheat gluten과 taurine을 주요 단백질원, α-전분과 덱스트린을 주요 탄수화물 및 어유를 주요 지질원으로 각각 사용하였다. 실험사료는 1일 8~12회까지 공급하였으며, 총 실험기간은 산출 후 29일령 자어기까지이었다.

실험사료내 단백질 함량이 증가함에 따라서 필수아미노산과 비필수아미노산 함량이 증가하였다. 사육실험 종료시 생존한 조피볼락의 생존율은 실험구간에 유의적인 차이를 보이지 않았다. 실험 종료시 생존한 조피볼락의 어체중 증가(Weight gain)와 성장률(Growth rate)은 단백질 함량이 54%인 사료(CP54)를 공급한 실험구에서 다른 사료 공급구보다 유의적으로 높게 나타났으며, 그 다음으로 CP58, CP50, CP46 및 CP42의 순으로 높게 나타났다. 실험종료시 생존한 조피볼락 자어기의 전장도 CP54를 공급한 실험구에서 유의적으로 길었다. 조피볼락 자어기의 적정 단백질 요구량은 Broken-line model에 의하여 54.0%인 것으로 평가되었다. 조피볼락 자어기 전어체의 아미노산 조성은 Histidine을 제외하고 실험사료내 단백질 함량에 따른 차이를 보이지 않았다. 자어기 전어체의 일반성분 분석 결과, 회분을 제외하고 수분, 조단백질과 조지질 함량은 차이가 없었다. 이상의 결과를 고려하면 조피볼락 자어기용 과립형 미립자 사료내 단백질 요구량은 54%인 것으로 판단된다.

KEY WORDS: Rockfish (*Sebastes schlegeli*) larvae 조피볼락 자어기; Granulated microdiet 과립형 미립자 사료; Protein requirement 단백질 요구량

1. Introduction

Rockfish (*Sebastes schlegeli*) has been one of the most important marine fish species in Korea due to its fast growth and resistance against disease over three decade ago. Annual aquaculture production in Korea has been rapidly increased and reached 17,996 tons in 2016 (KOSIS, 2017). A variety of studies, such as dietary nutrient requirement (Lee et al., 1993; Bai et al., 1996; Lee et al., 1998; Kim et al., 2001; Lee, 2001; Lee et al., 2002; Kim et al., 2004; Yan et al., 2007), alternative animal and/or plant protein sources for fish meal in the diet (Lim et al., 2004; Jeon et al., 2014; Lee & Choi, 2013), optimum feeding frequency and rate (Mizanur et al., 2014), feeding stimulants/attractants (Takaoka et al., 1990), inclusion effect of dietary supplements (Lee et al., 2008; Park et al., 2008; Lim et al., 2009) for rockfish have been reported to date. However, a few studies to produce larvae of rockfish fed with live prey have been reported (Cho et al., 2001). Securing healthy larvae is very important to keep increasing aquaculture production of fish.

Live prey is commonly supplied to larval marine fish to enhance quantity and quality of larvae in 2-3 days after hatching (DAH) for oviparous fish species or parturition (DAP) for viviparous fish species (Miyashita et al., 1997). However, enrichment of live prey is needed because of its unbalanced- or deficient-nutrition before supplying to larval fish (Conceicao et al., 1998, 2003; Aragão et al., 2004; Saavedra et al., 2006). To raise and produce larval production systems with live prey generally needs more space, cost, time and labor of larval fish producer than to feed microdiet to larvae unless the highly concentrated live prey is prepared and ready to use.

The study to rear marine larval fish successfully by supplying live prey, such as rotifer (*Brachionus* sp) or *Artemia* nauplii is well established (Miyashita et al., 1997; Stottrup & Mcevoy, 2003). Live prey is commonly supplied to larval rockfish in 2-4 days after parturition (Cho et al., 2001). Rotifers are commonly supplied to many marine fish species for first feeding when larval digestive system is not well or completely developed (Govoni et al., 1986; Cho et al., 2001; Teshima et al., 2004; Rønnestad & Conceição, 2005). Development of microdiets to replace live prey for larval marine fish, such as Pacific bluefin tuna (*Thunnus orientalis*) (Takeuchi & Haga, 2015), red sea bream (*Pagrus major*) (Teshima et al., 2004), red drum (*Sciaenops ocellatus*) (Brinkmeyer & Holt, 1995), olive flounder (*Paralichthys olivaceus*) (Bai et al., 2001; Takeuchi et al., 2003; Wang et al., 2004; Li et al., 2013; Ha et al., 2018), gilthead seabream (*Sparus aurata*) (Saleh et al., 2013), Atlantic cod (*Gadus morhua*) (Johnson et al., 2009) have been successfully reported.

Protein including amino acids is critical molecules because of the role they paly in the structure and metabolism of all living organisms. Fish species cannot synthesize all essential amino acids (EAA) and must be supplied with amino acids, through the consumption of protein or mixture of amino acids (National Research Council, NRC, 2011). In addition, protein is one of the most important components of fish diet because it provides the EAA and the nitrogen sources for energy to growth (Kim et al., 2001). Amino acids are also required as precursors for various metabolites, neurotransmitters, hormones, cofactors, etc. (NRC, 2011).

