Thesis for the Degree of Master of Science

Effects of dietary substitution of fishmeal with the combined dry microalgae, *Nannochloropsis oceanica* (NO) biomass residue and casein on growth and body composition of juvenile abalone (*Haliotis discus*)

Sung Hyo Myung

Department of Marine Bioscience and Environment

The Graduate School

Korea Maritime and Ocean University

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Advisor: Prof. Sung Hwoan Cho

Sung Hyo Myung

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A dissertation

by

Sung Hyo Myung

Certified by **Sung Hwaon Cho**

....

Thesis Advisor

Accepted by **Cheol Young Choi**

....

Chairman

In-Seok Park

....

Member

Sung Hwaon Cho

....

Member

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Sung Hyo Myung

The Department of Marine Bioscience & Environment, Graduate School,
Korea Maritime & Ocean University

요 약

본 연구에서는 국내 주요 전복 양식대상종 중의 하나인 까먹전복(*Haliotis discus*)을 대상으로 하여 미세조류 바이오매스 잔사와 카제인 혼합을 이용한 사료 내 어분대체에 따른 사료내 아미노산 변화와 전복의 성장 및 체조성에 미치는 영향을 조사하였다. 1,260마리의 전복 치패(시작시 평균 마리당 무게: 1.29 g)을 임의로 선별하여 18개의 70 L 플라스틱 수용기에 각각 70마리씩 수용하였다. 모든 실험구에는 손으로 만복시까지 1주일에 7일간 1일 1회 매일 사료를 공급하여 주었으며, 사육실험 기간은 총 16주간이었다. 실험에 이용된 실험사료는 5종류의 실험사료를 준비하였다. 어분을 28% 첨가한 대조구 사료(Con), 어분을 미세조류 바이오매스 잔사와 카제인 혼합으로 25% 대체한 사료(NO25), 어분을 미세조류 바이오매스 잔사와 카제인 혼합으로 50% 대체한 사료(NO50),

어분을 미세조류 바이오매스 잔사와 카제인 혼합으로 75% 대체한 사료(NO75) 및 어분을 미세조류 바이오매스 잔사와 카제인 혼합으로 100% 전량 대체한 사료(NO100)를 각각 두었다. 마지막으로 실험용사료의 효과를 비교하기 위해 자연산 먹이인 다시마(ST)를 공급한 실험구를 두었으며, 각 실험구는 3반복구를 두었다.

배합사료 내 어분이 미세조류 바이오매스 잔사로 대체됨에 따라서 사료 내 Isoleucine, Leucine, Phenylalanine, Threonine, Valine과 같은 필수아미노산 함량이 증가하는 경향을 나타내었다. 그러나 배합 사료 내 어분이 미세조류 바이오매스 잔사로 대체됨에 따라서 사료 내 Arginine은 감소하는 경향을 보였다.

16주간 사육실험에서 전복치패의 생존률은 실험용 배합사료를 공급한 실험구가 다시마(ST)를 공급한 실험구 보다 높았다. 전복치패의 증체량과 일일성장률(SGR)의 경우 NO100 사료가 다른 모든 실험구보다 높게 나타났으며, 사료 내 어분을 미세조류 바이오매스 잔사와 카제인으로 대체함에 따라 계속해서 증가하는 경향을 보였다. 또한 전복치패의 각장의 경우에도 어분을 미세조류 바이오매스 잔사와 카제인으로 대체할 경우 계속해서 증가하는 경향을 보였다.

본 사육 실험 후 임의로 선별한 전복치패 가식부의 일반성분분석 결과 수분을 제외한 조지질, 조단백질, 회분의 함량은 실험구간에 유의적인 차이를 보였으며 조지질, 조단백질, 회분의 함량은 실험용 배합사료의 직접적인 영향을 받은 것으로 나타났다.

본 연구 결과 전복치패의 배합사료 내 어분의 함량이 28%인 경우에는 미세조류 바이오매스 잔사와 카제인으로 어분을 완전히 대체할 수 있다.

