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Effects of astaxanthin produced by *Paracoccus haeundaensis* on growth and body color in *Epinephelus akaara*

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Abstract

The purpose of this study was to determine whether marine microorganism-derived astaxanthin has effects on coloration improvement and growth of the Hong Kong grouper (*Epinephelus akaara*). Juvenile and immature *E. akaara* were fed diets comprised of a mixture of commercial feed and biosynthetic astaxanthin obtained from *Paracoccus haeundaensis* to analyze changes in their growth and body color. Color variances in the skin and muscle were measured using spectrophotometry. High-performance liquid chromatography (HPLC) was used to measure the astaxanthin content in the muscle and skin, and to determine the amount of astaxanthin accumulated in the body. Astaxanthin did not affect growth but affected red coloration of the skin in both juvenile and immature *E. akaara*. Furthermore, the astaxanthin content in the muscle and skin of immature fish consistently increased as the concentration of astaxanthin supplemented in the feed increased. In conclusion, astaxanthin derived from the marine microorganism *Paracoccus haeundaensis* was effective in improving the coloration of both juvenile and immature *E. akaara*. Therefore, it is considered a feasible feed additive that improves the coloration of fish and increase the aquaculture business's income.

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Introduction

Carotenoids are yellow, orange, or red organic pigments that are produced by plants and microorganisms. They are responsible for the orange-red color of the skin and muscle of marine animals such as salmon, red seabream, lobster, and shrimp. However, fish species cannot synthesize carotenoid-based pigments internally but instead accumulate pigments synthesized from plants or microorganisms by ingestion of feed (Kim et al., 2006a). Carotenoid-based pigments provided with fish feed are extracted and purified from natural ingredients such as *Phaffia rhodozyma*, *Agrobacterium aurantiacum*, *Chlorococcum* sp., *Haematococcus pluvialis*, *Chlorella zofingiensis*, *Chlorella vulgaris*, and *Spirulina platensis* (Gouveia et al., 2003).

Astaxanthin, a red pigment belonging to carotenoids, has been widely used as a feed supplement to improve the red body color of fish. Many previous studies have reported an improvement in red coloration and increased astaxanthin content in fish, including snapper (*Pagrus auratus*), salmon, and rainbow trout (*Oncorhynchus mykiss*) (White et al., 2002; Booth et al., 2004; Tolasa et al., 2005). Astaxanthin has promoted the commercial value of some fish species and ensured higher market prices as a consequence of improving the coloration of those fish species. Another benefit to supplementation with astaxanthin is that fish with higher astaxanthin content have higher commercial value as a food product. In addition to these advantages, many studies have reported antioxidant effects of astaxanthin intake on the fish body (Waagbo et al., 2003; Scheikhzadeh et al., 2012). As astaxanthin has been reported to help increase lymphocyte counts, improve immune responses, and prevent inflammation, there have been several studies investigating the potential use of astaxanthin as a multi-functional additive to replace vitamins (Amar et al., 2004; Hamre et al., 2004; Ambati et al., 2014).

The global feed supplements market was estimated at approximately 28 billion dollars in 2017 and is projected to develop at an annual growth of 6.5% up to 2023 (Mordor Intelligence, 2018). Among many feed supplements, the world's carotenoid market is projected to account for 270 million dollars by 2023, with an annual growth of 4% from 2018 to 2023 (Mordor Intelligence, 2018). However, the high cost associated with the production of astaxanthin, which is mostly extracted from natural sources, limits its availability and therefore minimizes access to its benefits.

The Hong Kong grouper (*E. akaara*) is a stationary species that is widespread on the coast of the Korean Peninsula, south of central Japan, China, and Taiwan. It mainly inhabits the coral and rocky reefs off the coast (Masuda, 1984). The Hong Kong grouper is an appetizing red fish; it is sold at a high price in the greater Chinese markets, and the market demand increases every year (Heemstra and Randall, 1993; Froese and Pauly, 2014). Following the sharp decline in the population of wild *E. akaara*, it was listed in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Sadovy et al., 2018). Since then, efforts have been made, mainly in Korea, China, Taiwan, and Japan, to develop an aquaculture technology for the species. The market price of a cultured Hong Kong grouper is affected by the redness of its body color. A fish cultured in a cage for some time will exhibit lighter red skin and darker flesh; these properties decrease its product value. Thus, aquaculturists have made efforts to match the color of the cultured Hong Kong groupers to the red color of those inhabiting the wild prior to distribution in the market.

