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Short communication

Effects of dietary astaxanthin and xanthophylls on the growth and skin pigmentation of large yellow croaker *Larimichthys croceus*

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ABSTRACT

A 9-week feeding trial was conducted to investigate the effects of dietary astaxanthin and xanthophylls on the growth and skin coloration of large yellow croaker *Larimichthys croceus* (mean initial weight: 33.33 ± 1.67 g). There were five experimental diets: CD (control diet without carotenoid), AST37.5 (diet with 37.5 mg/kg of astaxanthin), AST75 (diet with 75 mg/kg of astaxanthin), X37.5 (diet with 37.5 mg/kg of xanthophylls) and X75 (diet with 75 mg/kg of xanthophylls). Results showed that the growth and feed efficiency ratio were not significantly affected by dietary treatments ($P > 0.05$). With the feeding period increased, the lightness (L^*) in the dorsal skin showed a slight increase. However, it decreased in the ventral skin ($P > 0.05$). Treatments of X37.5 and X75 had lower redness (a^*) in the dorsal skin. As to a^* values in the ventral skin, fish fed with carotenoid supplemented diets were higher than that in the CD group. Yellowness (b^*) values in the ventral and dorsal skins of fish fed with the carotenoid supplemented diets were elevated as the feeding period increased. Fish in the X37.5 and X75 groups had higher b^* values in the ventral and dorsal skins than those in the AST37.5 and AST75 groups ($P < 0.05$). Similarly, X37.5 and X75 groups provided higher carotenoid concentrations in the dorsal and ventral skins than the AST37.5 and AST75 groups. These results suggested that both xanthophylls (Wisdem®Golden Y-20) and astaxanthin (Carophyll® Pink) were effective carotenoid sources for the skin color improvement of large yellow croaker.

1. Introduction

Large yellow croaker (*Larimichthys croceus* R) is one of the most important mariculture fish in China, with more than 90,000 tons produced in 2012 (China Fishery Statistical Yearbook, 2013). It is a kind of benthopelagic fish species (Wang et al., 2013). Naturally, it has golden ventral skin, tan back and lateral skin, red lips and yellow fins. The skin color of large yellow croaker is greatly influenced by light. In the day time, fish farmed in floating sea cage turn to silver (Fig. 1A). While at night, they turn into their original color (Fig. 1B). Therefore, fish farmers harvest large yellow croaker at night in order to get the natural appearance. Under intensive culture conditions, however, the farmed large yellow croaker is susceptible to lose its natural color. This is one of the most important reasons leading to its low market price and low consumer acceptance. The price of farmed large yellow croaker is less than 1/20 that of wild one. Up to now, lots of studies have been done on the nutrition requirement of large yellow croaker. However, the problem of skin color is still not resolved.

Fish, as with other animals, cannot synthesize carotenoid *de novo* (Goodwin, 1984). Their color highly relies on carotenoid from the diet (Torrissen et al., 1990). As a very important carotenoid, astaxanthin is widely used as a red or pink pigment supplemented in diet for fish to improve the red color of the fillet or the skin. These fish species included Atlantic salmon (*Salmo salar* L.) (Storebakken et al., 1987), sea trout (*Salmo trutta* L.) (Foss et al., 1987), rainbow trout (*Oncorhynchus mykiss*) (Storebakken and Choubert, 1991), Australian snapper (*Pagrus auratus*) (Booth et al., 2004; Doolan et al., 2009) and red porgy (*Pagrus pagrus*) (Tejera et al., 2007). Meanwhile, astaxanthin is also an effective yellow pigment. In fact, gilthead seabream (*Sparus aurata*) can convert astaxanthin to lutein esters and epilutein esters and exhibit golden forefront (Gomes et al., 2002). Channel catfish (*Ictalurus punctatus*) is another kind of fish that can use astaxanthin as pigment source resulting in yellow pigment in the skin and muscle (Li et al., 2007). Xanthophylls, including zeaxanthin and lutein, are effective yellow pigment for poultry pigmentation (Hadden et al., 1999). Studies showed that xanthophylls can also be used as yellow colorants for fish to improve skin or muscle color, such as walking catfish (*Clarias fuscus*) (Leng et al., 2003), channel catfish (Li et al., 2007) and rainbow trout (Yanar et al., 2007).

