Thesis for the Degree of Master of Science

Effects of different feeding regimes on compensatory growth of juvenile abalone (*Haliotis discus hannai*) fed the formulated diet and dry sea tangle (*Laminaria japonica*)

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by

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요 약

본 연구에서는 주요 패류 양식종인 전복(*Haliotis discus hannai*)을 대상으로 하여 전복용 배합사료와 건조다시마(*Laminaria japonica*) 공급 시 다양한 사료 공급 방법에 따른 전복의 보상성장을 조사하였다.

실험 1에서는 전복용 배합사료 공급 시 사료 공급 방법을 달리한 6종류의 실험구를 설정하였으며 16주간 매일 사료 공급하는 대조구(Cont.), 1주 절식 이후 15주간 사료 공급 실험구(Starvation 1 treatment: S1), 2주 절식 이후 14주간 사료 공급 실험구(Starvation 2 treatment: S2), 3주 절식 이후 13주간 사료공급 실험구(Starvation 3 treatment: S3), 4주 절식 이후 12주간 사료공급 실험구(Starvation 4 treatment: S4), 6주 절식 이후 10주간 사료공급 실험구(Starvation 6 treatment: S6)였고 3반복구를 두었다.

사육실험 종료 시 전복의 체중 증가량은 절식기간에 비례하여 감소하는 경향을 보였으며, 1주간의 짧은 절식기간에도 완전한 보상성장을 나타내지 않았다. Cont., S1, S2 및 S3 실험구에서 전복의 생존율은 S4와 S6 실험구의 전복 생존율보다 유의적

으로 높게 나타났다(P<0.05). 전복의 각장과 각폭은 절식에 비례하여 감소하는 경향을 보였으며, 실험 종료 시 전복의 체중 증가 결과와 유사하였다. 반면 전복 체중에 대한 가식부의 비율은 Cont., S1 및 S4 실험구에서 가장 낮은 값을 보인 S6 실험구보다 유의적으로 높았으나(P<0.05), S2 와 S3 실험구와는 유의적인 차이를 보이지 않았다(P>0.05). 전복 가식부의 수분과 조지질 및 회분 함량은 실험구간 유의적인 차이를 보이지 않았다(P>0.05). 반면 Cont.에서 전복 가식부의 조단백질 함량은 S4와 S6 실험구에 비하여 유의적으로 높았으나(P<0.05), S1, S2와 S3 실험구와는 유의적 차이를 보이지 않았다(P>0.05).

실험 2에서는 먹이의 종류에 따른 전복의 보상성장 효과를 비교하기 위하여 건조다시마 공급 시 실험 1과 동일한 사료공급 방법으로 전복의 보상성장을 조사하였다. 실험 구간은 실험 1과 동일하며, 사료공급 방법은 6개의 실험구를 설정하였다. 실험구는 16주간 매일 건조다시마 공급 대조구(Cont.), 1주 절식이후 15주간 건조다시마 공급 실험구(Starvation 1 treatment: S1), 2주 절식이후 14주간 건조다시마 공급 실험구(Starvation 2 treatment: S2), 3주 절식 이후 13주 건조다시마 공급 실험구(Starvation 3 treatment: S3), 4주 절식 이후 12주간 건조다시마 공급 실험구(Starvation 4 treatment: S4), 6주 절식 이후 10주간 건조다시마공급 실험구(Starvation 6 treatment: S6)로 3반복구 실험하였으며, 먹이는 1일 1회 손으로 만복 시까지 공급 하였다.

실험종료 후 Cont. 에서 전복 치패의 성장은 우수하였으나(P<0.05), S1, S2 및 S3 실험구와 유의적 차이는 없었다(P>0.05). Cont. 와 S1, S2 및 S3 실험구에서 전복 각장은 S6 실험구에 비해 유의적으로 성장하였지만(P<0.05), S4 실험구와 유의적 차이는 없었다(P>0.05). 각폭, 가식부의 비율, 수분, 조단백질과 조지질 및 회분 함량은 모든 실험구간에 유의적 차이를 보이지 않았다(P>0.05).

본 연구결과 전복 치패에 있어서 전복용 배합사료와 건조다시마 공급 시, 본 연구에서 이용된 다양한 사료공급 방법 중 전복용 배합사료 공급시 실험 1에서는 완전한 보상성장은 나타나지 않았다. 건조 다시마를 공급 시 실험2에서 3주 절식 이후 사료를 공급하는 실험구에서는 전복의 보상성장 효과가 나타났다. 이러한 결과는 전복에

있어서 공급하는 먹이의 종류에 따라 보상성장 효과가 다르게 나타나는 것으로 판단된다.

I. General Introduction

Since abalone (*Haliotis discus hannai*) has been used for human consumption due to its high nutrient content such as vitamins and amino acids in Eastern Asia including Korea, its demand becomes high. As demand of abalone for human consumption increases, its farmed supply keeps rapidly increasing. Annual production of abalone takes up only 2-3% (6,207 MT) of the shellfish (326,544 MT) production in volume, but it is responsible for over 50% (233 billion Won) of the total value (494 billion Won) in 2008, being a highly valued species (MIFAFF 2009).