Keywords: 전복 (*Haliotis discus*), 영양분 대체, 어분, 미세조류 바이오매스 잔사 (*Nannochloropsis oceanica*), 카제인

Experiment

Effects of dietary substitution of fishmeal with the combined dry microalgae, *Nannochloropsis oceanica* (NO) biomass residue and casein on growth and body composition of juvenile abalone (*Haliotis discus*)

Abstract

The effects of dietary substitution of fishmeal with the combined dry *Nannochloropsis oceanica* (NO) biomass residue and casein on growth performance and carcass composition of juvenile abalone was determined. One thousand two hundred and sixty juvenile abalone were randomly distributed into the 18, 70 L plastic rectangular containers. Five experimental diets in triplicate were prepared. The 28% fishmeal was included into the control (Con) diet. The 25, 50, 75 and 100% fishmeal were substituted with the combination of graded levels of dry NO biomass residue and casein, referred to as the NO25, NO50, NO75 and NO100 diets, respectively. Finally, the salted sea tangle (ST), *Laminaria japonica* was prepared to compare the effect of the experimental diets on performance of abalone. The essential amino acids, such as isoleucine, leucine, phenylalanine, threonine and valine tended to increase with dietary substitution of fishmeal with NO biomass residue in the experimental diets. Arginine tended to decrease with dietary substitution of fishmeal with NO biomass residue. Survival of abalone fed the experimental diets was higher than that of abalone fed the ST diet for 16 weeks. Weight gain and specific growth rate (SGR) of abalone fed the NO100 diet were higher than those of abalone fed the all other diets. Weight gain and SGR of abalone linearly increased with dietary substation of fishmeal with NO. Shell length of abalone tended to increase with dietary substitution of fishmeal with the combined dry NO biomass residue and casein. The chemical composition of the soft body of abalone was different among treatments except for moisture content. In conclusion, fishmeal in the diets for abalone could be completely replaced with the combined dry NO biomass residue and casein when the 28% fishmeal was included.

Keywords: abalone (*Haliotis discus*), dietary substitution, fishmeal, *Nannochloropsis oceanica* (NO) biomass residue, casein

1. Introduction

Abalones are known to be herbivorous and feed mostly on macroalgae, which is usually low in lipid (Thongrod, Tamtin, Chairat & Boonyaratpalin 2003). However, availability of macroalgae commonly used for abalone, such as sea tangle *Laminaria japonicaand* and *Undariais* very limited during winter season in Eastern Asia. In addition, protein content of these macroalgae do not satisfy dietary protein requirement of abalone (Mai, Mercer & Donlon 1995b). Therefore, supplement of protein source is unavoidable in formulating abalone feed.

Casein has been known as a good protein source for abalone (Uki, Kemuyama & Watanabe 1985a; Uki, Kemuyama & Watanabe 1986), but it can not be practically used into the commercial diet due to its high price. The studies to develop the alternative animal and/or plant protein sources for casein in the diets for abalone have been performed (Uki, Kemuyama & Watanabe 1985b; Viana, Lopez & Salas 1993; Lee, Yun & Hur 1998a; Sales & Britz 2003; Gracia-Esquivel & Felbeck 2009; Cho 2010). Cho, Park, Kim & Yoo (2008) also showed that abalone (*H.discus hannai*) fed the combined fishmeal and soybean meal diet or the combined fishmeal, soybean meal and crustacean meal grew as well as abalone fed the casein-basal diet for 16 weeks.

Fishmeal is another good protein source in formulating the aquafeed including abalone. However, an international market price of fishmeal keeps increasing sharply due to an expansion of aquaculture and high demand for protein source in the aquafeed. Therefore, development of a new feed ingredient to replace fishmeal in the aquafeed is highly needed.

