Korean J. Malacol. 36(2): 63-70 2020
https://doi.org/10.9710/kjm.2020.36.2.63

The effect of the combined addition of astaxanthin and *Lactobacillus* on the growth and hemolymph characteristics of *Haliotis discus hannai*

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ABSTRACT

This study investigated the effect of astaxanthin, synthesized from a marine bacterium (*Paracoccus haeundaensis*), combined with a culture solution of *Lactobacillus* (*Lactobacillus helveticus*) on the growth and physiological responses of the abalone *Haliotis discus hannai*. The presence of astaxanthin in feed did affect the survival, whereas 100 ppm astaxanthin in feed positively affected the growth, of *H. discus hannai*. These results indicate that the optimal concentration of astaxanthin in feed has a positive effect on the growth of *H. discus hannai*, and *Lactobacillus* in feed has no effect on animal growth. Abalone treated with 100 ppm astaxanthin showed significantly lower glucose levels and superoxide dismutase and lysozyme activities in hemolymph compared with other treatments ($p < 0.05$). Therefore, the optimal level of astaxanthin in feed may have a positive effect on physiological homeostasis in *H. discus hannai*.

Key words: *Haliotis discus hannai*, Astaxanthin, Growth, *Lactobacillus*, Health

INTRODUCTION

Abalone (*Haliotis discus hannai*) aquaculture in Korea has rapidly expanded over the last decade; between 2000 and 2017, abalone production increased from 100 to 16,027 metric tons (KOSIS, 2017). Although the volume of abalone aquaculture accounts for only 4% of the total farmed shellfish in Korea, the value of farmed abalone comprises half that of total shellfish production. The rapid growth of abalone aquaculture can be attributed to several factors including increased production of seaweed fed to abalone and the development of sea-cage-farming techniques. However, the increase in production has also contributed to environmental deterioration, frequent disease outbreaks, and reduced survival of farmed abalone. Global changes in the ocean environment, such as rising temperatures, are also impeding productivity. Therefore, farmers and researchers are focusing their efforts to develop technologies to minimize this loss in productivity. Among various methods to improve survival, the abalone industry is interested in enhancing animal immunity to farm healthy abalone.

Abalone aquaculture in South Korea includes land-based hatcheries that raise juveniles, which are then moved to sea-cage farms where they are reared to a marketable size. At hatcheries, broodstock maturation is induced by regulating water temperature; fertilized eggs are then collected and reared on diatoms and formulated feed (NFRDI, 2008). One of the most applicable methods in the field to enhance the immunity of farmed animals is use of feed additives.

Received: June 15, 2020; Revised: June 25, 2020; Accepted: June 30, 2020
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1225-3480/24759
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Those additives should be abundant and inexpensive to ensure economic feasibility. However, most bioactive compounds that enhance immunity are expensive, so feed additives comprising these compounds are burdensome for farming businesses. Recently, techniques to mass produce highly priced bioactive compounds were developed using bioconversion technology. One major example of such compounds is astaxanthin (Seo *et al.*, 2015; Seo and Kim, 2017), a lipid-soluble carotenoid pigment that gives the meat or skin of marine animals such as salmons, sea breams, and crustaceans their red-orange color (Schiedt *et al.*, 1986; Storebakken *et al.*, 1987; Ha *et al.*, 1993; Okada *et al.*, 1994). Astaxanthin has free-radical-scavenging and antioxidant properties and is effective in preventing cancers (Kim *et al.*, 2009). For this reason, astaxanthin is recognized as an important defense against reactive oxygen species and effective inhibitor of lipid peroxidation in the liver, and it improves fish meat when added to the feed of farmed trout (*Oncorhynchus masou*) (Kim and Kang, 1998; Choi *et al.*, 2010).

Lactobacillus bacteria have been shown to improve fish growth and immunity (Yang *et al.*, 2003; Jhon *et al.*, 2009). Yang *et al.* (2003) demonstrated the antibacterial properties and pathogenic bacterial inhibition of *Lactobacillus sakei* BK19. Although limited data are available on the effects of these substances in abalone (Lim and Lee, 2003; Park *et al.*, 2016), they can potentially improve the health and growth of abalone when added to feed.

