Thesis for the Degree of Master of Fisheries Science

Optimum dietary seaweed (kelp) powder level in juvenile abalone,

Haliotis discus hannai

By

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KOICA-PKNU International Graduate Program of Fisheries Science

The Graduate School

Pukyong National University

February, 2012

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치하기 전복 *Haliotis discus hannai* 에 있어 최적의 사료내 해조류(켈프)가루의 수준에 관한 연구

Advisor: Professor Sungchul C. Bai

By

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A thesis submitted in partial fulfilment of the requirements for the degree of

Master of Fisheries Science

in KOICA-PKNU International Graduate Program of Fisheries Science

The Graduate School

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A Dissertation

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February 24, 2012

Table of Contents

List of tables ii

List of figures iii

Abstract v

I. Introduction 1

II. Materials and Methods 9

Experimental design and diets 9

Experimental condition 10

Experimental animals and feeding trial 12

Experimental parameters 14

Sample collections and analysis 14

Statistical Analysis 16

III. Results 20

IV. Discussion and Conclusion 31

Acknowledgement 40

References 43

Appendix……………………………………………………………………...51

List of Tables

Table 1. Composition of experimental diets (% of dry matter basis)[1] 17

Table 2. Proximate analysis of experimental diets. 18

Table 3. Proximate analysis of seaweed (Kelp) powder used in experimental diets. 19

Table 4. Growth performance of abalone, *Haliotis discus hannai* after 10 weeks. 22

Table 5. Proximate body composition of abalone, *Haliotis discus hannai*.. 23

List of Figures

Figure 1. Average weight gain (WG, %) of abalone, *Haliotis discus hannai* fed 7 diets. Values were means from triplicate groups where the bar have different superscript were significantly different (P <0.05). 24

Figure 2. Average Feed efficiency (FE, %) of abalone, *Haliotis discus hannai* fed 7 diets. Values were means from triplicate groups where the bar have different superscript were significantly different (P <0.05). 25

Figure 3. Average specific growth rate (SGR, %) of abalone, *Haliotis discus hannai* fed 7 diets. Values were means from triplicate groups where the bar have different superscript were significantly different (P <0.05). 26

Figure 4. Average protein efficiency ratio (PER, %) of abalone, *Haliotis discus hannai* fed 7 diets. Values were means from triplicate groups where the bar have different superscript were significantly different (P <0.05). 27

Figure 5. Average shell length increase (SLI, %) of abalone, *Haliotis discus hannai* fed 7 diets. Values were means from triplicate groups where the bar have different superscript were significantly different (*P* <0.05). 28

Figure 6. Average survival rate (SR, %) of abalone, *Haliotis discus hannai* fed 7 diets. Values were means from triplicate groups where the bar have different superscript were significantly different (*P* <0.05). 29

Figure 7. Broken line analysis of weight gain (WG, %) of abalone, *Haliotis discus hannai* fed 7 diets. 30

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Abstract

The present study was conducted to evaluate the optimum dietary seaweed (kelp) powder in juvenile abalone, *Haliotis discus hannai*. Juvenile abalone with average initial individual weight and shell length of 0.44 ± 0.01 g and 15.6 ± 0.7 mm, respectively were fed 7 formulated diets for 10 weeks at a temperature range of 20 ± 1 ^{0}C. The formulated diets contained 30% crude protein and 5.8 crude lipids. Dietary levels of seaweed powder were distributed in 7 diets from 25% (Con), 20% (SW20), 15% (SW15), 10% (SW10), 5% (SW5) to 0% (SW0) and 25% w/ 0.4% mushroom powder (SW25M), respectively. A formulated diet served as the control (Con). The diets were fed at 4% body weight once daily at 1700 h. Weight gain (WG),

feed efficiency (FE), specific growth rate (SGR), protein efficiency ratio (PER), shell length increase (SLI) and survival rate (SR) were evaluated. Highest weight gain (WG) was obtained by abalone fed diet Con (25% seaweed powder). This value was, however, not significantly different ($P < 0.05$) from those fed diets SW20, SW15, SW10, and SW5. Abalone fed SW0 (0% seaweed powder) showed significantly lower WG. The protein efficiency ratio (PER) and feed efficiency (FE) showed the same trend as the weight gain. The specific growth rate (SGR) was not significantly different from those fed diets Con, SW20, SW15, SW10, and SW5. Abalone fed diet SW0 showed significantly lower SGR. Survival rate was generally high ranging from 72 to 86% for all treatments. Diet SW25M, even though gained highest growth performances, was used just to find out the effect of mushroom powder in abalone growth compared with control diet as reference for further study in the future.

Proximate body composition of abalone, *Haliotis discus hannai* was shown in Table 5. Moisture content has no significant differences among abalone fed diets Con and SW20, however, there were significant differences among diets SW15 ~ SW0 ($P < 0.05$). It was observed that the decreasing concentration level of seaweed powder in the diet increased the moisture content in the whole body of abalone. Crude fat of abalone fed diets Con ~ SW5 has no significant differences except abalone fed diet SW0 which significantly lower than among other diets (P <0.05). Crude protein content of abalone meat fed diets Con ~ SW0 has no significant differences among all diets (P <0.05). Ash content of abalone meat fed diets SW10 was significantly higher than among abalone fed diets Con, SW20, SW15, SW5 and SW0 (P <0.05). Growth and feed utilization of abalone, *Haliotis discus hannai* fed diets containing 15% seaweed powder were significantly higher than those of animals fed the non-supplemented diet. Hence, faster growth could be achieved at 15% supplementation level. However, abalone, *Haliotis discus hannai* grows slowly. A lot would be saved at 5% supplementation compared to 15% over the long duration of culture, since no significant differences were recorded in WG among animals fed at 5% and 15%. Broken line regression analysis indicated that the optimum seaweed powder supplementation level could be 12.2% in juvenile abalone, *H. discus hannai*.

These results indicated that seaweed powder supplementation could improve growth performance of abalone, *H. discus hannai*. The observed growth improvement was not very pronounced, probably, due to the short duration of this study. Nevertheless, broken line regression analysis indicated that the optimum seaweed powder supplementation level could be 12.2% in juvenile abalone, *H. discus hannai*.

Introduction

Aquaculture became the fastest growing sector in the world with a growth rate of 10% annually and predicted to play a major and ever increasing role in meeting human needs for protein. Culture of abalone, a herbivorous marine shellfish with an increased production of 600% over the past 10 years has attracted the attention of researchers from worldwide (Gordon & Cook, 2001).

Abalones are small to very large-sized edible sea snails, marine gastropod molluscs inhabiting coastal reef zones. Abalone have long been a valuable food source for humans in every area of the world where a species is abundant and very popular with different common names like ear-shells, sea ears, as well as muttonfish or mutton shells in Australia, ormer in Great Britain, *perlemoen* and venus's-ears in South Africa and *pāua* in New Zealand.

About 90 species have been recorded in the world, where large species generally populate in temperate regions and small species populate in subtropical and tropical regions. About 20 of them are relatively large in size with a high market value and are captured by the commercial fishery (Uki, 1989a). The prime demand is in Asia for the preparation of popular traditional cuisine. They are cultured and harvested commercially in countries such as Japan, China, Australia, New Zealand, South Africa, Korea and Taiwan (Sales & Janssens, 2004). Over the past 10 years, the abalone fisheries have declined approximately 30% due to poaching,

Among different species, *Haliotis discus hannai* belong to the family Haliotidae and the genus *Haliotis* is a widely cultured mollusk in different Asian countries and fetching high price in domestic as well as international seafood market. *H. discus hannai* commonly known as Ezo abalone and locally popular as Jeonbok.

Meat of this abalone is considered a delicacy especially in China, Japan, and Korea. In Chinese speaking regions, abalone is commonly known as bao yu, and sometimes forms part of a Chinese banquet. Similar to shark fin soup or birds nest soup, it is considered a luxury item, and is traditionally reserved for special occasions such as weddings and other celebrations. In Japan, live and raw abalone are used in a wasabi sushi, or served steamed, salted, boiled, chopped, or simmered in soy sauce. Salted, fermented abalone entrails are the main component of tottsuru, a local dish from Honshū. Tottsuru is mainly enjoyed with sake. Apart from its demand for the food, the highly iridescent inner nacre layer of the shell of abalone has traditionally been used as a decorative item, in jewelry, buttons, and as inlay, in furniture and in musical instruments such as guitars, etc.

Abalones are herbivorous species with a nocturnal pattern of grazing behavior. They tend to remain inactive by day (Barkai & Griffiths, 1987), however, this does not mean that all abalone would not be active feeding at night. Once abalone became post-larvae, they are fed benthic (bottom growing) algae. At 4 to 6 months of age, they weaned on a diet of either seaweed or an artificial diet. Daily intake of artificial diets, depending on species, diet, body size and husbandry, varies from 0.9 to 5.7 of body weight (Fleming *et al.*, 1996).

As abalone consume natural diet consisting of 40-50% carbohydrate, they have many types of polysaccharide hydrolyses and bacteria (Fleming *et al.*, 1996) in the digestive system capable of hydrolyzing a variety of complex polysaccharides in algae. However, evidence shows that the digestive physiology of juvenile abalone can readily adapt to artificial diets (Knauer *et al.*, 1996).