Dietary protein requirement varies depending on fish size (Dabrowski, 1984; Ye, et al., 2015). Dietary protein requirement for young (small) fish is known to be higher than for old (large) one (Wilson & Halver, 1986; NRC, 1993; Einen & Roem, 1997). Dietary protein requirements of juvenile rockfish (initial weight of 3.2 g, 7.3 g and 21.9 g) were reported to be 50%, 48.6% and 42%, respectively (Lee et al., 2002; Kim et al., 2001; Cho et al., 2015). However, no study on nutrient requirements in diet for larval rockfish has been reported yet.

Granulated microdiet has been recently developed in Korea. However, protein requirement in granulated microdiet for larval rockfish has not been performed. The object of this study is, therefore, to determine protein requirement in granulated microdiet for larval rockfish.

2. Materials and Methods

2.1 Spawning and larval rearing conditions

Female rockfish broodstocks, which had fully distended in abdominal and appeared to be near parturition, were selected and transferred from Heaksando (Shinan-gun, Jeollanam-do, Korea) to Sinbi hatchery (Namhae-gun, Gyeongsangnam-do, Korea). Rockfish broodstocks were stocked into a 20-ton fiberglass reinforced plastic (FRP) circular tank that was filled with seawater sterilized by ultraviolet radiation. Broodstocks were taken out after parturition from the tank and newborn larvae were held in the tank until 9-DAP. Water temperature ranged from 16.9°C – 21.0°C (mean temperature ± SD: 18.9 ± 0.71°C) in the tank.

2.2 Feeding schedule for larval fish

Feeding schedule and feeding ration for larval rockfish after parturition are described in Table 1. Rotifer were fed for 1-DAP to 6-DAP larval rockfish, *Artemia* nauplii and granulated microdiets (#3 and #4) were fed for 6-DAP to 10-DAP and for 10-DAP to 29-DAP larval rockfish, respectively as larval fish grew. Rotifer and *Artemia* nauplii were enriched by S.presso (INVE, Dendermonde, Belgium) before feeding to larval fish. Granulated microdiets were carefully hand-fed to larvae 8-12 times a day between 06:00 and 18:00 h.

Table 1 Feeding schedule and ration for larval rockfish day after parturition (DAP) in this study

DAP (day after parturition)	Rotifer (Number/mL)	*Artemia* (Number/mL)	Amount of microdiet (#3) (g/time)	Amount of mixture of #3 and #4 microdiets at 1:1 (g/time)	Amount of microdiet (#4) (g/time)	Daily feeding frequency
0						
1-5	12					
6	6	6				
7-9		12				
10		4	0.02			8
11-14			0.04			12
15-20			0.20			12
21-23			0.47			12
24-27				0.50		12
28-29					0.58	12

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

2.3 Preparation of the experimental diets

Ingredients and nutrient contents of the granulated microdiets are presented in Table 2. Fish meal, soluble fish protein concentrate, krill meal, wheat gluten and taurine were used as the primary protein sources in the experimental diets. Alpha-starch and dextrin and fish oil were used as the carbohydrate and lipid sources, respectively, in the experimental diets. Five granulated microdiets containing different levels of crude protein ranging from 42% to 58% with 4% increments at the expense of dextrin and fish oil at constant estimated energy level (4.42 kcal/g diet) were prepared in triplicate. 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 granulated 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).

Table 2 Feed ingredients of the experimental microdiets (%, DM basis)

	Experimental diets				
	CP42	CP46	CP50	CP54	CP58
Ingredients (%)					
Sardine[a]	16	18	20	22	24
Soluble fish protein concentrate[b]	16	18	20	22	24
Krill meal	27	29	31	33	35
Wheat gluten	2	2	2	2	2
Taurine	2.5	2.5	2.5	2.5	2.5
α-starch	3	3	3	3	2
Dextrin	21	15.5	10	4.5	0
Fish oil	6	5.5	5	4.5	4
Soybean lecithin	0.65	0.65	0.65	0.65	0.65
Vitamin premix[c]	4	4	4	4	4
Choline chloride (50%)	0.85	0.85	0.85	0.85	0.85
Mineral premix[d]	1	1	1	1	1
Nutrients (%)					
Dry matter	98.2	98.3	98.7	99.1	98.5
Crude protein	42.4	46.5	50.3	54.2	58.2
Crude lipid	14.0	14.4	14.5	14.7	15.1
Ash	8.8	8.5	8.6	8.5	8.5
Estimated energy (kcal/g)[e]	4.42	4.42	4.42	4.42	4.42

[a]Sardine imported from Chile.

[b]Soluble fish protein concentrate from France

[c]Vitamin premix contained the following amount which were diluted in brewer's yeast (mg kg/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.

[d]Mineral premix contained the following amount which were diluted in brewer's yeast (mg kg/diet): $MgSO_4·7H_2O$, 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; $MnSO_4·H_2O$, 65.8.

[e]Estimated energy calculated based on 4 kcal/g for protein and carbohydrate, and 9 kcal/g for lipid (Garling & Wilson, 1976).