This study investigated the effects of marine-derived and cultured astaxanthin and *Lactobacillus* incorporated into abalone feed on *H. discus hannai*, in terms of growth and hemolymph characteristics.

Material and Methods

1. Experimental feed formulation

We isolated astaxanthin biosynthesized by the marine bacterium *Paracoccus haeundaensis* (Seo *et al.*, 2015). The astaxanthin culture solution was cryopreserved, and we used varying amount of cryopreserved solution was used to feed. We prepared feed formulations containing astaxanthin, astaxanthin and *Lactobacillus*, or neither. The astaxanthin-containing feed included either 0, 100 (A100), 1,000 (A1000), or 3,000 (A3000) ppm astaxanthin (Table 1). To investigate the combined effectiveness of *Lactobacillus* and astaxanthin, a *Lactobacillus*

Table 1. Composition of the experimental diets

	Diets (%)						
Ingredients	C	A100	A1000	A3000	A100 + L.h	A1000 + L.h	A3000 + L.h
Fermented soybean meal	40	40	40	40	40	40	40
Undaria powder	40	40	40	40	40	40	40
Wheat flour	10	10	10	10	10	10	10
Starch	5	5	5	5	5	5	5
Sodium alginate	5	5	5	5	5	5	5
Total	100	100	100	100	100	100	100
Astaxanthin	-	100 ppm	1,000 ppm	3,000 ppm	100 ppm	1,000 ppm	3,000 ppm
Lactic acid bacteria (*Lactobacillus helveticus*)	-	-	-	-	○	○	○

A100: 100 ppm astaxanthin; A1000: 1,000 ppm astaxanthin; A3000: 3,000 ppm astaxanthin; A100+L.h: 100 ppm astaxanthin + *Lactobacillus helveticus*; A1000+L.h: 1,000 ppm astaxanthin + *Lactobacillus helveticus*; A3000+L.h: 3,000 ppm astaxanthin +*Lactobacillus helveticus*; C: control.

Table 2. Nutrient contents of the experimental diets

Diets	Nutrients (%)				
	Water	Crude ash	Crude protein	Crude lipid	Carbohydrate
C	13.57	14.27	30.75	0.59	40.82
A100	12.4	15.19	30.97	0.67	40.77
A1000	12.86	16.14	31.11	0.78	39.11
A3000	14.16	15.32	30.08	1.12	39.32
A100 + L.h	13.57	14.99	30.41	0.78	40.25
A1000 + L.h	12.27	15.43	30.47	0.78	41.05
A3000 + L.h	13.24	15.28	30.51	1.07	39.9

A100: 100 ppm astaxanthin; A1000: 1,000 ppm astaxanthin; A3000: 3,000 ppm astaxanthin; A100 + L.h: 100 ppm astaxanthin + *Lactobacillus helveticus*; A1000 + L.h: 1,000 ppm astaxanthin + *Lactobacillus helveticus*; A3000 + L.h: 3,000 ppm astaxanthin + *Lactobacillus helveticus*; C: control.

helveticus (+L.h.) culture solution (2.2×10^8 CFU·mL^{-1}) was added with astaxanthin to the feed at a total concentration of 10% (Table 1).

Moisture, crude fat, and ash contents in the feeds were analyzed by drying (105℃), the Soxhlet extraction method, and a digestion method (550℃), respectively. To calculate the crude protein content, the total nitrogen level was quantified using an element analyzer (Flash 2000, Thermo Quest Inc, Austin, TX, USA) and then multiplied by a conversion factor of 6.25. Carbohydrate content was obtained by subtracting the combined level of moisture, crude protein, crude lipid, and ash from 100.

