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Substitution effect of fish meal and mixture of macroalgae with tunic meal of sea squirt (*Halocynthia roretzi*) in extruded pellet on growth performance and chemical composition of the soft body of abalone (*Haliotis discus*, Reeve 1846)

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Abstract

Substitution effect of fish meal (FM) and mixture of macroalgae (MA) with tunic meal of sea squirt (*Halocynthia roretzi*) (SS) in extruded pellet (EP) on growth performance and chemical composition of the soft body of juvenile abalone compared to *Undaria pinnatifida* was investigated. A total of 1260 juvenile abalone were randomly distributed into 18, 70-L plastic containers (seventy abalone per container). Six experimental diets were prepared in triplicate. Five diets were pelletized by an extruder pelleter like commercial EP. Fourteen percent FM and 25% mixture of MA (*U. pinnatifida*: *Saccharina japonica*: *Sargassum fusiforme* = 2:2:1) were included into the control (Con) diet. 50% and 100% of FM and mixture of MA were substituted with an equal amount of tunic meal of SS, referred to as the FM50, FM100, MA50 and MA100, respectively. Finally, dry *U. pinnatifida* was prepared to compare the

effect of EPs on growth performance of abalone. The experimental diets were fed to abalone once a day at a satiation level with a little leftover for 16 weeks. Weight gain and specific growth rate (SGR) of abalone fed the all EPs were greater than those of abalone fed the *Undaria*. Among the EPs, weight gain of abalone fed the MA50 and FM50 diets was greater than that of abalone fed the Con and FM100 diets, but not significantly different from that of abalone fed the MA100 diet. Crude protein and crude lipid contents of the soft body of abalone fed all EPs were higher than those of abalone fed the *Undaria*. In conclusion, FM and MA up to 50% and 100%, respectively, could be replaced with tunic meal of SS without retardation in growth performance of abalone when 14% FM and 25% mixture of MA was included in EP.

KEY WORDS: Abalone (*Haliotis discus*); Fish meal (FM); Macroalgae (MA); Tunic meal of sea squirt (*Halocynthia roretzi*); Extruded pellet (EP)

까막전복 (*Haliotis discus*) 치패용 EP (Extruded pellet) 사료내 어분 및 해조류 혼합분 대체원으로서 멍게 (*Halocynthia roretzi*) 피낭분의 첨가 효과

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요 약

까막전복 (*Haliotis discus*) 치패용 extruded pellet (EP)내 어분 및 해조류 혼합분 대체원으로서 멍게 (*Halocynthia roretzi*) 피낭분의 첨가가 전복의 생존율, 성장 및 체조성에 미치는 영향을 조사하였다. 전복 치패(평균 체중 ± SE: 3.1 ± 0.00 g) 1,260마리를 18개의 70-L 사각 수조에 각각 70마리씩 수용하였다. 총 5종류의 실험사료를 준비하였으며, 14%의 어분 및 25%의 해조류 혼합분을 첨가하여 제조한 대조구 사료(Con), Con 사료내 어분을 멍게 피낭분으로 50% 대체한 사료(FM50), 100% 대체한 사료(FM100), Con 사료내 해조류 혼합분을 멍게 피낭분으로 50% 대체한 사료(MA50), 100% 대체한 사료(MA100)와 자연산 먹이인 건조 미역(*Undaria*)을 준비하였으며, 실험구는 3반복을 두었다. 먹이는 전복 전체중의 2-3% 수준으로 1일 1회 (17:00) 충분히 공급하였으며, 총 16주간 공급하였다.

16주간의 사육실험 결과, 전복의 생존율은 82.9% 이상으로 나타났다. 증체량(weight gain)은 Con 사료를 공급한 실험구가 FM50 사료와 MA50 사료를 공급한 실험구에 비하여 낮게 나타났다. 그러나 FM100과 *Undaria*를

공급한 실험구보다 높게 나타났으며, MA100 사료를 공급한 실험구와는 차이가 나타나지 않았다. 일일성장률(specific growth rate, SGR)은 FM50과 MA100 사료를 공급한 실험구가 Con 사료와 MA50 사료를 공급한 실험구와는 차이가 없었으나, FM100 사료와 *Undaria*를 공급한 실험구보다 높게 나타났다. 모든 전복용 EP 사료구는 미역 공급구보다 우수한 성장을 보였다. 사육실험 종료시 생존한 전복의 각장, 각폭 및 가식부 무게는 전복의 성장 결과와 유사한 경향을 보였다. 전복의 가식부내 조단백질과 조지질 함량은 모든 EP 사료 공급구가 *Undaria* 공급구보다 높게 나타났다. 이상의 결과를 고려할 때 까막전복용 EP 사료내 어분 14% 및 해조류 혼합분 25% 첨가시 멍게 피낭분으로 50% 및 100%까지 각각 대체 가능하며, EP 사료내 해조류 혼합분 50%의 멍게 피낭분으로의 대체는 전복의 성장 향상에 효과적인 것으로 보인다.