Abalone farmers commonly supply macroalga such as sea tangle (*Laminaria japonica*) and *Undaria pinnatifida* which can be easily harvested in wild to abalone during winter season, on the other hand, they supply dried sea tangle (*L. japonica*) and *U. pinnatifida* for the rest of the year in Korea. However, these macroalga are known not to satisfy dietary nutrient requirement of abalone (Lee et al. 1997, Lee et al. 1998). Therefore, several feeding trials to develop artificial feed with nutrition-balanced for abalone, such as dietary protein requirement and sources (Uki et al. 1985; Uki, et al. 1986a; Mai et al. 1995b; Lee et al. 1998a; Bautista-Teruel et al. 2003; Cho et al. 2007), dietary lipid requirement and sources (Mai et al. 1995a; Lee and Park 1998), dietary fatty acid requirements (Uki et al. 1986), dietary carbohydrate sources (Lee et al. 1998b) and dietary pigment sources (Lim and Lee 2003) have been performed. Optimum dietary protein and lipid levels of abalone were estimated to be 25-35% and 3-7%, respectively when various levels of protein and lipid in the diets were tested on abalone (Mai et al. 1995a, 1995b; Fleming et al. 1996; Bautista-Teruel et al. 2003).

At occurrence of unfavourable environmental condition, such as coldwater mass or red tide, abalone which is a slow-moving shellfish is likely to be starved to minimize mortality at abalone farms. Sometimes this condition may last over several weeks and it eventually result to the economical loss of abalone farmers due to a decrease in production of abalone. Under this condition, application of compensatory growth which is a faster growth rate of animals after realimentation while undernutrition is one of the most effective methods to improve growth and production of abalone. A fewer studies on compensatory growth of donkey's ear abalone *H. asinina* (Fermin 2002), northern abalone *H. kamtschatkna* Carefoot et al. (1993) compared to fish (Bilton and Robins 1973; Quinton and Blake 1990; Damsgaard and Dill 1998; Rueda et al. 1998; Gaylord and Gatlin 2000, 2001; Wang et al. 2000; Tian and Qin 2003, 2004; Cho 2005b; Cho and Jo 2002) have been performed. Compensatory growth of fish varied depending on fish species, feed allowance, fish species, fish size, water temperature, feed allowance, dietary nutrient content, duration of feeding trial, feeding regimes and social factor (Bilton and Robins 1973; Hayward et al. 1997; Rueda et al. 1998; Gaylord and Gatlin 2000; Wang et al. 2000; Gaylord and Gatlin 2001; Zhu et al. 2001; Ali et al. 2003; Oh et al. 2007, 2008; Bavcevic et al. 2010).

Therefore, in this study, effects of different feeding regimes on compensatory growth of juvenile abalone fed the formulated diet and dry sea tangle were compared.

II. Experiment 1

Aquaculture Research

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SHORT COMMUNICATION

Compensatory growth of juvenile abalone *Haliotis discus hannai* with different feeding regime

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Compensatory growth of fish (a rapid or faster than normal growth rate resulting from refeeding after fasting or undernutrition) has been reported in many fish species and varies depending on fish species, fish size, water temperature, feed allowance, dietary nutrient composition, duration of feeding trial and feeding regime (Zhu, Cui, Ali & Wootton 2001; Cho 2005; Cho, Lee, Park, Ji, Lee, Bae & Oh 2006; Oh, Noh & Cho 2007; Oh, Noh, Kang, Kim, Cho & Jo 2008). However, there are few studies on the effect of alternate starvation and refeeding cycles on compensatory growth of donkey's ear abalone *Haliotis asinina* (Fermin 2002). Compensatory growth of fish is usually accompanied by an increase in food intake after refeeding, called hyperphagia (Fermin 2002; Cho *et al.* 2006; Oh *et al.* 2007, 2008).

Abalone (*Haliotis discus hannai* Ino) is the most commercially important marine shellfish species for aquaculture in Eastern Asia because of its high nutritional value for human consumption. Thus, many feeding trials on dietary protein requirements (Uki, Kemuyama & Watanabe 1986; Mai, Mercer & Donlon 1995b) and protein sources (Uki, Kemuyama & Watanabe 1985; Lee, Yun & Hur 1998; Bautista-Teruel, Fermin & Koshio 2003; Cho, Park, Kim & Yoo 2007), dietary lipid requirement and sources (Mai, Mercer & Donlon 1995a; Lee & Park 1998), dietary fatty acid requirements (Uki, Sugiura & Watanabe 1986), dietary carbohydrate sources (Lee, Yun, Min & Yoo 1998) and dietary pigment sources (Lim & Lee 2003) have been performed for abalone grow-out. Optimum abalone dietary protein and lipid requirements were estimated to be 25–35% and 3–7%, respectively, when various levels of protein and lipid in abalone diets were tested (Mai *et al.* 1995a, b; Fleming, Barneveld & Hone 1996; Bautista-Teruel *et al.* 2003).