Microalgae have received a lot of attention for biofuel production because it can produce oil in the cell body as well as carbohydrates and protein (Li, Horsman, Wu, Lan & Dubois-Calero 2008; Lam & Lee 2012) and their biomass residue still contains the high protein content after oil extraction. Thus microalgae biomass residue could be used as protein source in the aquafeed. In considering herbivorous feeding habit of abalone, algal-based protein source could be highly recommendable for abalone feed. *Nannochloropsis* was reported to be the good microalgae for a large-scale biodiesel production at the indoor conditions (Moazami, Ashori, Ranjbar, Tangestani, Eghtesadi & Nejad 2012). Dang, Li, Speck & Benkendorff (2011) also reported that supplementation of 10% cyanobacteria, *Arthrospira maxima* and microalgae, *Dunaliella salina* into the commercial feed was effective to improve growth rate of greenlip abalone (*H. laevigata*). In addition, Shipton & Britz (2001) reported that growth rate of abalone (*H. midae*) fed the diets substituting 50% fishmeal with by either soy meal or *Spirulina* was comparable to that of abalone fed the fishmeal-basal diet.

In this study, therefore, the effects of dietary substitution of fishmeal with the combined dry microalgae, *Nannochloropsis oceanica* (NO) biomass residue and casein on growth performance and carcass composition of juvenile abalone was determined.

2. Materials and Methods

2.1. Preparation of Abalone and Rearing Conditions

Juvenile abalone (*H. discus*) were purchased from a private hatchery and transferred to an abalone farm (Ocean and Fisheries Research Institute, Jeju Special Self-Governing Province, Jeju, Korea). Before an initiation of the feeding trial, abalone were acclimated to the experimental conditions for 4weeks and fed with the dry sea tangle (*Laminaria japonica*) once a day at the ratio of 2-3% of total biomass. One thousand two hundred and sixty juvenile abalone averaging 1.29 g (1.5 cm in shell length) were randomly distributed into the 18, 70 L plastic rectangular containers (120 cm × 36 cm: seventy juvenile per container). Nine containers were placed into each of two, 9 ton concrete flow-through raceway systems (water volume: 3 ton) at a flow rate of 48.3 L/min. The sand-filtered seawater at a temperature ranging from 16.7 to 21.8°C (mean ± SD: 18.4 ± 0.99°C) was supplied throughout the feeding trial. 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 2-3.5% biomass). Dead abalone were removed daily and the bottoms of the containers were siphon-cleaned daily. The feeding trial lasted for 16 weeks. At the end of the feeding trial, abalone were harvested and collectively weighed from each container.

2.2. Preparation of the Experimental Diets

Five experimental diets and the salted sea tangle (ST) in triplicate were prepared (Table 1). The 28% fishmeal and 13% soybean meal were included into the control (Con) diet as the primary protein source. The 12% wheat flour and 4% dextrin, and 3% squid liver oil and 2% soybean oil were used as the carbohydrate and lipid sources, respectively in the Con diets. The 25, 50, 75 and 100% fishmeal were substituted with the combined large-scale outdoor cultured microalgae, NO biomass residue supplied by NLP Co. Ltd (Busan, Korea) and casein, referred to as the NO25, NO50, NO75 and NO100 diets, respectively. NO biomass residue was prepared after the biofuel extraction from microalgal biomass using a modified method of Bligh & Dyer (1959), Xu, Miao & Wu (2006) and Lee, Yoo, Jun, Ahn & Oh (2010). The experimental diets were composed to satisfy the dietary nutrient requirements for abalone (Mai et al. 1995a, 1995b). Finally, the ST was prepared to compare the effect of the experimental diets on performance of abalone.

Next, 22% sodium alginate was added to all experimental diets. Thereafter, all the ingredients were mechanically mixed well and water added at a ratio of 1:1. A paste was made from each of the diets by using an electronic mixer and shaped into 0.15 cm thick sheets, which were then cut by hand into 1 cm^2 flakes. The flakes were then dipped into an aqueous solution of 5% $CaCl_2$ for 1 minute and the excess solution was drained naturally. The flakes were then dried naturally for 2 days and stored at -20℃ until use.

