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Effect of dietary substitution of sea tangle (ST), *Laminaria japonica* with rice bran (RB) on growth and body composition of juvenile abalone, *Haliotis discus*

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A dissertation

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요 약

본 연구에서는 전복용 배합사료내 다시마대체원으로서 생미강을 이용하여 전복의 성장 및 체조성에 미치는 영향을 조사하였다. 실험에 이용된 전복 치패는 실험조건에서 4주간 적응시킨 후, 마리당 평균 0.43 g의 전복 1,260마리를 무작위로 선별하여 18개의 70 L 플라스틱 수용기에 각각 70마리씩 수용하였다. 실험사료는 1일 1회 만복 수준으로 사료를 공급하였다. 총 사육실험 기간은 16주간이었다. 총 6종류의 실험사료를 준비하였다. 다시마 분말을 20% 첨가하여 제조한 대조구 사료(RB0), 다시마를 생미강으로 대체한 다시마대체 20% (RB20), 다시마대체 40% (RB40), 다시마대체 60% (RB60), 다시마대체 80% (RB80) 및 다시마대체 100% (RB100) 사료를 준비하였다. 전복의 생존률은 모든 실험용 배합사료를 공급한 실험구간의 유의적인 차이가 나타나지 않았으며, 증체량(weight gain)은 RB40을 공급한 실험구가 RB0을 공급한 실험구를 제외한 모든 실험구보다 유의적으로 높게 나타났다. 또한 RB0과 RB100을 공급한 실험구 사

이에는 유의적인 차이가 나타나지 않았다. 일일성장률(Specific growth rate, SGR)은 RB40을 공급한 실험구가 다른 모든 실험 사료를 공급한 실험구보다 유의적으로 높게 나타났다. 또한 RB0을 공급한 실험구의 일일성장률은 RB100을 공급한 실험구보다 유의적으로 높게 나타났으나, RB20, RB60 및 RB80을 공급한 실험구와는 유의적인 차이가 나타나지 않았다. 전복 가식부의 수분, 조단백질, 회분 함량은 실험용 배합사료에 의해 영향을 받은 것으로 나타났다. 본 연구 결과 전복용 배합사료내 다시마를 20% 첨가시 전복의 증체량에 부정적인 영향 없이 다시마를 생미강으로 100% 까지 완전히 대체 가능하며, 또한 다시마를 생미강으로 40% 대체시 가장 우수한 전복의 성장률을 보였다.

Keywords: 전복 (*Haliotis discus*), 대체원, 다시마, 생미강

Effect of dietary substitution of sea tangle (ST), *Laminaria japonica* with rice bran (RB) on growth and body composition of juvenile abalone, *Haliotis discus*

Abstract

Dietary substitution effect of sea tangle (ST) with rice bran (RB) on growth and carcass composition of juvenile abalone, *Haliotis discus* was determined. Juvenile abalone was acclimated to the experimental conditions for 4 weeks. Seventy Juvenile abalone averaging 0.43 g were randomly distributed into each of the 18, 70 L plastic rectangular containers. The experimental diets were fed to abalone once a day at a satiation level with a little leftover. The feeding trial lasted for 16 weeks. Survival of abalone was not affected by dietary substitution of ST with RB. However, weight gain of abalone fed the RB40 diet was higher than that of abalone fed the all other diets except for the RB0 diet. No significant difference in weight gain was found in abalone fed between the RB0 and RB100 diets. SGR of abalone fed the RB40 diet was higher than that of abalone fed all other diets. Also SGR of abalone fed the RB0 diet was higher than that of abalone fed the RB100 diet, but not different from that of abalone fed the RB20, RB60 and RB80 diets. Moisture, crude protein and ash content of the soft body of abalone were affected by dietary substitution of ST with RB. In conclusion, the 100% ST could be substituted with RB without a retardation of weight gain of abalone when the 20% ST was included into the experimental diet. The best growth performance was obtained in abalone fed the RB40 diet substituting 40% ST with RB.

