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Technical contribution

Effects of dietary xanthophylls/astaxanthin ratios on the growth and skin pigmentation of large yellow croaker *Larimichthys crocea* (Richardson, 1846)

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Summary

An 8-week feeding trial was conducted to evaluate the effects of dietary xanthophylls/ astaxanthin ratio on the growth and skin color of large yellow croaker, *Larimichthys crocea*. Five pigment-supplemented diets were formulated to contain 75/0, 50/25, 37.5/37.5, 25/50 and 0/75 mg kg^{-1} of xanthophylls/astaxanthin. The xanthophylls contain 89.31% lutein and 6.12% zeaxanthin. A diet without pigment supplementation was used as the control. The large yellow croaker juveniles (13.80 ± 0.03 g) were randomly distributed in 18 sea cages (1.0 × 1.0 × 1.5 m) at a density of 45 fish per cage. Water temperature ranged from 21 to 31°C during the feeding trial. To obtain results, the survival rate, specific growth rate, feed conversion ratio, skin redness, skin yellowness, skin lightness, skin carotenoid content and skin melanin content were measured. The results showed that the survival rate, specific growth rate and feed conversion ratio were not significantly affected by dietary treatments ($P > 0.05$). The ventral skin lightness was also not affected by dietary treatments ($P > 0.05$); however, the dorsal skin lightness of fish fed with the control diet was significantly lower than those fed with pigment-supplemented diets ($P < 0.05$). The lowest values of yellowness and carotenoid content both in the ventral skin and dorsal skin were found in the control group. Yellowness and carotenoid content increased with an increasing proportion of dietary xanthophylls in both the ventral and dorsal skin. Higher redness values were found in the compound pigment groups, either in the dorsal skin or ventral skin. Fish fed with the control diet showed a higher melanin content in the dorsal skin than those fed with pigment-supplemented diets, although differences were not significant ($P > 0.05$). Lightness and yellowness were linearly related to skin carotenoid content. Meanwhile, skin yellowness and carotenoid content were linearly related to the proportion of xanthophylls in dietary pigments.

Introduction

The farming of large yellow croaker *Larimichthys crocea* has been under rapid development since the 1990s, and is now the third most popular mariculture fish species in China, with 95, 118 metric tonnes produced in 2012 (Fishery Bureau, Ministry of Agriculture, 2013). Market size of farmed large yellow croaker is about 250 g. The rearing cycle is usually about 13 months. *Larimichthys crocea* normally has golden-yellow skin, red lips and yellow fins (Yi et al., 2014a); however, under the current intensive culture, the fish has lost its original coloring. One possible reason is that the farmers feed the large yellow croaker with chopped trash fish that usually lack sufficient amounts of pigment.

Fish body pigmentation depends entirely on the dietary carotenoid intake (Gomes et al., 2002) because fish cannot synthesize carotenoid *de novo* (Goodwin, 1984). As a very important carotenoid, astaxanthin can significantly improve the red color of the skin or fillet of fish, such as Atlantic salmon *Salmo salar* L. (Storebakken et al., 1987), rainbow trout *Oncorhynchus mykiss* (Storebakken and No, 1992), red porgy *Pagrus pagrus* (Tejera et al., 2007) and channel catfish *Ictalurus punctatus* (Li et al., 2007). Previous studies have shown that xanthophylls are also efficient pigments to improve the yellow color of the skin or fillet of the walking catfish *Clarias fuscus* (Leng et al., 2003) and rainbow trout (Yanar et al., 2007). Yi et al. (2014a) reported that dietary astaxanthin or xanthophylls (37.5 and 75 mg kg^{-1}) could significantly improve the skin color of farmed large yellow croaker; moreover, *L. crocea* fed with dietary astaxanthin is closer to the coloring in the wild. In addition, the optimal dietary lipid content for the skin pigmentation of large yellow croaker was estimated to be 12.00 and 13.19% for ventral skin yellowness and carotenoid content, respectively (Yi et al., 2014b). In consideration of the golden-yellow-red color style of the wild large yellow croaker, it was suggested that both yellow pigments (e.g. lutein and zeaxanthin) and red pigments (e.g. astaxanthin and canthaxanthin) are needed by this fish species.