In this study, we used a marine microorganism that biosynthesizes astaxanthin, with the aim of obtaining an affordable astaxanthin for use in culture feed supplementation. The astaxanthin derived from the microorganisms was directly supplemented into the *E. akaara* diets. To determine the effects of astaxanthin supplementation in the fish diet, changes in growth, body color, and internal astaxanthin content were investigated.

Materials and Methods

Experimental fish and culture conditions

Hong Kong groupers used in the experiments were juvenile and immature fish produced at Cheongsol Susan located in Muan-gun, Jeollanam-do, Korea. The juvenile fish used in the first experiment (EXP. I) were 12.0 ± 0.1 cm long and weighed 27.2 ± 0.5 g, while the

immature fish used in the second experiment (EXP. II) were 19.54 ± 0.04 cm long and weighed 115.9 ± 0.8 g.

The experiments were conducted at Aquaculture Research Center, Ocean and Fisheries Science Institute, located in Younggwang-gun, Jeollanam-do. The water tank used in the experiments was a 3-ton cylindrical polypropylene water tank (width, 2 m; depth, 0.9 m; and volume, 2.8 m3). Seawater filtered with a sand filter was supplied at a rate of 20 L/min; water temperature during the experiments was maintained at 25.0 ± 1.0℃, and dissolved oxygen was maintained at 7 ppm or above.

Addition of astaxanthin

In EXP. I, two cages were installed in each experimental tank where 60 juvenile fish were housed in each cage. There were three replicate groups in the experiment. The fish were cultured for 90 days. The experimental feed used in EXP. I was a compound feed of juvenile flounders with a size range of 2.0–2.6 mm (52% crude protein, 10% crude fat; Woosung Ltd, Korea). Four experimental groups were formed based on the amount of astaxanthin supplemented. Astaxanthin was supplemented at a final concentration of 100 ppm (AST100), 1000 ppm (AST1000), and 3000 ppm (AST3000), while the control group was fed a diet with no astaxanthin added. The astaxanthin applied to the formed diets was derived from *Paracoccus haeundaensis* (Seo et al., 2017). A specified amount of freeze-dried culture medium and water were added to the feed, and the fish were fed to satiation twice daily.

EXP. II comprised an experimental water tank with three replicates, each of which housed 20 fish that were cultured for 60 days. The experimental feed used in the experiment was a compound feed of flounders with a size of 7.5 mm (52% crude protein, 10% crude fat; Woosung Ltd, Korea). The culture conditions and astaxanthin development conditions were identical to EXP. I. Five experimental groups were studied: feed with no astaxanthin added (control), feed with 10, 50, and 100 ppm of marine microorganism-derived astaxanthin added (AST10, 50, and 100, respectively), and feed with 100 ppm of a commercial astaxanthin (DSM, Netherlands) added (C.AST 100).

Growth and survival rate

Throughout the experiment, the daily feed amount, and amount remaining were monitored, and the total length and weight of the experimental fish were measured to 0.1 cm and 0.1 g, respectively, using Vernier calipers and an electronic scale. Growth was determined based on the acquired growth data and feeding rate. Weight gain, Specific growth rate, Daily feed intake, Feed efficiency, Condition factor were calculated as shown in the following formula (**Table 1**). At the end of the experiment, 30 individual fish were randomly sampled for each experimental group, anesthetized for 1 min with 150 ppm of 3-aminobenzoic acid ethyl ester (Sigma, USA) and dissected to measure visceral and liver weights. The weight ratio of the liver and viscera collected was calculated using the hepatosomatic index (HIS) and visceral somatic index (VSI). Survival rate was obtained by calculating backwards from the daily mortality observed in the experimental water tank during the experiment.

Color measurements of fish skin and muscle

To measure the color of the skin and muscle, 30 individual fish were randomly selected from each experimental group. Measurements were taken using a colorimeter (CR-200, Minolta Co., Japan). The hunter scale was used to evaluate color and lightness (L-value, where +L indicates light), redness (+a: red, -a: green), and yellowness (+b: yellow, -b: blue).