2. Rearing of abalone

Juvenile abalone (10.82 ± 0.74 g) were raised at a private hatchery in Wando, Jellanamdo province, South Korea. They were housed in tanks for 7 days without feeding to acclimate to the experimental environment. Groups of 30 domesticated juveniles were placed in square plastic tanks with filtered seawater supplied at a rate of 1.5 L·min^{-1}. Water temperature, salinity, and dissolved oxygen were maintained at 21.1 ± 1.2℃, 32.1 ± 2.1 psu, and 7.9 ± 0.7 mg·L^{-1}, respectively. The daily feeding ratio (17:00) was 1.5-2% of the combined weight of all abalone individuals in each tank. Uneaten feed was removed the following day during, identical to those used at the source hatchery, were placed in the tanks. The natural photoperiod was applied the entire 17-week duration of the experiment.

To evaluate growth, the shell length and weight of all individuals were measured at the beginning and end of the experiment. Shell length was measured using digital vernier calipers (Mitutoyo, Japan) to the nearest 0.01 mm, and weight was measured using an electronic scale (MW-Ⅱ, CAS Co., China) to the nearest 0.01 g. Based on the growth and feeding data, feed efficiency (%) was calculated (weight gain/total amount eaten × 100). The number of dead abalone was counted daily to calculate the survival rate.

3. Preparation and analysis of hemolymph

To prepare hemolymph, we collected blood via cardiac puncture using an EDTA-treated (50 mM EDTA in PBS, pH 7.6) syringe to prevent hemocyte coagulation. The blood samples were centrifuged for 10 min at 12,000 rpm at 4℃, and the resulting supernatant was stored at −75℃ until analysis.

Plasma glucose was analyzed using a glucose assay kit (STA-681, Cell Biolabs Inc., San Diego, CA, USA) and the total protein content was measured using a biuret-based total protein kit (ll0128, Sigma-Aldrich, St. Louis, MO, USA). The plasma superoxide

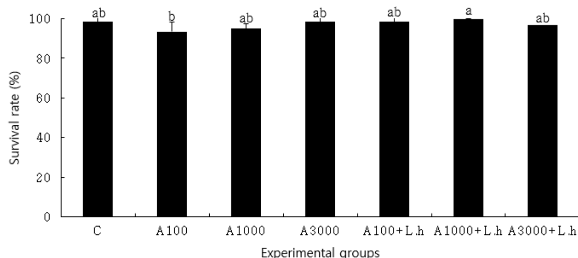

Fig. 1. Survival rate of *Haliotis discus hannai* fed the experimental diets for 17 weeks. Superscript letters indicate significant differences among the experimental groups (P0.05). A100: 100 ppm astaxanthin; A1000: 1,000 ppm astaxanthin; A3000: 3,000 ppm astaxanthin; A100 + L.h: 100 ppm astaxanthin + *Lactobacillus helveticus*, A1000 + L.h: 1,000 ppm astaxanthin + *Lactobacillus helveticus*: A3000 + L.h: 3,000 ppm astaxanthin + *Lactobacillus helveticus*; C: control.

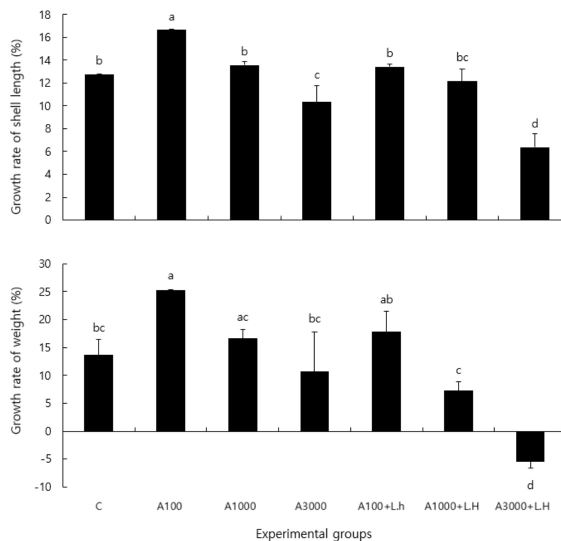

Fig. 2. Growth rate, according to shell length and weight of *Haliotis discus hannai*, fed the experimental diets for 17 weeks. See Fig. 1 for other details.

dismutase (SOD) level was analyzed using a fish SOD ELISA kit (E10146689, MyBioSource, San Diego, CA, USA). Lysozyme activity and catalase (CAT) activity in plasma were measured using a lysozyme ELISA kit (L04QLZ20, MyBioSource) and a CAT activity assay kit (STA-341, Cell Biolabs Inc.), respectively.