Astaxanthin is widely used for salmonid pigmentation (Storebakken and No, 1992) and can represent as much as 15–20% of the total feed cost (Torrissen, 1995). The market price of synthetic astaxanthin is above $2000 USD kg^{-1} (Li et al., 2011). However, the price of xanthophylls (e.g. lutein and zeaxanthin) at between $570–790 USD kg^{-1} (Prommuak et al., 2013) is lower than that of synthetic astaxanthin. Therefore,

the combination of astaxanthin and xanthophylls may be used as an alternative method to improve skin and/or fillet coloration, considering the costs associated with pigmentation in farmed fish species. Thus, the aim of this study was to evaluate the effects of different dietary xanthophylls/astaxanthin ratios on the growth and skin coloration in the on-growing stage of the large yellow croaker, *Larimichthys crocea*.

Materials and methods

Experimental diets

A diet formulated without pigment supplementation was used as the control (Diet-0). An analysis showed it to contain 46% protein and 12% lipid. Five pigment-supplemented diets were formulated to contain 75/0, 50/25, 37.5/37.5, 25/50 and 0/75 mg kg^{-1} xanthophylls/astaxanthin on a dry matter basis. These were named Diet-1, Diet-2, Diet-3, Diet-4 and Diet-5, respectively (Table 1). The pigments were Carophyll®pink (astaxanthin 10%; DSM, Netherland) and Wisdem®GoldenY-40 (xanthophylls 41.21 g kg^{-1}: lutein 89.31%, zeaxanthin 6.12%; Guangzhou Wisdom Bio-Technology, China).

All ingredients were first finely-ground into a powder. The pigments were then dissolved in fish oil and thoroughly mixed with the other ingredients. Distilled water (300 g kg^{-1}) was added to produce a stiff dough. Finally, the dough was pelleted by being pressed through a sieve in a feed mill with 4 mm holes. Diets were dried in a ventilated oven at 40°C until attaining a moisture level below 8%, and stored at −20°C in black bags.

Experimental procedure

Large yellow croaker juveniles (1200 fish) were obtained from a commercial hatchery and acclimatized in a floating sea cage (3.0 × 3.0 × 3.0 m) at a density of 0.61 kg m^{-3} for 2 weeks. Water temperature, salinity and dissolved oxygen were measured every 2 days. Environmental conditions (current speed < 0.5 m s^{-1}, temperature: 27–29°C, salinity 28–29 g L^{-1}, oxygen level: 6.3–7.5 mg L^{-1}) were good for acclimation. At the beginning of the trial, fish were starved for 24 h, and then weighed after being anesthetized with eugenol (1 : 10 000) (Shanghai Reagent Corp, China). Fish of similar size (13.80 ± 0.03 g) were randomly distributed into 18 sea cages (1.0 × 1.0 × 1.5 m). Each cage was stocked with 45 fish. Each diet was randomly assigned to three cages. Fish were handfed to apparent satiation twice daily (5.00 and 17.00) for 8 weeks. During the feeding period, water temperature ranged from 21 to 31°C, salinity from 28 to 32 g L^{-1} and dissolved oxygen from 6.2 to 8.3 mg L^{-1}.

Sample collection and analysis

At the end of the feeding trial, fish were starved for 24 h prior to sampling. After anesthetizing with eugenol, the numbers

Table 1
Feed formulation and proximate composition (dry matter basis, %)

	Diet-0	Diet-1	Diet-2	Diet-3	Diet-4	Diet-5
Xanthophylls (mg kg^{-1})	0	75	50	37.5	25	0
Astaxanthin (mg kg^{-1})	0	0	25	37.5	50	75
Fishmeal	43.00	43.00	43.00	43.00	43.00	43.00
Soybean meal	21.00	21.00	21.00	21.00	21.00	21.00
Wheat meal	20.90	20.72	20.75	20.77	20.79	20.82
Yeast	2.00	2.00	2.00	2.00	2.00	2.00
Lecithin	1.50	1.50	1.50	1.50	1.50	1.50
Fish oil	7.10	7.10	7.10	7.10	7.10	7.10
Vitamin premix[1]	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[2]	2.00	2.00	2.00	2.00	2.00	2.00
Attractant[3]	0.30	0.30	0.30	0.30	0.30	0.30
Mold inhibitor[4]	0.10	0.10	0.10	0.10	0.10	0.10
Ethoxyquin	0.10	0.10	0.10	0.10	0.10	0.10
Wisdem®GoldenY-40[5]	0.00	0.18	0.12	0.09	0.06	0.00
Carophyll®pink[6]	0.00	0.00	0.03	0.04	0.05	0.08
Chemical analysis						
Moisture (%)	5.73	6.19	5.80	5.28	5.64	6.26
Crude protein (%)	46.07	46.29	46.78	45.57	45.40	46.02
Crude lipid (%)	11.81	11.75	12.57	12.52	12.75	12.55
Ash (%)	12.20	12.78	12.78	13.20	12.70	12.71
Pigments (mg kg^{-1})	–	75.35	75.61	72.44	75.97	77.98

