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Effects of dietary lutein/canthaxanthin ratio on the growth and pigmentation of large yellow croaker *Larimichthys croceus*

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Abstract

This study was conducted to investigate the effects of dietary lutein/canthaxanthin ratio on the growth and skin coloration of large yellow croaker. Five carotenoids supplemented diets were formulated to contain 75/0, 50/25, 37.5/37.5, 25/50 and 0/75 mg kg^{-1} of lutein/canthaxanthin. The diet without carotenoids supplementation was used as the control. Fish of the similar size (13.83 ± 0.04 g) were fed with these experimental diets for 8 weeks in sea cages. Results showed that there were no significant differences in survival rate, specific growth rate and feed conversion ratio among the all treatments ($P > 0.05$). The ventral skin lightness was not affected by dietary treatments ($P > 0.05$). However, the dorsal skin lightness in the treatment of control was significantly lower than those in the treatments with supplemented dietary carotenoids ($P < 0.05$). The lowest values of yellowness, redness and carotenoid content both in ventral and dorsal skin were found in the control. Yellowness and carotenoid content both in ventral skin and in dorsal skin decreased with the decreasing of the proportion of dietary lutein. Meanwhile, the redness increased with the increasing of the proportion of dietary canthaxanthin. Fish fed with the control diet had higher melanin content in the dorsal skin, although no significant differences were found. Coloration parameters were linearly related to the carotenoid content in skin. Meanwhile, yellowness, redness and carotenoid content were linearly related to the proportion of dietary lutein. In conclusion, under present conditions, both lutein and canthaxanthin are needed in the diet for large yellow croaker. Compared to the lutein, higher dietary canthaxanthin contents are better for the skin redness.

KEY WORDS: canthaxanthin, diet, large yellow croaker, lutein, skin colour

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Introduction

Large yellow croaker *Larimichthys croceus* is a commercially important mariculture fish species in China. The production in 2012 was 95 118 metric tons (China Fishery Statistical Yearbook 2013). Naturally, this fish has golden ventral skin, red lips and tongue, tan back and lateral skin, and yellow fins. Most people like this fish just because of its golden–yellow–red colour, which means 'fortune' and 'happiness' in Chinese culture. Consequently, skin coloration is considered as one of the most important price criteria for large yellow croaker. It also determines the consumer's impression of other quality parameters, such as freshness and health. However, the fish lost its natural colour style under intensive culture, which leads to low market price and low acceptability.

Yi *et al.* (2014) reported that the skin colour of farmed large yellow croaker can be improved by adding 37.5–75.0 mg kg^{-1} astaxanthin or xanthophylls in diet for 9 weeks. The skin coloration of large yellow croaker fed with astaxanthin was more likely to the wild one due to the higher redness in skin. Meanwhile, higher yellowness and carotenoid content in skin were found in the fish fed with xanthophylls. Combined with the golden–yellow–red colour style of the wild large yellow croaker, the above study suggested that both yellow carotenoids (e.g. lutein and zeaxanthin) and red carotenoids (e.g. astaxanthin and canthaxanthin) are needed by this fish species. Similar researches were shown before. For example, Wang *et al.*

(2006) reported that the most efficient and cost-effective carotenoid formulation for characins *Hyphessobrycon callistus* was the mixed diet containing 20 mg kg^{-1} each of astaxanthin and β-carotene.

Achieving successful pigmentation in farmed large yellow croaker is one of the most important targets to formulate commercial feed. Meanwhile, the cost of pigment in commercial feeds is also considerable. In salmons, the cost of pigment represents 10–20% of the total cost of feed (Ingle de la Mora *et al*. 2006). The market price of synthetic astaxanthin is above \$2000 kg^{-1} (Li *et al*. 2011). Canthaxanthin, similar to astaxanthin, is another important red carotenoid widely used in salmon pigmentation. However, the market price of canthaxanthin was relative lower than that of astaxanthin (Baker *et al*. 2002). To reduce the cost of commercial feeds, a combination of canthaxanthin and yellow carotenoid can be an alternative method. Therefore, the aim of this study was to evaluate the effects of dietary lutein/canthaxanthin ratio on the skin coloration and to find a cost-effective carotenoid regiment for large yellow croaker.