Under unfavourable environmental conditions, such as an occurrence of coldwater mass or red tide, abalone is likely to be starved to minimize mortality at abalone farms. Sometimes this condition may last over several weeks during abalone culture, deteriorate its growth and eventually lead to a reduction in abalone production. In this study, therefore, the possibility of compensatory growth of abalone with different feeding regimes when the properly formulated diet was supplied was investigated.

Juvenile abalone were purchased from a private hatchery and transferred to an abalone farm (Korea Aquaculture Institute, Gyeongsangbuk-do, Korea). Before the initiation of the feeding trial, abalone were acclimated to the experimental conditions for 4 weeks and fed the dry sea tangle (*Laminaria japonica*) once a day at the ration of 1.5–2.0% of total biomass. Fifty juvenile abalone averaging 10.0 $\pm$ 0.06 g (mean $\pm$ SD) were randomly stocked into each of the 18, 50 L plastic rectangular containers. Six containers were placed into each of three 1.3 tonne concrete flow-through raceway systems at a flow rate of 120 L min^{-1}. Sand-filtered seawater at temperatures ranging from 16.7 to 26.5 °C (mean $\pm$ SD; 22.2 $\pm$ 2.76 °C) was supplied throughout the 16-week feeding trial. Aeration was supplied into each raceway and the photoperiod followed the natural condition. The experimental diets were fed to abalone once a

Table 1 Ingredients and nutrient composition of the experimental diet

	Composition ($g\,kg^{-1}$)
Ingredients	
Fishmeal*	380
Defatted soybean meal	150
Dextrin†	170
Sea tangle powder	30
Soybean oil	5
Fish oil	5
Sodium alginate‡	200
Vitamin premix§	20
Mineral premix¶	40
Nutrient (DM basis, $g\,kg^{-1}$)	
Dry matter	812
Crude protein	359
Crude lipid	50
Ash	155

*Fishmeal imported from Chile.
†Dextrin was purchased from Sigma Chemical (St Louis, MO, USA).
‡Sodium alginate was purchased from Sigma Chemical.
§Vitamin premix contained the following amounts of ingredients, which were diluted in cellulose ($g\,kg^{-1}$ 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.
¶Mineral premix contained the following ingredients ($g\,kg^{-1}$ 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.

day (17:00 hours) to satiation (about 1.5–2.0% biomass). Dead abalone were removed daily and the bottoms of the containers were siphon-cleaned every other day.

A 38% fishmeal and 15% defatted soybean meal combination was used as the protein source in the experimental diet (Table 1). Dextrin, and soybean oil and fish oil were used as carbohydrate and lipid sources, respectively, and a 20% sodium alginate was also added to the experimental diet. Thereafter, all the ingredients were mixed mechanically with water at 1:1 by volume. A paste was prepared with 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 dipped into an aqueous solution of $CaCl_2$ (5%) for 1 min and the excess of liquid was drained naturally. The flakes were then dried naturally for 2 days and stored at -20 °C until use.

Six treatments with triplicates were used. The abalone control group (Con) was fed once daily to satiation on the experimental diet for 16 weeks. Other abalone were fed once a day for 15 weeks after a 1-week feed deprivation (S1 treatment), for 14 weeks after a 2-week feed deprivation (S2 treatment), for 13 weeks after a 3-week feed deprivation (S3 treatment), for 12 weeks after a 4-week feed deprivation (S4 treatment) and for 10 weeks after a 6-week feed deprivation (S6 treatment) respectively.

At initiation, 20 abalone were sampled and frozen for chemical analysis and 15 abalone from each container at the end of the feeding trial were also sampled and frozen for chemical analysis. All samples were thawed and the shell and soft-body tissue were separated before examination. Shell length and width were measured in millimetres with a digital caliper (Mitutoyo, Kawasaki, Japan), and the ratio of the soft body weight to total body weight (the carcass weight+the excised shell's weight) of abalone was calculated to determine an index of the nutritional status of abalone.

The separated carcasses of all abalone from each container were 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 by an ether-extraction method, moisture was determined by oven drying at 105 °C for 24 h and ash was determined by a muffle furnace at 550 °C for 4 h. All these methods were adopted from AOAC (1990).

One-way ANOVA and Duncan's multiple range test (Duncan 1955) were used to determine the differences between the means of treatments through SAS version 9.1 (SAS Institute, Cary, NC, USA). A regression analysis was used for weight change of abalone over time during the 6-week food deprivation (SAS Institute).

The following linear relationship was observed between the change in weight ($g\ abalone^{-1}$) of abalone and the week of feed deprivation ($P<0.007$): Y (weight of abalone) $= -0.04X$ (week of feed deprivation)$+10.14$, $R^2=0.6108$. A reduction in weight in abalone with any number of weeks of feed deprivation was relatively lesser than that of fish reported in Cho (2005) and Cho *et al.*'s (2006) studies (Fig. 1).