[1]Vitamin premix (mg kg^{-1} or g kg^{-1} diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vit. B_{12}, 0.1 mg; vit. 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.
[2]Mineral premix (mg kg^{-1} or g kg^{-1} 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.
[3]Attractant: glycine and betaine.
[4]Mold inhibitor: 50% calcium propionic acid and 50% fumaric acid.
[5]Wisdem®GoldenY-40: total xanthophylls content 41.21 mg kg^{-1}, lutein 89.31%, zeaxanthin 6.12%, Guangzhou Wisdom Bio-Technology Co., Ltd, China.
[6]Carophyll®pink: astaxanthin10%, DSM, Netherlands.

and weights of all fish in each cage were collected. The body weight was obtained from bulk weighing cage by cage. The ventral skin and dorsal skin on the right side of six fish per cage (18 fish per treatment) were then dissected for carotenoid and melanin analysis. Skin samples were covered with aluminum foil and stored at −20°C. Additionally, between 19:30 and 22:00, six fish per cage (18 fish per treatment) were randomly sampled to evaluate the skin color using a portable Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan). Measurements were performed on the ventral skin and right side of the dorsal skin. The color parameters were L^*, a^* and b^* for lightness, redness and yellowness, respectively, according to the recommendations of the International Commission on Illumination (CIE, 1976).

Carotenoid contents in feed and skin were analyzed according to the method described by Cejas et al. (2003), with some modifications. Briefly, samples of skin (0.25 g) or feed (1.0 g) were finely homogenized with 10 ml ethyl acetate: ethanol (1 : 1 v/v) and centrifuged (4000 ***g***, 5 min). The supernatant was removed, and the sediment extracted with 5 ml ethyl acetate, followed by 10 ml hexane. The three supernatants were pooled together and dried under a stream of pure nitrogen. Samples were re-suspended in 4 ml acetone with 0.02% BHT and centrifuged (10 000 ***g***, 5 min). Carotenoid content was measured by spectrophotometer (UV-2401PC, Kyoto, Japan) in acetone. Carotenoid content was expressed using the extinction coefficients $E_{(1\%,\ 1\ cm)} = 1900$ at 474 nm (Foss et al., 1984) for diet astaxanthin and $E_{(1\%,\ 1\ cm)} = 2500$ (Schiedt and Liaaen-Jensen, 1995) at 448 nm for diet xanthophylls and skin carotenoid.

Six fish per cage (18 fish per treatment) were used to measure the skin melanin content by the method of Wilson and Dodd (1973). A sepia melanin synthetic standard (M-2649, Sigma-Aldrich, St. Louis, MO) was purchased from Sigma-Aldrich. The proximate composition of experimental diets was determined following methods of the Association of Official Analytical Chemists (AOAC (Association of Official Analytical Chemists), 1995).

Calculations and statistical analysis

$$\text{Survival rate (SR)} = 100\times \text{(final fish number/initial fish number)}$$

$$\text{Specific growth rate (SGR)} = (\text{Ln final weight} - \text{Ln initial weight}) \times 100/\text{days}$$

$$\text{Feed conversion ratio (FCR)} = \text{dry feed fed (g)}/\text{wet weight gain (g)}$$

$$\text{Carotenoid content } (\mu\text{g g}^{-1}) = 10\,000 \times V \times A/W/E_{(1\%,1\,\text{cm})}$$

where V is total volume of the extraction, A is the absorbance, and W is the weight of the sample, and $E_{(1\%,\ 1\ cm)}$ is the extinction coefficient. This formula was calculated per treatment.