Analysis of astaxanthin in skin and muscle

Three fish were randomly collected from each experimental group of EXP. II. High performance liquid chromatography (HPLC) was used to measure the astaxanthin content in skin and muscle samples. An Automated Biologic HR system (Bio-Rad Laboratories, USA)

was used. The fish were anesthetized with 150 ppm of 3-aminobenzoic acid ethyl ester (Sigma, USA) for 1 min, and the scales and blood were removed to isolate only the skin and muscle of the fish. To extract the pigment, tissue samples (1 g) were soaked in a mixture of chloroform-methanol (6:4) for 24 h. The pigment extract was centrifuged at 4000 rpm for 30 min and filtered through a 0.22 μm nylon filter in preparation for loading the sample onto the HPLC column (Poproshell 120 EC-C18 [4.6 × 250 mm]; Agilent Technologies, USA). The solvent used for analysis was methanol:acetonitrile:water (90:5:5). A sample of 20 μL was injected, and the separation time was 30 min. Post-running time was 10 min, and the concentration of the solvent was equal to that at the start. The flow rate inside the column was set to 1 mL per minute. The separated astaxanthin was measured at 470 nm; then, a comparison was made with the reference material, astaxanthin, purchased from Sigma-Aldrich.

Statistical analysis

Statistical significance of the measurements (mean ± SE) in each experiment was determined by ANOVA and Duncan test ($p < 0.05$) using the SPSS Statistics program (version 26).

Results

Growth and survival rate

No fish mortality (data not shown) nor abnormal symptoms of disease were observed in any of the experimental groups in EXP. I and II. In EXP. I, where fish were cultured for 90 days, the weight of the juvenile fish in the experimental group AST3000 was not significantly different ($p < 0.05$). Total length and weight of fish in the AST100 and AST1000 groups were not significantly different between the control and experimental groups ($p < 0.05$). Weight gain and daily growth rate were not significantly different between AST3000 and the control ($p < 0.05$). The fish in the AST100 group had the lowest weight gain and daily growth rate ($p > 0.05$). The daily ingestion rate was not significantly different in any of the experimental groups ($p > 0.05$) (**Table 1**).

After 60 days of cultivation, the fish in EXP. II had an average total length of 21.85 ± 0.08 cm and an average weight of 180.64 ± 2.53 g, and there was no significant difference observed between any of the experimental groups ($p < 0.05$). Feed efficiency was significantly low in AST10 compared with the control ($p > 0.05$), but there was no significant difference observed in the rest of the experimental groups. None of the experimental groups showed any significant difference in terms of weight gain, daily growth rate, and daily ingestion rate ($p < 0.05$) (**Table 2**).

Table 1 Growth performance of juvenile red spotted grouper reared at different pigment additive feed during the experimental period

	Group			
	Control	*AST 100ppm*	*AST 1,000ppm*	*AST 3,000ppm*
Final body length (cm)	15.05±0.23^{b}	15.25±0.09^{ab}	15.17±0.12^{ab}	15.66±0.12^{a}
Final body weight (g)	57.44±2.66^{ab}	54.86±0.47^{b}	54.07±1.05^{b}	60.95±0.82^{a}
Weight gain (%)	122.64±11.54^{a}	94.45±1.52^{b}	101.00±5.62^{ab}	122.40±3.25^{a}
Specific growth rate (%)	0.89±0.06^{a}	0.74±0.01^{b}	0.77±0.03^{ab}	0.89±0.02^{a}
Feed efficiency (%)	55.91±4.46	48.80±0.31	47.55±1.31	53.52±1.84
Daily feed intake (%)	1.51±0.06	1.46±0.01	1.57±0.05	1.58±0.04

* The values are mean±SE (n=30). The values within each column followed by the different alphabetic letter are significantly different (P<0.05). Weight gain: [(W2-W1)/W1X100], Specific growth rate: [In(W2)-In(W1)]X100/D, Feed efficiency: (G/F)X100, Daily feed intake: FX100/[(W1+W2+W3)XD/2]. D: days of rearing, F: feed intake, G: weight gain, W1: initial body weight, W2: Final body weight, W3: dead fish weight, AST: astaxanthin.

Table 2 Growth performance of red spotted grouper reared at different pigment additive feed during the experimental period

	Group				
	Control	*C. AST*	*AST 10ppm*	*AST 50ppm*	*AST 100ppm*
Final body length (cm)	21.71±0.07	21.82±0.21	21.88±0.17	22.09±0.11	22.06±0.31
Final body weight (g)	173.68±5.80	183.19±1.66	173.93±3.07	189.05±5.19	190.15±9.34
Weight gain (%)	53.12±4.50	56.53±1.21	47.57±3.14	63.35±2.23	61.75±10.31
Specific growth rate (%)	0.47±0.03	0.50±0.01	0.43±0.02	0.55±0.02	0.53±0.07
Feed efficiency (%)	85.00±6.87^{a}	75.74±0.52^{ab}	66.51±2.92^{b}	81.09±4.25^{ab}	78.25±4.70^{ab}
Daily feed intake (%)	0.56±0.07	0.65±0.01	0.64±0.02	0.66±0.04	0.66±0.05