4. Statistical analysis

The values from each experiment are expressed as means ± standard error (SE). The statistical significance of the experimental measurements and95% confidence limits ($p < 0.05$) were determined by one-way ANOVA and Duncan's multiple range test using SPSS Statistics (version 24).

RESULTS AND DISCUSSION

1. Survival rate

The final survival rates ranged from 93 ± 4.7 to 100% in all experimental groups (Fig. 1). The A1000 + L.h. and A100 groups showed the highest and lowest survival rates, respectively. However, neither group showed a significant difference from the control ($p > 0.05$). The low survival rate in the A100 group can be attributed to the death of juvenile abalone that failed to adapt to the tank environment early in the experiment. After excluding the early-stage results from the analysis, the A100group showed the same survival pattern as those of the other groups (data not shown). This result indicates that neither astaxanthin nor the astaxanthin-producing bacterium *P. haeundaensi* had any effect on the survival of *H. discus hannai*. According to Part *et al*. (2016), abalone fed *Lactobacillus*-enhanced feed showed an average survival rate of 85% with no significant difference from those fed commercial formulated feed. The present study also resulted in no significant difference in survival rate between the L.h.-treated and non-treated groups, indicating that *Lactobacillus* does not affect abalone survival.

2. Growth rate

The A100 group showed the greatest increases in both body weight (16.65 ± 0.04%) and shell length (25.24 ± 0.18%, Fig. 2). Growth rate decreased with increasing astaxanthin concentrations; the A3000 + L.h. group showed a decrease in body weight of −5.6 ± 4.7%. Ju and Dong (2016) reported that feed enriched with red algae and shell extract resulted in a change in the shell pigmentation of *H. discus hannai*, but without any observed change in growth. Another study indicated that abalone shell and meat coloration depend on feed type (James *et al*., 2016), but there are

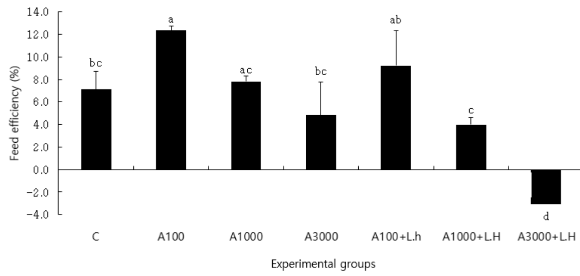

Fig. 3. Feeding efficiency of *Haliotis discus hannai* fed the experimental diets for 17 weeks. See Fig. 1 for other details.

very few studies on the effect of feed on abalone growth. Tsushima and Matsuno (1998) found that providing breeding abalone with astaxanthin-enriched feed accelerated larval hatching and early growth. In our study, a low astaxanthin concentration positively affected abalone growth but not shell pigmentation (data not shown). However, studies in fish reported that astaxanthin did not affect the growth of rainbow trout (*Oncorhynchus mykiss*), olive flounder (*Paralichthys olivaceus*), or red seabream (*Pagrus pagrus)* (Rehulka, 2000; Kalinowski *et al.*, 2005; Kim *et al.*, 2006). This difference may be caused by experimental conditions, varying bioavailability of astaxanthin among taxa, or different methods of culture and processing.

Our results suggest a negative relationship between astaxanthin content and feeding efficiency (Fig. 3). This indicates that food additives provided at inappropriate concentrations will be ineffective or harmful, regardless of their concentrations of otherwise 'beneficial' bioactive compounds (Kim *et al.*, 2006; Seo *et al.*, 2009). Therefore, determination of the optimal amounts of additives to use is necessary.