Materials and methods

The experimental diets

Fishmeal (659 g kg^{-1} crude protein, 74 g kg^{-1} crude lipid) and soybean meal (510 g kg^{-1} crude protein, 30 g kg^{-1} crude lipid) were used as the main dietary protein sources. Fish oil was used as the lipid source, and wheat meal (155 g kg^{-1} crude protein, 28 g kg^{-1} crude lipid) was used as the carbohydrate source. These ingredients were obtained from

Table 1 Feed formulation and proximate composition (g kg^{-1} dry basis)

	Diet 0	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Yellow carotenoid (mg kg^{-1})	0	75	50	37.5	25	0
Red carotenoid (mg kg^{-1})	0	0	25	37.5	50	75
Fish meal[1]	430	430	430	430	430	430
Soybean meal[1]	210	210	210	210	210	210
Wheat meal[1]	209	207	208	208	208	208
Beer yeast[1]	20	20	20	20	20	20
Fish oil[1]	71	71	71	71	71	71
Soybean lecithin[2]	15	15	15	15	15	15
Vitamin premix[3]	20	20	20	20	20	20
Mineral premix[4]	20	20	20	20	20	20
Attractant[5]	3	3	3	3	3	3
Mould inhibitor[6]	1	1	1	1	1	1
Ethoxyquin	1	1	1	1	1	1
Wisdem®GoldenY-40[7]	0	1.8	1.2	0.9	0.6	0
Wisdem®Red[8]	0	0	0.3	0.4	0.5	0.8
Chemical analysis, g kg^{-1}						
Moisture	57	62	58	60	58	63
Crude protein	461	463	466	457	458	456
Crude lipid	118	118	126	129	128	122
Ash	122	128	127	133	130	130
Carotenoids (mg kg^{-1})	–[9]	75.4	74.0	76.5	74.4	75.7

[1] Fish meal: 659 g kg^{-1} crude protein, 74 g kg^{-1} crude lipid; soybean meal: 510 g kg^{-1} crude protein, 30 g kg^{-1} crude lipid; wheat flour: 155 g kg^{-1} crude protein, 28 g kg^{-1} crude lipid; beer yeast: 519 g kg^{-1} crude protein, 33 g kg^{-1} crude lipid. These ingredients were obtained from Qingdao Great-Seven Bio-tech, Co., Ltd, China.

[2] Soybean lecithin: 50% phosphatidylcholine, Qingdao Great-Seven Bio-tech, Co., Ltd., China.

[3] Vitamin premix (mg or g kg^{-1} diet): thiamine, 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.

[4] Mineral premix (mg 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; Zeolite, 15.447 g.

[5] Attractant: glycine and betaine.

[6] Mould inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.

[7] Wisdem® GoldenY-40: lutein 3.66%, zeaxanthin 0.25%, Guangzhou Wisdom Bio-Technology Co., Ltd, China.

[8] Wisdem® Red: canthaxanthin 10%, Guangzhou Wisdom Bio-Technology Co., Ltd, China.

[9] '–': Not detectable.

Qingdao Great-Seven Bio-technology (Qingdao, China), Co., Ltd. Wisdom® red (canthaxanthin 10%) and Wisdom® golden Y-40 (lutein 3.66%, zeaxanthin 0.25%) were obtained from Guangzhou Wisdom Bio-Technology (Guangzhou, China) Co., Ltd. The diet without carotenoids supplementation was used as the control, which was named as Diet 0. Five carotenoid-supplemented diets were formulated to contain 75/0,50/25,37.5/37.5,25/50 and 0/75 mg kg^{-1} of lutein/canthaxanthin. They were named as Diet 1, Diet 2, Diet 3, Diet 4 and Diet 5, respectively (Table 1).

All ingredients were finely ground into powder. The pigments were then dissolved in fish oil and thoroughly mixed with 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 having 4-mm holes in an experimental feed mill. Diets were dried in a ventilated oven at 40 °C until reaching a moisture level below 8% and then stored at −20 °C in black bags.