* The values are mean±SE (n=30). The values within each column followed by the different alphabetic letter are significantly different (P<0.05). Weight gain: [(W2-W1)/W1X100], Specific growth rate: [In(W2)-In(W1)]X100/D, Feed efficiency: (G/F)X100, Daily feed intake: FX100/[(W1+W2+W3)XD/2]. D: days of rearing, F: feed intake, G: weight gain, W1: initial body weight, W2: Final body weight, W3: dead fish weight, C.AST: commercial astaxanthin, AST: astaxanthin.

Visceral somatic and hepatosomatic indices

In EXP. I, the condition factor of *E. akaara* was 1.62 ± 0.01 in the control, which was significantly higher than the rest of the experimental groups ($p < 0.05$). There was no significant difference in the visceral somatic index for all experimental groups ($p > 0.05$). The hepatosomatic index was the highest in AST1000 and the lowest in AST100 (**Table 3**).

Table 3 VSI and HSI of juvenile red-spotted grouper reared at different pigment additive feed during the experimental period

	Group			
	Control	*AST 100ppm*	*AST 1,000ppm*	*AST 3,000ppm*
Condition factor	15.05±0.23^{b}	15.25±0.09^{ab}	15.17±0.12^{ab}	15.66±0.12^{a}
Visceral somatic index (VSI, %)	57.44±2.66^{ab}	54.86±0.47^{b}	54.07±1.05^{b}	60.95±0.82^{a}
Hepatosomatic index (HSI, %)	122.64±11.54^{a}	94.45±1.52^{b}	101.00±5.62^{ab}	122.40±3.25^{a}

* The values are mean±SD (n=30). The values within each column followed by the different alphabetic letter are significantly different (P<0.05). Condition factor: ($W2/L^3$)X100. W2: Final body weight, L: final body length, AST: astaxanthin.

In EXP. II, none of the indices, including the fatness, visceral somatic, and hepatosomatic indices of the immature *E. akaara* showed significant differences between any of the experimental groups ($p > 0.05$) (**Table 4**).

Table 4 VSI and HSI of red-spotted grouper reared at different pigment additive feed during the experimental period

	Group				
	Control	*C. AST*	*AST 10ppm*	*AST 50ppm*	*AST 100ppm*
Condition factor	1.70±0.04	1.77±0.02	1.66±0.02	1.75±0.02	1.77±0.02
Visceral somatic index (VSI, %)	7.63±0.53	8.02±0.22	7.75±0.44	7.41±0.23	7.76±0.39
Hepatosomatic index (HSI, %)	2.73±0.11	2.50±0.20	2.52±0.38	2.29±0.08	2.71±0.33

* The values are mean±SE (n=30). The values within each column followed by the different alphabetic letter are significantly different (P<0.05). Condition factor: ($W2/L^3$)X100. W2: Final body weight, L: final body length, C.AST: commercial astaxanthin, AST: astaxanthin.

Color measurements of fish skin and muscle

In EXP. I, the L-values representing the color measurements of the skin of juvenile *E. akaara* did not show a significant difference between any of the experimental groups ($p > 0.05$). However, the a-values of the skin in AST100 and AST1000 were 4.02 ± 0.04 and 4.08 ± 0.03, respectively, both of which were significantly higher than the rest of the experimental groups ($p < 0.05$). The b-value of the control was -4.90 ± 0.28, which was significantly higher than the rest of the experimental groups ($p < 0.05$). According to the color measurements of the muscle of juvenile *E. akaara*, L- and b-values were not significantly different between any of the experimental groups ($p > 0.05$). However, the a-values in AST100, AST1000, and AST3000 were significantly higher than the control ($p < 0.05$) (**Figure 1**).