Keywords: abalone, *Haliotis discus*, dietary substitution, sea tangle, rice bran

1. Introduction

An annual global production from abalone culture has increasing since 2000 (FAO 2013). Macroalgae such as sea tangle (ST), *Laminaria japonica* or *Undaria* are commonly used as feed for abalone culture in eastern Asia, but availability of these macroalgae is exclusively limited during winter season in wild. Therefore, dry or salted macroalgae are commonly supplied to abalone except for winter season. During dry or salting process of these macroalgae, their nutrients like protein (essential amino acid) and lipid (fatty acid) are likely to be destroyed and deficient. To date, many feeding trials have reported that supply of macroalgae produced an inferior growth rate of abalone to the nutrient-balanced feed (Uki et al., 1985; Viana et al., 1993; Lee et al., 1997; Kim et al., 1998; Bautista-Teruel et al., 2003; Cho et al., 2006; Cho et al., 2008; Garcial-Esquivel and Felbeck 2009; Dang et al., 2011). Therefore, development of the nutrient-balanced diet for abalone culture is highly needed.

Animal protein source such as casein, fish meal and crustacean meal and plant protein source such as soybean meal were the good protein source for growth of abalone (Viana et al., 1993; Lee et al., 1998; Sales and Britz 2001; Cho et al., 2008; Cho 2010). Garcial-Esquivel and Felbeck (2009) recommended supplementation of kelp meal into the combined fish meal and soybean meal-based diet to improve growth of red abalone, *Arthrospira maxima* and microalgae, *Dunaliella salina* into the commercial diet improved growth rate greenlip abalone, *H. laevigata*.

An international market price of macroalgae commonly used as feed for abalone has recently increased due to an expansion of the biofuel industry to develop them for ethanol production around the world and high demand for human consumption. Because these macroalgae is no longer to be a cheap feed ingredient in the commercial diet for abalone, development of an alternative feed ingredient that is both cheap and available year-round is highly needed for abalone culture.

Donkey's ear abalone, *H.asinina* well utilized the high levels of carbohydrate rather than high levels of lipid in the diets to support its growth (Thongrod et al., 2003). Mai et al. (1995a) also explained that abalone, *H. tuberculata* and *H. discus hannai* seemed to have great potential for utilizing carbohydrate, which is a major component in its natural diet, for energy and perhaps other nutritional purposes. The combined terrestrial leaf meal, *Moringa oliefera* and freshwater aquatic fern, *Azolla pinnata* with animal protein (fish meal and shrimp meal) were the promising feed ingredients for the practical diet for the farmed abalone, *H. asinina* (Reyes and Fermin 2003).

Rice bran (RB), one of the agricultural wastes, has a high potential as feed ingredient that is rich in the nutrients such as crude protein and vitamins (Gao et al., 2008), and cheap as a feed ingredient for abalone. In this study, therefore, dietary substitution effect of ST with RB on growth performance and carcass composition of juvenile abalone, *Haliotis discus* was determined.

2. Materials and Methods

2.1. Preparation of Abalone and Rearing Conditions

Juvenile abalone 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 4 weeks and fed with the dry sea tangle once a day at the ratio of 2-3% total biomass. Seventy juvenile abalone averaging 0.43 g (about 1 cm in shell length) were randomly distributed into each of the 18, 70 L plastic rectangular containers (120 cm × 36 cm). Nine containers were placed into each of two 9 ton concrete flow-through raceway systems (water volume: 3 ton) as a flow rate of 48.3 L/min/raceway. The sand-filtered seawater at a temperature ranging from 16.7 to 21.8°C (mean ± SD: 18.4 ± 0.99°C) at 15:00 hours 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 hours) at a satiation level with a little leftover (about 2-3.5% biomass). Dead abalone was 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 was harvested and collectively weighed from each container.

2.2. Preparation of the Experimental Diets

Six experimental diets in triplicate were prepared (Table 1). The 30% fish meal and 7% soybean meal were included into the RB0 diet as the primary protein source. And the 5% wheat flour and 8% dextrin, and 3% squid liver oil and 1% soybean oil were used as the carbohydrate and lipid sources, respectively in the RB0 diet. A 20% ST powder was included into the RB0 diet. The 20, 40, 60, 80 and 100% of ST powder were substituted with RB, referred to as the RB20, RB40, RB60, RB80 and RB100 diets, respectively. The experimental diets were satisfied for dietary protein and lipid requirements for abalone (Mai et al., 1995a; Mai et al., 1995b).

Next, a 20% 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 minute and the excess solution was drained naturally. The flaked were then dried naturally for 2 days and stored at -20°C until use.