Results were presented as means ± SEM (Standard Error of the Means). All data were analyzed by one-way ANOVA using SPSS 15.0 for Windows (SPSS Statistics Inc., Chicago, IL). The level of significance was set at $P < 0.05$, and Tukey's test was used to compare the mean values. Regression analysis was conducted between color parameters and carotenoid content. For fish fed with pigment-supplemented diets, linear regression analysis between the percentage of xanthophylls in dietary pigments and resulting skin yellowness or the percentage of xanthophylls in dietary pigments and resulting skin carotenoid content were also performed.

Results

Effects of dietary pigments on growth performance and feed utilization are shown in Table 2. SGR ranged from 1.8 to 2.0, but no significant differences were found among treatments ($P > 0.05$). Similarly, there were no significant differences in SR and FCR among treatments ($P > 0.05$). The SR varied between 86.7 and 92.6%; FCR ranged from 1.5 to 1.8.

Data on skin coloring are presented in Table 3. There were no significant differences in ventral skin lightness (L^*) among treatments ($P > 0.05$). However, the dorsal skin lightness of fish fed with Diet-0 was significantly lower than those fed with pigment-supplemented diets ($P < 0.05$). The lowest yellowness (b^*) values were found in the ventral and dorsal skin of fish fed Diet-0. In fish fed pigment-supplemented diets, the highest yellowness values were observed in the fish fed Diet-1 in both sampled skin areas, and the lowest in fish fed Diet-5. There were no significant differences in ventral skin yellowness between Diet-1 and Diet-2 or between Diet-4 and Diet-5 test groups ($P > 0.05$). After 8 weeks, higher ventral skin redness was shown in fish fed Diet-2 and Diet-3; lowest values were found in fish fed Diet-0. As to the dorsal skin, the highest redness was observed in fish fed Diet-4 ($P < 0.05$), but with no significant differences found between fish fed Diet-3 and Diet-4 ($P > 0.05$).

Skin carotenoid content and melanin content data are reported in Table 4. Fish fed the control diet showed a higher dorsal skin melanin content than those fish fed pigment-supplemented diets, although no significant differences were observed among treatments ($P > 0.05$). No melanin was detected in the ventral skin. Lowest carotenoid contents in the ventral skin and dorsal skin were in fish fed Diet-0 ($P < 0.05$). Fish fed with a higher proportion of xanthophylls in the dietary pigment ratio obtained a higher carotenoid content in both the dorsal and ventral skin.

Table 5 shows the regression relationship between the color parameters and carotenoid content of the skin. Positive correlations between yellowness and carotenoid content were found both in dorsal skin ($y = -0.0217 + 5.0049x$, $R^2 = 0.950$, $P = 0.001$) and ventral skin ($y = -67.198 + 2.8425x$, $R^2 = 0.973$, $P = 0.000$). A linear increase of lightness was found with an increasing carotenoid content in dorsal skin ($y = -155.93 + 3.3482x$, $R^2 = 0.766$, $P = 0.022$). Meanwhile, a linear decrease of lightness was shown in ventral skin ($y = 3676.1 - 43.345x$, $R^2 = 0.675$, $P = 0.045$). However, there were no significant relationships

Table 2
Effects of dietary xanthophylls/astaxanthin ratio on survival rate, specific growth rate and feed conversion ratio in large yellow croaker, *Larimichthys crocea*, after an 8-week feeding trial

	Final fish number in each cage	Initial weight (g)	Final weight (g)	Survival rate (%)	Specific growth rate (% day^{-1})	Feed conversion ratio
Diet-0	38/40/42	13.6	40.2	88.9	1.9	1.7
Diet-1	37/39/41	13.9	41.0	86.7	1.9	1.8
Diet-2	39/38/40	13.9	40.6	86.7	2.0	1.7
Diet-3	43/41/41	13.9	40.0	92.6	1.9	1.6
Diet-4	41/43/41	13.9	38.6	92.6	1.8	1.7
Diet-5	40/41/44	13.8	40.4	92.6	2.0	1.5
Significance						
F-value		2.100	0.163	2.444	0.276	0.842
P-value		0.136	0.971	0.095	0.917	0.552
Pooled SEM		0.05	0.69	0.92	0.03	0.03