Survival of abalone in the Con, S1, S2 and S3 treatments was significantly ($P<0.05$) higher than that in the S4 and S6 treatments (Table 2). However, unlike this study, Carefoot, Qian, Taylor, West and Osborne (1993) reported that no mortality of abalone (*Haliotis kamtschatkana*) subjected to 27 days of starvation was observed and these periods of starvation led to no debilitating illness or excessive weight loss in abalone. Abalone subjected to a > 4-week starvation seemed to suffer a critically lower survival rate.

Weight gain of abalone tended to decrease in proportion to the number of weeks of feed deprivation, indicating that juvenile abalone do not have the ability to achieve full compensatory growth after being subjected to any number of weeks of feed deprivation under this experimental condition. Unlike this study, however, growth of abalone fed on the macro alga (*Gracilariopsis bailinae*) with a different feeding regime (alternate 5-day feed deprivation and 5-day refeeding or 10-day feed deprivation and 10-day refeeding for 120 days) was poorer than that of abalone fed the alga daily, although full compensatory growth was achieved in donkey's ear abalone subjected to alternate feed deprivation and refeeding, and then fed alga to *ad libitum* daily during the following 80 days (Fermin 2002). Based on the results of this study and Fermin's (2002) study, abalone seemed to have the ability to achieve differing compensatory growth depending on species, feeding regime and diet type. In comparison, some fish species

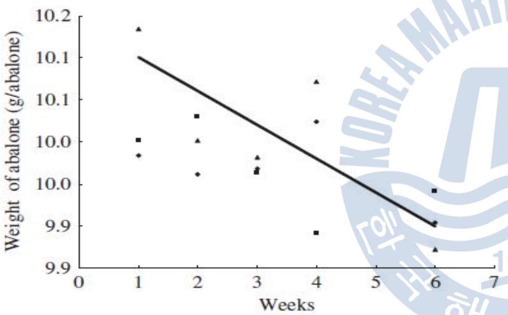

Figure 1 Change in weight (g abalone^{-1}) of juvenile abalone *Haliotis discuss hannai* with feed deprivation (mean ± SE, $n = 3$): Y (weight of abalone) $= -0.04X$ (week of feed deprivation)+ 10.14, $R^2 = 0.6108$. ($P < 0.007$).

had an ability to achieve full compensatory growth up to a 2-week feed deprivation in the feeding trials (Zhu *et al.* 2001; Cho 2005; Cho *et al.* 2006). The smaller fish seemed less able to tolerate feed deprivation (Bilton & Robins 1973).

Shell length of abalone in the Con and S1 treatments was significantly ($P < 0.05$) longer than that of abalone in the S2, S3, S4 and S6 treatments. Shell width of abalone in the Con treatment was not significantly ($P > 0.05$) different from that in the S1 treatment, but significantly ($P < 0.05$) greater than that in the S2, S3, S4 and S6 treatments. Both shell length and shell width tended to decrease with the number of weeks of feed deprivation, similar to the pattern of weight gain at the end of the 16-week feeding trial in this study. However, the ratio of the soft body weight to the total body weight of abalone in the Con, S1 and S4 treatments was significantly ($P < 0.05$) greater than that in the S6 treatment, which was the lowest, but not significantly ($P > 0.05$) different from that in the S2 and S3 treatments.

The moisture, crude lipid and ash content of abalone carcasses were not significantly ($P > 0.05$) different between treatments (Table 3). However, crude protein content of abalone in the Con treatment was significantly ($P < 0.05$) higher than that of abalone in the S4 and S6 treatments, but not significantly ($P > 0.05$) different from that of abalone in the S1, S2 and S3 treatments. The lowest crude protein content was obtained in abalone in the S6 treatment. Crude protein content of abalone tended to decrease with the number of weeks of feed deprivation except for abalone in the S2 treatment, partially agreeing with other studies, which showed that the crude protein content of fish decreased with the number of weeks of feed deprivation (Oh *et al.* 2007, 2008).

Because the nutrient (protein and lipid) content of macro-algae is poorer than that of the well-formulated diet, the compensatory growth of abalone

Table 2 Survival (%), weight gain (g abalone^{-1}), shell length (mm), shell width (mm) and the ratio of the soft body weight to the total body weight of abalone fed the experimental diet for 16 weeks

Treatments	Initial weight (g abalone^{-1})	Final weight (g abalone^{-1})	Survival (%)	Weight gain (g abalone^{-1})	Shell length (mm)	Shell width (mm)	Soft body weight/ total body weight
Con	10.0 ± 0.02	17.8 ± 0.40^{a}	98.0 ± 1.15^{a}	7.7 ± 0.38^{a}	52.7 ± 0.43^{a}	34.5 ± 0.47^{a}	0.70 ± 0.006^{a}
S1	10.1 ± 0.05	16.6 ± 0.25^{b}	98.0 ± 1.15^{a}	6.5 ± 0.30^{b}	51.7 ± 0.13^{a}	33.5 ± 0.23^{ab}	0.70 ± 0.005^{a}
S2	10.0 ± 0.02	14.4 ± 0.26^{cd}	100 ± 0.00^{a}	4.7 ± 0.09^{c}	49.7 ± 0.91^{b}	32.6 ± 0.11^{bc}	0.68 ± 0.005^{ab}
S3	10.0 ± 0.01	14.7 ± 0.10^{c}	98.7 ± 1.76^{a}	4.4 ± 0.24^{cd}	49.7 ± 0.65^{b}	31.8 ± 0.69^{cd}	0.68 ± 0.008^{ab}
S4	10.1 ± 0.04	14.1 ± 0.24^{cd}	93.3 ± 1.76^{b}	4.0 ± 0.23^{cd}	49.4 ± 0.43^{b}	31.7 ± 0.19^{cd}	0.69 ± 0.001^{a}
S6	10.0 ± 0.01	13.6 ± 0.15^{d}	90.0 ± 1.15^{b}	3.6 ± 0.15^{d}	48.7 ± 0.38^{b}	31.4 ± 0.22^{d}	0.67 ± 0.008^{b}