Table 1. Ingredients of the experimental diets

	Experimental diets					
	RB0	RB20	RB40	RB60	RB80	RB100
Ingredient (%, DM)						
Fish meal (CP:72.7%, CL:11.3%)	30	30	30	30	30	30
Soybean meal (CP:53.5%, CL:2.0%)	7	7	7	7	7	7
Wheat flour (CP:14.6%, CL:4.1%)	5	5	5	5	5	5
Dextrin	8	8	8	8	8	8
Sea tangle (ST) (CP:10.5%, CL:0.1%)	20	16	12	8	4	0
Rice bran (RB) (CP:14.9%, CL:16.5%)	0	4	8	12	16	20
Squid liver oil	3	3	3	3	3	3
Soybean oil	1	1	1	1	1	1
Sodium alginate	20	20	20	20	20	20
Mineral premix[1]	4	4	4	4	4	4
Vitamin premix[2]	2	2	2	2	2	2
Nutrients (%, DM)						
Dry matter	90.2	90.7	92.7	92.1	90.4	91.7
Crude protein	28.8	29.0	29.0	29.3	29.3	29.6
Crude lipid	7.4	7.8	8.3	8.9	9.4	9.7
Ash	18.8	17.8	16.1	15.3	14.5	13.7

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

[2]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; choline chloride, 200; cyanocobalamin, 0.003.

2.3. Analytical Procedures of the Diets and Carcass

Thirty abalone at the start and twenty 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 in 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 an index of nutritional status 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 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 standard AOAC (1990) practices.

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 (SAS Institute, Cary, NC, USA). Percentage data was arcsine transformed prior to statistical analysis.

3. Results

Survival of abalone was all over 80%, but not significantly ($P > 0.05$) affected by dietary substitution of ST with RB (Table 2). However, weight gain of abalone fed the RB40 diet was significantly ($P < 0.05$) higher than that of abalone fed the all other diets except for the RB0 diet. However, no significant difference in weight gain was found in abalone fed between the RB0 and RB100 diets substituting 100% ST with RB. SGR of abalone fed the RB40 diet was significantly ($P < 0.05$) higher than that of abalone fed the all other diets. And SGR of abalone fed the RB0 diet was significantly ($P > 0.05$) different from that of abalone fed the RB20, RB60 and RB80 diets.

However, none of shell length, shell width and the ratio of soft weight to total weight of abalone at the end of 16-week feeding trial was significantly ($P > 0.05$) affected by dietary substitution of ST with RB (Table 3).

Moisture content of the soft body of abalone fed the RB20 and RB100 diets was significantly ($P < 0.05$) higher than that of abalone fed the RB0, RB40, RB60 and RB80 diets (Table 4). Moisture content of the soft body of abalone fed the RB80 diet was significantly ($P > 0.05$) higher than that of abalone fed the RB0, RB40 and RB60 diets. Crude protein content of the soft body of abalone fed the RB60 diet was significantly ($P < 0.05$) higher than that of abalone fed the all other diets. The lowest crude protein content was obtained in abalone fed the RB80 diet. However, crude lipid content of the soft body of abalone fed the RB0 diet was significantly ($P < 0.05$) higher than that of abalone fed the all other diets. The lowest ash content was obtained in abalone fed the RB100 diet.

Table 2. Survival (%), weight gain (g/abalone) and specific growth rate (SGR) of juvenile abalone, *Haliotis discus* fed the experimental diets substituting sea tangle (ST) with rice bran (RB) for 16 weeks

Experimental diets	Initial weight (g/abalone)	Final weight (g/abalone)	Survival (%)	Weight gain (g/abalone)	SGR[1] (%/day)
RB0	0.43 ± 0.001	1.38 ± 0.010	83.3 ± 3.81	0.95 ± 0.009^{ab}	0.455 ± 0.0022^{b}
RB20	0.43 ± 0.001	1.38 ± 0.002	82.4 ± 2.08	0.95 ± 0.001^{b}	0.455 ± 0.0006^{bc}
RB40	0.43 ± 0.002	1.41 ± 0.008	85.2 ± 2.08	0.98 ± 0.007^{a}	0.464 ± 0.0009^{a}
RB60	0.43 ± 0.003	1.38 ± 0.014	84.3 ± 0.82	0.94 ± 0.011^{b}	0.452 ± 0.0010^{bc}
RB80	0.43 ± 0.002	1.36 ± 0.009	82.4 ± 1.72	0.93 ± 0.008^{b}	0.451 ± 0.0016^{bc}
RB100	0.43 ± 0.001	1.35 ± 0.015	84.3 ± 1.65	0.92 ± 0.015^{b}	0.448 ± 0.0038^{c}
F-value			0.3	4.1	7.0
P-value			$P > 0.9$	$P < 0.03$	$P < 0.0003$

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

[1]Specific growth rate (SGR) = [(Ln(Wf) - Ln(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.