Table 3
Effects of dietary xanthophylls/astaxanthin ratio on skin color of large yellow croaker after an 8-week feeding trial

	Ventral skin[1]			Dorsal skin[2]		
	L^*[3]	a^*[3]	b^*[3]	L^*	a^*	b^*
Diet-0	82.99	-2.26^{a}	43.19^{a}	57.94^{a}	-2.37^{a}	7.03^{a}
Diet-1	81.84	-1.18^{b}	69.13^{d}	70.21^{b}	-1.65^{a}	19.05^{d}
Diet-2	82.05	0.33^{de}	67.88^{cd}	70.59^{b}	-2.29^{a}	16.81^{c}
Diet-3	81.93	0.81^{e}	65.17^{c}	72.93^{b}	-1.31^{ab}	16.71^{c}
Diet-4	82.98	-0.61^{bc}	59.94^{b}	73.17^{b}	-0.31^{b}	16.14^{c}
Diet-5	82.54	-0.10^{cd}	58.27^{b}	68.76^{b}	-2.20^{a}	14.04^{b}
Significance						
F-value	1.577	52.218	156.151	34.336	15.185	167.427
P-value	0.252	0.000	0.000	0.000	0.000	0.000
Pooled SEM	0.18	0.26	2.20	1.37	0.20	0.96

Means not bearing same superscript letters significantly different ($P < 0.05$).
[1]Sample area of ventral skin: between pelvic fin and anal fin.
[2]Sample area of dorsal skin: below dorsal fin and above lateral line.
[3]L^*: lightness, a^*: redness, b^*: yellowness.

Table 4
Effects of dietary xanthophylls/astaxanthin ratio on carotenoid content and melanin content in ventral skin and dorsal skin of large yellow croaker after an 8-week feeding trial

	Carotenoid (μg g^{-1})		Melanin (μg g^{-1})	
	Dorsal skin[1]	Ventral skin[2]	Dorsal skin	Ventral skin
Diet-0	37.32^{a}	58.24^{a}	83.82	ND
Diet-1	97.16^{c}	131.80^{d}	73.56	ND
Diet-2	84.67^{bc}	124.94^{cd}	65.30	ND
Diet-3	80.24^{bc}	120.60^{cd}	66.21	ND
Diet-4	87.12^{c}	105.19^{bc}	63.80	ND
Diet-5	62.70^{b}	89.52^{b}	75.12	ND
Significance				
F-value	21.943	29.944	2.255	
P-value	0.000	0.000	0.128	
Pooled SEM	4.97	6.28	2.99	

ND, not detectable.
Means not bearing same superscript letters = A significantly different ($P < 0.05$).
[1]Sample area of dorsal skin: below dorsal fin, above lateral line.
[2]Sample area of ventral skin: between pelvic fin and anal fin.

between redness (a^*) and carotenoid content in the ventral skin ($y = 112.8 + 15.455x$, $R^2 = 0.387$, $P = 0.187$) or dorsal skin ($y = 99.495 + 14.587x$, $R^2 = 0.286$, $P = 0.274$).

In fish fed with pigment-supplemented diets, a positive correlation between yellowness and the proportion of xanthophylls in dietary pigments was found both in ventral skin

Table 5
Regression analysis between color parameters (x) and carotenoid content (y) in dorsal and ventral skin of large yellow croaker

	Color parameters	Equation	R^2	P
Dorsal skin	L^*	$y = -155.93 + 3.3482x$	0.766	0.022
	a^*	$y = 99.495 + 14.587x$	0.286	0.274
	b^*	$y = -0.0217 + 5.0049x$	0.950	0.001
Ventral skin	L^*	$y = 3676.1-43.345x$	0.675	0.045
	a^*	$y = 112.8 + 15.455$x	0.387	0.187
	b^*	$y = -67.198 + 2.8425x$	0.973	0.000

Color parameters: L^*: lightness, a^*: redness, b^*: yellowness.

($y = 58.001 + 0.1215x$, $R^2 = 0.896$, P = 0.015) and dorsal skin ($y = 14.202 + 0.047x$, $R^2 = 0.960$, P = 0.003) (Fig. 1). Similarly, a positive correlation between skin carotenoid content and the proportion of xanthophylls in dietary pigments was also detected both in ventral skin ($y = 92.451 + 0.4392x$, $R^2 = 0.931$, P = 0.008) and dorsal skin ($y = 67.307 + 0.3014x$, $R^2 = 0.794$, P = 0.042) (Fig. 2).