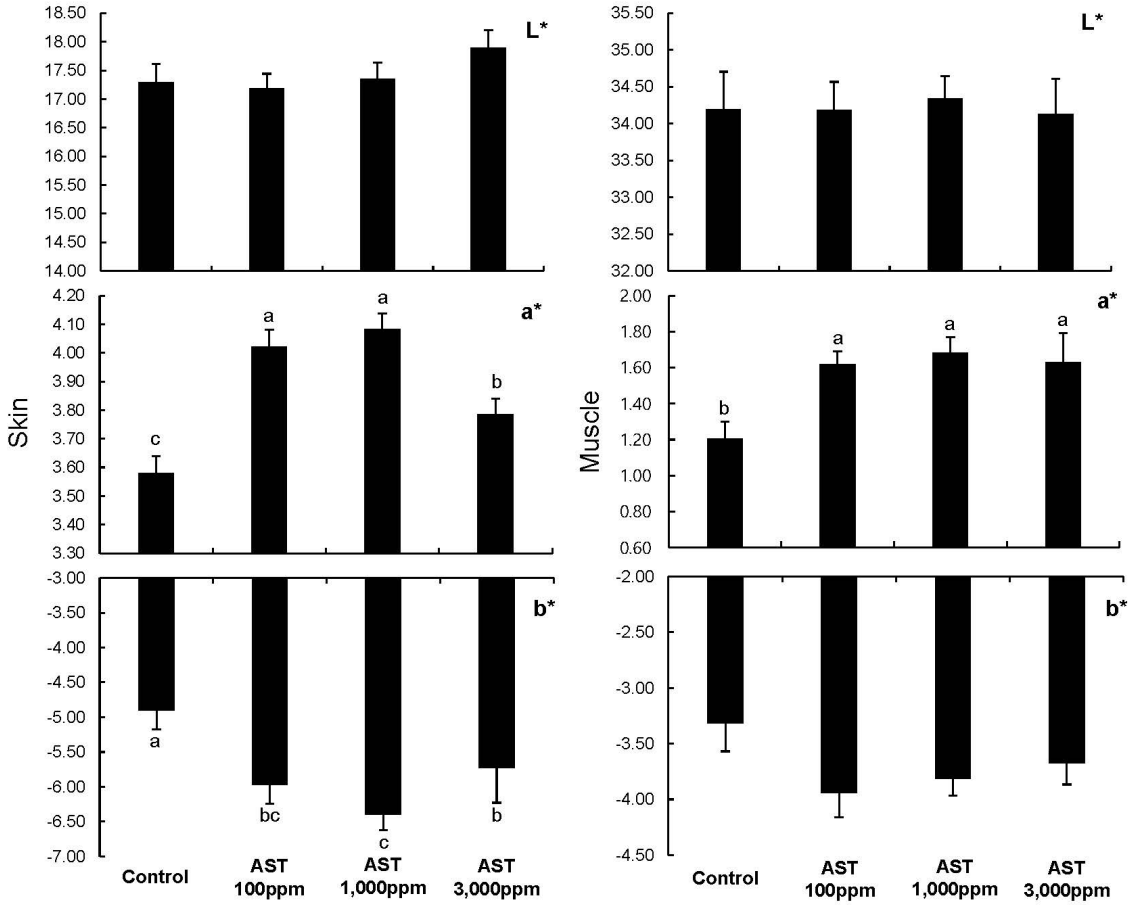

Figure 1 Skin and muscle color of juvenile red-spotted grouper fed the diets containing different experimental diets. Values are presented as mean ± S.E (n=30). Values having different alphabetic letters are significantly different (P<0.05). L, a, and b value with asterisk indicate lightness (L-value, where +L indicates light), redness (+a: red, -a: green), and yellowness (+b: yellow, -b: blue), respectively.

In EXP. II, according to the color measurements of the skin of immature *E. akaara*, the highest L-value of 33.03 ± 0.72 was observed in the control but did not show a significant difference with C.AST, AST50, and AST100 ($p > 0.05$). The a-value was the highest in C.AST (5.36 ± 0.21), but did not show a significant difference with AST10 ($p > 0.05$). The b-value did not show a significant difference between any of the experimental groups ($p > 0.05$) (**Figure 2**). According to the color measurements of the muscle samples, the L-value was the lowest in the control and highest in AST50. The a-value was the highest in AST100 but did not show a significant difference between any of the experimental groups ($p >$

0.05). The b-values in the rest of the experimental groups were significantly higher than the b-value in the control ($p < 0.05$) (**Figure 2**).