In our study, the addition of L.h. culture to astaxanthin-enriched feed tended to result in lower growth rates in abalone, compared with the astaxanthin only and control groups, indicating that L.h. negatively affects abalone body weight. Park *et al.* (2016) compared the effects of commercial feed versus *Lactobacillus* (*Bacillus amyloliquefaciens*)-supplemented feed on abalone growth and also found no significant difference. Jhon *et al.* (2009) found that herbal ingredients and various *Lactobacillus* species (*L. acidophilus*, *L. brevis*, and *L. plantarum*) did not affect feeding efficiency in rock seabream (*Oplgnathus fasciatus*). On the other hand, Byun *et al.* (1997) observed greater weight gain in flounder fed *Lactobacillus* sp. than in control flounder. Peak *et al.* (2001) also found that olive flounder fed *L. brevis*, *L. plantarum*, or *Enterococcus faecium* gained 50% more weight than did the control. This difference may derive from different bioavailabilities of *Lactobacillus* among species, as well as different feed compositions and experimental conditions. Different species of *Lactobacillus* may also vary in their effects on animal growth.

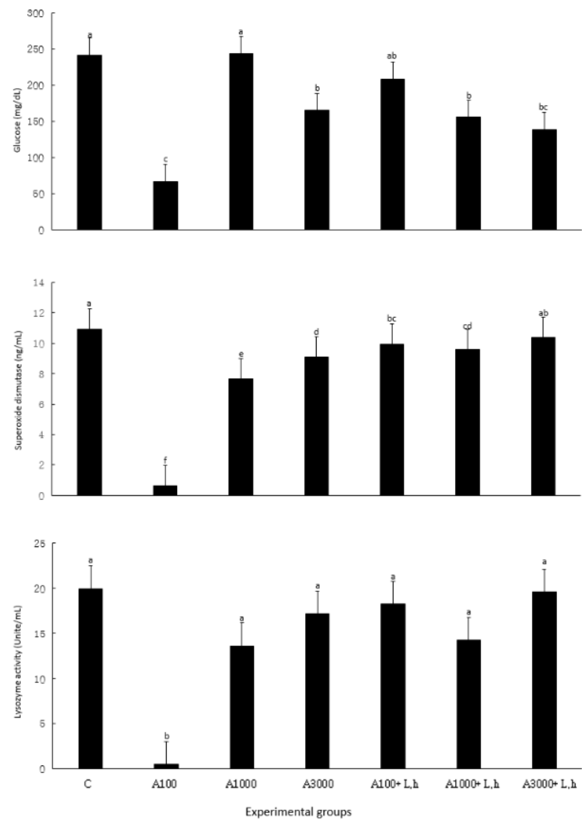

Fig. 4. Glucose level, superoxide dismutase (SOD) activity, and lysozyme activity in the hemolymph of *Haliotis discus hannai* fed the experimental diets for 17 weeks. See Fig. 1 for other details.

3. Hemolymph analyses

Stress increases hemolymph glucose and total protein contents in farmed animals (Hontela *et al.*, 1996; Cheng *et al.*, 2004; Baeck *et al.*, 2014). To

investigate the effects of astaxanthin and L.h. on the physiology of *H. discus hannai*, we measured hemolymph glucose and total protein contents. The A100 treatment resulted in significantly lower levels of hemolymph glucose (67.29 ± 8.64 mg · dL^{-1}) in abalone compared with all other groups including the control ($p < 0.05$), indicating that only certain concentrations of astaxanthin in feed can lower hemolymph glucose levels. It is thought that biologically appropriate levels of astaxanthin are absorbed by abalone to help sustain normal physiological functions and homeostasis. The A100 treatment group also had the lowest total protein content, but not significantly so ($p > 0.05$, data not shown). The total protein content in blood is an important clinical indicator of health, nutrition, and disease in both terrestrial and aquatic animals. In fish, the primary repose to a stressor includes elevation of plasma cortisol, which then stimulates a secondary response that affects protein metabolism and increases blood protein levels (Davidson, 2000; Riche, 2007). Abalone have a different stress response to that offish, but only a limited number of studies are available on this topic, and it is difficult to conclude whether the total protein content in blood can indicate physiological homeostasis in abalone. However, the lack of a significant difference in total protein content could indicate that astaxanthin does not negatively affect abalone physiology.