Fish and feeding trial

The feeding trial was conducted in Xihu bay, Ningbo, China. Large yellow croaker juveniles (1200 fish) were purchased from a local 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 half month. During the acclimation, fish were fed with the control diet twice daily. Water temperature, salinity and dissolved oxygen were measured every 2 days throughout the trial. 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 fasted for 24 h and then weighed after being anesthetized with eugenol (1:10000) (Shanghai Reagent Corp., Shanghai, China). Fish with the similar size (13.83 ± 0.04 g) were randomly distributed into 18 sea cages (1.0 × 1.0 × 1.5 m) at a density of 45 fish per cage. Each diet randomly was assigned to triplicate cages. Fish were hand fed to apparent satiation twice daily (5:00 and 17:00) for 8 weeks. During the feeding trial, water temperature ranged from 21 to 31 °C, salinity from 28 to 32 g L^{-1}, and the dissolved oxygen from 6.2 to 8.3 mg L^{-1}.

Analysis and measurement

Dorsal skin on the right side and ventral skin of 6 fish per cage were sampled for carotenoid and melanin analysis. Skin samples were covered with aluminium foil and stored at −20 °C. At night, between 20:00 and 23:00, six fish per cage were randomly collected to evaluate their skin colour using a portable Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan). The skin colour measurement was performed on ventral skin and right side of the dorsal skin. The colour parameters were L^*, a^* and b^* for lightness, redness and yellowness, respectively, according to the recommendation of the International Commission on Illumination (CIE 1976).

Carotenoid content in feed and skin was measured by the method of Cejas *et al.* (2003) with some modifications. Briefly, samples of skin (0.5 g) and feed (1.0 g) were finely homogenized in 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 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 (10000 ***g***, 5 min). Carotenoid contents were quantified by spectrophotometer (UV-2401PC, Kyoto, Japan) in acetone. Acetone was used as the blank to adjust the background absorption before measuring. Carotenoid content was expressed using the extinction coefficients $E_{(1\%,\ 1\ cm)}$ = 1900 at 470 nm (Buttle *et al.* 2001) for diet canthaxanthin and $E_{(1\%,\ 1\ cm)}$ = 2500 (Schiedt & Liaaen-Jensen 1995) at 448 nm for skin carotenoid and diet lutein.

Dry matter, crude lipid, crude protein, moisture and ash of dietary ingredients and the experimental diets were determined following the methods of Association of Official Analytical Chemists (AOAC (Association of Official Analytical Chemist) 1995). Melanin content of dorsal and ventral skin was quantified by the method of Dong *et al.* (2011). Melanin standard (M-2649) was purchased from Sigma.

Calculations and statistical analysis

Survival rate (SR) = 100× (final fish number/initial fish number).

Specific growth rate (SGR) = (Ln final weight − Ln initial weight) × 100/days.

Feed conversion ratio (FCR) = dry feed fed (g)/wet weight gain (g).

Carotenoid content = $10000 \times V \times A/W/E_{(1\%,\ 1\ cm)}$

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

Results were presented as means ± SEM. All data were analysed by one-way ANOVA by SPSS 15.0 for Windows. The level of significance was set at $P < 0.05$, and Tukey's test was used to compare the mean values. Linear regression

analysis was conducted between colour parameters and carotenoid content. As to the fish fed with pigment-supplemented diets, linear regression analysis between the percentage of lutein in dietary pigments and yellowness, redness or carotenoid content was also performed.

Results

Effects of dietary carotenoid on growth performance and feed utilization of large yellow croaker are presented in Table 2. Carotenoid supplementation had no significant effects on survival rate, specific growth rate and feed conversion ratio among groups ($P > 0.05$). The survival rate ranged from 87.30 to 94.54%. The specific growth rate fluctuated from 1.76 to 2.00. And the feed conversion ratio varied between 1.53 and 1.84.