Table 3. shell length (mm), shell width (mm), shell height (mm), soft body weight (g/individual) and the ratio of soft body weight to total weight of abalone, *Haliotis discus* at the end of the 16-week feeding trial

Experimental diets	Shell length (mm)	Shell width (mm)	Shell height (mm)	Soft body weight (g)	Soft body weight/total weight
RB0	27.0 ± 0.18	18.4 ± 0.16	5.3 ± 0.15	1.3 ± 0.02	0.61 ± 0.008
RB20	27.0 ± 0.58	18.4 ± 0.47	5.3 ± 0.24	1.3 ± 0.10	0.61 ± 0.008
RB40	27.3 ± 0.18	18.5 ± 0.19	5.3 ± 0.04	1.3 ± 0.05	0.60 ± 0.009
RB60	26.6 ± 0.83	17.9± 0.58	5.2 ± 0.12	1.2 ± 0.09	0.60 ± 0.002
RB80	26.8 ± 0.97	18.1 ± 0.60	5.2 ± 0.28	1.1 ± 0.13	0.61 ± 0.005
RB100	27.1 ± 0.57	18.2 ± 0.41	5.2 ± 0.14	1.2 ± 0.11	0.62 ± 0.009
F-value	0.2	0.3	0.1	0.9	1.0
P-value	$P > 0.9$	$P > 0.9$	$P > 0.9$	$P > 0.5$	$P > 0.4$

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

None of biological parameters of abalone measured was significantly different among treatments.

Table 4. Chemical composition (%, wet weight basis) of the soft body of abalone, *Haliotis discus* fed the experimental diets substituting sea tangle (ST) with rice bran (RB) for 16 weeks

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
RB0	78.4 ± 0.09^{c}	18.9 ± 0.06^{bc}	2.7 ± 0.02^{a}	3.4 ± 0.06^{a}
RB20	80.1 ± 0.37^{a}	18.8 ± 0.05^{bc}	2.6 ± 0.04^{a}	3.2 ± 0.06^{b}
RB40	78.5 ± 0.08^{c}	18.9 ± 0.03^{b}	2.6 ± 0.05^{a}	3.2 ± 0.03^{b}
RB60	78.3 ± 0.09^{c}	19.2 ± 0.04^{a}	2.5 ± 0.03^{a}	3.2 ± 0.02^{bc}
RB80	79.2 ± 0.10^{b}	18.7 ± 0.03^{c}	2.7 ± 0.01^{a}	3.2 ± 0.02^{bc}
RB100	80.0 ± 0.15^{a}	18.9 ± 0.10^{bc}	2.7 ± 0.05^{a}	3.0 ± 0.02^{c}
F-value	21.6	10.2	2.2	7.7
P-value	$P < 0.001$	$P < 0.0005$	$P > 0.1$	$P < 0.002$

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

4. Discussion

A commercial formulated diet might be suitable for juvenile greelip abalone, *H. laevigata* larger than 4 mm in shell length (Daume and Ryan 2004). Later, Daume et al. (2007) also recommended that moving greenlip abalone into a tank system to feed formulated feed rather than an algal diet consisting of diatoms or macroalgae once they reach 7 mm in shell length. Since an initial size of abalone used in this study was 1 cm in shell length, abalone grew well on the experimental diets.