Abalone farming is highly capital-intensive with high operating costs. Dependence on natural seeds, slow growth rate and lack of knowledge of appropriate feeding technology are some of the important constraints restricting the further expansion of culture of this species. Animals are stocked at high densities in small shore-based, man-made structures and reared on seaweeds and/or artificial diets. Fish meal has been a major ingredient in fish diets because of its high protein quality and palatability. Substituting less expensive protein sources for high-price fish meal in salmonid feeds and abalone as well is one way to lower production costs (Hardy, 1996).

An ideal system for the culture of abalone juveniles should provide sufficient surface area for use by the abalone, free access to food, minimum contact between animals and fecal matter, adequate flows of seawater and aeration, and minimum of handling (Hahn, 1989). Abalone production systems need to optimize the related factors of water quality, production technology, type and size of cages and culture tanks, abalone size, quality and quantity of food (Mai *et al.*, 1994).

Seaweed powder is considered as one of main ingredients in abalone artificial diets. It is made from natural seaweed by modern technology. It contains rich carbohydrates, algal polysaccharides, vitamins, amino acids, iodine, many kinds of mineral elements and trace elements, such as calcium, phosphorus, sodium, iron, potassium, etc. It is widely used as feed additive for aquatic products and livestock farming, which can promote the growth of animals. It can promote growth of animal, prevent from diseases, enhance rate of yielding eggs, improve quality of meat products and taste, fasten speed of aquatic animal weight and reduce feed consumption. However, a regular supply of large volumes of seaweeds often presents logistics problems. Because of this, and also due to the high proportion of feed in production costs, the development of a convenient and cost-effective, formulated diet for abalone has received major attention from the start of research on abalone farming (Britz, 1996).

Formulated feeds of abalone must contain sufficient protein, carbohydrates, lipids and essential amino acids as adequate nutritional requirements (Fleming *et al.*, 1996; Durazo-Beltran *et al.*, 2003). The appropriate levels and ratios of protein, lipid and carbohydrate are important for the development of practical animal feeds in order to reduce the catabolism of protein for energy (Suarez and Mommsen, 1987; Cowey and Walton, 1989; Wilson, 1994). Feed costs for animal production in aquaculture can be minimized by determining requirements of a given animal for high-cost essential nutrients and by maximizing the use of low-cost energy sources. Carbohydrates are relatively inexpensive and readily available energy sources for animals compared to dietary protein and lipid. However, the ability of fish or shellfish to utilize carbohydrate varies among species (NRC, 1993). It may be very important to provide an adequate level of carbohydrate in the diet in order to reduce the catabolism of protein for energy which reduces protein retention and increases nitrogen effluent to the environment.

Energy in commercial artificial abalone diets is supplied primarily in the form of carbohydrate (wheat flour, maize flour, sodium alginate, dextrin, starch, bran), making up between 30 and 60% of the diet (Fleming *et al.*, 1996).

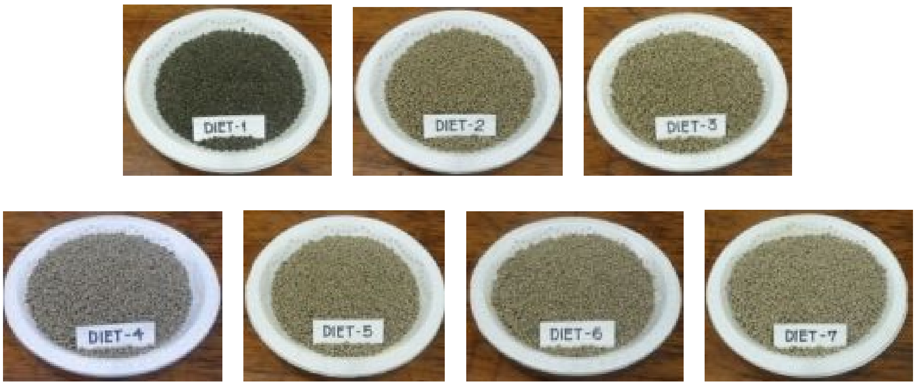
Uki *et al*., (1986a) showed that growth of *Haliotis discus hannai* fed diets containing 20% to 30% crude protein was better than those diets having protein content outside this reported range. To date, nutrition research on *Haliotis discus hannai* has identified dietary requirements of protein (Uki *et al*., (1986a, Mai *et al*., 1995a), lipids, and essential fatty acids (Uki *et al*., 1986b, Mai *et al*., 1995b, Mai *et al*., 1996), vitamins (Tan & Mai 2001), and minerals (Tan *et al*., 2001) for normal growth. Other studies have also been conducted to investigate the utilization of dietary sources for protein (Uki, Kemuyama & Watanabe 1995; Lee, Yun & Hur, 1998; Bautista-Teruel, Fermin & Koshio, 2003; Cho, Park, Kim & Yoo, 2007), 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, Fleming, Barneveld & Hone, 1996; Bautista-Teruel *et al*., 2003).

Most of the studies conducted were focused on protein and lipid requirements for *Haliotis discus hannai* growth but less emphasis was given to the quantitative carbohydrate requirements for abalone being a herbivore shellfish species and highly dependent on seaweeds as their natural food. Thus, this study aim to determine the optimum dietary seaweed (kelp) powder level in juvenile abalone, *Haliotis discus hannai*.

Materials and Methods

Experimental design and diets

Ingredients composition and proximate analysis of the experimental diets were shown in Table 1 and 2. Seven (7) formulated diets were used containing 30% crude protein, 5.8% crude lipid. Fish meal, dehulled soybean meal, wheat gluten meal, and feather meal were used as protein sources, squid liver oil was used as lipid source and seaweed powder, wheat flour and corn starch for carbohydrate sources and binder as well. Experimental diet ingredients were mixed mechanically at a ratio of 50 ml water/100 g feed, pelletized using a laboratory pelletizing machine, and dried at room temperature overnight. Pellets were ground using an electric grinder and stored at -30^{o}C until used.



Experimental diets used in the study.

Experimental condition

Experimental condition used in the study include 525 abalone juveniles as the test animals, 21 tanks measuring 28x18x14 cm suspended in 40L aquaria as stocking place of abalone juveniles, 7 formulated diets, weighing scale, thermometer, digital calliper, etc. The experiment was conducted at the Nutrition and Metabolism Laboratory, Pukyong National University, Busan, Korea for a period of 10 weeks.

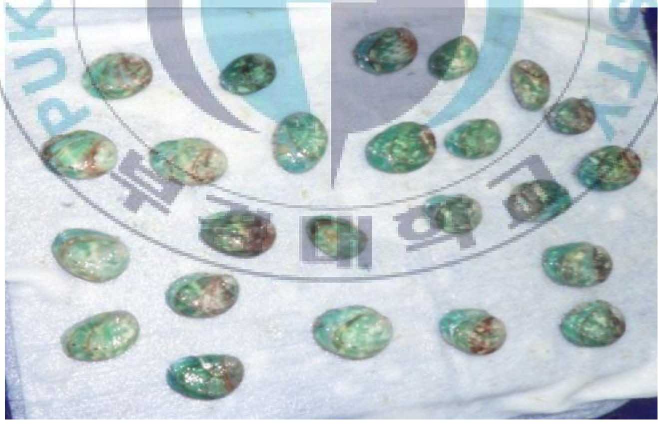
The experimental tanks used in the study.

Digital weighing scale used to measure abalone weight.

Digital calliper used to measure abalone shell length.

Experimental animals and feeding trial

Juvenile abalone, *Haliotis discus hannai* were procured from a local hatchery farm in Ulsan, Korea and the feeding trial was conducted at the Nutrition and Metabolism Laboratory, Pukyong National University, Busan, Korea. Prior to the start of feeding trial, abalone juveniles were fed *ad libitum* with commercial diet for 2 weeks to acclimate them to the experimental diets and laboratory condition. The feeding trial was conducted in an indoor semi-recirculating system in a 28x18 x14cm rectangular plastic tanks suspended in a 40L aquaria. Supplemental aeration was provided to maintain dissolved oxygen levels (≥5mg/L). Other conditions were conducted at 20±1°C (mean ± SD) water temperature, 34±2ppt water salinity during whole experimental period. Experimental abalone juveniles averaging 0.44±0.01g (mean ± SD) were randomly distributed in each aquarium as a group of 25 abalone. The feeding trial was conducted for 10 weeks. The experimental diets were fed to triplicate groups of abalone at a fixed rate of 4% at 5pm everyday on a dry matter basis. Each tank was cleaned by siphoning every 2 days and change of water was done twice a week. Total fish weight in each tank was determined every 4 weeks, and the feeding rate was adjusted accordingly.



Abalone juveniles used as test animals in the study.

Experimental parameters

Experimental parameters in this study include growth performance and proximate body composition of abalone juveniles, *Haliotis discuss hannai*.