When exposed to stress, organisms produce reactive oxygen species, which have a strong oxidizing potential to combine with other bodily substances and reduce or inhibit cell functions. Thus, as a defense mechanism, antioxidative enzymes such as SOD, CAT, glutathione peroxidase, and glutathione S-transferase are elevated in stressed organisms, including abalone to defend against external stress induced by high water temperature and low salinity (Min *et al*., 2015). In our study, the A100 + L.h. treatment had a significantly lower SOD levels (0.63 ± 0.38ng · mL^{-1}) compared to other experimental groups ($p < 0.05$). We assumed that the highly antioxidative astaxanthin enhanced potential defense in the abalone, reducing SOD activity. However, in groups without L.h., astaxanthin was positively correlated with SOD activity. This indicates that only appropriate levels of astaxanthin can contribute to homeostasis in abalone. It is generally believed that *Lactobacillus* has antioxidative defenses against reactive oxygen species (Kim and Ham, 2003). In our study, however, the SOD value was higher in groups treated with both astaxanthin and L.h. than in groups treated with astaxanthin only. Further experiments are needed to understand the causes of this negative effect and to identify the optimal count of L.h. that produces probiotic benefits.

Levels of CAT activity in hemolymph were not significantly different among the experimental groups ($p > 0.05$, data now shown). CAT catalyzes the reduction of $2H_2O_2$ to $2H_2O$ and O_2, preventing peroxides from damaging cell membranes (Chance *et al*., 1979; Wendel and Feuerstein, 1981). Therefore, we assumed that the combination of astaxanthin and L.h. did not induce sufficient stress to affect CAT activity. Lysozymes play an important role in immune defenses against pathogens, and *H. discus hannai* exhibit elevated levels of lysozyme activity under stress induced by high water temperatures (Min *et al*., 2015). In our experiments, the A100 treatment induced the lowest lysozyme activity (0.46 ± 0.51 U · mL^{-1}; $p < 0.05$). We assume that astaxanthin can enhance potential immunity and disease resistance to diseases, and the optimum concentration can be much more effective. However, further experiments using direct inoculation of pathogens are needed to better understand immune defense mechanisms.

In conclusion, the combined addition of astaxanthin and *Lactobacillus* in feed did not have a significant effect on the survival of *H. discus hannai* compared with the control or astaxanthin only treatments. However, astaxanthin concentrations outside the optimal range had negative effects on physiological homeostasis. Finally, the addition of L.h to feed did not affect the growth or physiological status of *H. discus hannai*.

ACKNOWLEDGEMENTS

This study was carried out under the Golden Seed Project (213008-05-4-SB710), a joint program by the

Ministry of Agriculture, Food and Rural Affairs; Ministry of Oceans and Fisheries; Rural Development Administration; Korea Forest Service; and the 2018 project of development of aquaculture products with improved physiological activity and pigmentation using marine microorganisms producing Korean traditional colors, supported by the Korea Institute of Marine Science and Technology Promotion funded by the Ministry of Oceans and Fisheries.

REFERENCE

Baeck, S.K., Min, E.Y., and Kang, J.C. (2014) Combined effects of copper and temperature on hematological constituents in the rock fish, *Sebastes schlegeli. Journal of Fish Pathology*, **27**(1): 57-65.

Byun, J. W., Park, S. C., Beno, Y., and Oh, T. K. (1997) Probiotics effect of *Lactobacillus* sp. DS-12 in flounder (*Paralichthys olivaceus*). *The Journal of General and Applied Microbiology*, **43**(5): 305-308.

Chance, B., Sies, H., and Boveris, A. (1979) Hydroperoxide metabolism in mammalian organs. *Physiological Reviews*, **59**(3): 527-605.

Choi, C.S., Eom, S.H., Lee, M.S., and Kim, Y.M. (2010) Effect of an astaxanthin-supplemented diet on the nutritional composition of rainbow trout (*Oncorhynchus mykiss). Korean Journal of Fisheries and Aquatic Sciences*, **43**(2): 109-116.

Davidson G.W., Davie P.S., Young G., and Fowler R.T. (2000) Physiological responses of rainbow trout *Oncorhynchus mykiss* to crowding and anesthesia with AQUI-STM. *Journal of World Aquaculture Society*, **31**(1): 105-114.