As mentioned above, previous studies on fish pigmentation focused on the red skin or fillet color. Little information is available on the yellow

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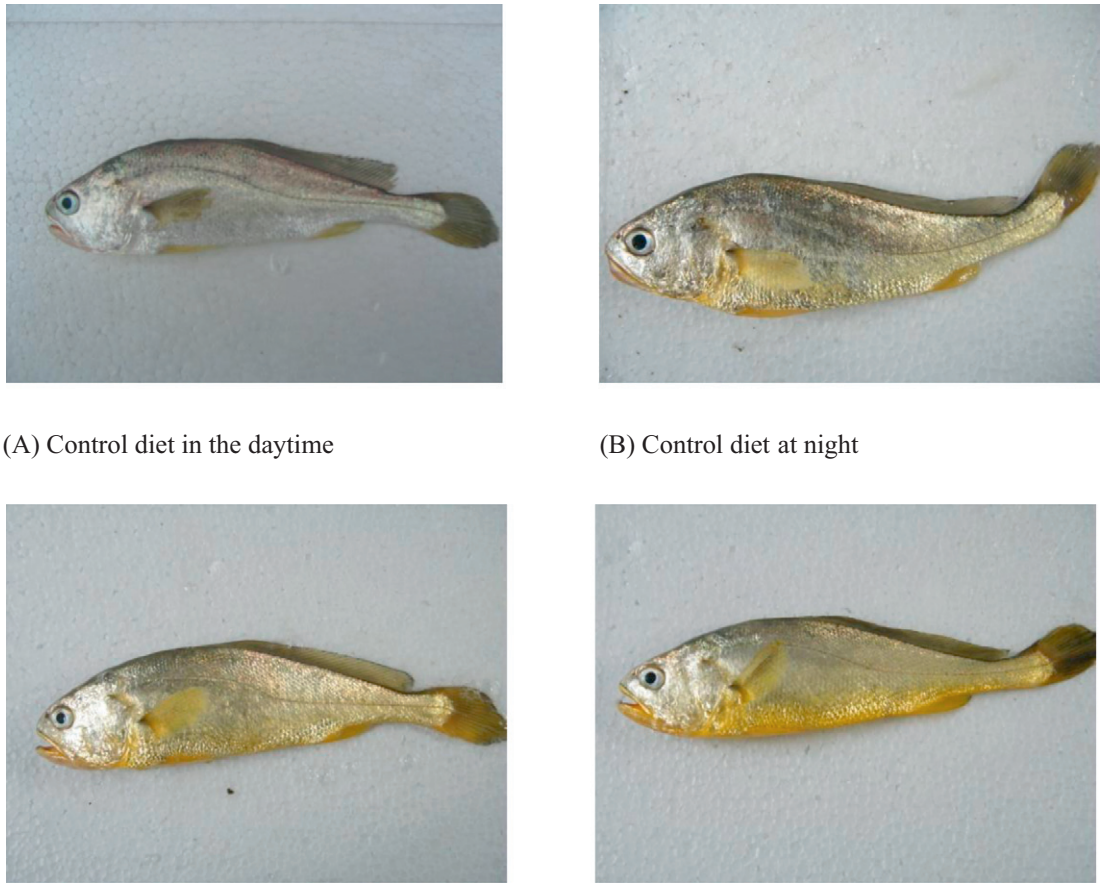

(A) Control diet in the daytime (B) Control diet at night

(C) Astaxanthin diets at night (AST37.5 and AST75) (D) Xanthophylls diets at night (X37.5 and X75)

Fig. 1. Pigmentation of farmed fish in the daytime without carotenoids added and the pigmentation of large yellow croaker *Larimichthys croceus* fed with different carotenoid supplementation diets at night.

color of the mariculture species. The aim of the present study was to investigate the effects of astaxanthin and xanthophylls as carotenoid sources on growth and skin color of large yellow croaker.

2. Materials and methods

2.1. Experimental diets

Five experimental diets were designed to contain approximately 43% crude protein and 13% crude lipid. The control diet without carotenoid supplementation was named as CD. Two diets contained 37.5 and 75 mg/kg synthetic astaxanthin were named as AST37.5 and AST75, respectively. The other two diets supplemented with 37.5 and 75 mg/kg xanthophylls were named as X37.5 and X75, respectively. The ingredients and compositions of the experimental diets are shown in Table 1.

All ingredients were ground into fine powder through a 320 μm mesh. Synthetic astaxanthin (Carophyll® Pink, astaxanthin 8%, F. Hoffman La-Roche Basel, Switzerland) or xanthophylls (Wisdem® Golden Y-20, extracted from marigold flower, Guangzhou Wisdom Bio-Technology Co., Ltd., China) were dissolved in oil premix (fish oil and soybean oil), and then thoroughly mixed with other ingredients. After that, water was added to produce stiff dough. Finally, the dough was pelleted with an experimental feed mill and dried in a ventilated oven at 40 °C until the moisture level below 10%. The dry pellets were broken up and sieved into proper pellets size (3 mm × 5 mm) and stored at −20 °C in black bags until use.