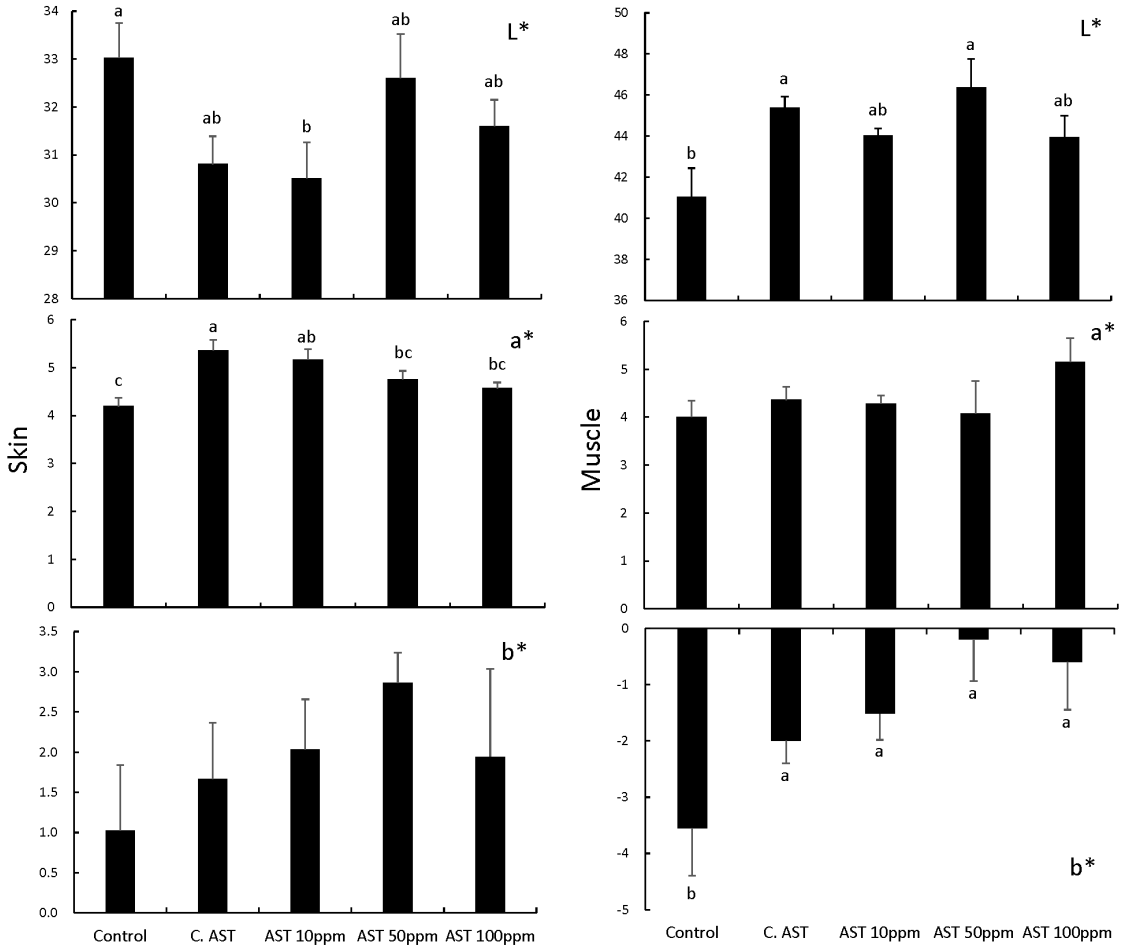

Figure 2 Skin and muscle color of red-spotted grouper fed the diets containing different experimental diets. Values are presented as mean ± S.E (n=30). Values having different alphabetic letters are significantly different (P<0.05). L, a, and b value with asterisk indicate lightness (L-value, where +L indicates light), redness (+a: red, -a: green), and yellowness (+b: yellow, -b: blue), respectively.

Concentration of astaxanthin in skin and muscle

In EXP. II, the highest concentration of astaxanthin in the skin of *E. akaara* was 0.07 ± 0.004 μg/g, which was observed in the AST100 group. C.AST exhibited the second highest astaxanthin concentration of 0.058 ± 0.001 μg/g. The concentrations detected in the AST10 and AST50 groups were 0.007 ± 0.001 μg/g and 0.045 ± 0.003 μg/g, respectively. As the concentration of AST in the feed increased, the concentration of astaxanthin in the skin increased as well ($p < 0.05$) (**Figure 3**).

Astaxanthin could not be detected in the muscle samples of the *E. akaara* control group. However, the concentrations of astaxanthin detected in the C.AST, AST10, AST50, and AST100 groups were 0.049 ± 0.003, 0.004 ± 0.003, 0.040 ± 0.003, and 0.061 ± 0.002 μg/g, respectively. Similar to what was observed for the skin samples, the astaxanthin content in muscle tended to increase as the astaxanthin content in the feed increased (**Figure 3**).