KEY WORDS: 까막전복(*Haliotis discus*); 어분; 해조류 혼합분; 멍게 (*Halocynthia roretzi*) 피낭분; 상업용 사료

1. Introduction

With the growing global population and increasing demand for abalone as a luxury food source, the growth of abalone aquaculture has been stimulated (Bostock et al. 2010; Wang et al. 2014). Worldwide farmed abalone production has been increased sharply up to 153445 metric tons in 2016 (FishstatJ 2018), whereas Korea is the second largest abalone producing country in the world with 16027 metric tons in 2017 (KOSIS 2018). Because of the ecological and economical value of abalone, aquaculture of abalone has encouraged to produce more abalone with a minimum cost and high profit (Zhao et al. 2018).

Abalone are anatomically and biochemically adapted to digest macroalgae (MA), which forms an essential component of their natural diet in the wild (Garcia-Carreno et al. 2003). However, availability of MA, such as sea tangle *Saccharina japonica* and *Undaria pinnatifida* are exclusively available during winter season in Eastern Asia including Korea (Kim et al. 2016). In addition, protein [amino acid (AA)] and lipid [fatty acids (FA)] content of these MA do not satisfy nutrient requirement of abalone (Uki et al. 1986; Mai et al. 1995 a,b). The poor growth performance of abalone fed a single macroalgae compared to well formulated feed has been reported by many studies (Cho et al. 2006; Garcia-esquivel and Felbeck 2006; Cho et al. 2008; Cho and Kim 2012; Jung et al. 2016; Myung et al. 2016; Jang et al. 2017; Lee et al. 2017; Ansary et al. 2018a, b; Choi et al. 2018). Since these MA are expensive (US $3-4/kg) feed ingredient in commercial abalone diet and an international market price of MA has recently increased sharply due to high demand for human consumption and expansion of abalone aquaculture (Jang et al. 2017), development of alternative source for MA is highly needed in abalone feed.

Fish meal (FM) has been commonly represented as an ideal nutritional source of protein in aquafeeds (Mahmoud et al. 2015). FM contains indispensible AA and is

a rich source of long-chain omega-3 fatty acids, vitamin and minerals (Olsen and Hasan 2012). However, global prices of FM in 2016 have increased 215% since 2000 (FishstatJ 2018) due to increasing demand, unstable supply and the continuous expansion of aquaculture (Mahmoud et al. 2015). Therefore, it is very crucial to explore an alternative protein sources for FM in aquafeeds (Ayoola 2010; Hixson 2014; Henry et al. 2015; Djissou et al. 2016; Zhang et al. 2018).

Since tunic meal of sea squirt (SS) (*Halocynthia roretzi*) is usually left to the sea after deshelling for human consumption, it is regarded as a pollution source. Annual sea squirt aquaculture production in Korea was 2336 metric tons in 2000 and reached 26273 metric tons in 2017 (KOSIS 2018). The production of sea squirt in Korea is anticipated be continuously increased in future. In addition, the availability of crude protein (ca. 40%) and carbohydrate (ca. 46%) (Lee et al. 1998) content in tunic meal of SS seems to be relatively high. Choi et al. (2018) reported that FM could be replaced with tunic of SS up to 80% without retardation in growth of abalone when 20% FM was included into the experimental diet. Jang et al. (2017) also reported that *S. japonica* could be completely (100%) substituted with tunic of SS without retardation in performance of abalone when 20% MA was included into the experimental diet. However, inclusion effect of tunic meal of SS in extruded pellet (EP) on abalone is still unknown.

The aim of this study was, therefore, to investigate the effect of substituting FM and MA with tunic meal of SS on growth performance and the soft body composition of juvenile abalone (*H. discus*, Reeve 1846), which is endemic to the waters off Japan and Eastern Asia, including South Korea's Jeju Island (Han, 1998).

2. Materials and methods

2.1 Preparation of abalone and rearing conditions

Juvenile abalone (*H. discus*) were purchased from a private hatchery (Daegeon Fisheries, Jeju, Korea) and transferred to an abalone farm (Ocean and Fisheries Research Institute, Jeju Special Self-Governing Province, Jeju, Korea). Prior to initiation of the feeding trial, abalone were acclimated to the experimental conditions for two weeks and fed with the dry *Undaria japonica* once a day at the ratio of 2-3% of total biomass. A total of 1260 juvenile abalones (mean ± SD: 3.14 ± 0.00 g) were randomly distributed into 18, 70-L plastic rectangular containers (72.5 cm × 47 cm × 32 cm; 70 abalone per container). Six containers were placed into 3, 10-ton concrete flow-through raceway systems (water volume: 2.8 ton) at a flow rate of 110 L/min/raceway.