Sample collections and analysis

At the end of the feeding trial, all abalone juveniles were weighed, measured and counted to calculate percent weight gain (WG), feed efficiency (FE), specific growth rate (SGR), protein efficiency ratio (PER), shell length increase (SL), survival rate (SR) and body composition analysis.

Measuring weight gained of the test animals.

Measuring shell length of abalone.

Shedding off abalone shell for proximate body composition analysis.

Statistical Analysis

All data were analyzed by one-way ANOVA to test the effects of diet treatments. When a significant treatment effect was observed, a Least Significant Difference (LSD) test was used to compare mean values. Treatment effect was considered with the significant level at $P < 0.05$.

Table 1. Composition of experimental diets (% of dry matter basis)[1]

	Diets[2]						
Ingredients	Con	SW20	SW15	SW10	SW5	SW0	SW25M
Fish meal[3]	12	12	12	12	12	12	12
Dehulled soy bean meal[4]	7.4	7.4	7.4	7.4	7.4	7.4	7.4
Wheat gluten meal[5]	5	5	5	5	5	5	5
Feather meal[6]	8.1	8.5	8.9	9.3	9.7	10.1	8.2
Wheat flour[7]	23.4	23.4	23.4	23.4	23.4	23.4	23.4
Seaweed (Kelp) powder[8]	25	20	15	10	5	0	25
Corn starch[9]	13	17.6	22.2	26.9	31.5	36.1	12.5
Mushroom powder[10]	0	0	0	0	0	0	0.4
CaP[11]	1	1	1	1	1	1	1
Squid liver oil[12]	3	3	3.1	3	3	3	3.1
Vitamin mix[13]	1	1	1	1	1	1	1
Mineral premix[14]	1	1	1	1	1	1	1
TOTAL	100	100	100	100	100	100	100

[1] Feed stuffs not mentioned here are the same feed stuffs as the domestic aquaculture feed company are using currently.

[2] Con, 25% seaweed powder; SW20, 20% seaweed powder; SW15, 15% seaweed powder; SW10, 10% seaweed powder; SW5, 5% seaweed powder; SW0, 0% seaweed powder; SW25M, 25% seaweed powder with 0.4% mushroom powder.

[3,4,5,6,7,8,9,11,12,13,14] Busan , Korea

[10] Seaweed (Kelp) powder Suhyup Feed Company, Gyeongsangnam-do, Korea

Table 2. Proximate analysis of experimental diets.

	Diets[1]						
	Con	SW20	SW15	SW10	SW5	SW0	SW25M
Crude protein	26.7	27.73	27.12	26.5	26.42	26.78	27.16
Crude lipid	4.36	4.09	3.82	3.68	3.5	4.18	4.51
Crude ash	11.32	10.38	8.75	7.61	5.9	4.44	11.47
Moisture	13.38	10.46	10.04	10.49	11.46	10.87	12.14
Energy (joule/g)	15817.9	16219.6	16424.8	16926.6	15797.8	15817.9	15805.4

[1] Refer to Table 1.

Table 3. Proximate analysis of seaweed (Kelp) powder used in experimental diets.

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Calorie (cal/g)
Seaweed powder (Kelp)	6.99	12.24	1.37	25.4	2784.4

Results

Results of growth performance were summarized in Table 4. After 10 weeks of feeding trial, weight gain (WG), feed efficiency (FE) and protein efficiency ratio (PER) of abalone, *Haliotis discus hannai* fed Con, SW_{15} and SW_{20} diets were significantly higher than those of animals fed SW_0 diet ($P < 0.05$). However, there were no significant differences in these parameters among abalone, *H. discus hannai* fed Con, SW_5, SW_{10}, SW_{15} and SW_{20} diets or among those fed SW_0, SW_5 and SW_{10} diets. Specific growth rate (SGR) of abalone, *H. discus hannai* fed SW_0 diet was significantly lower than those of animals fed the control diet and those fed seaweed powder-supplemented diets. There were no significant differences in SGR among animals fed the control diets and those fed the seaweed powder-supplemented diets. There were no significant differences in survival (SR) and shell length increase (SLI) of abalone, *H. discus hannai* fed all the experimental diets.

Results of body composition analysis of abalone, *Haliotis discus hannai* as to moisture, crude fat, crude protein and ash content were shown in Table 5. Moisture content has no significant differences among abalone fed diets Con and SW20, however, there were significant differences among diets SW15 to SW0 (*P* <0.05). It was observed that the decreasing percentage of seaweed powder in the diet increased the moisture content in the whole body of abalone. Crude fat of abalone fed diets Con to SW5 has no significant differences except abalone fed diet SW0 which significantly lower than among other diets (*P* <0.05). Crude protein content of abalone meat fed diets Con to SW0 has no significant differences among all diets (*P* <0.05). Ash content of abalone meat fed diets SW10 was significantly higher than among abalone fed diets Con, SW20, SW15, SW5 and SW0 (*P* <0.05).

Table 4. Growth performance of abalone, *Haliotis discus hannai* after 10 weeks[1].

	Diets[2]							
(%)	Con	SW20	SW15	SW10	SW5	SW0	SW25M	Pooled SEM[3]
WG[4]	114.56[a]	107.85[a]	103.76[a]	95.87[ab]	94.80[ab]	61.94[b]	133.01[a]	5.99
FE[5]	68.37[a]	70.42[a]	65.51[a]	58.12[ab]	61.21[ab]	40.19[b]	80.23[a]	3.48
SGR[6]	2.54[a]	2.42[a]	2.37[a]	2.24[a]	2.22[a]	1.61[b]	2.80[a]	0.1
PER[7]	1.00[a]	1.05[a]	0.96[a]	0.84[ab]	0.90[ab]	0.58[b]	1.18[a]	0.05
SLI[8]	20.02[a]	18.48[a]	17.27[a]	17.13[a]	17.97[a]	13.39[a]	20.14[a]	0.94
SR[9]	78.67[a]	88.00[a]	84.00[a]	78.00[a]	72.00[a]	74.00[a]	86.67[a]	2.13

[1] Means of triplicate groups; Values in the same row with different superscripts are significantly different (*P* □0.05).

[2] Refer to Table 1.

[3] Pooled standard error of mean: SD/ √n.

[4] WG (%) = Weight gain (%): [(final wt. – initial wt.) / initial wt.] x 100.

[5] FE = Feed efficiency: (wet weight gain / dry feed intake) x 100.

[6] SGR = Specific growth rate: [(loge final wt. – loge initial wt.) / days] x 100.

[7] PER = Protein efficiency ratio: (wet weight gain / protein intake).

[8] SLI = Shell length increase (%): [(final wt.-initial length) / initial length] x 100.

[9] SR = Survival rate: (no. of animals at end of experiment / no. of animals stock) x 100.

Table 5. Proximate body composition of juvenile abalone, *Haliotis discus hannai*.

	Diets[1]						
	Con	SW20	SW15	SW10	SW5	SW0	SW25M
Moisture	72.75^{bc}	74.05^{ab}	75.36^{a}	75.2^{a}	75.28^{a}	74.98^{a}	74.46^{ab}
Crude fat	6.26^{ab}	5.82^{bc}	6.08^{b}	5.69^{bc}	5.84^{bc}	5.18^{c}	5.56^{bc}
Crude protein	60.76^{a}	61.36^{a}	52.1^{a}	62.62^{a}	61.36^{a}	62.93^{a}	61.89^{a}
Ash	7.21^{cd}	7.47^{c}	8.89^{b}	10.33^{a}	7.52^{c}	7.81^{bc}	6.21^{d}

[1] Refer to Table 1.

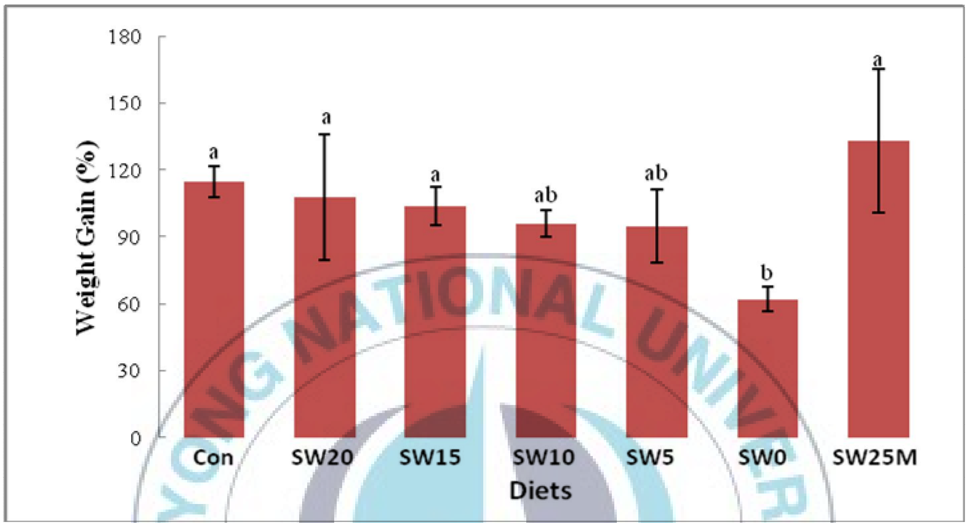


Figure 1. Average weight gain (WG, %) of abalone, *Haliotis discus hannai* fed 7 diets. Values were means from triplicate groups where the bar have different superscript were significantly different ($P < 0.05$).