Discussion

Under satiation feeding the supplementation of carotenoid did not significantly influence survival, growth performance or feed utilization of large yellow croaker. This result agrees with other studies (Amar et al., 2001; Baker et al., 2002; Olsen and Baker, 2006; Tejera et al., 2007; Kop and Durmaz, 2008; Doolan et al., 2009; Pan and Chien, 2009).

The differences of color lightness between the dorsal and ventral skin (compared to the control group and carotenoid-supplemented groups) could be mainly due to the difference in melanin content in the skin, whereby a higher melanin level would result in lower lightness. Fish fed with the control diet showed higher melanin content in the dorsal skin than those fish fed with pigment-supplemented diets, although no significant differences were found. Melanin was not detected in ventral skin of any dietary treatments (Table 4). Hearing (2005) reported that melanin was the main pigment responsible for the dark color of cultured fish.

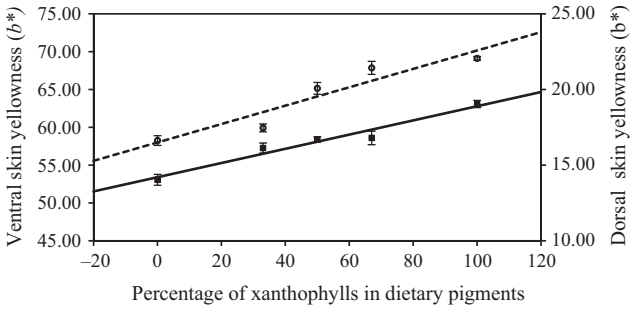

Fig. 1. Effects of dietary xanthophylls/astaxanthin ratio on dorsal skin yellowness (■) and ventral skin yellowness (○), large yellow croaker, *Larimichthys crocea*. Values expressed as mean ± SEM (n = 18). Data fitted by linear regression (dorsal skin $y = 14.202 + 0.047x$, $R^2 = 0.960$, P = 0.003, and ventral skin $y = 58.001 + 0.1215x$, $R^2 = 0.8955$, P = 0.015).

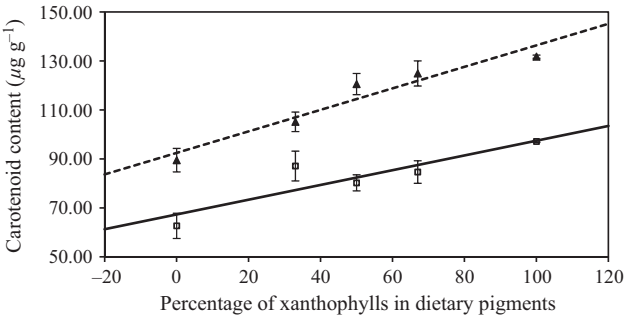

Fig. 2. Effects of dietary xanthophylls/astaxanthin ratio on dorsal skin carotenoid content (□) and ventral skin carotenoid content (▲) large yellow croaker, *Larimichthys crocea*. Values expressed as mean ± SEM (n = 18). Data fitted by linear regression (dorsal skin $y = 67.307 + 0.3014x$, $R^2 = 0.794$, P = 0.042, and ventral skin $y = 92.451 + 0.4392x$, $R^2 = 0.931$, P = 0.008).

Xue et al. (2011) found that the darkbarbel catfish *Pelteobagrus vachelli* contained higher melanin in the dorsal skin, which exhibited lower lightness. Pavlidis et al. (2006) and Dong et al. (2011) also reported that skin melanin reduced the skin lightness in fish. In this study, the observed difference between the control and pigment-supplemented groups could be related to the skin carotenoid contents (Table 4). Melanin and carotenoids play similar roles in a variety of physiologically important metabolic pathways (Griffith et al., 2006). Therefore, the lack of dietary carotenoid in the control group might enhance the generation of melanin, resulting in abnormally dark coloration in the dorsal skin.