The sand-filtered seawater at temperature ranging from 16.8 to 20.7℃ (mean ± SD: 18.0 ± 0.71℃) and aeration was supplied into each raceway and the photoperiod followed natural conditions. The experimental diets were fed to abalone once a day (17:00 h) at a satiation level with a little leftover (about 2-3% biomass). Dead abalone was removed daily and the bottoms of the containers were cleaned daily. At the end of the 16-week feeding trial, abalone were harvested and collectively weighed from each container.

2.2 Preparation of the experimental diets

The experimental diets were pelletized by an extruder pelleter (Jyoda, Japan) in Ewha Oil and Fat Industry Co. Ltd (Busan, Korea). EPs were round-shapes and their sizes were 5 mm in diameter (1.5 mm in thickness). Six experimental diets in triplicate were prepared (Table 1). Fourteen percent FM and 25% mixture of MA (*U. pinnatifida*:*S. japonica*:*Sargassum fusiforme* = 2:2:1) were included in the

Table 1. Ingredient and chemical composition of the experimental diets (%, DM basis)

	Experimental diets					
	Con	FM50	FM100	MA50	MA100	*Undaria*
Ingredient (%)						
Fish meal (CP: 68.5%, CL: 8.9%)	14	7	0	14	14	
Soybean meal (CP: 56.1%, CL: 2.0%)	25	30	35	25	25	
Tunic of sea squirt (CP: 44.8%, CL: 1.1%)	0	3.5	7	12.5	25	
Macroalgae[1] (CP: 15.0%, CL: 2.1%)	25	25	25	12.5	0	
Corn gluten meal	4.4	4.4	4.4	4.4	4.4	
Shrimp meal	3	3	3	3	3	
Wheat flour	20	18.5	17	20	20	
Spirulina	0.6	0.6	0.6	0.6	0.6	
Yeast	0.2	0.2	0.2	0.2	0.2	
Dextrin	5	5	5	5	5	
Choline chloride (50%)	0.5	0.5	0.5	0.5	0.5	
Sea aroma	0.1	0.1	0.1	0.1	0.1	
Vitamin premix[2]	0.2	0.2	0.2	0.2	0.2	
Mineral premix[3]	2	2	2	2	2	
Nutrient (%)						
Dry matter	98.9	99.2	99.3	99.4	99.0	86.0
Crude protein	32.9	33.1	31.5	35.0	36.7	20.7
Crude lipid	2.9	2.2	3.5	3.7	3.6	0.8
Ash	12.9	12.2	11.2	11.6	10.0	36.1

[1]Macroalgae (MA) are the mixture of *Undaria pinnatifida*, *Saccharina japonica* and *Sargassum fusiforme* at a ratio of 2:2:1.

[2]Vitamin premix contained the following amount which were diluted in cellulose (g/ kg mix): excipient, 317; riboflavin, 23.8; pyridoxine, 4.7; niacin, 95.2; Ca-pantothenate, 33.3; inositol, 476.9; folic acid, 1.5; p-amino benzoic acid, 47.6.

[3]Mineral premix contained the following ingredients (g/kg mix): Excipient, 45.5; $MgSO_4$, 140.8; NaH_2PO_4, 92.4; KH_2PO_4, 246; $Ca(H_2PO_4)_2$, 139.5; $ZnSO_4$, 22.5; Ca-lactate, 310; $AlCl_3$, 0.15; KI, 0.15; $MnSO_4$, 2; $CoCl_2$, 1.

control (Con) diet. Fifty and one hundred percent of each FM and MA were substituted with tunic meal of SS, referred to as the FM50, FM100, MA50 and MA100 diets, respectively. All EPs were formulated to satisfy dietary nutrient requirements of abalone (Uki et al. 1986; Mai et al. 1995a, b). Finally, the dry *U. pinnatifida* was prepared to compare effect of the EPs on the growth performance of abalone.

2.3 Analytical procedures of feed ingredients, diets and the soft body of abalone

At the end of the 16-week feeding trial, thirty abalones from each container were randomly sampled and frozen at –20℃ for the measurement of the shell growth, soft body weight, and chemical analysis of the soft body of abalone. Prior to examination, all samples were slightly thawed at room temperate, followed by separation of the shell and soft body tissue. Shell length, width and height were measured in millimeters with a digital caliper (Mitutoyo Corporation, Kawasaki, Japan), and the ratio of the soft body weight to body weight (the soft body weight + the excised shell's weight) was calculated to determine an index of biological status of abalone. Specific growth rate (SGR, % body weight gain/day) was calculated using the formula of Britz (1996): SGR = [(ln(W_f) - ln(W_i))/days of feeding] × 100, where ln(W_f) = natural log of the final mean weight of abalone and ln(W_i) = natural log of the initial mean weight of abalone.