2.2. Experimental procedure

The feeding trial was conducted at Xihu bay of Ningbo, Zhejiang province, China. It started from September and ended in November. Large yellow croaker juveniles were purchased from a local commercial hatchery. Prior to the experiment, fish were stocked into floating sea cage (3.0 × 3.0 × 3.0 m) and fed with the control diet for two weeks. At the end of acclimation, fish were not fed for 24 h and weighed after being anesthetized with eugenol (1:10,000) (Shanghai Reagent Corp., China). Fish of similar size (33.33 ± 1.67 g) were randomly distributed into 15 sea cages (1.5 × 1.5 × 2.0 m) at a density of 60 fish per cage. Each diet was randomly assigned to triplicate cages. Fish were hand-fed to apparent satiation twice daily (05:00 and 17:00) for nine weeks. During this period, water temperature ranged from 19.5 to 31.5 °C, salinity from 28 to 32‰ and the dissolved oxygen was approximately 7 mg/l. At the end of the feeding trial, fish were fasted for 24 h, and then were weighed and counted.

2.3. Skin color measurement and carotenoid concentration analysis

Skin color measurement was performed in ventral skin and right dorsal skin at night (20:00–22:00). A portable Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan) was used. At the beginning of the feeding trial, fifteen fish were measured. Then at the 21st, 42nd and 63rd days, five fish in each cage were assessed, respectively. The color parameters were L^* for lightness, a^* for redness or greenness, and

Table 1
Formulation and chemical proximate composition of the experimental diets (% dry matter).

Ingredient	CD	AST37.5	AST75	X37.5	X75
White fish meal[a]	44.00	44.00	44.00	44.00	44.00
Soybean meal[a]	10.00	10.00	10.00	10.00	10.00
Wheat meal	29.30	29.25	29.21	29.11	28.93
Beer yeast	3.00	3.00	3.00	3.00	3.00
Fish oil	4.00	4.00	4.00	4.00	4.00
Soybean oil	2.70	2.70	2.70	2.70	2.70
Lecithin	2.50	2.50	2.50	2.50	2.50
Mineral premix[b]	2.00	2.00	2.00	2.00	2.00
Vitamin premix[c]	2.00	2.00	2.00	2.00	2.00
Attractant [d]	0.30	0.30	0.30	0.30	0.30
Mold inhibitor[e]	0.10	0.10	0.10	0.10	0.10
Ethoxyquin	0.10	0.10	0.10	0.10	0.10
Synthetic astaxanthin[f]	0.00	0.05	0.09	0.00	0.00
Xanthophylls[g]	0.00	0.00	0.00	0.17	0.35
Proximate analyses					
Dry matter (%)	91.79	90.88	90.41	90.51	90.64
Crude protein (%)	42.70	42.66	42.49	42.54	42.63
Crude lipid (%)	12.84	12.16	12.98	12.88	13.13
Ash (%)	12.82	12.62	13.11	12.24	12.69
Carotenoids (mg/kg)	0.00	38.71	73.27	39.40	64.46

[a] Fish meal: crude protein 74.60% dry matter, crude lipid 9.20% dry matter; soybean meal: crude protein 48.42% dry matter, crude lipid 0.98% dry matter.
[b] Mineral premix (mg or g/kg diet): NaF, 2 mg; KI, 0.8 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; Ca $(H_2PO_4)_2 \cdot H_2O$, 3000 mg; NaCl, 100 mg; Zoelite, 15.447 g.
[c] Vitamin premix (mg or g/kg diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B_{12}, 0.1 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.20 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; alpha-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2500 mg; ethoxyquin, 150 mg; wheat middling, 14.012 g.
[d] Attractant: glycine and betaine.
[e] Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.
[f] Synthetic astaxanthin: Carophyll® Pink, astaxanthin 8%, F. Hoffman La-Roche Basel, Switzerland.
[g] Xanthophylls: Wisdem®Golden Y-20, total xanthophyll content 21.65 g/kg, lutein 88.20%, zeaxanthin 5.33%, Guangzhou Wisdom Bio-Technology Co., Ltd.

b^* for yellowness or blueness, respectively, in according to the recommendation of the International Commission on Illumination (CIE, 1976).

For the analysis of carotenoid concentration, five fish per cage were sampled at the end of the feeding trial. The samples were covered with aluminium foil and stored at −20 °C. Carotenoid in feed and skin were extracted according to the method of Cejas et al. (2003) with some modifications. Briefly, samples of skin (0.25 g) and feed (1 g) were finely homogenized with 10 ml ethyl acetate:ethanol (1:1 v/v) and centrifuged (4000 *g*, 5 min). The supernatant was collected, and then the sediment was extracted with 5 ml ethyl acetate first and followed by 10 ml hexane. The three supernatants were pooled together and dried under a stream of pure nitrogen. The samples were re-suspended in 4 ml acetone with 0.02% BHT and centrifuged (10,000 *g*, 5 min). Carotenoid concentration was measured by spectrophotometer (UV-2401PC, Kyoto, Japan) in acetone. It was expressed as the extinction coefficients $E_{(1\%,\ 1\ cm)} = 1900$ at 474 nm (Foss et al., 1984) for astaxanthin and $E_{(1\%,\ 1\ cm)} = 2500$ at 448 nm (Schiedt and Liaaen-Jensen, 1995) for xanthophylls.