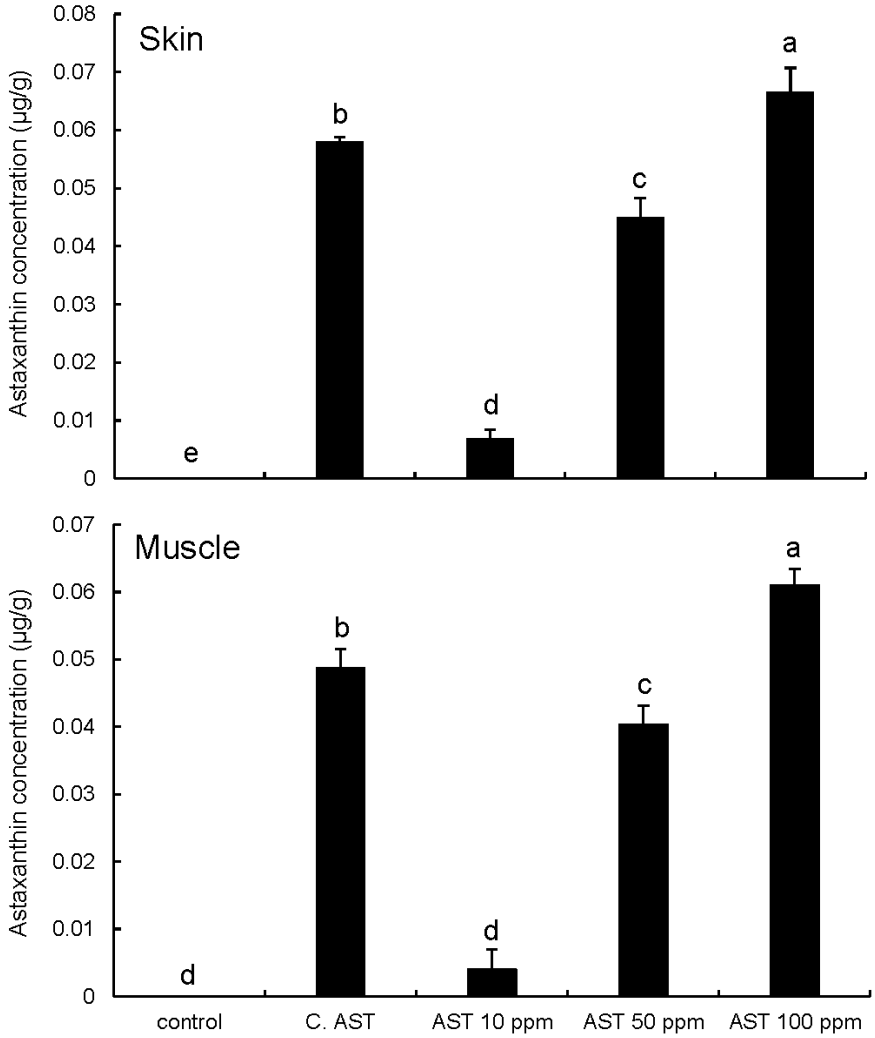

Figure 3 Skin and muscle astaxanthin concentration of red-spotted grouper fed the diets containing different experimental diets. Values are presented as mean ± S.D (n=3). Values having different alphabetic letters are significantly different (P<0.05). C.AST: commercial astaxanthin, AST: astaxanthin.

Discussion

Carotenoids have been reported to improve fish growth due to their positive effect on the intermediate metabolism of aquatic animals (Sengner et al., 1989). In this study, however, feed supplemented with astaxanthin—a carotenoid-based pigment—had no positive effect on the growth of juvenile and immature *Epinephelus akaara*. Kalionwski et al. (2005) reported a similar observation. They used a carotenoid-based pigment to supplement red porgy (*Pagrus pagrus*) diets to study the effects on growth and feed efficiency. Similar to our observations, they observed no significant differences between the experimental groups. Furthermore, supplementing astaxanthin in a rainbow trout (*Oncorhynchus mykiss*) or flounder (*Paralichthys olivaceus*) diet was reported to have no effect on fish growth and feed efficiency (Rehulka, 2000; Kim et al., 2006b). On the other hand, supplementing astaxanthin to Oscar (*Astronotus ocellatus*), large yellow croaker (*Larimichthys crocea*), or golden pompano diets was reported to have a positive effect on fish growth (Alishahi et al., 2015; Li et al., 2014; Xie et al., 2017). A plausible reason for the conflicting results concerning the effect of astaxanthin supplementation on fish growth, as referred to in Kop and Durmaz (2008), is that the effects of carotenoids are species specific, and carotenoid metabolic pathways are not identical among fish species. As growth improvement was not evident among *E. akaara* fed diets supplemented with

astaxanthin, it is inferred that astaxanthin did not have a positive effect on the metabolism of *E. akaara*. Otherwise, considering the growth rate of *E. akaara*, the time span of the experiments may have been comparatively short, noting that *Epinephelus akaara*, apart from other Serranidae species, is a very slow-growing species. Thus, the effect of astaxanthin may not have been fully reflected in their growth.