Proximate analysis of feed ingredients, experimental diets and the soft body of abalone were conducted using standard AOAC (1990) practices. The separated soft body tissue of abalone from each container were then homogenized and used for proximate analysis. Crude protein content was determined by the Kjeldahl method (Buchi B-324/435/412; Auto Kjeldahl System, Flawil, Switzerland), crude lipid was determined using an ether-extraction method, moisture was determined by oven drying at 105℃ for 24 h, and ash was determined using a muffle furnace at 550℃ for 4 h.

For the analysis of AAs (except for methionine and cysteine), feed ingredients and the experimental diets were hydrolyzed with 6N HCl for 24 h at 110℃ followed by ion exchange chromatography with an AA analyzer (L-8800 Auto-analyzer; Hitachi, Tokyo, Japan). For the analysis of methionine and cysteine, the samples were oxidized with performic acid at below 5℃ for 24 h to obtain the methionine sulfone and cysteic acid, then freeze dried twice with deionized water. The freeze-dried samples were hydrolyzed and analyzed as in the process of other AAs.

Lipid for FAs analysis of feed ingredients and the experimental diets was extracted by a mixture of chloroform and methanol (2:1 v/v) according to the method of Folch et al. (1957), and fatty acid methyl esters were prepared by transesterification with 14% BF_3-MeOH (Sigma, St. Louis, MO, USA). Fatty acid methyl esters were analyzed using gas chromatography (Trace GC; Thermo, Waltham, MA, USA) with flame ionization detector, equipped with SP^{TM}-2560 capillary column (100 × 0.25 mm inner diameter, film thickness 0.20 μm; Supelco, Bellefonte, PA, USA).

2.4 Statistical analysis

One-way ANOVA and Duncan's multiple range test (Duncan 1955) were used to determine the significant differences among the means of treatments using SPSS program version 19.0 (SPSS Michigan Avenue, Chicago, IL, USA). Percentage data were arcsine-transformed prior to statistical analysis.

3. Results

AA profiles and fatty acid composition of feed ingredients are presented in Tables 2 3, respectively. All essential and nonessential AAs contents, except for cystine, in tunic meal of SS were higher than mixture of MA. However, FM contained lower levels of all essential AAs compared to tunic meal of SS. A sum of n-3 highly unsaturated fatty acid (HUFA) content were considerably high in FM compared to mixture of MA and tunic meal of SS, especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Linoleic acid (18:2n-6), linolenic acid (18:3n-3) and EPA contents were high in mixture of MA compared to FM and tunic meal of SS. In contrast, DHA content in tunic meal of SS was higher than mixture of MA.

AA profiles of the experimental diets are given in Table 4. There were no significant difference in AA profiles of the EPs, but all essential and nonessential AAs were relatively high compared to the *U. pinnatifida*. The essential AAs, such as leucine, lysine, methionine and valine tended to decrease with an increased amount of substitution of FM with tunic meal of SS in the EPs. All essential and nonessential AAs content in the EPs tended to increase with an increased amount of substitution of MA with tunic meal of SS and the highest all AAs content was obtained in the MA100 diet.

FA profiles of the experimental diets are presented in Table 5. A sum of n-3 HUFA contents were relatively high in the Con diet compared to that of the FM50 and FM100 diets, especially EPA and DHA. Linoleic acid content in the MA100 diet was relatively high compared to the Con and MA50 diets. However, linolenic acid content tended to decrease with increased amount of substitution of mixture of MA with tunic meal of SS.

Table 2. Amino acid profiles (%, DM basis) of feed ingredient

	Feed ingredient		
	FM (Crude protein: 68.5%)	Mixture of MA (*U. pinnatifida*: *S. japonica*: *S. fusiforme* = 2:2:1) (Crude protein: 15.0%)	Tunic meal of SS (Crude protein: 44.8%)
Alanine	4.18	0.59	1.42
Aspartic acid	6.31	0.97	4.00
Cystine	0.60	0.18	1.37
Glutamic acid	8.70	1.15	3.90
Glycine	4.28	0.49	1.82
Proline	2.75	0.35	1.44
Serine	2.75	0.44	1.80
Tyrosine	1.93	0.27	1.52
Arginine	3.92	0.38	2.00
Histidine	1.92	0.13	0.66
Isoleucine	2.92	0.38	1.43
Leucine	4.94	0.61	1.73
Lysine	5.37	0.44	2.25
Methionine	1.96	0.22	0.53
Phenylalanine	2.78	0.44	1.60
Threonine	2.99	0.48	1.75
Valine	3.47	0.50	1.70