2.4. Calculations and statistical analysis

$$\text{Survival rate (SR)}(\%) = 100 \times (\text{final fish number}/\text{initial fish number})$$
$$\text{Specific growth rate (SGR)} = (\ \ln\text{final weight} - \ln\text{initial weight}) \times 100/\text{days}$$
$$\text{Feed efficiency ratio (FER)} = \text{wet weight gain (g)}/\text{feed consumed (g)}$$
$$\text{Carotenoid concentration (CC) (mg/kg)} = 10{,}000 \times V \times A/W/E_{(1\%,1\ cm)}$$

where V is total volume of the extract, A is the absorbance, and W is the weight of sample, $E_{(1\%,\ 1\ cm)}$ is the extinction coefficients.

Results were presented as means ± S.E.M. All data were analyzed by one-way ANOVA by SPSS 15.0 for windows (Kalinowski et al., 2005). Regression analysis was done to determine the relationship between b^* values and the skin carotenoid concentrations. The level of significance was set at $P < 0.05$, and Tukey's test was used to compare the mean values.

3. Results

3.1. Growth performance and feed utilization

The results showed that survival rate (SR), specific growth rate (SGR) and feed efficiency ratio (FER) were not significantly affected by dietary treatments ($P > 0.05$) (Table 2). The survival rate ranged from 91% to 95%, the specific growth rate fluctuated from 1.28 to 1.40 and the feed efficiency ratio was in the range of 0.48–0.54.

3.2. Skin color parameters

Fish fed with astaxanthin or xanthophyll supplemented diets showed a stronger golden-yellow color in skin (Fig. 1C and D) than that fed with the control diet (Fig. 1B; dorsal skin: $L^* = 54.56$, $a^* = -0.47$, $b^* = 8.10$; ventral skin: $L^* = 82.44$, $a^* = -0.81$, $b^* = 58.10$). The fish fed with xanthophyll diets (dorsal skin: $L^* = 54.93$, $a^* = -2.35$, $b^* = 18.92$; ventral skin: $L^* = 80.49$, $a^* = 1.56$, $b^* = 83.29$) (Fig. 1D) got higher coloration than the fish fed with astaxanthin diets (dorsal skin: $L^* = 55.09$, $a^* = 0.78$, $b^* = 14.77$; ventral skin: $L^* = 79.04$, $a^* = 3.53$, $b^* = 71.05$) (Fig. 1C).

Lightness (L^*) values of ventral and dorsal skins are shown in Fig. 2. No significant differences were observed in L^* values of ventral skin or dorsal skin for all treatments during the feeding trial ($P > 0.05$). Nevertheless, the ventral skin lightness of fish fed with carotenoid supplemented diets showed a slight decrease with increasing feeding period, but the dorsal skin lightness showed a slight increase.

Redness (a^*) values of the ventral skin of all treatments tended to increase during the experiment, while this phenomena did not appear on the dorsal skin (Fig. 3). X37.5 and X75 groups had lower a^* values on the dorsal skin than those of AST37.5, AST75 and CD groups at the end of the experiment. As to the redness of ventral skin, fish fed with carotenoid supplemented diets were redder than those of CD group.

Yellowness (b^*) values of fish fed with carotenoid supplemented diets tended to increase throughout the experiment both in ventral

Table 2
Growth performance, survival rate and feed utilization of large yellow croaker *Larimichthys croceus* fed with the experimental diets for 9 weeks.

Diets	CD	AST37.5	AST75	X37.5	X75
Initial body weight (g)	33.39 ± 0.44	33.67 ± 0.75	32.69 ± 0.83	33.30 ± 0.77	33.58 ± 0.75
Final body weight (g)	80.45 ± 3.06	78.21 ± 3.41	76.63 ± 1.94	73.57 ± 1.43	78.51 ± 2.93
SGR (%/day)[a]	1.40 ± 0.05	1.33 ± 0.09	1.28 ± 0.03	1.23 ± 0.06	1.35 ± 0.09
FER[b]	0.49 ± 0.02	0.53 ± 0.02	0.48 ± 0.01	0.49 ± 0.02	0.54 ± 0.02
Survival (%)	92.78 ± 2.82	93.67 ± 2.96	91.00 ± 1.00	92.00 ± 2.00	95.00 ± 2.51

Values are mean ± S.E.M. of three replicates.
[a] SGR: specific growth rate.
[b] FER: feed efficiency ratio.