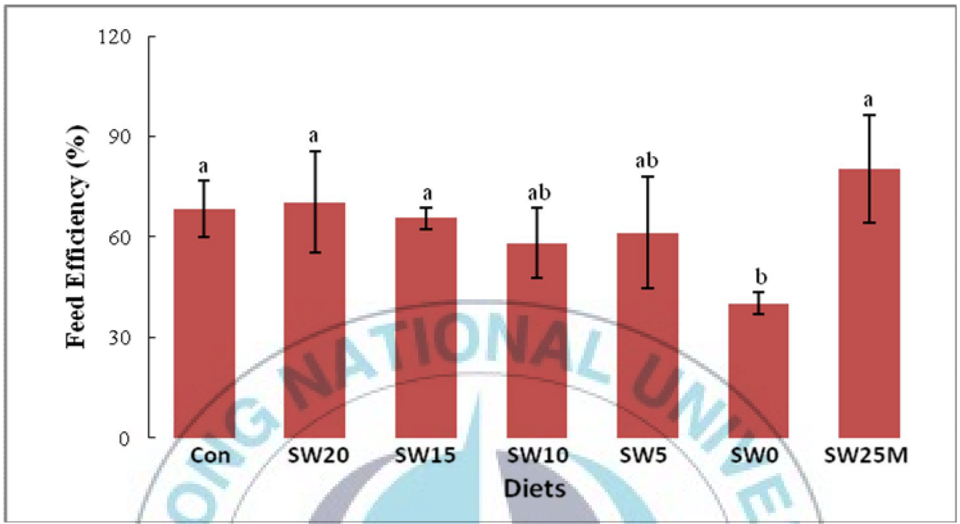


Figure 2. Average Feed efficiency (FE, %) of abalone, *Haliotis discus hannai* fed 7 diets. Values were means from triplicate groups where the bar have different superscript were significantly different (P <0.05).

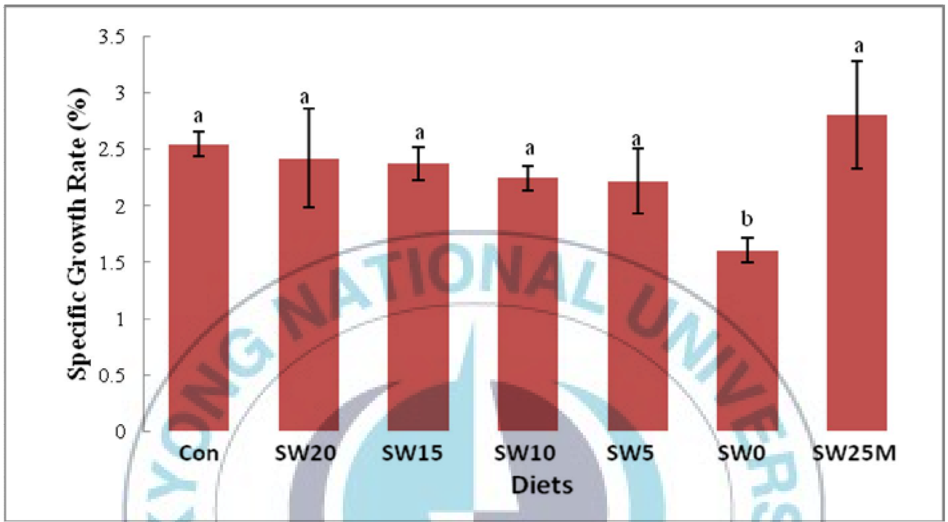


Figure 3. Average specific growth rate (SGR, %) of abalone, *Haliotis discus hannai* fed 7 diets. Values were means from triplicate groups where the bar have different superscript were significantly different (P <0.05).

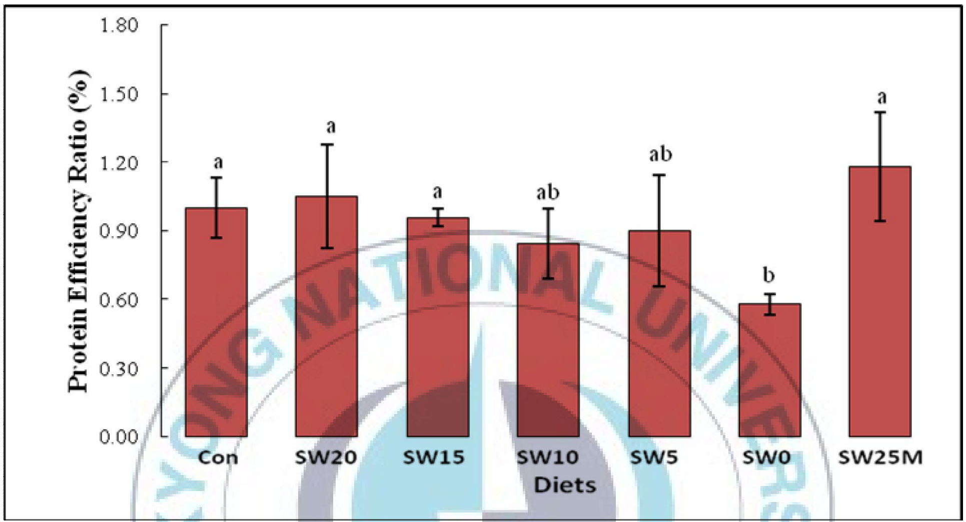


Figure 4. Average protein efficiency ratio (PER, %) of abalone, *Haliotis discus hannai* fed 7 diets. Values were means from triplicate groups where the bar have different superscript were significantly different (P <0.05).

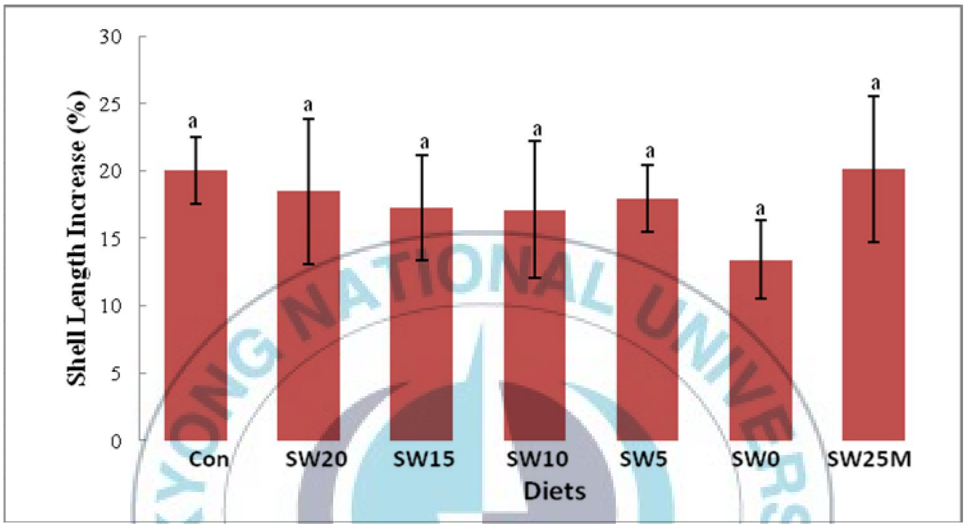


Figure 5. Average shell length increase (SLI, %) of abalone, *Haliotis discus hannai* fed 7 diets. Values were means from triplicate groups where the bar have different superscript were significantly different (P <0.05).

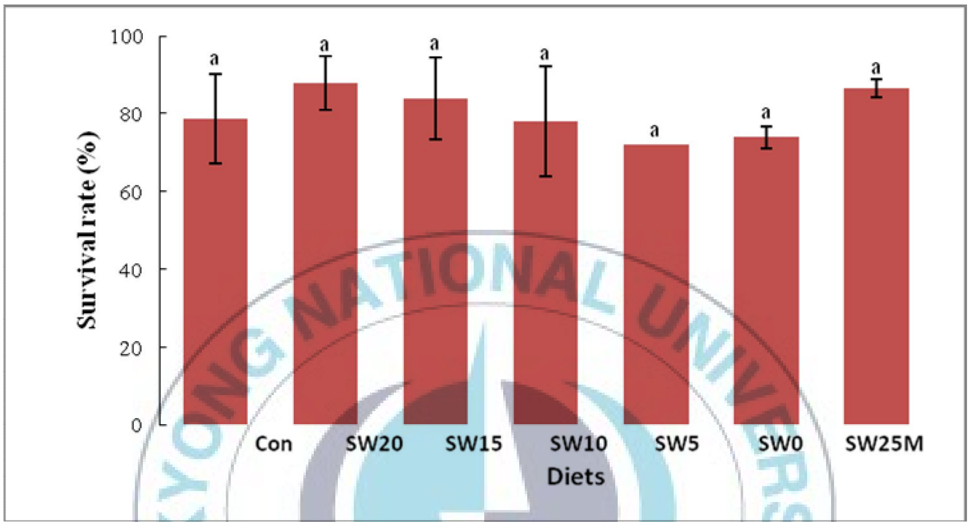


Figure 6. Average survival rate (SR, %) of abalone, *Haliotis discus hannai* fed 7 diets. Values were means from triplicate groups where the bar have different superscript were significantly different (*P* <0.05).