Values (mean ± SE) in the same column sharing a common superscript are not significantly different ($P < 0.05$).

Table 3 Chemical composition (g kg^{-1}) of carcass of abalone with different feeding strategy

Treatments	Moisture	Crude protein	Crude lipid	Ash
Con	807 ± 2.5	136 ± 1.6^{a}	13 ± 0.6	23 ± 0.3
S1	815 ± 6.1	126 ± 4.9^{abc}	13 ± 1.4	23 ± 1.5
S2	806 ± 2.2	130 ± 1.4^{ab}	16 ± 1.0	23 ± 0.2
S3	808 ± 1.1	127 ± 1.4^{abc}	13 ± 1.0	23 ± 0.3
S4	813 ± 4.6	122 ± 4.7^{bc}	12 ± 1.6	24 ± 0.2
S6	815 ± 3.3	119 ± 2.4^{c}	15 ± 2.3	24 ± 0.4

Values (mean ± SE, $n = 3$) in the same column sharing the same letter are not significantly different ($P < 0.05$).

can potentially be quite different. Therefore, a comparison of compensatory growth of abalone subjected to fasting when fed on macro-algae and the well-formulated diet is urgently needed in the future.

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Keywords: abalone *Haliotis discus hannai*, compensatory growth, feed deprivation, refeeding

III. Experiment 2

Effect of feeding regime on compensatory growth of juvenile abalone (*Haliotis discus hannai*) fed the dry sea tangle (*Laminaria japonica*)

Abstract

Effect of feeding regime on compensatory growth of juvenile abalone (*Haliotis discus hannai*) fed the dry sea tangle (*Laminaria japonica*) was determined. Six treatments with triplicate were used in this study: abalone were fed the dry sea tangle once a day at a satiation level for 16 weeks as a control (Cont.) and other five groups of abalone were fed the dry sea tangle once a day at a satiation level for 15 weeks after 1-week starvation (Starvation 1 treatment: S1), 14 weeks after 2-week starvation (Starvation 2 treatment: S2), 13 weeks after 3-week starvation (Starvation 3 treatment: S3), 12 weeks after 4-week starvation (Starvation 4 treatment: S4), and 10 weeks after 6-week starvation (Starvation 6 treatment: S6), respectively.

The highest survival of abalone was achieved in the S2 treatment, but not different from that of abalone in the Cont., S1 and S3 treatments (P>0.05). Weight gain of abalone in the Cont. treatment was higher than that of abalone in the S4 and S6 treatments (P<0.05), but not different from that of abalone in the S1, S2 and S3 treatments (P>0.05). Chemical composition

of abalone was not affected by different feeding regimes (P>0.05).

1. Introduction

Abalone (*Haliotis discus hannai*) is one of the most commercially important marine shellfish species for aquaculture in Korea due to its high market value and nutrient content for human consumption, hence, its annual aquaculture production has recently kept in creasing sharply (KNSO 2009). It has been commonly raised by feeding macroalga, such as sea tangle (*Laminaria japonica*) or *Undaria pinnatifida* in commercial farming, but this macroalga is naturally grown and harvested during the winter season. Therefore, dry sea tangle or *U. pinnatifida* harvested during the winter season and prepared by dry processing is commonly supplied to abalone during year-round culture except for the winter season.

Since macroalgae were known to be limiting in dietary nutrient requirements for the growth of abalone probably due to its low protein and/or lipid content (Viana et al. 1993; Lee et al. 1998; Bautista-Teruel and Millamena 1999; Cho et al. 2008), it could eventually lead to poor production of abalone. Mai et al. (1994) reported that amino acids which were likely to be deficient in 6 species of macroalgae could be the limiting factors for growth of abalone. Therefore, Fleming et al. (1996) suggested the development of artificial diets for abalone culture. However, farmers prefer feeding abalone by macroalgae in place of artificial diets in commercial farming due to easy management and less water pollution sources discharged.