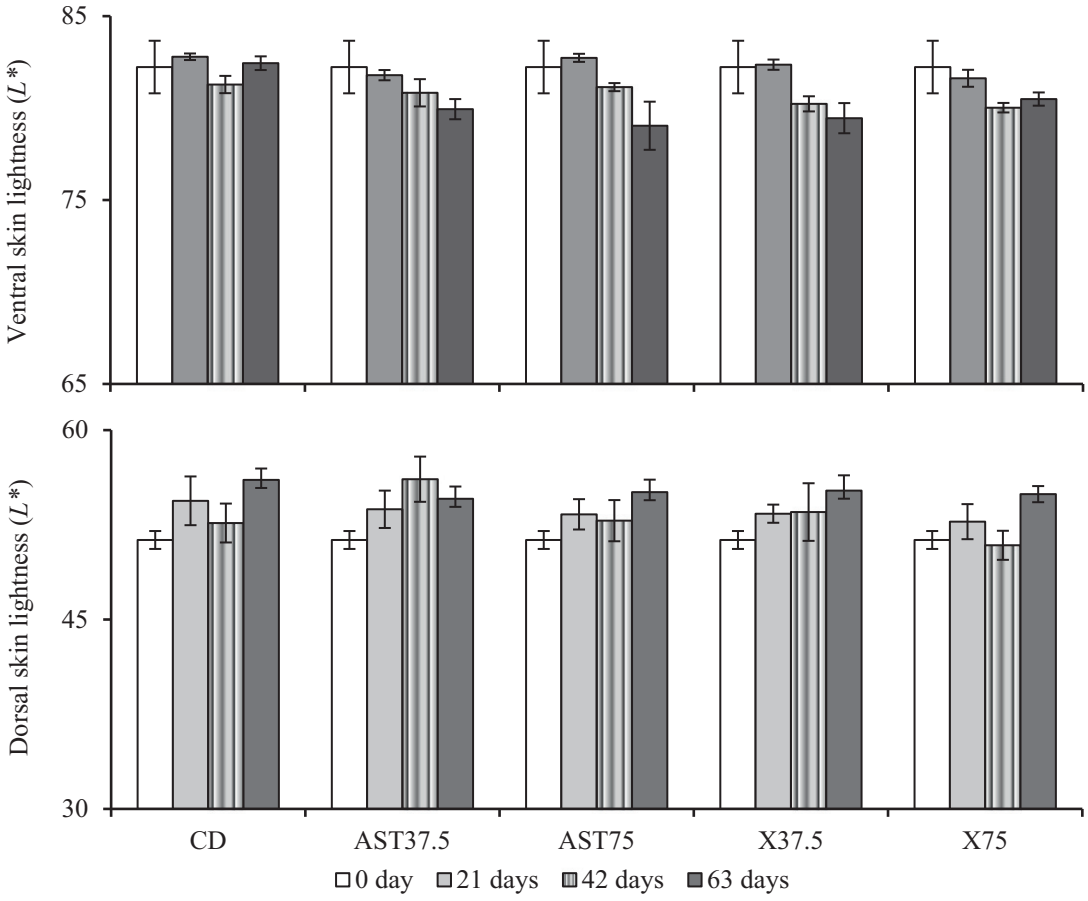

Fig. 2. Lightness (L^*) values of ventral skin and dorsal skin at the beginning and at the 21st, 42nd, 63rd days. Each bar is the mean value of three replicates, five fish per replicate.

and dorsal skins (Fig. 4). However, the b^* values of fish fed with control treatment have no significant difference during the feeding trial ($P > 0.05$). In contrast to AST37.5 and AST75 groups, X37.5 and X75 groups exhibited significantly higher b^* values both in dorsal and ventral skins and the lowest b^* values was in the CD group ($P < 0.05$). AST75 group had higher b^* values compared to AST37.5 group in the two different sample areas at the 21st, 42nd and 63rd days, although no significant differences were observed ($P > 0.05$). The ventral skin yellowness of X75 group was significantly higher than those of X37.5 group at the 21st, 42nd and 63rd days ($P < 0.05$). The b^* values of dorsal and ventral skins of each diet were increased rapidly during the first three weeks, and slowly for the following six weeks.

3.3. Skin carotenoid concentration

Carotenoid concentrations of dorsal and ventral skins are presented in Table 3. No significant differences of ventral skin carotenoid concentrations were observed between AST37.5 group and AST75 group or X37.5 group and X75 group ($P > 0.05$). Nevertheless, ventral skin carotenoid concentrations of X37.5 and X75 groups were significantly higher than those of AST37.5 and AST75 groups and the lowest value was in the CD group ($P < 0.05$). As to the carotenoid concentration of dorsal skin, no significant difference was found between AST37.5 group and AST75 group ($P > 0.05$), while X75 group was significantly higher than that of X37.5 group and the lowest value was also in the CD group ($P < 0.05$).

3.4. Regression analysis

Regression analysis suggested that b^* values were responded to the skin carotenoid concentrations both in ventral skin ($Y = -154.5 + 3.0307X$, $r = 0.926$, $P = 0.024$) and dorsal skin ($Y = -4.4696 + 1.7937X$, $r = 0.935$, $P = 0.020$) (Table 4).