Data of skin colour are presented in Table 3. No significant differences in ventral skin lightness (L^*) were found among groups ($P > 0.05$). However, the dorsal skin lightness of fish fed with Diet 0 was significantly lower than those fed with carotenoid-supplemented diets ($P < 0.05$). The lowest redness (a^*) values and yellowness (b^*) values were found in fish fed with Diet 0 both in ventral skin and in dorsal skin. Yellowness of dorsal skin tended to decrease with the decreasing of the proportion of dietary lutein. Nevertheless, redness of dorsal skin tended to increase with the increasing of the proportion of dietary canthaxanthin. Meanwhile, fish fed with lutein-only diet (Diet 1) showed significantly lower redness in ventral skin than those of fed with diets containing canthaxanthin (Diet 2 to Diet 5) ($P < 0.05$). Fish fed with canthaxanthin-only diet (Diet 5) showed significantly lower yellowness in ventral skin than those of fed with diets containing lutein (Diet 1 to Diet 4) ($P < 0.05$).

Data on skin carotenoid content and melanin content are shown in Table 4. No significant differences in dorsal skin melanin content were found among treatments ($P > 0.05$). However, in contrast to the dorsal skin, no melanin was detected in ventral skin. Dorsal skin carotenoid content in Diet 4 group and Diet 5 group was significantly lower than those in Diet 1 group, Diet 2 group and Diet 3 group. The lowest value was found in Diet 0 group ($P < 0.05$). Ventral skin carotenoid content in Diet 2 group and Diet 3 group was significantly higher than that in Diet 4 group and Diet 5 group. The lowest one was also shown in Diet 0 group ($P < 0.05$).

Regression analysis between carotenoid content and colour parameters is reported in Table 5. A linear decrease of lightness was observed with the increasing of the carotenoid content in ventral skin ($y = 83.864–0.0173x$, $R^2 = 0.876$, $P = 0.006$). However, a linear increase of lightness was shown in the dorsal skin ($y = 50.791 + 0.2426x$, $R^2 = 0.782$, $P = 0.019$). In addition, yellowness was highly correlated with carotenoid content in ventral skin ($y = 26.092 + 0.312x$, $R^2 = 0.904$, $P = 0.004$) and dorsal skin ($y = -0.2591 + 0.2074x$, $R^2 = 0.981$, $P = 0.000$). Nevertheless, redness presented low correlation with carotenoid content both in ventral skin ($y = -3.2082 + 0.0254x$, $R^2 = 0.465$, $P = 0.135$) and in dorsal skin ($y = 1.875–0.0103x$, $R^2 = 0.039$, $P = 0.707$).

As to fish fed with carotenoid-supplemented diets, positive correlation between yellowness and the percentage of

Table 2 Effects of dietary lutein/canthaxanthin ratio on the growth performance and feed utilization of large yellow croaker after the 8-week feeding trial

	IBW(g)[1]	FBW(g)[1]	SR (%)[2]	SGR (% d^{-1})[3]	FCR[4]
Diet 0(L/C[5] = 0/0)	13.64	40.19	88.89	1.93	1.66
Diet 1(L/C = 75/0)	13.91	40.98	86.90	1.92	1.65
Diet 2(L/C = 50/25)	13.71	42.18	94.54	2.00	1.53
Diet 3(L/C = 37.5/37.5)	13.91	40.55	89.29	1.91	1.58
Diet 4(L/C = 25/50)	13.89	37.18	87.30	1.76	1.84
Diet 5(L/C = 0/75)	13.93	38.94	87.30	1.83	1.75
Significance					
F-value	2.761	1.954	0.960	2.122	1.010
P-value	0.069	0.159	0.482	0.133	0.456
Pooled SEM ($n = 3$)	0.04	0.79	1.25	0.04	0.04

[1] IBW, initial body weight; FBW, final body weight.
[2] SR: Survival rate = 100 × (final fish number/initial fish number).
[3] SGR: Specific growth rate = (Ln final weight – Ln initial weight) × 100/days.
[4] FCR: Feed conversion ratio = dry feed fed (g)/wet weight gain (g).
[5] L/C: lutein (mg kg^{-1})/canthaxanthin (mg kg^{-1}).