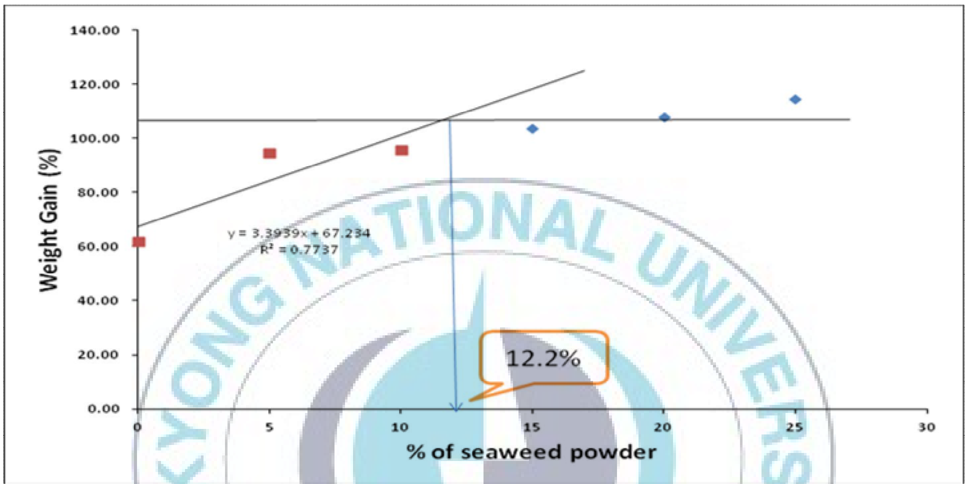


Figure 7. Broken line analysis of weight gain (WG, %) of juvenile abalone, *Haliotis discus hannai* fed 7 diets.

Discussion and Conclusion

Abalone is one of the most highly valued seafood in the world. There are approximately 90 species of abalone worldwide. About 15 are harvested commercially in countries such as Japan, China, Australia, New Zealand, South Africa, Korea and Taiwan (Sales & Janssens, 2004). Over the past 10 years, the abalone fisheries have declined approximately 30%, mainly due to poaching, whereas cultured abalone production has increased by 600% (Gordon & Cook, 2001). Abalone farming is highly capital-intensive with high operating costs. Animals are stocked at high densities in small shore-based, man-made structures and reared on seaweeds and/or artificial diets. During the early phases of abalone cultivation, most abalone farms had used natural algal feeds. However, a regular supply of large volumes of seaweeds often presents logistics problems. Because of this, and also due to the high proportion of feed in production costs, the development of a convenient and cost-effective, formulated diet for abalone has received major attention from the start of research on abalone farming (Britz, 1996).

The economic aquaculture of abalone requires the use of stable inexpensive formulated feeds that will meet the abalones dietary requirements, and maintain high animal growth rates and good health. To keep feed production costs down, protein content needs to be optimized. Ideally, the protein should be used primarily as a source of amino acids for growth (protein deposition in muscles), while the animal energy requirements for maintenance, activity and somatic growth should be sourced from lipid (concentrated energy ingredient) and carbohydrate (usually a less expensive ingredient). At high levels of dietary protein, the efficiency with which protein is utilized by abalone decreases and the excess protein is catabolized, especially if other important nutrients are being displaced (Britz, 1996; Go´ mez-Montes *et al.*, 2003). In formulating feeds for aquaculture, increasing the protein content of the feed results in the reduction of the content of other nutrients, which are usually carbohydrates. Abalone is an herbivorous gastropod whose natural diet is carbohydrate-rich marine plants and whose main form of energy storage are carbohydrates (as glycogen). Carbohydrates are also very important precursors for mucopolysaccharide and glycoprotein production used in locomotion and reproduction. If not enough carbohydrate is supplied in the diet, it can be readily synthesized via the catabolism of proteins and lipids (Livingstone & de Zwaan, 1983). Relative to carbohydrates, protein is generally considered a more expensive ingredient in formulated feeds. To avoid the abalone from using dietary protein as an energy source (i.e. protein sparing), suitable types and amounts of an alternative energy source must be supplied in the feed. While the protein of the feed can be spared by increasing the feeds energy concentration or by increasing lipid levels for a variety of species (Wilson, 1989; Jantrarotai *et al*., 1998), previous studies have shown that increasing the lipid levels of abalone feeds (with protein level constant) can decrease the growth rates in abalone (e.g. Britz & Hecht, 1997). Because a gastropods energy metabolism is carbohydrate-based (Livingstone & de Zwaan, 1983), it is carbohydrate that is a more suitable energy source than lipid.

Uki *et al*., (1986a) showed that growth of *Haliotis discus hannai* fed diets containing 20% to 30% crude protein was better than those diets having protein content outside this reported range. To date, nutrition research on *Haliotis discus hannai* has identified dietary requirements of protein (Uki *et al*., 1986a, Mai *et al*., 1995a, lipids, and essential fatty acids (Uki *et al*., 1986b, Mai *et al*., 1995b, Mai *et al*., 1996), vitamins (Tan & Mai, 2001), and minerals (Tan *et al.*, 2001) for normal growth. Other studies have also been conducted to investigate the utilization of dietary sources for protein (Uki *et al.*, 1985, Lee *et al.*, 1998a, Lee *et al.*, 1999a), lipid (Lee & Park *et al.*, 2000, Lira & Lee, 2003) in the same species.

Depending upon these previous reports, 7 experimental diets were formulated containing 30% crude protein, 5.8% crude lipid. Dietary levels of seaweed powder was used varying from 0 – 25% in 7 different diets (Con, SW20, SW15, SW10, SW5, SW0, SW25M). 0.4% of mushroom powder was added in a diet containing 25% seaweed powder to study the effect of mushroom powder (SW25M). Formulated feed containing 25% seaweed powder was used as the control.

While comparing the results obtained from the present study with previous findings reported for abalone in various experiments, it seems to be highly encouraging. The previous survival rate reported by K. Mai *et al.*, 1995, (92 – 98%), Zhanhuiqi *et al.*, 2010 (100%) in *Haliotis discus hannai.* As initial body weight and other experimental conditions are some of the major factors directly affecting the survival of animals. The survival rate 72-86% obtained from the very small size abalone (average initial individual weight and shell length of 0.44 ± 0.01 g and 15.6 ± 0.7 mm, respectively), in the present study seems to be satisfactory. Abalones are highly sensitive to stress, needs careful handling. Increased mortality was observed during the experiment, immediately after the intermediate measurement. It can be considered as another major reason associated with slightly less survival obtained from the present study, differed with other experiments. Although mortality due to stress was noticed after midterm measurement, but the purpose was to evaluate the correct nutritional and feeding requirement and to study the difference in growth pattern with increasing weight.

However, in the present study, there were no significant differences in percentage weight gain among abalone, *H. discus hannai* fed Con (114.56), SW5 (94.80), SW10 (95.87), SW15 (103.76) and SW20 (107.85) diets but almost similar with the previous researches. In a recent 90 days study conducted by M.N. Bautista, Teruel et.al., 2011 to develop an appropriate feed for the *Haliotis asinine Linne*, the obtained highest weight gain was 238 ± 4.44. The highest weight gain obtained by S. Thongrod et.al., 2003 in a 28 weeks long experiment with *Haliotis asinine Linne* was 779.53 ± 16.94. While comparing these weight gains with the present 10 weeks experiment, results are satisfactory. The weight gain reported for *H. discus hannai* in different experiments are generally calculated in terms of daily weight gain. Mai *et al*., 1995 reported 567.68 mg/abalone as the highest weight gain from his 100 days experiment with *H. discus hannai.* While comparing with the weight gain obtained from the present study, results are highly encouraging.

Specific growth rate (SGR) of abalone, *H. discus hannai* fed SW0 diet (1.61) was significantly lower than those of animals fed the control diet (2.54) and those fed seaweed powder-supplemented diets (2.22 to 2.42). There were no significant differences in SGR among animals fed the control diets and those fed the seaweed powder-supplemented diets. Specific Growth Rate (SGR) reported in previous studies conducted by K. Mai. et al., 2011 (1.34± 0.06) for *H. discus hannai* from 16 weeks of experiment. The SGR reported for *Haliotis midae,* by P. J. Britz, 1996 in a 95 days experiment ranged from 1 to 1.2. Bautista, Teruel *et al*., 2011 obtained the highest SGR 1.39 ± 2.23 for *H. asinina linne*, in 90 days of experiment. While comparing these reports with the SGR obtained from the present 70 days of experiment, results are highly encouraging.