Table 1 Ingredients of the experimental diets (%, dry matter basis)

	Experimental diets					
	Con	NO25	NO50	NO75	NO100	Sea tangle (ST)
Ingredients (%, DM)						
Fishmeal (CP:72.7%, CL:11.3%)	28	21	14	7	0	
Soybean meal	13	13	13	13	13	
Nannochloropsis oceanica (NO) biomass residue* (CP:39.6%, CL:9.9%)	0	7	14	21	28	
Casein (CP:89.3%, CL:6.3%)	0	3	6	9	12	
Wheat flour (CP:14.6%, CL:4.1%)	12	9	6	3	0	
Dextrin	4	4	4	4	4	
Sea tangle	10	10	10	10	10	
Squid liver oil	3	3	3	3	3	
Soybean oil	2	2	2	2	2	
Sodium alginate	22	22	22	22	22	
Vitamin premix§	2	2	2	2	2	
Mineral premix¶	4	4	4	4	4	
Nutrients (%, dry matter)						
Dry matter	87.8	88.6	88.3	89.3	89.0	35.6
Crude protein	31.3	30.7	31.4	30.9	30.8	7.6
Crude lipid	9.2	9.4	8.9	8.5	8.7	1.2
Ash	15.6	15.0	14.6	14.6	14.4	65.5

[*]Crude protein and lipid content of *Nannochloropsis oceanica* (NO) was 31.5% and 32.8%, respectively, before biofuel extraction.

[§]Mineral premix contained the following ingredients (g/kg mix): NaCl, 10, $MgSO_4$. $7H_2O$, 150; NaH_2PO_4. $2H_2O$, 250; KH_2PO_4, 320; $CaH_4(PO_4)_2$. H_2O, 200; Ferriccitrate, 25; $ZnSO_4$.$7H_2O$, 4; Ca-lactate, 38.5; CuCl, 0.3; $AlCl_3$. $6H_2O$, 0.15; KIO_3, 0.03; $Na_2Se_2O_3$, 0.01; $MnSO_4$. H_2O, 2; $CoCl_2$. $6H_2O$, 0.1.

[¶]Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 200; α-tocopheryl acetate, 20; thiamin hydrochloride, 5; riboflavin, 8; pyridoxine, 2; niacin, 40; Ca-D-pantothenate, 12; myo-inositol, 200; D-biotin, 0.4; folic acid, 1.5; p-amino benzoic acid, 20; K_3, 4; A, 1.5; D_3,0.003; cholinechloride, 200; yanocobalamin, 0.003.

2.3. Analytical Procedures of the Diets and Carcass

Twenty abalone at the start and ten abalone from each container at the termination of the feeding trial were sampled and frozen for chemical analysis. Prior to examination, all samples were slightly thawed, followed by separation of the shell and soft-body tissue. Shell length and shell width were measured to a precision of 1.0 mm with a digital caliper (Mitutoyo Corporation, Kawasaki, Japan), and the ratio of soft body weight to body weight (the soft body weight+the excised shell's weight) was calculated to determine a condition index for abalone. Specific growth rate (SGR, % body weight gain/day) was calculated using the formula of Britz (1996): SGR = [(ln(Wf) - In(Wi))/days of feeding]×100, where In(Wf) = natural log of the final mean weight of abalone and In(Wi) = natural log of the initial mean weight of abalone.

The pooled separated soft body tissue from all abalone from each container was then homogenized and used for proximate analysis. 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 according to AOAC (1990) practices. Amino acid composition of the experimental diets 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.

2.4. Statistical Analysis

One-way ANOVA and Duncan`s multiple range test (Duncan 1955) were used to determine the significance of the differences among the means of treatments by using SAS version 9.3 program (SAS Institute, Cary, NC, USA). Regression analysis for weight gain and SGR of abalone were also conducted. Percentage data was arcsine-transformed prior to statistical analysis.