Table 3 Effects of dietary lutein/canthaxanthin ratio on the skin colour of large yellow croaker after the 8-week feeding trial

	Ventral skin			Dorsal skin		
	L^*[1]	a^*[1]	b^*[1]	L^*	a^*	b^*
Diet 0 (L/C[2] = 0/0)	82.99	−2.26[a]	43.19[a]	57.94[a]	−2.31[a]	7.03[a]
Diet 1 (L/C = 75/0)	81.84	−1.18[b]	69.13[c]	70.21[b]	−1.65[ab]	19.05[d]
Diet 2 (L/C = 50/25)	81.54	0.39[c]	67.55[c]	73.76[b]	−1.34[b]	18.66[d]
Diet 3 (L/C = 37.5/37.5)	81.43	0.39[c]	66.71[c]	73.02[b]	−1.52[b]	18.56 [cd]
Diet 4 (L/C = 25/50)	81.92	0.20[c]	65.78[c]	70.64[b]	−0.51[c]	16.13[b]
Diet 5 (L/C = 0/75)	81.71	0.47[c]	56.60[b]	72.94[b]	0.90[d]	16.27[bc]
Significance						
F-value	1.875	120.158	61.666	46.728	53.392	89.691
P-value	0.179	0.000	0.000	0.000	0.000	0.000
Pooled SEM (n = 3)	0.13	0.14	1.17	0.42	0.24	0.35

Means not bearing the same superscript letters are significantly different ($P < 0.05$).
[1] L^*: lightness; a^*: redness; b^*: yellowness.
[2] L/C: lutein (mg kg^{-1})/canthaxanthin (mg kg^{-1}).

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

	Carotenoid (μg kg^{-1})		Melanin (μg kg^{-1})	
	Dorsal skin	Ventral skin	Dorsal skin	Ventral skin
Diet 0 (L/C[1] = 0/0)	37.32[a]	58.24[a]	83.82	ND[2]
Diet 1 (L/C = 75/0)	97.16[c]	131.80 [cd]	73.56	ND
Diet 2 (L/C = 50/25)	89.67[c]	136.46[d]	63.65	ND
Diet 3 (L/C = 37.5/37.5)	91.88[c]	138.89[d]	72.13	ND
Diet 4 (L/C = 25/50)	77.72[b]	109.97[bc]	74.50	ND
Diet 5 (L/C = 0/75)	75.19[b]	105.43[b]	75.30	ND
Significance				
F-value	47.127	81.048	1.160	
P-value	0.000	0.000	0.387	
Pooled SEM (n = 3)	7.25	5.05	2.51	

Means not bearing the same superscript letters are significantly different ($P < 0.05$).
[1] L/C: lutein (mg kg^{-1})/canthaxanthin (mg kg^{-1}).
[2] ND: Not detectable.

Table 5 Regression analysis between carotenoid content (x) and colour parameters (y) of the dorsal skin and ventral skin

Sample area	Colour parameters[1]	Equation	R^2	P
Dorsal skin	L^*	$y = 50.791 + 0.2426x$	0.782	0.019
	a^*	$y = -1.875 + 0.0103x$	0.039	0.707
	b^*	$y = -0.2591 + 0.2074x$	0.981	0.000
Ventral skin	L^*	$y = 83.864 - 0.0173x$	0.876	0.006
	a^*	$y = -3.2082 + 0.0254x$	0.465	0.135
	b^*	$y = 26.092 + 0.312x$	0.904	0.004

[1] Colour parameters: L^*: lightness, a^*: redness, b^*: yellowness.

dietary lutein was detected both in ventral skin ($y = 59.278 + 0.118x$, $R^2 = 0.791$, $P = 0.043$) and in dorsal skin ($y = 16.108 + 0.0327\ x$, $R^2 = 0.740$, $P = 0.061$) (Fig. 1). Similarly, positive correlation between carotenoid content and the percentage of dietary lutein was also found in ventral skin ($y = 108.73 + 0.3169x$, $R^2 = 0.569$, $P = 0.141$) and dorsal skin ($y = 74.663 + 0.2341x$, $R^2 = 0.850$, $P = 0.026$) (Fig. 2). However, redness presented negative correlation in ventral skin ($y = 0.769–0.0144x$, $R^2 = 0.588$, $P = 0.130$) and dorsal skin ($y = 0.4432–0.0254x$, $R^2 = 0.797$, $P = 0.041$) (Fig. 3).