2.4 Experimental conditions

7,200 larvae were randomly transferred to 24, 80-L square plastic tanks (300 larvae per tank) at 9-DAP for the feeding trial. Two size of granulated microdiets (0.31-0.48 and 0.48-0.63 μm) were fed to larvae for 20 days. #3 diet were fed 10-27-DAP and #4 diet were fed 24-29-DAP as the fish grew. MIC-F (INVE, Dendermonde, Belgium) and PRO-W (INVE, Dendermonde, Belgium) were applied to each tank to purify water and to enhance larvae condition at 1 g per tank throughout the feeding trial. Seawater sterilized by ultraviolet radiation was supplied at a flow rate of 0.45 L/min/tank. The photoperiod followed natural condition during the feeding trial. At the end of the feeding trial, all surviving fish from each tank were collectively weighed and sampled for measurement of growth and nutritional analysis. All sample were stored at −70°C before analysis.

2.5 Analytical procedures for the microdiets and larval rockfish

All surviving larval fish from each tank had been frozen and then thawed for chemical analysis. The fifty larval fish that had been randomly chosen from each tank were measured for total 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 composition of the experimental microdiets and larval fish were determined by using a high speed amino acid analyzer (Hitachi L-8800, Tokyo, Japan) after which the samples were hydrolyzed in 6 N HCl for 24 h at 110°C.

2.6 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 SPSS version 19.0 program (SPSS Michigan Avenue, Chicago, IL, USA). Broken-line analysis (Robbins et al., 1979) was used to determine dietary protein requirement of larval rockfish using SAS version 9.3 program (SAS Institute, Cary, NC, USA). Percentage data were arcsine-transformed before statistical analysis.

3. Results

3.1 Amino acid profiles of the experimental diets

Amino acid profiles, total amino acid (TAA), sum of EAA, the ratio of EAA to TAA (EAA/TAA) in the main protein sources and experimental diets were presented in Table 3. In the granulated microdiets, as the protein levels increased, all EAA contents increased. Aspartic and glutamic acids, and leucine and lysine were the most abundant EAA and nonessential amino acids (NEAA) in the experimental diets, respectively. EAA/TAA in experimental diets ranged from 0.51 to 0.52.

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

	Sardine meal	Soluble fish protein concentrate	Krill meal	Experimental diets				
				CP42	CP46	CP50	CP54	CP58
Alanine	4.16	4.61	2.96	2.52	2.70	2.92	3.12	3.35
Arginine	4.27	4.45	3.34	2.65	2.81	3.00	3.20	3.42
Aspartic acid	7.17	6.10	5.77	4.14	4.38	4.68	4.99	5.28
Cystine	0.82	0.60	0.39	0.40	0.42	0.50	0.51	0.59
Glutamic acid	9.23	8.55	7.22	6.17	6.48	6.90	7.30	7.63
Glycine	3.49	6.98	2.46	2.71	2.86	3.09	3.31	3.56
Histidine	1.52	1.40	1.13	0.95	1.03	1.13	1.21	1.28
Isoleucine	3.29	2.51	2.92	2.01	2.12	2.28	2.42	2.58
Leucine	5.52	4.28	4.47	3.27	3.47	3.74	3.95	4.23
Lysine	5.89	4.81	4.06	3.11	3.34	3.60	3.87	4.14
Methionine	2.37	1.96	1.58	1.17	1.22	1.36	1.47	1.60
Phenylalanine	3.35	2.59	2.53	1.82	1.92	2.07	2.20	2.32
Proline	2.41	3.73	2.06	1.82	1.92	2.07	2.20	2.32
Serine	3.04	3.03	2.19	1.77	1.87	1.99	2.12	2.25
Threonine	3.11	2.76	2.41	1.86	1.97	2.11	2.25	2.37
Tyrosine	2.30	1.67	1.21	1.63	1.70	1.83	1.93	2.05
Valine	3.84	3.18	2.83	2.12	2.26	2.44	2.60	2.76
TAA	65.8	63.2	49.5	40.1	42.5	45.7	48.7	51.7
∑EAA	35.5	29.6	26.5	20.6	21.8	23.6	25.1	26.8
EAA/TAA	0.54	0.47	0.53	0.51	0.51	0.52	0.52	0.52

3.2 Growth performance of rockfish

Survival (%), weight gain (mg/fish) and growth rate (%) of the larval rockfish fed the granulated microdiets are given in Table 4. The survival of larval rockfish was not significant ($P > 0.05$) different among the experimental diets. Weight gain of larval rockfish fed the CP54 diet was significantly ($P < 0.05$) higher than that of larval fish fed the all other diets (CP42, CP46, CP50 and CP58 diets) (Fig. 1). Growth rate (%) of larval rockfish fed the CP54 diet was significantly ($P < 0.05$) higher than that of larval fish fed the all other diets (CP42, CP46, CP50 and CP58 diets) (Fig. 2). Weight gain and growth rate of larval rockfish fed the CP58 diet were significantly ($P < 0.05$) higher than those of larval fish fed the CP50, CP46 and CP42 diets. Weight gain and growth rate of larval rockfish fed the CP50 diet were also significantly ($P < 0.05$) higher than those of larval fish fed the CP42 and CP46 diets. The total length of larval rockfish fed the CP54 diet was significantly ($P < 0.05$) longer than that of larval fish fed the all other diets (CP42, CP46, CP50 and CP58 diets) (Fig. 3).