4. Discussion

Beyond as pigment source, carotenoid also has various other functions in fish, including enhancement of the resistance of diseases (Amar et al., 2001), promotion of broodstock performance (Verakunpiriya et al., 1997) and improvement of growth (Christiansen et al., 1995; Kalinowski et al., 2010; Torrissen, 1984). In the present study, however, growth and feed efficiency ratio were not significantly affected by dietary treatments. This result was in agreement with those in previous studies on Atlantic salmon (Baker et al., 2002; Olsen and Baker, 2006), rainbow trout (Amar et al., 2001), gilthead seabream (Gomes et al., 2002), characins (*Hyphessobrycon callistus*) (Wang et al., 2006), Australian snapper (Doolan et al., 2009),red porgy (Kalinowski et al., 2005; Tejera et al., 2007) and flame-red dwarf gourami (*Colisa lalia*) (Baron et al., 2008).

There were no significant differences in ventral or dorsal skin lightness (L^*) of fish fed with different diets (Fig. 2), in agreement with previous studies. Kalinowski et al. (2005) found that skin lightness of red porgy was not influenced by carotenoid supplemented diets. Doolan et al. (2009) also found that skin lightness of Australian snapper was not affected by astaxanthin supplemented diets. However, the ventral skin lightness in the present study tended to reduce along the feeding trial when fish were fed with carotenoid supplemented diets, while the dorsal skin lightness tended to increase. This may be likely caused by the deposition of pigments in chromatophore. Similar results were also reported in skin color of Australian snapper (Doolan et al., 2008, 2009) and flesh color of coho salmon (Smith et al., 1992). In addition to pigments in chromatophore, environment factors could affect fish

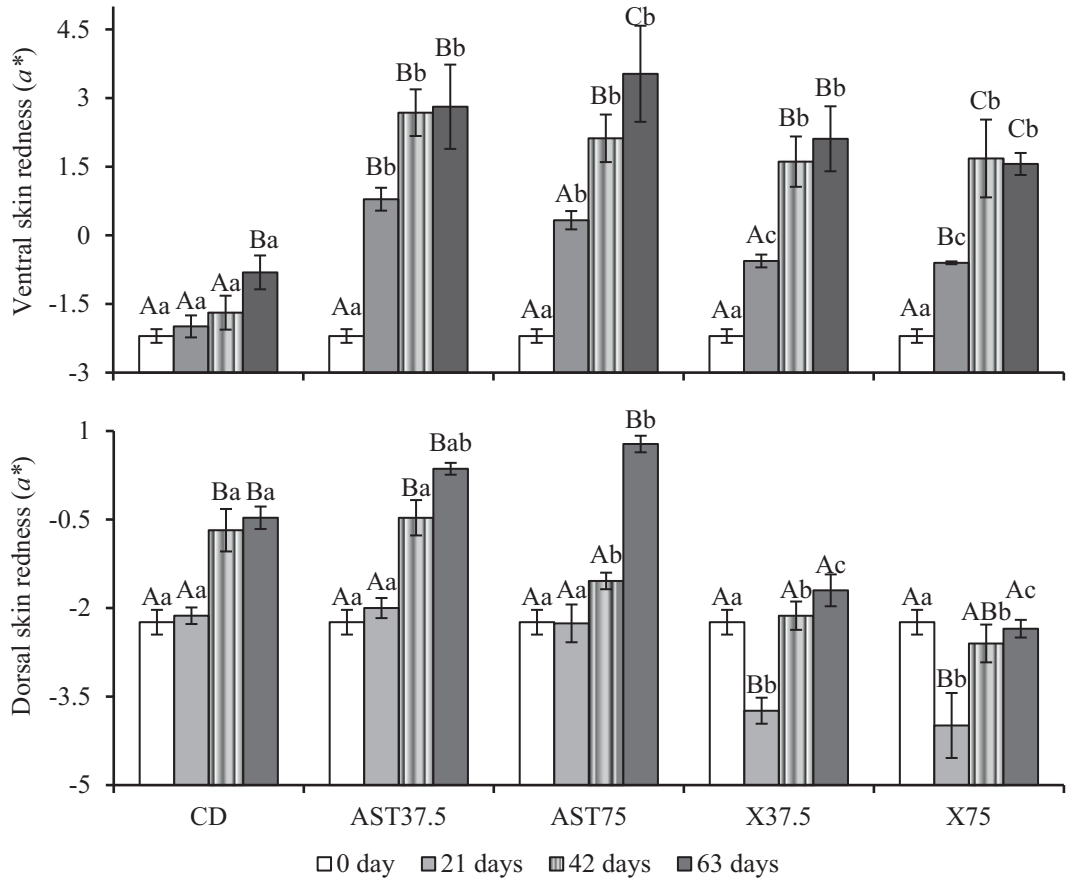

Fig. 3. Redness (a^*) values of ventral skin and dorsal skin at the beginning and at the 21st, 42nd, 63rd days. (A, B, C) Average values of the same diet at different times with different letters are significantly different ($P < 0.05$). (a, b, c) Average values of different diets at the same sampling time with different letters are significantly different ($P < 0.05$). Each bar is the mean value of three replicates, five fish per replicate.

skin lightness. These factors included background color, illumination and light spectrum (Kalinowski et al., 2007; Matsui et al., 1992; Papoutsoglou et al., 2000; Rotllant et al., 2003). Nutrition is another factor that can influence the lightness of skin. Chatzifotis et al. (2005) reported that high dietary protein/carbohydrate ratio enhanced the melanin content in the skin of red porgy. Melanin is the main pigments responsible for the dark coloration of cultured fish (Hearing, 2005).