Table 3. Fatty acid compositions (%, of total fatty acids) of feed ingredient

	Feed ingredient		
	FM (Crude lipid: 8.9%)	Mixture of MA (*U. pinnatifida: S. japonica*: *S. fusiforme* = 2:2:1) (Crude lipid: 2.1%)	Tunic meal of SS (Crude lipid: 1.1%)
C14:0	7.08	11.28	8.68
C16:0	24.70	18.43	28.06
C18:0	6.32	1.01	10.93
C22:0			
∑Saturates	38.1	30.72	47.67
C16:1n-9	6.89	1.97	5.97
C18:1n-9	12.53	24.75	21.65
C20:1n-9	1.40		
∑Monoenes	20.82	26.72	27.62
C18:2n-6	1.10	7.41	10.76
C20:2n-6		1.26	
C18:3n-3		4.39	
C18:3n-6		1.06	
C18:4n-3	1.42	5.59	
C20:3n-3	2.07	11.64	
C20:5n-3 (EPA)	14.18	7.36	7.41
C22:5n-3	2.34		
C22:6n-3 (DHA)	16.94	1.10	6.54
∑n-3 HUFA	35.53	20.10	13.95
Unknown	3.03	2.77	

∑n-3 HUFA: Sum of n-3 highly unsaturated fatty acids

Table 4. Amino acid profiles of the experimental diets (%, DM basis)

	Experimental diets					
	Con	FM50	FM100	MA50	MA100	*Undaria*
Alanine	1.82	1.75	1.55	1.89	1.92	1.53
Aspartic acid	3.16	3.22	3.15	3.47	3.72	1.31
Cystine	0.47	0.51	0.55	0.61	0.73	0.15
Glutamic acid	5.51	5.53	5.35	5.84	6.00	2.12
Glycine	1.69	1.59	1.36	1.81	1.97	0.75
Proline	1.79	1.77	1.69	1.91	2.03	0.61
Serine	1.56	1.58	1.49	1.71	1.82	0.59
Tyrosine	0.98	1.00	0.99	1.19	1.26	0.40
Arginine	1.91	1.91	1.83	2.10	2.26	0.65
Histidine	0.73	0.73	0.69	0.78	0.83	0.23
Isoleucine	1.39	1.39	1.33	1.50	1.58	0.55
Leucine	2.70	2.67	2.47	2.82	2.85	0.99
Lysine	1.82	1.77	1.61	1.97	2.12	0.65
Methionine	0.63	0.55	0.45	0.66	0.68	0.27
Phenylalanine	1.54	1.56	1.52	1.65	1.73	0.63
Threonine	1.34	1.35	1.27	1.47	1.58	0.62
Valine	1.62	1.61	1.53	1.75	1.87	0.74

Table 5. Fatty acid compositions of the experimental diets (% of total fatty acids)

	Experimental diets					
	Con	FM50	FM100	MA50	MA100	*Undaria*
C14:0	2.57	2.44	2.33	2.25	2.12	5.76
C16:0	16.02	15.70	16.34	16.17	16.49	28.63
C18:0	2.93	2.92	2.90	3.22	3.48	2.18
C20:0	0.26	0.28		0.26		
∑Saturates	21.78	21.34	21.57	21.9	22.09	
C16:1n-9	2.75	2.44	1.92	2.76	2.77	
C18:1n-9	19.78	19.48	19.57	20.10	20.12	14.18
C20:1n-9	3.47	3.03	2.11	3.79	4.43	
C24:1n-9	0.28			0.25		
∑Monoenes	26.28	24.95	23.6	26.9	27.32	
C18:2n-6	28.45	30.81	35.91	31.08	32.61	8.29
C20:2n-6	0.30	0.37	0.30	0.25		
C22:2n-6		0.74	0.25			
C18:3n-3	4.53	4.76	5.54	4.28	3.94	6.10
C18:3n-6	0.35	0.34	0.37	0.29		
C18:4n-3	2.65	2.51	2.78	1.71	0.77	11.03
C20:3n-3	2.35	2.16	2.26	1.42	0.66	12.79
C20:4n-3	0.24					
C20:5n-3	5.24	4.49	3.50	4.65	4.46	6.03
C22:5n-3	0.38	0.31		0.39	0.47	
C22:6n-3	4.10	2.97	1.24	4.10	4.94	
∑n-3 HUFA	12.31	9.93	7.00	10.56	10.53	18.82
Unknown	3.39	4.27	2.66	3.04	2.76	5.01