Results of body composition analysis of abalone, *Haliotis discus hannai* as to moisture, crude fat, crude protein and ash content were shown in Table 5. Moisture content had no significant differences among abalone fed diets Con and SW20, however, there was significant differences between diets SW15 to SW0 (P <0.05). It was observed that the decreasing percentage of seaweed powder in the diet increased the moisture content in the whole body of abalone. Crude fat of abalone fed diets Con to SW5 has no significant differences except abalone fed diet SW0 (5.18) which is significantly lower than Con (6.26) and SW15 (6.08) diets (P <0.05). While comparing with the proximate analysis of previous researches, the body fat content are supporting the report of low lipid requirement for abalone. Uki et al., 1985 have shown that the optimum lipid requirement of *H. discus hannai* is 5% in the artificial diet. Mai et al., (1996) have shown that *H. tuberculata* grew best when fed diet containing 3% lipid. Thongrod et al., (2003) have also shown that lipid to carbohydrate ratio of 1.3 to 47.8% provided good growth for the *H. asinina*. Montano-Vegas et al., (2005) have shown that since carbohydrate are preferred energy source of N. corruguta, lipid levels to feed this species of abalone should be minimized to the extent that essential fatty acid requirement are met. The low lipid requirement of abalone may probably be a result of their food origin since they mostly feed on low lipid food such as macro algae in their natural environment (Yong *et.al*., 1985).

Crude protein content of abalone flesh fed diets Con to SW0 has no significant differences among all diets (P <0.05). Millikin, (1982) suggested that a shorter experimental duration may account for a lack of differences in whole-body protein concentration of Striped bass. In our opinion, the manifestation of the effect of dietary protein on carcass protein content depends largely on the range of dietary protein levels, or of energy/protein ratio (E/P ratio): the wider the range, the more significant the effect. Study conducted by K-W Kim, *et al* (2004) showed that proximate composition of whole body of juvenile olive flounder indicated that crude protein of whole body increased with the increase of dietary CP and energy levels. Ash content of abalone meat fed diets SW10 was significantly higher than among abalone fed diets Con, SW20, SW15, SW5 and SW0 (P < 0.05).

Growth and feed utilization of abalone, *Haliotis discus hannai* fed diets containing 15% seaweed powder were significantly higher than those of animals fed the non-supplemented diet. Hence, faster growth could be achieved at 15% supplementation level. However, abalone, *Haliotis discus hannai* grows slowly. A lot would be saved at 5% supplementation compared to 15% over the long duration of culture, since no significant differences were recorded in WG among animals fed at 5% and 15%. Broken line regression analysis indicated that the optimum seaweed powder supplementation level could be 12.2% in juvenile abalone, *H. discus hannai*.

These results indicated that seaweed powder supplementation could improve growth performance of abalone, *H. discus hannai*. The observed growth improvement was not very pronounced, probably, due to the short duration of this study. Nevertheless, broken line regression analysis indicated that the optimum seaweed powder supplementation level could be 12.2% in juvenile abalone, *H. discus hannai*.

Acknowledgement

This dissertation would not have been possible without the guidance and the help of several individuals who in way or another contributed and extended their valuable assistance in the preparation and completion of this study.

First and foremost, I offer my sincerest gratitude to my thesis supervisor, Prof. Sungchul C. Bai, who has supported me throughout my thesis with his patience and knowledge whilst allowing me the room to work in my own way. I attribute the level of my Masters degree to his encouragement and effort and without him this thesis, too, would not have been completed or written. One simply could not wish for a better or friendlier supervisor.

Deepest gratitude was also due to the members of the thesis evaluation committee, Prof, Yong-Ki Hong, Prof. Sungchul C. Bai and Prof. Jae-Yoon Jo for without their knowledge, suggestions and assistance this study would not have been successful.

The generous support from Korean International Cooperation Agency (KOICA) and Pukyong National University (PKNU) were greatly appreciated for without their acceptance and financial support, my inclusion to this scholarship program and to study abroad have not been realized. Likewise, I would like to acknowledge also the untiring support, concern and guidance of Prof. Yong-Ki Hong, Dr. Kyoung Mi Kang, KOICA-PKNU International Graduate Program of Fisheries Science (GFS) Program Chairperson and Coordinator, respectively, and their assistants, whom made my 14 month stay in Korea a very enjoyable, fruitful and memorable one.

I acknowledged deeply my debt to Dr. So Hung Ko, KOICA-KOV (Korean Official Volunteer) in the Philippines, for his generous help and invaluable support in my acceptance to this scholarship program. I felt proud to have a Korean friend like him whom I considered as my real brother.

Special thanks to Dr. Joel P. Limson, NIPSC College President, Mr. Juan A. Longno, College Administrator II, NIPSC Concepcion Campus for their unselfish approval of my official trip to Korea. I also convey my thanks to all Faculty and Staff of NIPSC Concepcion Campus, Concepcion, Iloilo, Philippines for their prayers, encouragement and moral support.

All my lab mates at the PKNU Fish and Food Nutrition Research Centre (FFNRC) made it a convivial place to work. In particular, I would like to express my wholehearted thanks to Kumar Katya (my lab brother), Jun-Ho Lee, Gunhyun Park, Yong-HyeonYun, Hyeon-Ho Yun, Liu Yi, Jin-Hyeok Lee, Seunghan Lee, Sera Choi, Gilran Kang, Dr. Okorie Eme Okorie, Dr. Junyoung Bae, Mahmoud Mohseni, Suguen Ko and Mizanur Rahman for their friendship, love, constant help and support whenever I needed them. And to all whom, knowingly or unknowingly, have contributed to this thesis by their ideas, discussions or by concern.

My most deepest gratitude to my beloved wife, kids, parents (deceased), brothers and sisters for their unflagging love, inspiration and moral support throughout my life…without them I would not reached this far.

Lastly, to God the Father, for the gift of life, grace, opportunity, strength and wisdom for completing this study successfully and for keeping me alive and healthy all the time.

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Appendix

RAW DATA:

A. Diet Formulation

Diet 1 SW25(Control)									
Ingredients	% in diet	Protein %	Fat %	Energy kj/kg	g in 1 kg	for DM 1 kg	dry matter %	Carbohydrate %	Ash %
Fish meal	12	62	8.5	10406.69856	130.25073	120	0.9213	9.5	20
Dehulled soybean meal	7.4	51.6	1.4	6766.220096	81.47985	74	0.9082	40.8	6.2
Wheat gluten meal	5	79.7	1.9	4840.909091	53.821313	50	0.929	17.2	0.6
Feather meal	8.1	85.79	4.71	8102.906699	89.099109	81	0.9091	8.15	1.35
Wheat flour	23.4	14.57	2.9	24652.85167	271.36727	234	0.8623	89	0.3
Seaweed powder	25	13.77	0.82	20264.35407	301.82301	250	0.8283	69.09	27.5
Corn starch	13	7.3	1.3	13251.91388	131.31313	130	0.99	96.3	0
CaP	1	0	0	0	10.10101	10	0.99		
Squid liver oil	3.1	0	100	6674.641148	31	31	1	0	0
Vitamin premix	1	0	0	0	11.337868	10	0.882		
Mineral premix	1	0	0	0	10.019036	10	0.9981		
TOTAL	100	30.0	5.8	94960.5	1121.6	1000			

Diet 2 (SW20)									
Ingredients	% in diet	Protein %	Fat %	Energy kj/kg	g in 1 kg	for DM 1 kg	dry matter %	Carbohydrate %	Ash %
Fish meal	12	62	8.5	10406.69856	130.25073	120	0.9213	9.5	20
Dehulled soybean meal	7.4	51.6	1.4	6766.220096	81.47985	74	0.9082	40.8	6.2
Wheat gluten meal	5	79.7	1.9	4840.909091	53.821313	50	0.929	17.2	0.6
Feather meal	8.5	85.79	4.71	8503.050239	93.499065	85	0.9091	8.15	1.35
Wheat flour	23.4	14.57	2.9	24652.85167	271.36727	234	0.8623	89	0.3
Seaweed powder	20	13.77	0.82	16211.48325	241.45841	200	0.8283	69.09	27.5
Corn starch	17.6	7.3	1.3	17941.05263	177.77778	176	0.99	96.3	0
CaP	1	0	0	0	10.10101	10	0.99		
Squid liver oil	3.1	0	100	6674.641148	31	31	1	0	0
Vitamin premix	1	0	0	0	11.337868	10	0.882		
Mineral premix	1	0	0	0	10.019036	10	0.9981		
TOTAL	100	30.0	5.8	95996.9	1112.1	1000			

Diet 3 (SW15)									
Ingredients	% in diet	Protein %	Fat %	Energy kj/kg	g in 1 kg	for DM 1 kg	dry matter %	Carbohy drate %	Ash %
Fish meal	12	62	8.5	10406.69856	130.25073	120	0.9213	9.5	20
Dehulled soybean meal	7.4	51.6	1.4	6766.220096	81.47985	74	0.9082	40.8	6.2
Wheat gluten meal	5	79.7	1.9	4840.909091	53.821313	50	0.929	17.2	0.6
Feather meal	8.9	85.79	4.71	8903.19378	97.899021	89	0.9091	8.15	1.35
Wheat flour	23.4	14.57	2.9	24652.85167	271.36727	234	0.8623	89	0.3
Seaweed powder	15	13.77	0.82	12158.61244	181.09381	150	0.8283	69.09	27.5
Corn starch	22.2	7.3	1.3	22630.19139	224.24242	222	0.99	96.3	0
CaP	1	0	0	0	10.10101	10	0.99		
Squid liver oil	3.1	0	100	6674.641148	31	31	1	0	0
Vitamin premix	1	0	0	0	11.337868	10	0.882		
Mineral premix	1	0	0	0	10.019036	10	0.9981		
TOTAL	100	30.0	5.8	97033.3	1102.6	1000			