Many fish species are reported to retain their characteristic red color when fed diets containing carotenoid-based pigments (Kalinowski et al., 2005). In this study, *Epinephelus akaara* fed diets supplemented with astaxanthin exhibited a darker red color, regardless of fish size, indicating that supplementation helped improve the body color. A study done on the effects of astaxanthin supplementation on color improvement of gilthead seabream (*Sparus aurata*) also suggested an analogous result to that shown in the present study (Gomes et al., 2002). Supplementing astaxanthin improved the body color of most fish species, which effectively improved the red and yellow color (Li et al., 2014). This study was able to demonstrate a darker red color of *Epinephelus akaara* by supplementing astaxanthin derived from the addition of microorganisms to the feed. However, a lighter red color was observed in the experimental group AST3000 where a higher concentration of astaxanthin was supplemented to the juvenile *E. akaara* diet. This suggests that the excessive supply of astaxanthin can be a negative effect similar to Tilapias studies (Shiau and Yu, 1999). Therefore, supplementing astaxanthin to the *E. akaara* diet can be effective in improving its skin color, but based on the results of this study, a concentration of 1000 ppm is estimated to be the threshold; an opposite effect is expected at higher concentrations. To verify our assumption, more detailed studies need to be done to determine the minimum concentration at which the maximum color improvement can be achieved.

Astaxanthin was detected in all the skin and muscle samples from immature fish used in the experimental groups AST10, AST50, AST100, and C.AST. The greater the astaxanthin content was in the feed, the greater the level of astaxanthin detected in the muscle and skin samples. A comparison of astaxanthin content in the skin and muscle suggested that astaxanthin content in the muscle was much lower than that in the skin. The level of astaxanthin accumulated in the skin and muscle samples of *E. akaara* tended to show a concentration dependent increase based on the level of astaxanthin supplemented in the diet, and this study indicates that the main target tissue of astaxanthin accumulation is the skin. These are promising results indicating potential for further skin color improvement of *Epinephelus akaara*, which is a practical method for enhancing the commercial value of the species. One study reported that increased accumulation of astaxanthin in Atlantic salmon (*Salmo salar*) muscle was dependent on the level of astaxanthin supplemented in the feed (Baker et al., 2002). Tizkar et al. (2015) demonstrated a concentration-dependent accumulation of astaxanthin in goldfish semen by supplementing astaxanthin in the diet. Taken together, these results indicate that astaxanthin contained in the diet accumulates not only in fish skin and muscle but also in various places in the body.

No differences in red color were observed among skin samples of immature *E. akaara* in the 10–100 ppm experimental groups. However, the astaxanthin content in skin samples increased in a concentration-dependent manner. Thus, the level of redness was not proportional to astaxanthin content in the skin. However, a study using the clown anemone fish (*Amphiprion ocellaris*) showed that the red color appeared darker as the level of astaxanthin increased; at the same time, the level of astaxanthin accumulated in the skin showed a concentration-dependent increase (Ho et al., 2013). Further studies are needed to understand the mechanism whereby astaxanthin causes the skin to turn red.

This study was conducted to investigate the potential for marine microorganism-derived astaxanthin to replace the costly astaxanthin used as a fish feed additive, currently available on the market. According to experiments on the red sea bream (*P. major*) conducted by the same researchers who conducted the experiments on *Epinephelus akaara*, the cost can be reduced by 15% or more in comparison to the common astaxanthin extracted and refined from micro-algae (data not shown). Using astaxanthin derived from

marine microorganisms is of high industrial value as it will reduce production costs, which in turn will help aquaculture business reduce their operating costs while increasing the commercial value of cultured fish.

In conclusion, using a diet supplemented with marine microorganism-derived astaxanthin had no positive effect on the growth of *E. akaara*, but was effective in improving its red color. This study investigated the feasibility of the use of astaxanthin derived from marine microorganisms in fish aquaculture industry.

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