In the present study, the lowest yellowness and carotenoid content were shown in the control group, both in the ventral skin and the dorsal skin. It is suggested that both astaxanthin and xanthophylls can significantly improve the skin color of large yellow croaker. Similar results were found in other fish species, such as salmons, sparids and catfish (Torrissen, 1989; Baker et al., 2002; Tejera et al., 2007; Kalinowski et al., 2011). Higher skin yellowness and carotenoid content was seen in fish fed with higher proportions of xanthophylls. This suggests that large yellow croaker could use xanthophylls more efficiently than astaxanthin, and is in agreement with previous studies. Choubert (2010) reported that rainbow trout use astaxanthin more efficiently than canthaxanthin. Li et al. (2007) showed that channel catfish prefer to deposit yellow pigments (e.g. lutein and zeaxanthin) rather than red pigments in the skin (e.g. canthaxanthin and astaxanthin). This indicates a highly species-specific utilization of different pigment sources in terms of absorption, deposition and skin or fillet pigmentation (Pavlidis et al., 2006). Results from the present study showed that large yellow croaker prefer to deposit yellow pigment, thus resulting in yellow skin. Pavlidis et al. (2006) also suggested that this fish species has a limited capacity to convert astaxanthin to yellow pigment, as is shown by the decreased ventral and dorsal skin yellowness in experimental diets with low xanthophyll/astaxanthin ratios.

In the present study, higher redness was shown in fish fed greater proportions of dietary astaxanthin. *Larimichthys crocea* is a species characterized by a golden-yellow-red color

style; *L. crocea* fed with dietary astaxanthin was more comparable to wild fish because of the resulting increase in skin redness (Yi et al., 2014a), as was also found in the present study. Therefore, the compound pigment feed supplement seems to be better for skin redness than the previous single pigment supplement for large yellow croaker pigmentation.

Several different mathematical models can be used to describe the relationship between color parameters and carotenoid content (Bjerkeng, 2000). Some studies reported linear relationships (Buttle et al., 2001; Baker et al., 2002; Ingle de la Mora et al., 2006), others employed non-linear models when the carotenoid content was high (Christiansen et al., 1995; King, 1996). The reason for this is because our color perception becomes saturated asymptotically when the carotenoid content exceeds certain limits (Bjerkeng, 2000). In the present study, both yellowness and lightness were linearly related (Table 5). This indicates that both parameters can be used to predict the skin carotenoid content. However, in the two sample areas (dorsal, ventral fin), redness did not have a significant linear relationship with the carotenoid content. This has been shown in previous studies on *Pethia conchonius* (Hamilton, 1822; Teimouri and Amirkolaie, 2013) and rainbow trout (Ingle de la Mora et al., 2006).

For fish feed producers, one of the most important targets in the formulation of large yellow croaker feed is achieving successful skin pigmentation. However, the cost of supplemental pigments in many commercial feeds is also considerable: accounting for 10–20% of the total cost of feed in the case of salmon (Ingle de la Mora et al., 2006). This stems from a market price of synthetic astaxanthin at or above \$2000 USD kg^{-1} (Li et al., 2011). However, the price of xanthophylls (e.g. lutein) at \$570–790 USD kg^{-1} (Prommuak et al., 2013) is significantly lower than that of astaxanthin. In the present study, yellowness and carotenoid content in ventral and dorsal skin were linearly related to the proportion of dietary xanthophylls (Figs 1 and 2). Results of the present study show that there is a measurable benefit of dietary xanthophylls or astaxanthin in terms of yellowness and carotenoid content in large yellow croaker, *Larimichthys crocea*.

Conclusions

In the present study, fish fed with pigment-supplemented diets had significantly higher ventral and dorsal skin coloration and carotenoid content than those fed with the control diet. Moreover, fish fed the mixture of astaxanthin and xanthophylls had higher redness in the dorsal skin and/or ventral skin than those fed an astaxanthin-only diet or a xanthophylls-only diet. The redness in the skin of fish fed the mixture of astaxanthin and xanthophylls was more similar to that of the wild large yellow croaker. Based on the present results, the pigments composition of Diet-3 (astaxanthin: xanthophylls = 1 : 1) can be used under farming conditions for juvenile *L. crocea* to obtain desirable skin coloration at around 8 weeks. However, further studies are needed using market size large yellow croaker in order to find the optimal pigment composition, pigment concentrations and feeding period.

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