Broken-line model [$Y = 198.4 - 2.18 (R - X_{LR})$, $R=54.0 \pm 1.77$ (SE)] showed that dietary protein requirement was estimated to be 55.4% based on weight gain of larval rockfish (Fig. 4).

Table 4 Survival (%), weight gain (mg/fish), growth rate (%) and total length (mm) of larval rockfish at the end of feeding trial

Experimental diets	Initial weight (mg/fish)	Final weight (mg/fish)	Survival (%)	Weight gain (mg/fish)	Growth rate[1] (%)	Total length (mm)
CP42	13.7 ± 0.00	187.8 ± 0.33^{b}	54.3 ± 2.65	174.1 ± 0.33^{d}	1273.8 ± 2.41^{d}	22.2 ± 0.01^{d}
CP46	13.7 ± 0.00	190.9 ± 0.88^{b}	54.6 ± 1.56	177.2 ± 0.88^{d}	1296.7 ± 6.47^{d}	22.2 ± 0.00^{cd}
CP50	13.7 ± 0.00	201.9 ± 1.00^{ab}	54.9 ± 1.82	188.2 ± 1.00^{c}	1377.3 ± 7.34^{c}	22.2 ± 0.01^{c}
CP54	13.7 ± 0.00	217.2 ± 1.07^{a}	55.2 ± 0.91	203.5 ± 1.07^{a}	1488.9 ± 7.86^{a}	22.5 ± 0.00^{a}
CP58	13.7 ± 0.00	211.0 ± 1.57^{a}	55.2 ± 1.13	197.3 ± 1.57^{b}	1443.7 ± 11.51^{b}	22.3 ± 0.01^{b}