∑n-3 HUFA: Sum of n-3 highly unsaturated fatty acids

At the end of the 16-week feeding trial, survival of abalone fed all experimental diets ranging from 82.9 to 89.5% was not significantly ($P > 0.05$) affected by the dietary substitution of FM and mixture of MA with tunic meal of SS (Table 6). Weight gain of abalone fed the MA50 and FM50 diets was significantly ($P < 0.05$) greater than that of abalone fed the Con and FM100 diets and *Undaria*, but not significantly SGR of abalone fed the MA50 diet was significantly ($P < 0.05$) higher than that of abalone fed the Con and FM100 diets and *Undaria*, but not significantly ($P > 0.05$) different from that of abalone fed the FM50 and MA100 diets. Weight gain and SGR of abalone fed all EPs (Con, FM50, FM100, MA50 and MA100 diets) were significantly ($P < 0.05$) greater than those of abalone fed the *Undaria*.

The longest shell length was obtained in abalone fed the MA50 diet, followed by the MA100, FM50, Con and FM100 diets and *Undaria* (Table 7). Shell width of abalone fed the Con, FM50, MA50 and MA100 diets was significantly ($P < 0.05$) wider than that of abalone fed the FM100 diet and *Undaria*. The soft body weight of abalone fed the Con, FM50, MA50 and MA100 diets was heavier than that of abalone fed the FM100 and *Undaria*. Shell height and the ratio of the soft body weight to total weight of abalone were not significantly ($P > 0.05$) affected by the experimental diets.

No significant differences in the moisture and ash content of the soft body of abalone was observed (Table 8). However, crude protein and crude lipid content of the soft body of abalone fed the all EPs were significantly ($P < 0.05$) higher than those of the soft body of abalone fed the *Undaria*. Crude protein and crude lipid content of the soft body of abalone was not significantly ($P < 0.05$) different among the EPs.

Table 6. Survival, weight gain and specific growth rate (SGR) of juvenile abalone fed the experimental diets substituting fish meal (FM) and mixture of macroalgae (MA) with tunic meal of sea squirt (SS) for 16 weeks

Experimental diets	Initial weight (g/abalone)	Final weight (g/abalone)	Survival (%)	Weight gain (g/abalone)	SGR[1] (%/day)
Con	3.2 ± 0.01^{a}	5.2 ± 0.06^{b}	85.2 ± 1.72^{a}	2.0 ± 0.06^{b}	0.45 ± 0.011^{b}
FM50	3.2 ± 0.02^{a}	5.3 ± 0.03^{a}	83.8 ± 4.15^{a}	2.2 ± 0.04^{a}	0.47 ± 0.009^{ab}
FM100	3.1 ± 0.01^{a}	5.0 ± 0.04^{c}	82.9 ± 2.18^{a}	1.9 ± 0.04^{c}	0.41 ± 0.008^{c}
MA50	3.1 ± 0.01^{a}	5.4 ± 0.01^{a}	88.1 ± 1.72^{a}	2.3 ± 0.02^{a}	0.49 ± 0.004^{a}
MA100	3.2 ± 0.01^{a}	5.3 ± 0.03^{a}	89.5 ± 1.26^{a}	2.2 ± 0.02^{ab}	0.47 ± 0.002^{ab}
Undaria	3.1 ± 0.01^{a}	4.5 ± 0.05^{d}	88.6 ± 2.18^{a}	1.4 ± 0.05^{d}	0.33 ± 0.011^{d}
P-value	$P > 0.7$	$P < 0.0001$	$P > 0.3$	$P < 0.0001$	$P < 0.0001$

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

[1]Specific growth rate (SGR) = [(Ln(W_f) - Ln(W_i))/days of feeding]×100, where Ln(W_f) = natural log of the final mean weight of abalone and Ln(W_i) = natural log of the initial mean weight of abalone.

Table 7. Shell length, shell width, shell height, soft body weight and the ratio of soft body weight to total weight of abalone (*Haliotis discus*) fed the experimental diets substituting fish meal (FM) and mixture of macroalgae (MA) with tunic meal of sea squirt (SS) for 16 weeks

Experimental diets	Shell length (mm)	Shell width (mm)	Shell height (mm)	Soft body weight (g/individual)	Soft body weight/total weight
Con	38.1 ± 0.08^{cd}	25.2 ± 0.08^{a}	8.0 ± 0.03^{a}	3.3 ± 0.06^{a}	0.6 ± 0.02^{a}
FM50	38.1 ± 0.18^{bc}	25.4 ± 0.17^{a}	8.0 ± 0.02^{a}	3.3 ± 0.06^{a}	0.6 ± 0.01^{a}
FM100	37.8 ± 0.03^{d}	24.6 ± 0.10^{b}	8.0 ± 0.07^{a}	3.1 ± 0.04^{b}	0.6 ± 0.00^{a}
MA50	38.6 ± 0.09^{a}	25.5 ± 0.06^{a}	8.0 ± 0.02^{a}	3.5 ± 0.06^{a}	0.6 ± 0.01^{a}
MA100	38.3 ± 0.05^{ab}	25.4 ± 0.05^{a}	8.1 ± 0.07^{a}	3.3 ± 0.06^{a}	0.6 ± 0.01^{a}
Undaria	37.9 ± 0.43^{d}	24.7 ± 0.28^{b}	8.0 ± 0.52^{a}	3.0 ± 0.14^{b}	0.6 ± 0.01^{a}
P-value	$P < 0.0001$	$P < 0.001$	$P > 0.9$	$P < 0.003$	$P > 0.4$