Compensatory growth of fish, a rapid or faster than normal growth rate resulting from refeeding after fasting or undernutrition, commonly occurs in the wild due to limited food availability. A feeding strategy leading to compensatory growth of abalone could be useful to abalone farming. Carefoot et al. (1993) reported that 27 days of starvation led to no debilitating illness or excessive weight loss and no loss in meat quality in Northern abalone (*H. kamtschatkana*). Also, donkey's ear abalone (*H. asina*) fed on the seaweed (*Gracilariopsis bailinae*) for 60 days after subjected to alternate feeding regimes (5-day starving and 5-day refeeding or 10-day starving and 10-day refeeding) for 140 days achieved full compensatory growth (Fermin 2002). However, abalone achieved only partial compensatory growth when fed a nutrition-balanced diet after 1-, 2-, 3-, 4- and 6-week starvation in the previous study (Cho et al. 2009). Dietary nutrient composition could largely affect compensatory growth of abalone as well.

In this study, the effect of feeding regime on compensatory growth of abalone fed on the dry sea tangle was determined.

2. Materials and Methods

2.1. Abalone and the Experimental Conditions

Juvenile abalone of the same age (approximately 12 months old) and similar size were purchased from a private hatchery (Harim Abalone Farm, Jeollanam-do, Korea) and transferred to an abalone farm (Korea Aquaculture Institute, Gyeongsangbuk-do, Korea). Before initiation of the feeding trial, abalone were acclimated to the experimental conditions for 2 weeks and fed the dry sea tangle once a day at 1-2% biomass. Thirty juvenile abalone (an initial body weight: 15.7±0.07 g) were randomly stocked into each of the 18, 50 plastic rectangular containers (water volume: 25 L). Six containers were placed into each of three 1.3 ton concrete flow-through raceway systems (water volume: 600 L) at a flow rate of 120 L/min. A mixture of the sand-filtered seawater and power plant effluent was supplied to maintain constant temperature, ranging from 21.0 to 23.0 °C (mean±SD: 22.3±0.48 °C). Aeration was supplied into each raceway and the photoperiod followed the natural condition. Dead abalone were removed daily and the bottoms of the containers were siphon-cleaned on every other day.

2.2. Design of the Feeding Trial

Six treatments with triplicates were used in this study. Abalone were fed the dry sea tangle (Crude protein 10.5%, crude lipid 0.1% and ash 22.8%) once a day (17:00) at a satiation level with a little left over (1.5-2.0% of biomass) for 16 weeks as a control (Cont.). Other abalone were fed the dry sea tangle once a day at the satiation level with a little leftover for 15 weeks after 1-week starvation (Starvation 1 treatment: S1), 14 weeks after 2-week starvation (Starvation 2 treatment: S2), 13 weeks after 3-week starvation (Starvation 3 treatment: S3), 12 weeks after 4-week starvation (Starvation 4 treatment: S4), and 10 weeks after 6-week starvation (Starvation 6 treatment: S6), respectively.

2.3. Analysis of Proximate Composition of Abalone

Ten abalone at the initiation of the experiment and fifteen abalone from each container at the end of the feeding trial were sampled and frozen at − 40 °C for chemical analysis. All samples were thawed, followed by separation of the shell and soft-body tissue prior to examination. Shell length and shell width were measured in mm with a digital caliper (Mitutoyo Corporation, Kawasaki, Japan), and the ratio of the soft body weight to total body weight (the carcass weight+the excised shell's weight) of abalone was calculated to determine an index of the nutritional status of abalone.

The separated carcass 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 determined by an ether-extraction method (Soxtec TM 2043 Fat Extraction System, Foss Tecator, Sweden), moisture determined by oven drying at 105 °C for 24 h, and ash determined by a muffle furnace at 550 °C for 4 h. All these methods were adopted from AOAC (1990).

2.4. Statistical Analysis

One-way ANOVA and Duncan`s multiple range test (Duncan 1955) were used to determine the differences between the means of treatments through

SAS version 9.1 (SAS Institute, Cary, NC, USA). Also, a regression analysis was used for weight change of abalone over time during the 6-week starvation (SAS Institute, Cary, NC, USA).

3. Results and Discussion

A linear relationship between weight change of abalone (g/abalone) and week of starvation was observed ($P<0.0001$): Y (weight of abalone)= − 0.17X (week of starvation)+15.89, $R^2=0.9462$ (Fig. 1). Weight loss of juvenile abalone averaging 15.7 g was estimated to be 3.2% at mean temperature of 22.3±0.48 °C for 4-week starvation in this study, whereas that of juvenile green abalone averaging 38.8 g was estimated to be 13.4% at 21.5±0.5 °C for 27-day starvation (Viana et al. 2007). This difference could have resulted from the greater weight loss in the larger abalone than the smaller one during starvation. Cho et al. (2009) reported weight loss of smaller abalone averaging 10.0 g at 22.2 °C for 4-week starvation was less (ca. 1%) than this study. Also 27 days of starvation led to no debilitating illness, excessive weight loss or deterioration of meat quality in Northern abalone (Carefoot et al. 1993).

The highest survival of abalone was achieved in the S2 treatment, but not significantly different ($P>0.05$) from that of abalone in the Cont., S1 and S3 treatments (Table 1).