Generally, astaxanthin is considered as a red or pink pigment, which results in red or pink coloration. Meanwhile, lutein and zeaxanthin are considered as yellow pigments, which result in yellow coloration (Li et al., 2007). Some fish can convert one carotenoid pigment to another pigment. For example, common carp (*Cyprinus carpio*) can convert zeaxanthin to astaxanthin (Hardy and Barrows, 2002). In the present study, fish fed with astaxanthin supplemented diets exhibited more reddish both in dorsal and ventral skins than those fed with xanthophylls (Fig. 3). However, fish fed with xanthophylls got 1.10–1.20 times higher yellowness values in ventral skin and 1.25–1.35 times higher in dorsal skin than those fed with astaxanthin (Fig. 4). The highest yellowness value was found in the treatment with 75 mg/kg of dietary xanthophylls. Large yellow croaker is a kind of fish which is characterized by yellow skin or fins. Results from the present study showed that the body color of large yellow croaker may mainly rely on the deposition of yellow pigment. Meanwhile, it is also suggested that this fish species has limited ability to convert astaxanthin to yellow pigment.

The change of ventral skin yellowness suggested that there would be color saturation in ventral skin yellowness for the increase rate tended to decrease during the nine weeks feeding trial (Fig. 4). Similarly, the improvement of skin reddish hue and chroma of red porgy tended to reduce during the 15 weeks of feeding trail (Kalinowski et al., 2005). Also, an apparent color saturation point was observed in Australian snapper fed with more than 39 mg/kg astaxanthin after 42 days (Doolan et al., 2009). The appearance of coloration parameters plateau depends on genetical factors (Blanc and Choubert, 1985; Torrissen and Naevdal, 1984), carotenoid concentration, feeding time, fish size and age (Torrissen, 1995). In contrast to the ventral skin, no yellowness saturation was found in dorsal skin during the experiment, as there were significant differences in dorsal skin yellowness between the 42nd day and the 63rd day (Fig. 4). The occurrence of yellowness saturation in dorsal skin may need longer time than the present feeding trial period. This needs further studies.

In the present study, higher carotenoid concentrations were found in dorsal and ventral skins of fish fed with xanthophylls than those fed with astaxanthin, regardless of dietary supplemental levels (Table 3). It was suggested that large yellow croaker could use xanthophylls more efficiently than astaxanthin. Li et al. (2007) reported that channel catfish accumulate yellow pigments lutein and zeaxanthin more efficiently than the red pigments astaxanthin and canthaxanthin in the skin. In salmonids, astaxanthin is more efficient than canthaxanthin for flesh pigmentation (Buttle et al., 2001; Storebakken and No, 1992; Torrissen et al., 1989). The differences in carotenoid utilization could be related to the absorption, metabolism and bonding affinity process in the skin or muscle. Compared to canthaxanthin, for example, rainbow trout had higher capacity to deposit astaxanthin in the muscle. This