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

[1]Growth rate (%) = Final weight of fish/initial weight of fish × 100

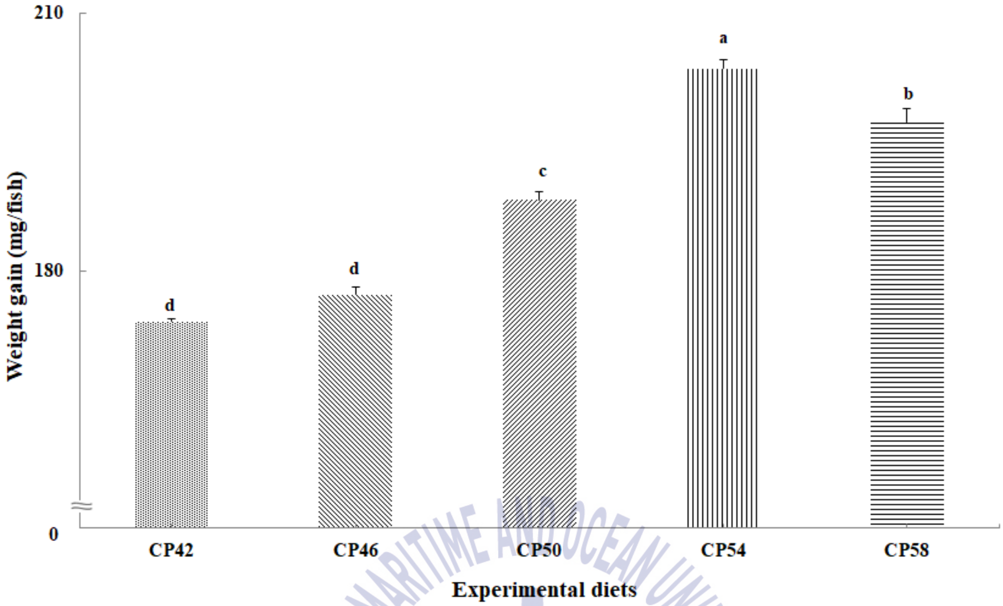

Figure 1 Weight gain (mg/fish) of rockfish at the end of feeding trial

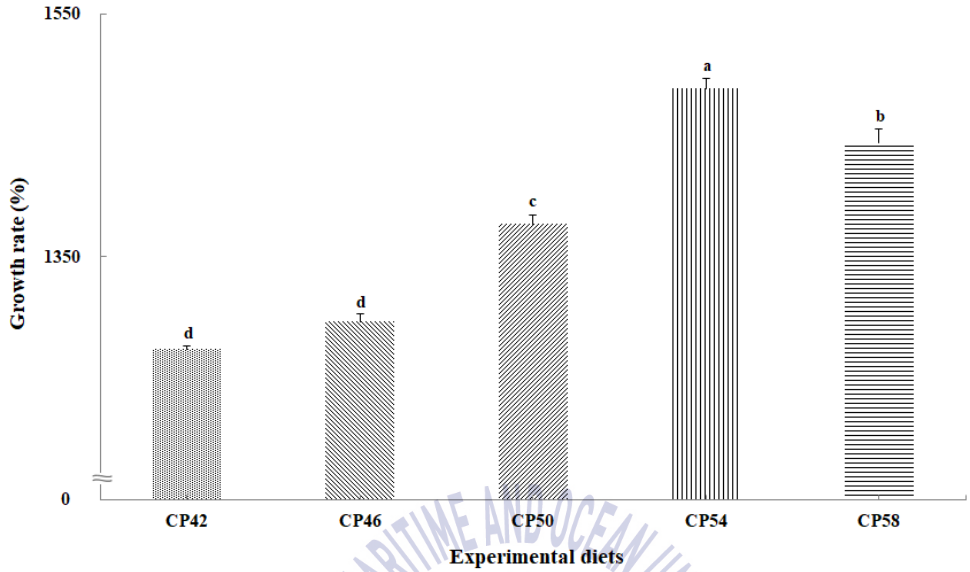

Figure 2 Growth rate (%) of rockfish at the end of feeding trial

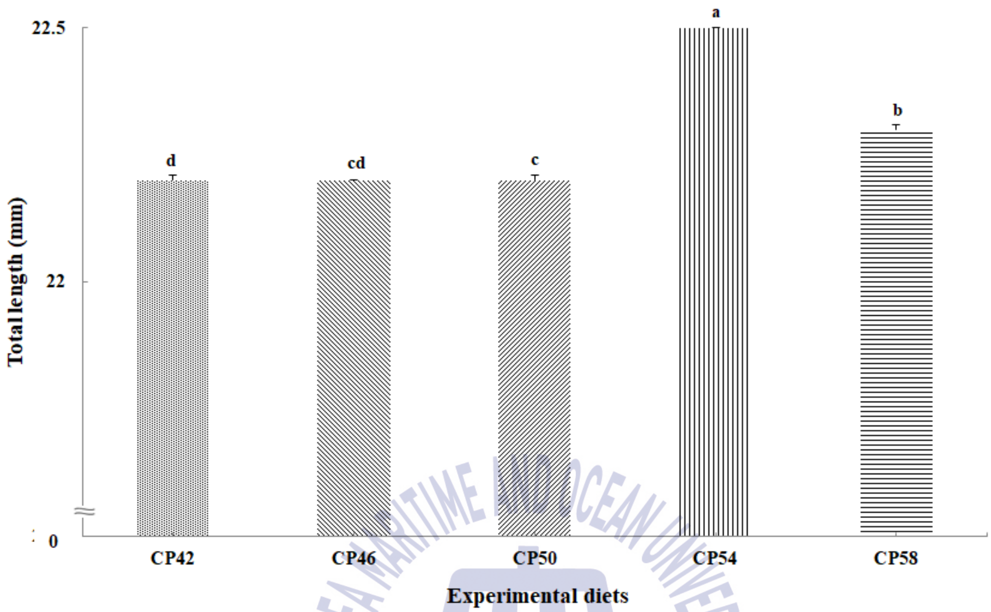

Figure 3 Total length (mm) of rockfish at the end of feeding trial

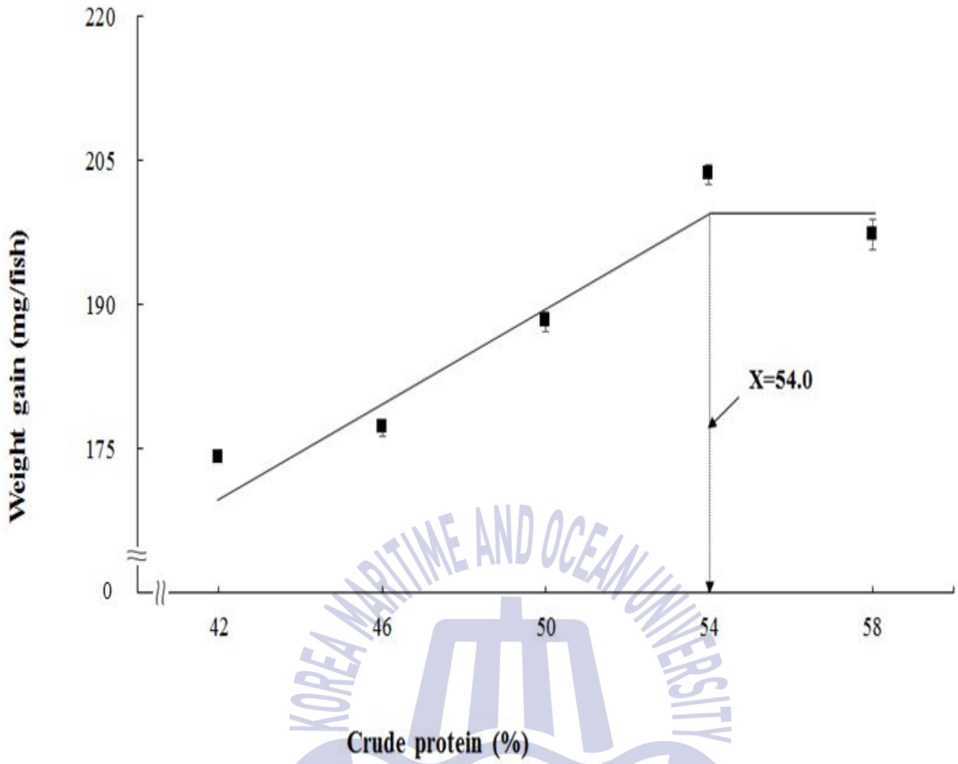

Figure 4 Effect of protein levels in granulated microdiets on weight gain (mg/fish) of rockfish larvae (means of triplicate ± SE). $Y = 198.4 - 2.18\ (R - X_{LR})$, $R = 54.0 \pm 1.77$ (SE).