Dhawan, V. and Jain, S. (2005) Garlic supplementation prevents oxidative DNA damage in essential hypertension. *Molecular and Cellular Biochemistry*, **275**(1-2): 85-94.

Ferraris, M., Radice, S., Catalani, P., Francolini, M., Marabini, L., and E. Chiesara, E. (2002) Early oxidative damage in primary cultured trout hepatocytes: a time course study. *Aquatic Toxicology*, **59**(3-4): 283-296.

Ha, B.S., Kang, D.S., Kim, J.H. Choi, O.S., and Ryu, H.Y. (1993) Metabolism of dietary carotenoids and effects to improve the body color of cultured flounder and red sea bream. *Journal of the Korean Fisheries Society,* **26**(2): 91-101.

Harris, J.O., Hoang, T.H., Qin, J. G., Stone, D.J., Duong, D. N., and M. S. Bansemer (2016) Colour changes of greenlip abalone (*Haliotis laevigata* Donovan) fed fresh macroalgae and dried algal supplement. *Journal of Aquaculture*, **456**: 16-23.

Hontela, A.,Daniel, C., and A.C. Ricard (1996) Effects of acute and subacute exposures to cadmium on the interrenal and thyroid function in rainbow trout, *Oncorhynchus mykiss*. *Aquatic Toxicology*, **35**(3-4): 171-182.

Jhon, B.K., Kim, M.C., Kim, Y.H., and Heo, M.S. (2009) Effects of the culture broth of lactic acid bacteria cultured in herb extracts on growth promotion and nonspecific immune responses of aquacultured fish. *Journal of Life Science*, **19**(1): 87-93.

Ju, Z.Y. and Deng, D.F. (2016) Effects of algae and shell pigment extract-supplemented diets on shell pigmentation and growth performance of pacific abalone, *Haliotis discus hannai. Journal of the World Aquaculture Society*, **48**(1): 93-102.

Kalinowski, C.T., Robaina, L.E., Fernandez-Palacios, Schuchardt, D., and Izquierdo, M.S. (2005) Effect of different carotenoid sources and their dietary levels on red porgy (*Pagrus pagrus*) growth and skin color. *Aquaculture*, **244**(1-4): 223-231.

Kim, H. and Kang, J.O. (1998) Determination of astaxanthin, á-tocopherol and TBARS in the liver and muscle of rainbow trout supplemented with red yeast containing astaxanthin. *Journal of the Korean Society of Food Science and Nutrition*, **27**(5): 935-939.

Kim, H.S. and Ham, J.S. (2003) Antioxidative ability of lactic acid bacteria. *Korean Journal for Food Science of Animal Resources*, **23**(2): 186-192.

Kim, S.S., Galaz, G.B., Lee, K.J., and Lee, Y.D. (2006) Effects of dietary supplementation of spirulina and astaxanthin for juvenile olive flounder*, Paralichthys olivaceusin* low temperature season. *Korean Journal of Aquaculture*, **19**(1): 57-63.

Kim, S.Y., Cho, E.A., Yoo, G.J., Yoo, J.M., Son, S.M., In, M.J., Kim, D.C., and Chae, H.J. (2009) Physiological activity of astaxanthin and its inclusion complex with cyclodextrin. *Journal of the KSBB*, **24**(6): 570-578.

KOSIS (2017), Korean statistical information service, Retrieved from http://kosis.kr/index/index.do on August 24.

Lim, T.J. and Lee, S.M. (2003) Effect of dietary pigment sources on the growth and shell color of abalone (*Haliotis discus hannai*). *Journal of the Korean Fisheries Society*, **36**(6): 601-605.

Min, E.Y., Kim, S.H., Hwang, I.K., Kim, K.W., Park, B.M., Lee, J.S., and Kang, J.C. (2015) Influence of elevated temperatures on the physiological response of hemolymph from two species of abalone, *Haliotis gigantea* and *Haliotis discus* (Reeve, 1846). T*he Korean Journal of Malacology*, **31**(3): 187-194.