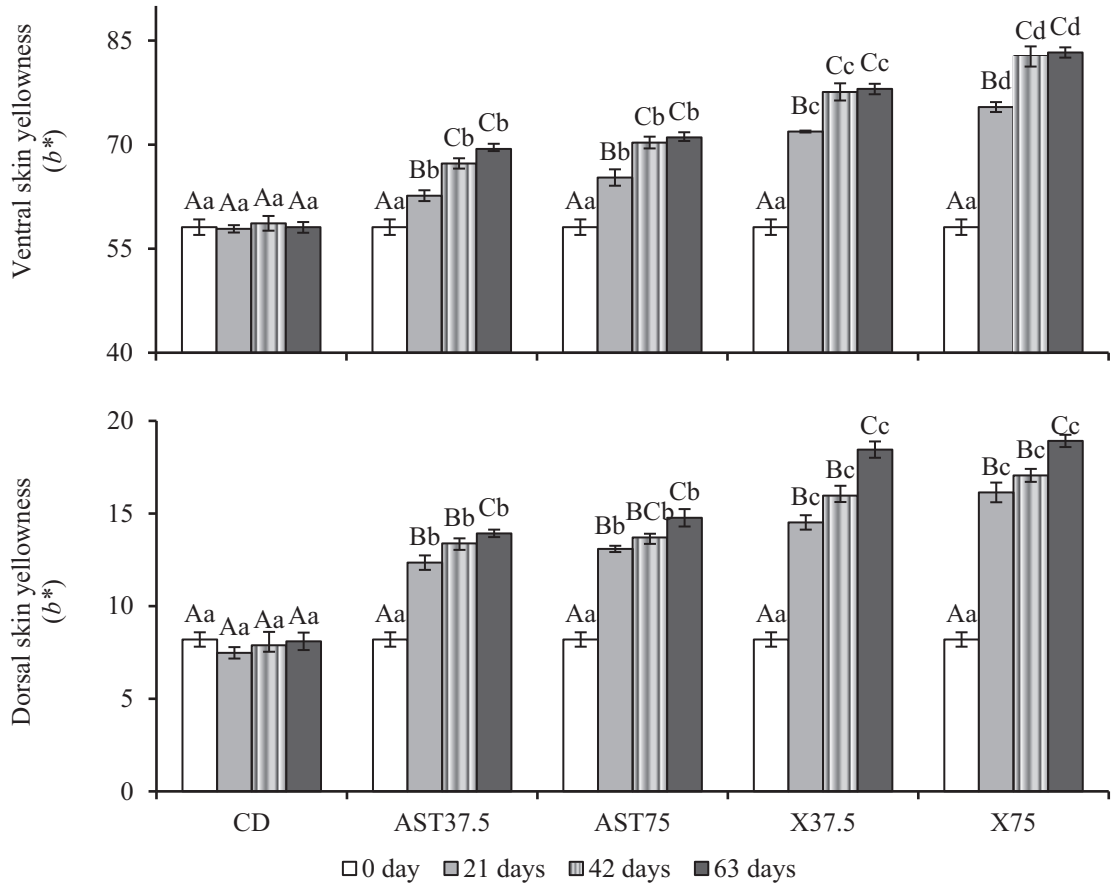

Fig. 4. Yellowness (b^*) values of ventral skin and dorsal skin at the beginning and at the 21st, 42nd, 63rd days. (A, B, C) Average values of the same diet at different times with different letters are significantly different ($P < 0.05$). (a, b, c) Average values of different diets at the same sampling time with different letters are significantly different ($P < 0.05$). Each bar is the mean value of three replicates, five fish per replicate.

could be ascribed to a higher absorption at the intestinal level (Gobantes et al., 1997), better digestibility (Choubert and Storebakken, 1996), lower metabolic transformation (Bjerkeng, 2000) or a higher bonding affinity (Henmi et al., 1987) of astaxanthin. Further study is needed to explain why large yellow croaker can use xanthophylls better than astaxanthin.

It was reported that color parameters (e.g., a^*, b^* and L^*) and carotenoid concentrations were linearly dependent (Baker et al., 2002; Bjerkeng et al, 1997; Hatlen et al., 1998; Ingle de la Mora et al., 2006; Skrede and Storebakken, 1986a,b). In the present study, b^* values and the carotenoid concentrations both in the dorsal and ventral skins were significantly linearly dependent (Table 4). It means that b^* value could be used as an indicator of skin carotenoid concentration in large yellow croaker. However, several reports showed that higher correlation can be achieved by employing non-liner relationships when the carotenoid concentration was on saturation (Bjerkeng et al., 1997; Kalinowski et al., 2007; King, 1996). The reason is that the response of color parameter values diminished as increasing carotenoid levels (Bjerkeng, 2000).

In summary, both xanthophylls and astaxanthin enhanced the skin coloration and skin carotenoid content of large yellow croaker. Xanthophylls provided higher yellowness value and more carotenoid content both in dorsal and ventral skins than astaxanthin. It seemed that large yellow croaker can use xanthophylls more efficiently than astaxanthin. In contrast to the xanthophyll treatments, astaxanthin treatments showed more reddish in ventral and dorsal skins.

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Table 3
Carotenoid concentrations in dorsal and ventral skins of large yellow croaker *Larimichthys croceus* fed with the experimental diets for 9 weeks.

	CD	AST37.5	AST75	X37.5	X75
Dorsal skin (mg/kg)	11.84 ± 0.91^{a}	18.43 ± 1.69^{b}	20.14 ± 1.46^{bc}	26.41 ± 1.85^{c}	33.87 ± 0.40^{d}
Ventral skin (mg/kg)	30.67 ± 2.75^{a}	46.07 ± 0.93^{b}	47.15 ± 2.32^{b}	96.05 ± 0.83^{c}	98.21 ± 0.54^{c}

Values are mean ± S.E.M. of three replicates, five fish per replicate.
Means not bearing the same superscript letters are significantly different ($P < 0.05$).

Table 4
Regression analysis between b^* values (X) and skin carotenoid concentrations (Y) of large yellow croaker *Larimichthys croceus* fed with the experimental diets for 9 weeks ($n = 15$).

	Model	r	P
Ventral skin	$Y = -154.5 + 3.0307X$	0.926	0.024
Dorsal skin	$Y = -4.4696 + 1.7937X$	0.935	0.020

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