3. Results

Amino acid profiles of the experimental diets are given in Table 2. The essential amino acids, such as isoleucine, leucine, phenylalanine, threonine and valine tended to increase with dietary substitution of fishmeal with NO biomass residue in the experimental diets. However, another essential amino acid, arginine tended to decrease with dietary substitution of fishmeal with NO. All essential and non-essential amino acid content in the ST diet was relatively low.

Survival of abalone was not significantly ($P > 0.05$) affected by dietary substitution of fishmeal with NO biomass residue (Table 3). However, survival of abalone fed the experimental diets was significantly ($P < 0.05$)

Table 2 Amino acid profiles of the experimental diets (dry matter % in the diet)

	Experimental diets					
	Con	NO25	NO50	NO75	NO100	ST
Alanine	1.49	1.48	1.38	1.31	1.33	0.07
Arginine	1.44	1.47	1.39	1.39	1.34	0.04
Aspartic acid	2.54	2.59	2.52	2.47	2.58	0.13
Cystine	0.27	0.26	0.25	0.24	0.22	0.02
Glutamic acid	4.07	4.26	4.32	4.34	4.78	0.17
Glycine	1.45	1.37	1.24	1.12	1.08	0.06
Histidine	0.58	0.61	0.61	0.60	0.67	0.02
Isoleucine	1.13	1.14	1.17	1.21	1.35	0.05
Leucine	1.96	2.12	2.19	2.23	2.52	0.08
Lysine	1.79	1.86	1.83	1.78	1.94	0.05
Methionine	0.55	0.56	0.57	0.55	0.54	0.01
Phenylalanine	1.14	1.20	1.24	1.26	1.43	0.05
Proline	1.22	1.42	1.64	1.83	2.25	0.04
Serine	1.05	1.22	1.26	1.28	1.41	0.05
Threonine	1.06	1.15	1.16	1.18	1.27	0.06
Tyrosine	0.69	0.81	0.87	0.95	1.00	0.04
Valine	1.32	1.37	1.42	1.47	1.67	0.06

Table 3 Survival (%) and weight gain (g/abalone) of juvenile abalone (*Haliotis discus*) fed the experimental diets substituting fishmeal with the combined dry microalgae *Nannochloropsis oceanica* (NO) biomass residue and casein for 16 weeks

Experimental diets	Initial weigh (g/abalone)	Final weight (g/abalone)	Survival (%)	Weight gain (g/abalone)
Con	1.29 ± 0.001	2.87 ± 0.009	85.7 ± 5.15[a]	1.57 ± 0.007[d]
NO25	1.29 ± 0.002	2.88 ± 0.018	87.1 ± 0.82[a]	1.59 ± 0.017[d]
NO50	1.29 ± 0.001	3.06 ± 0.033	83.3 ± 1.26[a]	1.77 ± 0.033[c]
NO75	1.29 ± 0.001	3.17 ± 0.013	84.8 ± 1.26[a]	1.88 ± 0.013[b]
NO100	1.29 ± 0.000	3.28 ± 0.023	81.0 ± 1.26[a]	1.99 ± 0.024[a]
ST	1.29 ± 0.002	2.08 ± 0.006	69.5 ± 1.26[b]	0.79 ± 0.007[e]

Values in the same column sharing a common superscript are not significantly different (P > 0.05).

higher than that of abalone fed the ST diet. Weight gain (Table 3) and SGR (Fig. 1) of abalone fed the NO100 diet were significantly ($P < 0.05$) higher than those of abalone fed the all other diets. Weight gain and SGR of abalone linearly increased with dietary substitution of fishmeal with NO biomass residue ($P < 0.0001$ for both measurements). However, no significant difference in weight gain and SGR was observed between abalone fed the Con and NO25 diets. The poorest weight gain and SGR were observed in abalone fed the ST diet.