Mai et al. (1995a) reported that improvement in weight gain of abalone, *H. discus hannai* fed the diets containing 3.11-7.09% lipid levels was observed when abalone was fed by one of the 25% protein diets with 0.63% to 11.58% lipid levels. Similarly, Green et al. (2011) reported that South African abalone, *H. midae* was unable to effectively utilize lipid as an energy source at levels in excess of 7% due to inherent physiological constraints. Therefore, abalone fed the RB0 diet containing 28.8% crude protein and 7.4% crude lipid seemed to grow relatively well in this study. No significant difference in weight gain of abalone fed the RB100 diet compared to the RB0 diet in this study indicated that complete substitution of ST with RB could be made without retardation of growth of abalone when the 20% ST was included into the combined fish meal and soybean meal-basal diet. However, no difference and significant difference in SGR of abalone fed the RB80 and RB100 diets compared to that of the RB0 diet, respectively in this study indicated that the 80% ST could be substituted with RB in the diet in terms of SGR of abalone. In addition, slightly and significantly improvement in weight gain and SGR abalone fed the RB40 diet compared to those of abalone fed the RB0 diet, respectively indicated that abalone grew better on the diet substituting 40% ST with RB rather than the RB0 diet containing the 20% ST exclusively.

Abalone species, *H. discus* used in this study seemed to utilize RB as well as ST in the experimental diets. Abalones are known to be herbivorous and feed mostly on macroalgae, which is usually low in lipid but high in carbohydrate, 40-50% (Thongrod et al., 2003). Because RB contained 40.5% carbohydrate calculated by the equation of the difference between 100 and sum of crude protein, crude lipid and ash content, RB seemed to be a good feed ingredient to replace ST of abalone feed. Fleming et al. (1996) also reported that abalone has various enzymes capable of hydrolyzing complex carbohydrates. Similarly the leaf meal, *M. oliefera* and freshwater aquatic fern, *A. pinnata* culture in terns of local availability of year round in Philippines (Reyes and Fermin 2003). Because macroalgae used for abalone culture in the past is not a cheap feed ingredient anymore, the development of the alternative feed ingredient (plant meal) that is both cheap and year-round available keeps being needed, In the earlier study (Cho et al., 2008), the possibility of dietary supplementation of byproduct of green tea for abalone (*H. discus hannai*) was reported.

Three different species abalone, *H. discus*, *H. sieboldii* and *H. discus hannai* grew better on the commercial or formulated diet than the macroalgae feed, *Undaria* reared at 17.2-26.2°C in the 9-week feeding (Kim et al., 1998). The reason for poorer growth of abalone fed the latter compared to the former might be that nutrient content in the latter does not satisfy dietary nutrient requirement for abalone, such as protein and lipid. Similarly, growth of abalone fed the formulated or commercial diet was faster than that of abalone fed the single macroalgae (Lee et al., 1997; Viana et al., 1993; Garcia-Esquivel and Felbeck 2009). However, supplementation of microalgae, *Arthrospira maxima* and macroalgae, *Dunaliella salina* into the commercial feed improved weight gain and immunity of greenlip abalone, whereas *Ulva lactuca* and *Spyridia filamentosa* could be useful supplements for abalone aquaculture, especially in areas with high risk of herpesvirus infection (Dang et al., 2011).

Unlike growth performance of abalone, the distinctive biological parameters of abalone (shell length, shell width, shell height, soft body weight and the ratio of soft body weight to total weight of abalone) was not affected by dietary substitution of ST with RB in this study. Similarly, the biological parameters of abalone would not be different among treatments although weight gain of abalone was significantly affected by dietary lipid level (Green et al., 2011). Unlike this study, However, the biological parameters of abalone measured agreed with weight gain of abalone (Bautista-teruel et al., 2003; Cho 2010).

Proximate composition of the soft body of abalone was affected by dietary substitution of ST with RB except for crude lipid content in this. Ash content of the soft body of abalone was relatively well reflected from that of the experimental diets. Proximate composition (Mai et al., 1995a, Thongrod et al., 2003; Cho et al., 2008; Gracia-Esquivel and Felbeck 2009;

Cho 2010).

Substitution effect of ST with RB in the commercial diet for abalone farm will be reported shortly. In conclusion, the 100% ST could be substituted with RB without a retardation of weight gain of abalone when the 20% ST was included into the experimental diet. However, the best growth performance was obtained in abalone fed the RB40 diet substituting 40% ST with RB.

Ⅱ. Conclusion

The 100% sea tangle (ST) could be substituted with rice bran (RB) without a retardation of weight gain of juvenile abalone, *H. discus* when the 20% ST was included into the experimental diet. The best growth performance was obtained in abalone fed the RB40 diet substituting 40% ST with RB.

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