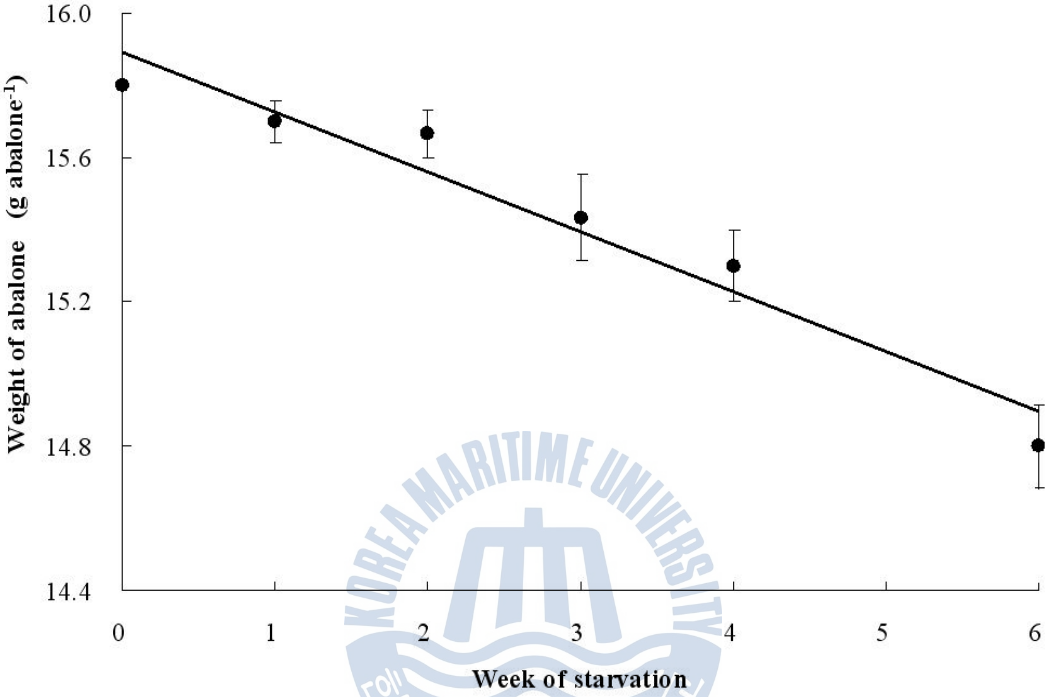

Figure 1. Change in weight (g abalone^{-1}) of juvenile abalone with starvation (means±SE, n=3): Y = − 0.17X+15.89, R^2=0.9462 (P<0.0001) X= (week of starvation), Y= (weight of abalone).

Also, no significant (P>0.05) difference in survival of abalone was observed in the Cont., S1, S3 and S4 treatments. This might indicate that abalone subjected to more than 4-week starvation could lead to decreased survival. The lowest survival of abalone was observed in the S6 treatment in this study. Similarly, Cho et al. (2009) reported that abalone subjected to longer feed deprivation had lower survival at the end of the feeding trial. Weight gain of abalone in the Cont. treatment was significantly (P<0.05) higher than that of abalone in the S4 and S6 treatments, but not significantly (P>0.05) different from that of abalone in the S1, S2 and S3 treatments. This might indicate that abalone fed on the dry sea tangle after up to 3-week starvation could achieve full compensatory growth in this study. Also weight gain of abalone in the S4 treatment was significantly (P<0.05) higher than that of abalone in the S6 treatment. Lower weight gain was achieved in abalone fed on the dry sea tangle than nutrition-balanced diet, agreeing with other studies (Lee et al. 1998; Bautista-Teruel and Millamena 1999; Cho et al. 2008). However, unlike this study, abalone did not achieve full, but partial compensatory growth in proportion to week of starvation (Cho et al. 2009). According to the results of both studies, the ability of juvenile abalone to achieve compensatory growth seemed to vary depending on feed nutrient content (or type). Growth rate of control group in this study was lower than that of abalone fed on the nutrition-balanced diet in Cho et al. (2009)'s study (35% vs 77%).

Hence abalone fed on the sea tangle 3-weeks of starvation could fully catch up with growth of control abalone.

Table 1. Survival (%), weight gain (g abalone^{-1}), shell length (mm), shell width (mm) and the ratio of the soft body weight to total body weight of abalone fed the experimental diet for 16 weeks[1]