Min, E.Y., Lee, J. S, Kim, J. W., Jeon, M. A.,and Kang, J.C. (2015) Influence of elevated temperatures on the physiological response of hemolymph from two species of the abalone. *Haliotis discus hannai* and *H. discus*. *The Korean Journal of Malacology*, **31**(1): 1-8.

National Fisheries Research Development Institute. (2008) Standard manual of abalone culture. pp. 225.

Okada, S., Nur-E-Borhan, S.A., and Yamaguchi, K. (1994) Carotenoid composition in the commercial black tiger prawns. *Journal of Fisheries Science*,

60(2): 213-215.

Park, J.Y., Kim, W.S., Kim, H.Y., and Kim, E. (2016) Potential use of *Bacillus amyloliquefaciens* as a probiotic bacterium in abalone culture. *The Korean Journal of Fish Pathology*, **29**(1): 35-43.

Peak, N. S., Lim, Y. B.,and Kim, Y. M. (2001) Antibacterial activity and growth promotion in aquacultured fish by probiotics. *Korean Journal of Applied Microbiology and Biotechnology*, **29**(1): 56-61.

Rehulka, J. (2000) Influence of astaxanthin on growth rate, condition, and some blood indices of rainbow trout. *Oncorhynchus mykiss*, *Aquaculture*, **190**: 27-47.

Riche, M.(2007) Analysis of refractometry for determining total plasma protein in hybrid striped bass (*Morone chrysops* × *M. saxatilis*) at various salinities. *Aquaculture*, **264**(1-4): 279-284.

Schiedt, K., Vecchi, M., and Glinz, E.(1986) Astaxanthin and its metabolites in wild rainbow trout (*Salmo gairdneri* R.). *Comparative Biochemistry* and Physiology Part B: *Comparative Biochemistry*, **83**B(1); 9-12.

Seo, J.Y., Kim, K.D., and Lee, S.M. (2009) Effects of supplemental herb medicines in the diets on growth, feed utilization and body composition of juvenile and grower rockfish *Sebastes schlegeli*. *Journal of Aquaculture*, **22**(1): 112-117.

Seo, Y.B and Kim, G.D. (2017) Microbial production of carotenoids: biological functions and commercial applications. *Journal of Life Science*, **27**(6): 726-737.

Seo, Y.B., Lee, J.K., Jeong, T.H., Nam, S.W. and Kim, G.D. (2015) Enhanced production of astaxanthin by Archaea chaperonin in *Escherichia coli*. *Korean Journal of Life Science*, **25**(12): 1339-1346.

Storebakken, T., Foss, P., Schiedt, Austreng, E., Liaaen-Jensen, S., and Manz, U.(1987) Carotenoids in diets for salmonids: IV. Pigmentation of Atlantic salmon with astaxanthin, astaxanthin dipalmitate and canthaxanthin. *Aquaculture*, **65**(3-4): 279-292.

Tsushima, M. and Matsuno, T. (1998) The role of β, β-carotene on growth and survival of juvenile Japanese abalone *Haliotis discus*. *The Journal of Fisheries Science*, **64**(4): 660-661.

Wendel, A. and Feuerstein, S. (1981) Drug-induced lipid peroxidation in mice-l modulation by monooxygenase activity, glutathione and selenium status. *Biochemical Pharmacology*, **30**(18): 2513-2520.

Winton, C., Chiu-Hsiam, L., and Jiann-Chu, C. (2004) Effect of dissolved oxygen on the immune responses of *Haliotis diversicolor supertexta* and its susceptibility to *Vibrio parahaemolyticus*. *Aquaculture*, **232**: 103-115.

Yang, B.G., Lee, J.H., and Heo, M.S. (2003) Antibacterial activity of *Lactobacillus sakei* BKl9 against fish pathogenic bacteria. *The Korean Journal of Microbiology*, **39**(1): 56-61.

Yang, B.G., Jeon, Y.J., and Heo, M.S. (2003) Screening and characterization of probiotic strains for prevention of bacterial fish diseases. *Korean Journal of Microbiology and Biotechnology*, **31**(2): 129-134.