3.3 Proximate composition of the whole-body of rockfish

The proximate composition of the whole-body of larval rockfish at the end of the feeding trial was given in Table 5. Moisture, crude protein and crude lipid content of the whole-body of larval rockfish were not significantly ($P > 0.05$) different among the experimental diets. However, ash content of the whole-body of fish fed the CP46 diet was significantly ($P < 0.05$) higher than that of fish fed the all other diets (CP42, CP50, CP54 and CP58 diets).

Table 5 Proximate composition (% of wet weight) of the whole-body of larval rockfish at the end of feeding trial

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
CP42	80.4 ± 0.10	12.4 ± 0.12	2.4 ± 0.10	3.0 ± 0.02^{bc}
CP46	80.3 ± 0.20	12.5 ± 0.09	2.5 ± 0.09	3.2 ± 0.08^{a}
CP50	80.5 ± 0.15	12.5 ± 0.08	2.5 ± 0.11	3.0 ± 0.05^{bc}
CP54	80.3 ± 0.12	12.5 ± 0.12	2.5 ± 0.06	2.9 ± 0.02^{c}
CP58	80.2 ± 0.20	12.6 ± 0.09	2.5 ± 0.13	3.0 ± 0.06^{c}

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

3.4 Amino acid profiles of whole-body of rockfish

Amino acid profiles of the whole-body of larval rockfish fed the experimental diets at the end of the feeding trial were not significantly ($P > 0.05$) affected by protein levels in the granulated microdiets, except for histidine (Table 6). No distinctive trend in amino acid profiles of the whole-body larval fish was observed.

Table 6 Amino acid profiles of the whole-body larval rockfish at the end of the feeding trial (% of wet weight)

	Experimental diets				
	CP42	CP46	CP50	CP54	CP58
Alanine	0.71 ± 0.030	0.75 ± 0.046	0.71 ± 0.017	0.70 ± 0.017	0.67 ± 0.021
Arginine	0.73 ± 0.025	0.79 ± 0.042	0.71 ± 0.021	0.71 ± 0.023	0.67 ± 0.040
Aspartic acid	1.13 ± 0.042	1.12 ± 0.012	1.10 ± 0.019	1.09 ± 0.024	1.07 ± 0.042
Cystine	0.13 ± 0.009	0.14 ± 0.003	0.13 ± 0.000	0.17 ± 0.044	0.12 ± 0.003
Glutamic acid	1.55 ± 0.059	1.54 ± 0.009	1.53 ± 0.012	1.51 ± 0.032	1.48 ± 0.054
Glycine	0.76 ± 0.022	0.76 ± 0.020	0.76 ± 0.010	0.75 ± 0.023	0.74 ± 0.030
Histidine	0.27 ± 0.012[ab]	0.29 ± 0.006[a]	0.25 ± 0.006[b]	0.26 ± 0.003[b]	0.26 ± 0.010[b]
Isoleucine	0.48 ± 0.020	0.51 ± 0.025	0.47 ± 0.006	0.47 ± 0.010	0.52 ± 0.088
Leucine	0.82 ± 0.035	0.82 ± 0.009	0.81 ± 0.013	0.81 ± 0.019	0.77 ± 0.021
Lysine	0.74 ± 0.025	0.74 ± 0.032	0.74 ± 0.015	0.72 ± 0.007	0.71 ± 0.036
Methionine	0.34 ± 0.018	0.34 ± 0.015	0.33 ± 0.007	0.34 ± 0.012	0.34 ± 0.012
Phenylalanine	0.47 ± 0.015	0.46 ± 0.009	0.46 ± 0.000	0.45 ± 0.009	0.45 ± 0.017
Proline	0.51 ± 0.009	0.51 ± 0.009	0.50 ± 0.012	0.50 ± 0.017	0.48 ± 0.019
Serine	0.55 ± 0.017	0.55 ± 0.006	0.55 ± 0.003	0.54 ± 0.012	0.53 ± 0.022
Threonine	0.55 ± 0.019	0.55 ± 0.020	0.54 ± 0.003	0.54 ± 0.007	0.53 ± 0.015
Tyrosine	0.34 ± 0.012	0.41 ± 0.050	0.36 ± 0.026	0.37 ± 0.020	0.33 ± 0.007
Valine	0.58 ± 0.023	0.58 ± 0.007	0.57 ± 0.006	0.57 ± 0.015	0.56 ± 0.018

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

4. Discussion

The several studies reported that fish larvae have very high instantaneous growth rates compared to adult stages (Conceição, 1997; Kamler, 1992; Otterlei et al., 1999). Amino acids are absorbed at different rates in fish larvae and absorption efficiency vary between amino acids, and may also change with species and developmental stage (Rønnestad et al., 2001; Conceição et al., 2002; Saavedra et al., 2008). In larval stage, amino acids are used for major energy source (Rønnestad et al., 1999) and larval fish required high amino acid contents to satisfy growing biomass (Rønnestad et al., 2003). As dietary protein level increased, all EAA content increased in the experimental diets in this study (Table 3).