Diet 4 (SW10)									
Ingredients	% in diet	Protein %	Fat %	Energy kj/kg	g in 1 kg	for DM 1 kg	dry matter%	Carbohy drate %	Ash %
Fish meal	12	62	8.5	10406.69856	130.25073	120	0.9213	9.5	20
Dehulled soybean meal	7.4	51.6	1.4	6766.220096	81.47985	74	0.9082	40.8	6.2
Wheat gluten meal	5	79.7	1.9	4840.909091	53.821313	50	0.929	17.2	0.6
Feather meal	9.3	85.79	4.71	9303.337321	102.29898	93	0.9091	8.15	1.35
Wheat flour	23.4	14.57	2.9	24652.85167	271.36727	234	0.8623	89	0.3
Seaweed powder	10	13.77	0.82	8105.741627	120.7292	100	0.8283	69.09	27.5
Corn starch	26.9	7.3	1.3	27421.26794	271.71717	269	0.99	96.3	0
CaP	1	0	0	0	10.10101	10	0.99		
Squid liver oil	3	0	100	6459.330144	30	30	1	0	0
Vitamin premix	1	0	0	0	11.337868	10	0.882		
Mineral premix	1	0	0	0	10.019036	10	0.9981		
TOTAL	100	30.0	5.8	97956.4	1093.1	1000			

Diet 5 (SW5)									
Ingredients	% in diet	Protein %	Fat %	Energy kj/kg	g in 1 kg	for DM 1 kg	dry matter%	Carbohy drate %	Ash %
Fish meal	12	62	8.5	10406.69856	130.25073	120	0.9213	9.5	20
Dehulled soybean meal	7.4	51.6	1.4	6766.220096	81.47985	74	0.9082	40.8	6.2
Wheat gluten meal	5	79.7	1.9	4840.909091	53.821313	50	0.929	17.2	0.6
Feather meal	9.7	85.79	4.71	9703.480861	106.69893	97	0.9091	8.15	1.35
Wheat flour	23.4	14.57	2.9	24652.85167	271.36727	234	0.8623	89	0.3
Seaweed powder	5	13.77	0.82	4052.870813	60.364602	50	0.8283	69.09	27.5
Corn starch	31.5	7.3	1.3	32110.4067	318.18182	315	0.99	96.3	0
CaP	1	0	0	0	10.10101	10	0.99		
Squid liver oil	3	0	100	6459.330144	30	30	1	0	0
Vitamin premix	1	0	0	0	11.337868	10	0.882		
Mineral premix	1	0	0	0	10.019036	10	0.9981		
TOTAL	100	30.0	5.8	98992.8	1083.6	1000			

Diet 6 (SW0)									
Ingredients	% in diet	Protein %	Fat %	Energy kj/kg	g in 1 kg	for DM 1 kg	dry matter%	Carbohy drate %	Ash %
Fish meal	12	62	8.5	10406.69856	130.25073	120	0.9213	9.5	20
Dehulled soybean meal	7.4	51.6	1.4	6766.220096	81.47985	74	0.9082	40.8	6.2
Wheat gluten meal	5	79.7	1.9	4840.909091	53.821313	50	0.929	17.2	0.6
Feather meal	10.1	85.79	4.71	10103.6244	111.09889	101	0.9091	8.15	1.35
Wheat flour	23.4	14.57	2.9	24652.85167	271.36727	234	0.8623	89	0.3
Seaweed powder	0	13.77	0.82	0	0	0	0.8283	69.09	27.5
Corn starch	36.1	7.3	1.3	36799.54545	364.64646	361	0.99	96.3	0
CaP	1	0	0	0	10.10101	10	0.99		
Squid liver oil	3	0	100	6459.330144	30	30	1	0	0
Vitamin premix	1	0	0	0	11.337868	10	0.882		
Mineral premix	1	0	0	0	10.019036	10	0.9981		
TOTAL	100	30.0	5.8	100029.2	1074.1	1000			

Diet 7 (SW25M)									
Ingredients	% in diet	Protein %	Fat %	Energy kj/kg	g in 1 kg	for DM 1 kg	dry matter%	Carbohy drate %	Ash %
Fish meal	12	62	8.5	10406.69856	130.25073	120	0.9213	9.5	20
Dehulled soybean meal	7.4	51.6	1.4	6766.220096	81.47985	74	0.9082	40.8	6.2
Wheat gluten meal	5	79.7	1.9	4840.909091	53.821313	50	0.929	17.2	0.6
Feather meal	8.2	85.79	4.71	8202.942584	90.199098	82	0.9091	8.15	1.35
Wheat flour	23.4	14.57	2.9	24652.85167	271.36727	234	0.8623	89	0.3
Seaweed powder	25	13.77	0.82	20264.35407	301.82301	250	0.8283	69.09	27.5
Corn starch	12.5	7.3	1.3	12742.22488	126.26263	125	0.99	96.3	0
Mushroom Powder	0.4	34.86	0.71	139.5502392		4			6.09
CaP	1	0	0	0	10.10101	10	0.99		
Squid liver oil	3.1	0	100	6674.641148	31	31	1	0	0
Vitamin premix	1	0	0	0	11.337868	10	0.882		
Mineral premix	1	0	0	0	10.019036	10	0.9981		
TOTAL	100	30.0	5.8	94690.4	1117.7	1000	10.2183	330.04	62.04

B. Growth Performance

Abalone Weight (g)

Diet	Tank	Initial weight (g)	4 weeks weight (g)	8 weeks weight (g)	10 weeks weight (g)
1	11	11	14.4	20.7	21.9
Con	18	10.8	14.1	15.7	16.1
(SW25M)	26	11.1	13.3	16.9	17.6
2	25	11.2	13.7	16.6	17.7
(SW20)	17	11.1	14.3	19.5	20.8
	1	11.2	14.1	20.2	22.6
3	10	11.1	14.3	16.8	17.4
(SW15)	7	10.9	14.4	19.6	20.3
	22	10.9	13.9	17.4	18.4
4	12	10.9	13.5	15.2	14.2
(SW10)	29	11.2	12	13.8	14
	27	10.9	14.3	18.9	19.2
5	2	10.8	12.8	15.2	16.8
(SW5)	4	11	13.5	20.3	20.9
	23	11	12.8	14.7	14.5
6	8	11	12.7	17.1	16.9
(SW0)	30	10.8	12.9	13.6	13.8
	20	10.8	11.5	12.9	12.3
7	3	11	15.3	23.8	25.1
(SW25M)	28	11	14.5	19.3	19.1
	5	11	15.1	21.7	22.4

Abalone Shell Length (mm)

Diet	Tank	Initial SL (mm)	10 weeks (mm)	SL increment (mm)
1 Con (SW25)	11	15.64	19.21	3.57
	18	15.51	18.32	2.81
	26	15.75	18.76	3.01
2 (SW20)	25	15.53	17.77	2.24
	17	15.86	18.47	2.61
	1	15.51	19.32	3.81
3 (SW15)	10	15.9	18.4	2.5
	7	15.9	18.18	2.28
	22	15.4	18.75	3.35
4 (SW10)	12	15.77	17.9	2.13
	29	14.91	17.73	2.82
	27	15.52	18.74	3.22
5 (SW5)	2	15.45	17.95	2.5
	4	15.17	18.16	2.99
	23	15.47	17.98	2.51
6 (SW0)	8	15.73	18.06	2.33
	30	15.7	17.48	1.78
	20	15.48	17.87	2.39
7 (SW25M)	3	15.56	19.67	4.11
	28	15.65	18.25	2.6
	5	15.47	18.16	2.69

Abalone Survival

Diet	Tank	Initial Stock	10 Weeks	Survival (%)
1 Con (SW25)	11	25	23	92
	18	25	18	72
	26	25	18	72
2 (SW20)	25	25	21	84
	17	25	24	96
	1	25	21	84
3 (SW15)	10	25	19	76
	7	25	24	96
	22	25	20	80
4 (SW10)	12	25	17	68
	29	25	18	72
	27	25	22	88
5 (SW5)	2	25	18	72
	4	25	23	92
	23	25	18	72
6 (SW0)	8	25	20	80
	30	25	19	76
	20	25	18	72
7 (SW25M)	3	25	22	88
	28	25	22	88
	5	25	21	84