Shell length of abalone fed the NO100 diet was the longest (Table 4). Shell length of abalone tended to increase with dietary substitution of fishmeal with the combined dry NO biomass residue and casein. Shell width of abalone fed the NO50, NO75 and NO100 diets was significantly ($P < 0.05$) wider than that of abalone fed the Con and ST diets, but not significantly ($P > 0.05$) different from that of abalone fed the NO25 diet. Shell height of abalone fed the Con, NO25, NO50, NO75 and NO100 diets was significantly ($P < 0.05$) higher than that of abalone fed the ST diet.

The soft body weight of abalone tended to increase with dietary substitution of fishmeal with NO biomass residue. However, the soft body weight of abalone fed the ST diet was the lightest. The ratio of softy body weight to total weight of abalone fed the Con, NO25, NO50, NO75 and NO100 diets was significantly ($P < 0.05$) higher than that of abalone fed the ST diet.

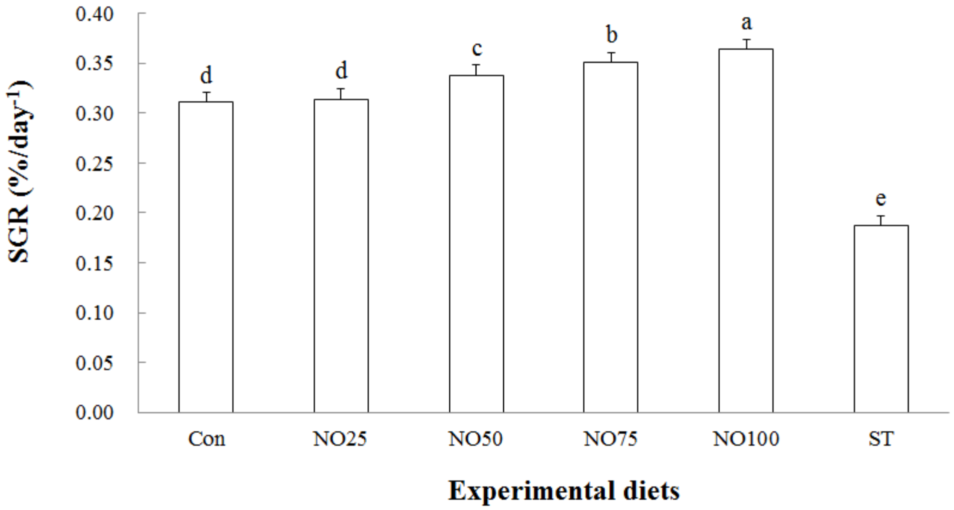

Figure 1 Specific growth rate (SGR, %/day) of juvenile abalone (*Haliotis discus*) fed the experimental diets substituting fishmeal with the combined dry microalgae *Nannochloropsis oceanica* (NO) biomass residue and casein for 16 weeks. Values are statistically significant ($P < 0.05$) where letters are different.

Table 4 Shell length (mm), shell width (mm), shell height (mm) and the ratio of soft body weight to total weight of abalone (*Haliotis discus*) at the end of the16-week feeding trial

Experimental diets	Shell length (mm)	Shell width (mm)	Shell height (mm)	Soft body weight (g)	Soft body weight/total weight
Con	34.0 ± 0.23[d]	23.2 ± 0.04[b]	7.2 ± 0.19[a]	2.3 ± 0.03[e]	0.62 ± 0.004[a]
NO25	34.5 ± 0.22[d]	23.5 ± 0.15[ab]	7.2 ± 0.09[a]	2.4 ± 0.06[d]	0.60 ± 0.032[a]
NO50	35.1 ± 0.02[c]	24.1 ± 0.05[a]	7.2 ± 0.18[a]	2.7 ± 0.03[c]	0.61 ± 0.008[a]
NO75	35.3 ± 0.28[b]	24.0 ± 0.27[a]	7.2 ± 0.06[a]	2.9 ± 0.07[b]	0.60 ± 0.002[a]
NO100	35.7 ± 0.68[a]	24.1 ± 0.37[a]	7.2 ± 0.17[a]	3.1 ± 0.04[a]	0.63 ± 0.010[a]
ST	31.8 ± 0.27[e]	21.1 ± 0.19[c]	6.7 ± 0.06[b]	1.7 ± 0.04[f]	0.56 ± 0.003[b]

Values in the same column sharing a common superscript are not significantly different ($P > 0.05$).