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

Table 8. Proximate composition (%) of the soft body of juvenile abalone fed the experimental diets substituting fish meal (FM) and mixture of macroalgae (MA) with tunic meal of sea squirt (SS) for 16 weeks

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
Con	74.5 ± 0.05[a]	18.5 ± 0.12[a]	1.3 ± 0.02[a]	2.5 ± 0.02[a]
FM50	74.3 ± 0.05[a]	18.4 ± 0.04[a]	1.3 ± 0.05[a]	2.6 ± 0.05[a]
FM100	74.4 ± 0.07[a]	18.3 ± 0.06[a]	1.3 ± 0.03[a]	2.5 ± 0.05[a]
MA50	74.3 ± 0.06[a]	18.4 ± 0.10[a]	1.3 ± 0.07[a]	2.5 ± 0.06[a]
MA100	74.2 ± 0.15[a]	18.2 ± 0.23[a]	1.3 ± 0.06[a]	2.5 ± 0.03[a]
Undaria	74.4 ± 0.06[a]	16.3 ± 0.04[b]	0.8 ± 0.06[b]	2.5 ± 0.04[a]
P-value	$P > 0.2$	$P < 0.0001$	$P < 0.0001$	$P > 0.8$

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

4. Discussion

Since tunic meal of SS is a noble source of protein, AA, carbohydrate and other nutrition (Choi et al. 1996; Kang et al. 1996; Lee et al. 1998; Kim 2002), they seem to have high potential as a substitute for FM and mixture of MA in abalone feed. Previous studies showed FM up to 80% and MA (*Saccharina japonica*) up to 100% could be replaced with tunic meal of SS in the experimental diets for the same species of abalone (Jang et al. 2017; Choi et al. 2018). As this is the first time report on substitution effect of tunic meal of SS for FM and mixture of MA in EP for abalone, its feasibility and suitability needs to be proved in commercial scale farm.

The essential AAs, such as arginine, histidine, leucine, lysine, methionine, phenylalanine, threonine and valine, tended to decrease with an increased amount of tunic meal of SS as alternative source for FM in the EPs. Increased amount of all essential and nonessential AAs with increased amount of substituting mixture of MA with tunic meal of SS in the EPs was observed. This results agreed with AA profiles of feed ingredient. Nevertheless, tendency of AAs did not cause any side-effect on performance of abalone in this study.

The requirement of n-3 HUFA for abalone (*H. discus hannai*) was reported to be ca. 1% in the 5% lipid diet (Uki et al. 1986). Similarly, in this study, n-3 HUFA content ranging from 7.0% (FM100 diet containing 3.5% lipid) to 12.3% (Con diet containing 2.9% lipid) of the total FAs satisfied dietary n-3 HUFA requirement for abalone. Bautista-Teruel et al. (2011) demonstrated that the highest (1.6%) supplementation of the combined linoleic acid, linolenic acid and n-3 HUFA in the 3.7% lipid diet improved weight gain of abalone (*H. asinina*). An increased tendency of linoleic acid and DHA was well reflected the FA compositions of feed ingredients.

High essential and nonessential AA and FA content in all EPs compared to *Undaria* probably resulted in improved weight gain and SGR of abalone in this study. This results agreed with the previous study indicated that the essential protein (AAs) and lipid (FAs) content in nutritionally-balanced formulated diets were sufficient to enhance the growth performances of abalone compared to natural diet (Viana et al. 1993; Lee et al. 1997; Kim et al. 1998; Cho et al. 2008; Garcia-Esquivel and Felbeck 2009; Lee et al. 2016; Jung et al. 2016; Jang et al. 2017; Lee et al. 2017; Ansary et al. 2018a, b; Choi et al. 2018; Lee et al. 2018).