Total Feed

Diet	Tank	4 weeks (g)	8 weeks (g)	10 weeks (g)	Total Feed (g)
1	11	8.47	17.28	9.13	34.88
Con	18	8.316	16.92	7.26	32.496
(SW25M)	26	8.547	15.96	7.81	32.317
2	25	8.624	16.44	7.22	32.284
(SW20)	17	8.547	17.16	8.58	34.287
	1	8.624	16.92	8.91	34.454
3	10	8.547	17.16	7.37	33.077
(SW15)	7	8.393	17.28	8.58	34.253
	22	8.393	16.68	7.7	32.773
4	12	8.393	16.2	6.63	31.223
(SW10)	29	8.624	14.4	6.05	29.074
	27	8.393	17.16	8.24	33.793
5	2	8.316	15.36	6.71	30.386
(SW5)	4	8.47	16.2	8.91	33.58
	23	8.47	15.36	6.41	30.24
6	8	8.47	15.24	7.48	31.19
(SW0)	30	8.316	15.48	5.94	29.736
	20	8.316	13.8	5.6	27.716
7	3	8.47	18.36	10.45	37.28
(SW25M)	28	8.47	17.4	8.47	34.34
	5	8.47	18.12	9.49	36.08

C. Proximate body composition analysis result

Moisture

Diet	Tank	Sample weight	Dish weight	Last weight		Avg. (%)
1	11	0.36	1.98	2.09	72.03	72.03
Con	18	0.32	1.89	1.98	72.35	72.35
(SW25)	26	0.37	1.94	2.04	73.87	73.87
2	25	0.40	1.98	2.08	73.46	73.46
(SW20)	17	0.34	1.89	1.97	74.79	74.79
	1	0.38	2.08	2.18	73.90	73.90
3	10	0.47	1.98	2.10	75.16	75.16
(SW15)	7	0.54	1.88	2.01	73.62	73.62
	22	0.49	1.95	2.06	77.28	77.28
4	12	0.30	1.97	2.04	75.14	75.14
(SW10)	29	0.36	1.87	1.96	75.37	75.37
	27	0.45	1.93	2.04	75.09	75.09
5	2	0.43	1.88	1.99	74.67	74.67
(SW5)	4	0.71	1.99	2.17	74.88	74.88
	23	0.47	1.95	2.06	76.27	76.27
6	8	0.48	1.97	2.10	73.37	73.37
(SW0)	30	0.33	1.97	2.05	75.55	75.55
	20	0.41	1.82	1.91	76.00	76.00
7	3	0.40	1.93	2.02	76.21	76.21
(SW25M)	28	0.39	1.98	2.08	74.21	74.21
	5	0.48	1.96	2.08	72.96	72.96

Freezed Moisture

Diet	Tank	Sample weight	Dish weight	Last weight		Avg. (%)
1	11	0.20	1.93	2.12	4.21	4.21
Con	18	0.20	1.93	2.12	2.94	2.94
(SW25)	26	0.20	1.96	2.16	3.59	3.59
2	25	0.20	1.91	2.11	6.73	6.73
(SW20)	17	0.21	1.97	2.17	4.36	4.36
	1	0.20	1.96	2.15	4.11	4.11
3	10	0.21	1.99	2.19	5.62	5.62
(SW15)	7	0.20	1.95	2.14	4.23	4.23
	22	0.20	2.00	2.19	4.86	4.86
4	12	0.20	1.89	2.08	4.76	4.76
(SW10)	29	0.20	1.99	2.19	5.75	5.75
	27	0.20	1.87	2.06	4.05	4.05
5	2	0.20	1.92	2.11	4.49	4.49
(SW5)	4	0.20	1.82	2.01	3.32	3.32
	23	0.20	1.87	2.06	5.02	5.02
6	8	0.20	1.88	2.07	4.30	4.30
(SW0)	30	0.15	1.98	2.12	5.43	5.43
	20	0.15	1.91	2.05	4.00	4.00
7	3	0.20	1.88	2.07	4.78	4.78
(SW25M)	28	0.15	1.88	2.03	4.41	4.41
	5	0.20	1.88	2.07	4.36	4.36

Crude Lipid

Diet	Tank	Sample weight	Dish weight	Last weight		DM	Avg. (%)
1 Con (SW25)	11	0.50	24.61	24.64	6.14	6.41	6.41
	18	0.50	26.32	26.34	5.68	5.85	5.85
	26	0.50	24.61	24.64	6.30	6.53	6.53
2 (SW20)	25	0.51	25.90	25.93	5.39	6.05	6.05
	17	0.50	25.85	25.88	5.54	5.79	5.79
	1	0.50	26.31	26.34	5.64	5.62	5.62
3 (SW15)	10	0.51	26.28	26.31	5.72	6.42	6.42
	7	0.50	26.44	26.47	6.06	5.97	5.97
	22	0.50	26.30	26.33	5.56	5.85	5.85
4 (SW10)	12	0.50	25.64	25.67	6.10	6.40	6.40
	29	0.50	26.28	26.30	4.59	5.88	5.88
	27	0.50	26.37	26.40	5.54	4.78	4.78
5 (SW5)	2	0.50	26.04	26.07	5.76	6.03	6.03
	4	0.51	26.30	26.33	5.55	5.74	5.74
	23	0.50	26.44	26.47	5.45	5.74	5.74
6 (SW0)	8	0.51	25.64	25.66	5.15	5.39	5.39
	30	0.50	26.28	26.30	4.68	4.95	4.95
	20	0.50	26.39	26.42	5.00	5.21	5.21
7 (SW25M)	3	0.50	24.61	24.64	5.21	5.47	5.47
	28	0.50	25.64	25.66	5.32	5.64	5.64
	5	0.50	26.28	26.31	5.39	5.56	5.56

Crude protein

Diet	Tank	Sample weight	HCl	Blank		질소보정계수		DM	Avg. (%)
1	11	0.10	6.72	0.10	56.50	1.03	58.28	64.80	64.80
Con	18	0.10	6.54	0.10	56.28	1.03	58.05	59.81	59.81
(SW25)	26	0.10	6.68	0.10	57.62	1.03	59.43	61.64	61.64
2	25	0.10	6.71	0.10	57.59	1.03	59.40	62.72	62.72
(SW20)	17	0.10	6.41	0.10	55.09	1.03	56.82	59.41	59.41
	1	0.10	6.68	0.10	56.71	1.03	58.50	61.95	61.95
3	10	0.10	6.66	0.10	57.21	1.03	59.01	60.70	60.70
(SW15)	7	0.10	6.50	0.10	55.54	1.03	57.29	61.62	61.62
	22	0.10	6.88	0.10	59.01	1.03	60.87	63.98	63.98
4	12	0.10	6.91	0.10	59.81	1.03	61.69	64.78	64.78
(SW10)	29	0.10	6.62	0.10	55.97	1.03	57.73	62.91	62.91
	27	0.10	6.79	0.10	57.49	1.03	59.30	60.17	60.17
5	2	0.10	6.80	0.10	57.35	1.03	59.15	61.94	61.94
(SW5)	4	0.10	6.62	0.10	56.92	1.03	58.71	60.73	60.73
	23	0.10	6.70	0.10	56.55	1.03	58.33	61.41	61.41
6	8	0.10	6.71	0.10	57.31	1.03	59.11	61.77	61.77
(SW0)	30	0.10	6.93	0.10	57.89	1.03	59.72	63.15	63.15
	20	0.10	6.99	0.10	59.44	1.03	61.31	63.87	63.87
7	3	0.10	6.80	0.10	56.63	1.03	58.41	61.34	61.34
(SW25M)	28	0.10	6.60	0.10	56.86	1.03	58.65	62.99	62.99
	5	0.10	6.78	0.10	58.38	1.03	60.21	61.32	61.32

Ash

Diet	Tank	Sample weight	Dish weight	Last weight		DM	Avg. (%)
1	11	0.30	21.85	21.88	7.92	8.27	8.27
Con	18	0.31	21.78	21.80	6.58	6.78	6.78
SW25M	26	0.30	23.88	23.90	6.35	6.59	6.59
2	25	0.30	24.58	24.60	6.77	7.57	7.57
SW20	17	0.30	23.35	23.38	7.44	7.78	7.78
	1	0.30	24.84	24.86	7.06	7.06	7.06
3	10	0.30	23.55	23.57	8.45	8.85	8.85
SW15	7	0.31	23.66	23.68	8.35	8.82	8.82
	22	0.30	23.90	23.92	8.56	8.99	8.99
4	12	0.30	22.51	22.54	10.96	11.51	11.51
SW10	29	0.30	23.90	23.92	8.93	10.17	10.17
	27	0.30	25.23	25.26	9.59	9.30	9.30
5	2	0.30	22.51	22.53	6.74	7.05	7.05
SW5	4	0.31	23.55	23.57	7.35	7.61	7.61
	23	0.30	23.89	23.92	7.51	7.91	7.91
6	8	0.30	23.66	23.68	6.96	7.27	7.27
SW0	30	0.20	22.11	22.13	7.42	7.85	7.85
	20	0.20	25.84	25.86	7.99	8.33	8.33
7	3	0.30	23.66	23.68	6.33	6.65	6.65
SW25M	28	0.30	23.88	23.90	5.86	5.85	5.85
	5	0.30	24.79	24.81	5.59	6.13	6.13