Discussion

In the present study, dietary carotenoid supplementation had no significant effects on growth performance and feed utilization. This is in agreement with the previous studies on rosy barb *Pethia conchonius* (Hamilton, 1822) (Teimouri *et al.* 2013), cichlids *Cichlasoma severum* sp (Heckel 1840) (Kop & Durmaz 2008), rainbow trout *Oncorhynchus mykiss*

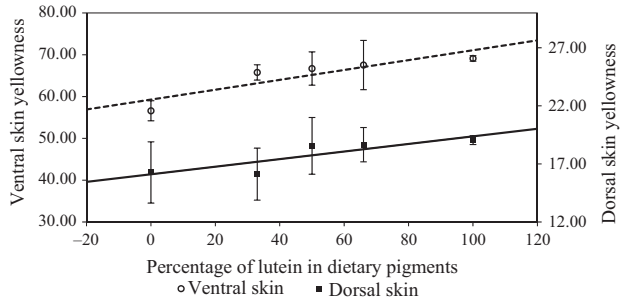

Figure 1 Effects of dietary lutein/canthaxanthin ratio on the dorsal skin yellowness (■) and the ventral skin yellowness (○) of large yellow croaker. Values are means and standard errors of three replicates. Data are fitted by linear regression (dorsal skin $y = 16.108 + 0.0327x$, $R^2 = 0.740$, $P = 0.061$, and ventral skin $y = 59.278 + 0.118x$, $R^2 = 0.791$, $P = 0.043$).

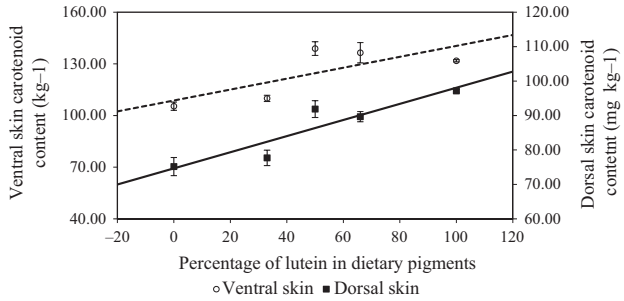

Figure 2 Effects of dietary lutein/canthaxanthin ratio on the carotenoid content in the dorsal skin (■) and the ventral skin (○) of large yellow croaker. Values are means and standard errors of three replicates. Data are fitted by linear regression (dorsal skin $y = 74.663 + 0.2341x$, $R^2 = 0.850$, $P = 0.026$, and ventral skin $y = 108.73 + 0.3169x$, $R^2 = 0.569$, $P = 0.141$).

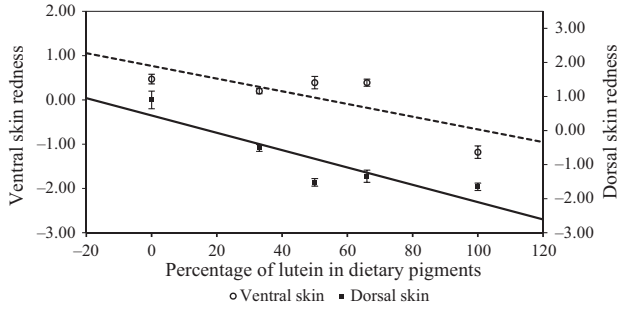

Figure 3 Effects of dietary lutein/canthaxanthin ratio on the dorsal skin redness (■) and the ventral skin redness (○) of large yellow croaker. Values are means and standard errors of three replicates. Data are fitted by linear regression (dorsal skin $y = 0.4432–0.0254x$, $R^2 = 0.797$, $P = 0.041$ and ventral skin $y = 0.769–0.0144x$, $R^2 = 0.588$, $P = 0.130$).