Treatments	Initial weight (g abalone^{-1})	Final weight (g abalone^{-1})	Survival (%)	Weight gain (g abalone^{-1})	Shell length (mm)	Shell width (mm)	Soft body weight/total body weight
Cont.	15.8 ± 0.01	21.3 ± 0.56^{a}	74.4 ± 4.01^{ab}	5.5 ± 0.54^{a}	57.7 ±1.42^{a}	36.8 ± 1.12^{a}	0.59 ± 0.010^{a}
S1	15.7 ± 0.05	20.1 ± 0.19^{ab}	67.8 ± 1.11^{ab}	4.3 ± 0.16^{ab}	56.3 ± 0.19^{a}	36.3 ± 0.33^{a}	0.59 ± 0.007^{a}
S2	15.7 ± 0.06	19.7 ± 0.64^{b}	75.6 ± 2.22^{a}	4.0 ± 0.59^{ab}	56.0 ± 0.61^{a}	36.2 ± 0.56^{a}	0.568 ± 0.014^{a}
S3	15.7 ± 0.01	19.9 ± 0.48^{ab}	66.7 ± 0.00^{abc}	4.2 ± 0.48^{ab}	56.4 ± 1.70^{a}	36.3 ± 1.46^{a}	0.58 ± 0.019^{a}
S4	15.7 ± 0.02	18.8 ± 0.45^{b}	64.4 ± 6.19^{bc}	3.2 ± 0.47^{b}	55.2 ± 0.20^{ab}	35.8 ± 0.27^{a}	0.60 ± 0.009^{a}
S6	15.8 ± 0.01	17.3 ± 0.33^{c}	56.7 ± 0.00^{c}	1.5 ± 0.34^{c}	52.3 ± 0.21^{b}	34.2 ± 0.52^{a}	0.59 ± 0.009^{a}

[1]Values (means±SE, n=3) in the same column sharing a same superscript letter are not significantly different (*P*>0.05).

However, growth of donkey's ear abalone subjected to alternate 5-day starvation and 5-day refeeding or 10-day starvation and 10-day refeeding was less than that of abalone fed on the seaweed daily for the first 140 days, but it accelerated after feeding *ad libitum* daily for the following 60 days and eventually resulted to full compensation (Fermin 2002). Fish achieving full compensatory growth is commonly accompanied by hyperphagia (Rueda et al. 1998; Gaylord and Gatlin 2000; Wang et al. 2000; Cho et al. 2006; Oh et al. 2007).

Shell length of abalone in the Cont., S1, S2 and S3 treatments was significantly (P<0.05) longer than that of abalone in the S6 treatment, but not significantly (P>0.05) different from that of abalone in S4 treatment (Table 1). However, shell width and the ratio of the soft body weight to total body weigh to fab alone was not significantly (P>0.05) different among treatments.

Moisture content ranged from 78.1% to 80.1%, crude protein content ranged from 12.4% to 13.5%, crude lipid content ranged from 0.9% to 1.1% and ash content ranged from 2.3% to 2.5% of carcass of abalone was not significantly (P>0.05) different among treatments at the end of 16-week feeding trial (Table 2), agreeing with other studies showing that body composition of fish achieving full compensatory growth was not different among treatments (Gaylord and Gatlin 2000; Xie et al. 2001; Zhu et al. 2004). Since muscle protein is the primary energy source used for survival of green abalone (*H. fulgens*) while starved (Durazo-Beltran et al. 2004; Viana et al. 2007), dietary nutrient composition should be carefully considered to improve compensatory growth after starvation.

We observed full compensatory growth of abalone after 3-weeks starvation in this study with partial compensatory growth of abalone in proportion to the week of starvation (Cho et al. 2009) indicated that abalone had a different ability of compensatory growth depending on feed nutrient content when fed after starvation. Moreover, abalone subjected to more than 4-week starvation did not achieve full compensatory growth in the 12-week feeding trial when fed the dry sea tangle in this experimental condition. Based on the results of this study, abalone fed on the dry sea tangle seemed to be able to achieve full compensatory growth up to 3-week starvation

Table 2. Chemical composition (g kg^{-1}) carcass of abalone at the end of 16 weeks[1]

Treatments	Moisture	Crude protein	Crude lipid	Ash
Cont.	78.1 ± 0.77^a	13.5 ± 0.33^a	1.1 ± 0.14^a	2.5 ± 0.04^a
S1	80.1 ± 0.23^a	12.9 ± 0.22^a	1.0 ± 0.03^a	2.3 ± 0.05^a
S2	79.4 ± 0.29^a	13.1 ± 0.18^a	1.0 ± 0.09^a	2.4 ± 0.08^a
S3	79.1 ± 0.16^a	12.9 ± 0.20^a	0.9 ± 0.06^a	2.4 ± 0.06^a
S4	79.1 ± 0.92^a	13.4 ± 0.47^a	1.0 ± 0.04^a	2.4 ± 0.07^a
S6	79.7 ± 0.38^a	12.4 ± 0.32^a	1.0 ± 0.07^a	2.3 ± 0.11^a

[1]Values (means±SE, n=3) in the same column sharing a same superscript letter are not significantly different (P>0.05).

V. Conclusion

Effects of different feeding regimes on compensatory growth of juvenile abalone (*Haliotis discus hannai*) fed the formulated diet and dry sea tangle (*Laminaria japonica)* were determined.

In the experiment 1, compensatory growth of juvenile abalone with different feeding regimes was determined. Juvenile abalone did not have an ability to achieve full compensatory growth when abalone was fed the nutrition-balanced formulated diet.

In the experiment 2, effect of feeding regime on compensatory growth of juvenile abalone fed the dry sea tangle was determined. However, unlike the results of the experiment 1, abalone subjected to 3-week feed deprivation achieved full compensatory growth when abalone was fed the dry sea tangle. Based on these results, it can be concluded that compensatory growth of abalone varied depending on feed nutrient (type).

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