Several dietary EAA requirements for some larval marine fish are presented in Table 7. Requirements of arginine content ranged from 1.8 to 3.1% of diet in several fish, such as red sea bream, European sea bass (*Dicentrarchus labrax*), Asian sea bass (*Lates calcarifer*), olive flounder, black sea bream (*Sparus macrocephalus*) (López-Alvarado & Kanazawa, 1994; Tibaldi et al., 1994; Murillo-Gurrea et al., 2001; Alam et al., 2002a, b; Zhou et al., 2010). These values were relatively lower than one measured (3.2% in the CP54 diet) in this study. Requirements of lysine (Zhou et al., 2007; Zhang et al., 2008), methionine (Coloso et al., 1999; Zhou et al., 2006), threonine (Tibaldi & Tulli, 1999) and valine (Rahimnejad & Lee, 2013) in several marine fish were reported to be 2.33-2.48, 1.0-1.2, 1.1-1.3 and 0.9% of diets, respectively, but relatively lower than 3.87%, 1.47%, 2.25% and 2.60% of the CP54 diet in this study. This was in partially agreement with other studies showing that the smaller fish required higher dietary nutrient content than the larger one (Wilson & Halver, 1986; Lee et al., 1993; NRC, 1993; Einen & Roem, 1997; Kim et al., 2001; Lee et al., 2002;

Cho et al., 2015).

The EAA/TAA in the experimental diets for larval rockfish ranged from 0.51 to 0.52 in this study. Similarly, Green et al. (2002) reported that the EAA/TAA of 0.57 in the diet achieved the best growth in rainbow trout (*Oncorhynchus mykiss*) when the diets containing various levels of EAA/TAA ranged from 0.23 to 0.66 were fed for 6 weeks.

Survival of larval rockfish was not affected by dietary protein levels in the experimental diets in this study. Not only survival, but also growth rate of larval fish is the important factors to determine successful larval production of fish. Weight gain and growth rate of larval rockfish increased with the dietary protein level increased from 42% to 54%, but decreased in a further increase in protein level (58%) in this study. The broken-line model has been the most widely used method of evaluating nutrient requirement with aquatic species. This linear model using two straight lines to model the dose-response relationship (Robbins et al., 1979). Several amino acids and weight gain are apparently linearly related so that broken-line model was selected for nutrition requirement in this study. Protein requirement for larval rockfish was estimated to be 54.0% based on the broken-line model (Fig. 4).

When fish fed diets containing protein levels above the requirements, plateaus or decreases in weight gain are reported in some species (Siddiqui et al., 1988; Santiago & Reyes, 1991; El-Sayed & Teshima, 1992; Kim et al., 2003; Lee et al., 2003; Zhang et al., 2010; Ha et al., 2018). Similarly, Ha et al. (2018) estimated protein requirement of 55.4% in granulated microdiets for larval olive flounder (initial weight of 12.5 mg) were fed with one of five granulated microdiets containing 42% to 58% protein levels. Dietary protein requirements for commercially important larval marine fish are presented in Table 8. Biswas et al. (2009) reported that Pacific bluefin tuna juvenile (initial weight of 0.26 g) required 61.9% protein diet with 17.9% lipid, but higher dietary protein level resulted to decline in growth rate when larvae fish were fed with one of five experimental diets containing different protein to lipid level (72.8%/9.2%, 66.8%/14.8%, 61.9%/17.9%, 57.2%/21.9% and 53.3%/27.0%). These plateaus or deceases in weight gain of fish are directly resulted from high dietary protein level over requirement. As dietary protein level increased over requirement of fish, ammonia excretion increased in several fish carp (*Cyprinus carpio*), Indian major carp, (*Labeo rohita*), silver perch (*Bidyanus bidyanus*) (Zorm, 1984; Chakraborty & Chakraborty, 1998; Yang et al., 2002). Many studies of ammonia toxicity of several fish, such as gilthead sea bream, on weight loss or growth stagnation, were reported (Wajsbrot et al., 1993; Fivelstad et al., 1995; Ruyet et al., 1997; Lemarié et al., 2004).