Moisture content of the soft body of abalone was not significantly ($P > 0.05$) different among treatments (Table 5). However, crude protein content of the soft body of abalone fed the Con and NO50 diets was significantly ($P < 0.05$) higher than that of abalone fed the NO25, NO75, NO100 and ST diets. The lowest crude protein content was obtained in abalone fed the ST diet. Crude lipid content of the soft body of abalone fed the Con, NO25 and NO50 diets was significantly ($P < 0.05$) higher than that of abalone fed the NO75 and ST diets, but not significantly ($P > 0.05$) different from that of abalone fed the NO100 diet. Ash content of the soft body of abalone fed the Con, NO25, NO50, NO75 and NO100 diets was significantly ($P < 0.05$) lower than that of abalone fed the ST diet.

Table 5 Proximate composition (%, wet weight basis) of the soft body of abalone (*Haliotis discus*) fed the experimental diets substituting fishmeal with the combined dry microalgae *Nannochloropsis oceanica* (NO) biomass residue and casein for 16 weeks

Diets	Moisture	Crude protein	Crude lipid	Ash
Con	77.4 ± 0.21[a]	24.0 ± 0.04[a]	2.4 ± 0.08[a]	3.2 ± 0.07[b]
NO25	77.2 ± 0.15[a]	22.8 ± 0.06[b]	2.5 ± 0.06[a]	3.2 ± 0.10[b]
NO50	77.3 ± 0.31[a]	23.6 ± 0.25[a]	2.3 ± 0.12[a]	3.2 ± 0.07[b]
NO75	78.0 ± 0.39[a]	23.0 ± 0.09[b]	2.0 ± 0.05[b]	3.1 ± 0.05[b]
NO100	77.8 ± 0.51[a]	23.0 ± 0.16[b]	2.2 ± 0.09[ab]	3.1 ± 0.01[b]
ST	77.2 ± 0.04[a]	19.4 ± 0.10[c]	1.6 ± 0.09[c]	3.6 ± 0.08[a]

Values in the same column sharing a common superscript are not significantly different (P > 0.05).

4. Discussion

Because dietary protein requirement of two abalones (*H. tuberculata* and *H. discus hannai*) were estimated to be 22.3-32.3% and 23.3-35.6%, respectively based on weight gain (Mai et al. 1995b), all the experimental diets had over 30% crude protein content except for the ST diet, which was much lower and probably why they didn't perform so well on this. This was also well supported by improvement in survival and weight gain (SGR) of abalone fed the experimental diets compared to those of abalone fed the ST diet, which is commonly used for the commercial abalone farm in Korea.