Improved and reduced weight gain of abalone fed the FM50 and FM100 diets, respectively, compared to the Con diet in this study indicated that substitution of FM up to 50% could be replaced with tunic meal of SS to improve weight gain of abalone, but further substitution retarded growth performance of abalone when 14% FM was included in EP. Low content in most of essential AA, such as arginine, histidine, isoleucine, leucine, lysine, methionine and valine, in the FM100 diet compared to the Con diet could be reasons for lower weight gain of abalone fed the former compared to the latter in this study. In Mai et al. (1995b)'s study, addition of a mixture of the synthetic amino acids, such as arginine, methionine and threonine in abalone diet improved growth performance of abalone, *H. tuberculata* and *H. discus hannai*.

Similarly, tuna by-product meal and the combined dry macroalgae (*Nannochloropsis oceanica*) biomass residue and casein could replace FM up to 75% and completely (100%), respectively, in formulated diet without retardation in growth performance of the same species abalone, *Haliotis discus* (Jung et al. 2016; Myung et al. 2016). Guzman & Viana (1998) also reported that feeding a diet containing abalone viscera silage as alternative source FM resulted in improved growth performance of abalone (*H. fulgens*). In addition, the availability of various animal and/or protein source as alternative source for FM in diet for abalone was reported by Shipton and Britz (2001)'s study and Cho (2010)'s study.

No difference in weight gain and SGR of abalone fed the MA100 and Con diets and the best growth performance was obtained in abalone fed the MA50 diet in this study indicated that mixture of MA could be completely replaced with tunic meal of SS without negative effect on growth performance of abalone when 25% mixture of MA was included and substituting 50% mixture of MA with tunic meal of SS could promote weight gain and SGR of abalone. These results were attributed to the fact that the tunic meal of SS contains an appropriate amount of carbohydrates. Britz et al. (1994) reported that the gross maintenance energy metabolism of abalone is carbohydrate based. In addition, Mai et al. (1995a) also demonstrated that two species of abalone, *H. tuberculata* and *H. discus hannai*, have a great potential for utilizing carbohydrate, which is a abundant in natural diet, for energy and perhaps for other nutritional purposes. As tunic meal of SS contains 32.7% carbohydrate compared to MA [32.7% in *S. japonica* (Jang et al. 2017), 42.4% in *U. pinnatifida* and 66.83% in *S. fusiforme* (Kim et al. 2014)] in this study, it appears to be a proper ingredient for alternative source for mixture of MA, which were the highest component and expensive ingredient in abalone feed. Similarly, Kim et al. (2016) demonstrated the agricultural byproduct with high carbohydrate content, rice bran, was the good alternative source for *L. japonica* in abalone feed. The availability of white radish byproduct (33.6%) (Lee et al. 2018) and fouling MAs, such as *Sargassum horneri* (60.8% carbohydrate) and *Ulva australis* (58.0% carbohydrate) (Ansary et al. 2018a, b) were also reported as appropriate ingredient to replace *Undaria* in diet.

SGR values ranging from 0.33 to 0.49%/day of abalone (an initial weight 3.1 g) fed the EPs and *Undaria* in this study was relatively low compared to the same species abalone (the initial weight of 3.3 g) fed the formulated diets ranging from 0.40 to 0.59, from 0.40 to 0.60 %/day, respectively (Jang et al. 2017; Choi et al. 2018). The differences could have resulted from MA used in study. MA used in this study are the mixture of *U. pinnatifida*, *S. japonica* and *S. fusiforme* at a ratio

of 2:2:1, but a single *U. pinnatifida* and *S. japonica* was used in the latter, respectively. Similarly, Lee et al. (2017)'s study reported that the mean SGR of abalone fed the EPs substituting mixture of MA (*U. pinnatifida*: *S. fusiforme* = 1:1) with rice bran was lower than that of abalone fed the formulated diets substituting *S. japonica* with rice bran in Kim et al. (2015)'s study.

Biological parameters (shell length and width, and the soft body weight) measured in this study were well reflected from growth performance of abalone. In previous study, the increased biological criteria of abalone agreed with better growth performance of abalone (Cho 2010; Myung et al. 2016; Lee et al. 2017; Jang et al. 2017; Ansary et al. 2018a; Choi et al. 2018).

Higher crude protein and lipid content in the soft body of abalone fed the EPs compared to *Undaria* in this study could be explained by the poor nutritional quality and quality of essential amino and fatty acids in the latter, and agreed with other studies showing nutritional balance diet positively affect chemical composition of abalone (Mai et al. 1995a, b; Cho et al. 2008; Myung et al. 2016; Lee et al. 2017; Jang et al. 2017; Choi et al. 2018).

5. Conclusion

In conclusion, FM up to 50% could be replaced with tunic meal of SS without retardation in growth performance of abalone when 14% FM was included. The substitutability of tunic meal of SS for MA seems to be high in extruded pellet in experimental condition. The all EPs substituting FM and MA with tunic meal of SS, respectively, achieved better performance of abalone over the *Undaria*.

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