Dabrowski (1984) explained that nutritional requirements change because do mechanisms of digestion and absorption during larval development. Cahu & Infante (2001) reported that the nutritional requirements of larval and juvenile fish are not similar and dietary formulation inducing good growth in juveniles leads poor larval growth and survival. Comparison of dietary protein requirement of 54.0% for larval rockfish in this study with juvenile rockfish (initial weight of 3.2 g, 7.3 g and 21.9 g) estimated to be approximately 50%, 48.6% and 42%, respectively (Lee et al., 2002; Kim et al., 2001; Cho et al., 2015) indicates that the younger (smaller) rockfish require the higher protein level than older (larger) one. This is in agreement with other studies showing that dietary protein requirement for young (small) fish was higher than for old (large) one (Wilson & Halver, 1986; NRC, 1993; Einen & Roem, 1997). Lee et al. (1993) also reported that daily protein requirements per 100 g of rockfish, were 0.99 g and 0.35 g respectively for small (initial weight of 8 g) and large size (initial weight of 300 g) groups. Mangalik (1986) reported that 3 g channel catfish (*Ictarulus punctatus*) required almost 4 times more protein per day than 250 g fish for best growth. Sweilum et al. (2005) showed that small fish (initial weight of 22.9 g) required a high-protein and low-energy (30%/10.5 kJ) diet, whereas large fish (initial weight of 38.9 g) required a low-protein and high-energy (25%/12.6 kJ) diet to achieve the best production of Nile tilapia (*Oreochromis niloticus*).

The proximates (moisture, crude protein and lipid) of the whole-body of larval rockfish fed the experimental diets was not affected by dietary protein level, except for ash in this study. Similarly, dietary protein level did not affect body composition of experimental fish (Lee et al., 2001; Lee et al., 2003; Ozorio et al., 2006; Sá et al., 2006; Wang et al., 2016).

Amino acid profiles of the whole-body of larval rockfish fed the experimental diets were not affected by protein level in the experimental diets, except for histidine. Similarly, differences in dietary protein levels did not change amino acid profiles of the whole-body of experimental fish.

Since digestion systems and sensory organs in the early stages of larval fish are not fully developed, more studies to detect supplied feed, increase consumption of microdiet supplied by inclusion of feeding stimulants/attractants or increase water stability of microdiet supplied are needed to minimize waste of microdiet, but to improve water quality in rearing tank and secure successful larval production of fish in future.

Table 7 Requirements of several dietary essential amino acids (% of the diet) for marine larval fish species

Essential amino acids	Fish species	Initial body weight (g/fish)	Estimated requirement (% of the diet)	References
Arginine	Olive flounder (*Paralichthys olivaceus*)	1.9	2.0-2.5	Alam et al., (2002a, b)
	European sea bass (*Dicentrarchus labrax*)	2.1	1.8	Tibaldi et al., (1994)
	Asian sea bass (*Lates calcarifer*)	2.6	1.8	Murillo-Gurrea et al., (2001)
	Black sea bream (*Sparus macrocephalus*)	10.5	2.8-3.1	Zhou et al., (2010)
	Red sea bream (*Pagrus major*)	15-DAH	2.5	López-Alvarado & Kanazawa, (1994)
	Rockfish (*Sebastes schlegeli*)	0.0137	3.2	This study
Lysine	Cobia (*Rachycentron canadum*)	1.25	2.33	Zhou et al., (2007)
	Yellow croaker (*Pseudosciaena crocea*)	1.23	2.48	Zhang et al., (2008)

	Rockfish	0.0137	3.87	This study
Methionine	Asian sea bass (*Lates calcarifer*)	2.59	1.0 (with 0.6% Cysteine)	Coloso et al., (1999)
	Cobia (*Rachycentron canadum*)	11.6	1.2 (with 0.7% Cysteine)	Zhou et al., (2006)
	Rockfish (*Sebastes schlegeli*)	0.0137	1.47	This study
Threonine	European sea bass	7.5	1.1-1.3	Tibaldi & Tulli, (1999)
	Rockfish	0.0137	2.25	This study
Valine	Red sea bream	32.04	0.9	Rahimnejad & Lee, (2013)
	Rockfish	0.0137	2.60	This study

Table 8 Requirements of dietary protein levels (%) for larval marine fish species

Fish species	Initial weight (mg) or day after hatching (DAH)	Feed type	Main protein source	Requirement (%)	References
Olive flounder (*Paralichthys olivaceus*)	12.5 mg	Granulated microdiet	Pollack meal, krill meal, wheat gluten, taurine	55.4	Ha et al., (2018)
Rockfish (*Sebastes schlegeli*)	13.7 mg	Granulated microdiet	Fish meal, soluble fish protein concentrate, krill meal, wheat gluten, taurine	54.0	This study
Pacific bluefin tuna (*Thunnus orientalis*)	260 mg	Moist pellet	Enzyme treated fish meal	61.9	Biswas et al., (2009)
Gilthead sea bream (*Sparus aurata*)	800 mg	ND[a]	Sardine meal	55.0	Vergara et al., (1996)
Sea bass (*Dicentrarchus labrax*)	15-DAH	Microencapsulated diet	Fish meal, casein hydrolysate	50.0	Péres et al., (1996)

ND[a]: not described

5. Conclusion

EAA and NEAA content increased as protein level increased in the experimental diets. Survival of larval rockfish was not affected by dietary protein level. However, weight gain and growth rate of larval fish fed the CP54 diet was higher than those of larval fish fed the other diets (CP42, CP46, CP50 and CP58 diets), followed by the CP58, CP50, CP46 and CP42 diets, in order. The proximates and amino acid profiles of the whole-body of larval rockfish fed the experimental diets was not affected by dietary protein level, except for ash and histidine. Protein requirement of the experimental diets was estimated to be 54.0% for 29-DAP larval rockfish based on weight gain (broken-line model).

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