The linearly increased weight gain and SGR of abalone fed the experimental diets proportion to substitution of fishmeal with the combined dry NO biomass residue and casein in this study indicated that NO biomass residue after biofuel extraction could be a good alternative protein source for fishmeal in the abalone feed. This was well supported by the fact that the several essential amino acid (isoleucine, leucine, phenylalanine, threonine and valine) contents of the experimental diets substituting fishmeal with NO biomass residue increased and corresponded with a superior weight gain and SGR of abalone compared to abalone fed the ST diet. Similarly, dietary supplementation of 10% microalgae (*A. maxima* and *D. salina*) improved weight gain and immunity of greenlip abalone, whereas a single macroalgae, *Ulva lactuca* or *Spyridia filamentosa* produced poor weight gain, but could be useful supplements for abalone aquaculture, especially inareas with high risk of herpesvirus infection (Dang et al. 2011). However, unlike this study, Shipton & Britz (2001) reported that growth rate of abalone (*H. midae*) fed the diets substituting 50% fishmeal with either soy meal or *Spirulina* was comparable to that of abalone fed the fishmeal-basal diet, but decreased when the 75 or 100% of fishmeal was substituted with the combined plant protein sources (*Spirulina*, soymeal and sunflower meal). In addition, the combined plant (soybean meal and *Spirulina*) basal diet produced poorer weight gain of abalone (*H. asinine*) compared to fishmeal-basal or the combined animal and plant protein diet because of low methionine content in the former (Bautista-Teruel, Fermin & Koshio 2003). These authors recommended use of the combined animal and plant protein diet to attain the best growth rate of abalone. However, in this study, methionine content was not reduced with dietary supplementation of fishmeal with NO biomass residue due to an increase in casein (animal protein) content to adjust constant protein level in the experimental diets.

Mai, Mercer & Donlon (1994) reported that two species of abalone (*H. tuberculata* and *H. discushannai*) had the similar amino acid requirements and some essential amino acid, such as arginine, methionine, threonine or histidine might be the limiting factor for growth of both abalones fed with macroalgae. To determine substitutable protein sources for fishmeal in the diet for abalone, the essential amino acids such as lysine and/or methionine were the primary consideration to be likely to be deficient (Shipton & Britz 2001; Bautista-Teruel et al. 2003; Cho et al. 2008). Cho (2010) also demonstrated that histidine was another amino acid, which must be carefully considered to determine suitability of an alternative protein source for fishmeal in the diets for abalone (*H. discus hannai*). Growth rate of South African abalone was also affected by both lysine and leucine contents in the diets, but protein efficiency ratio was mostly affected by both lysine and histidine contents (Shipton & Britz 2001). The poorest weight gain of abalone fed the ST diet in this study could be explained by the above studies. Three different abalone (*H. discus, H. sieboldii* and *H. discus hannai*) grew better on the both commercial diet and formulated diet than *Undaria* (Kim, Lee, Han, Kim & Park 1998). Similarly, a single macroalgae produced poorer weight gain of abalone than the well-formulated or commercial diet probably due to poor nutrient content in the former (Viana et al. 1993; Cho et al. 2008; Gracia-Esquivel & Felbeck 2009; Dang et al. 2011).

Biological parameters (shell length and the soft body weight of abalone) measured in this study were well reflected from growth of abalone. This might explain that abalone fed the experimental diets substituting fishmeal with the combined dry NO biomass residue and casein improved shell length rather than either shell width or shell height of abalone. However, all biological parameters of abalone fed the ST diet were poorer than those of abalone fed the experimental diets substituting fishmeal with NO biomass residue. Similarly, dietary nutrient content affected shell length, shell width and/or the ratio of the soft body weight to total body weight of abalone (Cho 2010).

The chemical composition of the soft body of abalone such as crude protein, crude lipid and ash content was relatively well reflected from that of the experimental diets. Similarly, dietary substitution of fishmeal with the animal and/or plant protein sources affected proximate composition of the soft body of abalone (Mai et al. 1995a, b; Thongrod et al. 2003; Cho et al. 2008; Garcia-Esquivel & Felbeck 2009; Cho 2010).

Although availability of microalgae biomass residue after biofuel extraction are very limited at this point, it could be a good protein source to replace fishmeal in the diets for abalone with further industry development in the future.

Ⅱ. Conclusion

In this study, effects of dietary substitution of fishmeal with the combined dry microalgae, *Nannochloropsis oceanica* (NO) biomass residue and casein on growth and body composition of juvenile abalone (*Haliotis discus*) were determined. Fishmeal in the diets for abalone could be completely replaced with the combined dry NO biomass residue and casein when the 28% fishmeal was included. Furthermore, weight gain and SGR of abalone linearly increased with dietary substitution of fishmeal with the combined dry NO biomass residue and casein.

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