(Amar *et al.* 2001), Australian snapper *Pagrus auratus* (Doolan *et al.* 2009), red porgy *Pagrus pagrus* (Kalinowski *et al.* 2005) and Atlantic salmon *Salmo salar* L. (Olsen & Baker 2006).

In the present study, no melanin was detected in ventral skin, while high melanin contents were found in dorsal skin among all treatments. Moreover, higher melanin content was found in the dorsal skin of fish fed with the control diet, although no significant differences were shown among all treatments (Table 4). Hearing (2005) reported that melanin was the main pigment responsible for the dark colour of cultured fish. Dong *et al.* (2011) found that the Chinese longsnout catfish *Leiocassis longirostris* (Günther) showed lower lightness when fish contain higher melanin in the skin. Similar results were also found in sparidae *Pagrus pagrus*, *Pagrus caeruleostictus* and *Dentex gibbosus* (Pavlidis *et al.* 2006).

In the present study, fish fed with the control diet also showed the lowest yellowness values, redness values and carotenoid contents both in ventral skin and in dorsal skin. It is suggested that both canthaxanthin and lutein can significantly improve the skin colour of large yellow croaker. This is in line with the results on rosy barb, channel catfish *Ictalurus punctatus*, rainbow trout and Atlantic salmon (Olsen & Baker 2006; Li *et al.* 2007; Choubert 2010; Teimouri *et al.* 2013). Fish cannot synthesize carotenoid *de novo* (Goodwin 1984), so their body colour highly relies on carotenoid from the diet (Torrissen *et al.* 1990). Canthaxanthin, similar to astaxanthin, is widely used to improve the fish skin colour and fillet colour. Zeaxanthin and lutein are also effective pigments for fish pigmentation.

In the present study, higher yellowness and carotenoid content were shown in fish fed diets with greater proportion of dietary lutein. It was suggested that large yellow croaker could use lutein more efficiently than canthaxanthin. This was in agreement with previous studies. Olsen & Baker (2006) reported that Atlantic salmon accumulated astaxanthin more efficiently than lutein in the fillet. Channel catfish, however, prefer to deposit lutein and zeaxanthin in the skin, compared to astaxanthin and canthaxanthin (Li *et al.* 2007). This could be because the utilization of carotenoid source in terms of absorption, deposition and pigmentation is species specific (Pavlidis *et al.* 2006). Large yellow croaker is a kind of fish which is characterized by golden–yellow skin, red lips and yellow fins. Results from the present study showed that large yellow croaker prefer yellow carotenoids, resulting in yellow skin. Moreover, it was also implied that large yellow croaker has the ability to convert the canthaxanthin to yellow carotenoids, as is

shown by the fish fed with canthaxanthin-only diet (Diet 5) that showed significantly higher skin carotenoid content and yellowness than fish fed with control diet (Diet 0). Similarly, Yi *et al*. (2014) reported that large yellow croaker has the ability to convert astaxanthin to yellow carotenoids.

In the present study, higher redness was shown in fish fed with diets containing canthaxanthin. This finding showed that canthaxanthin can improve the redness value of large yellow croaker both in ventral skin and in dorsal skin. Generally, canthaxanthin, similar to astaxanthin, is considered as red carotenoids supplemented in diet to improve the red colour of fish skin or fillet, such as Atlantic salmon (Storebakken *et al*. 1987), rainbow trout (Storebakken & Choubert 1991) and Australian snapper (Booth *et al*. 2004; Doolan *et al*. 2009). In contrast, lutein and zeaxanthin are considered as yellow carotenoids for fish to improve yellow skin or muscle colour, such as channel catfish (Li *et al*. 2007) and walking catfish *Clarias fuscus* (Leng *et al*. 2003). The golden–yellow–red colour style of large yellow croaker implies that both red and yellow carotenoids are needed in the diet for pigmentation. In our previous study, it was shown that the coloration of large yellow croaker fed with astaxanthin diet was more likely to the wild fish for higher redness in the skin (Yi *et al*. 2014). In the present study, the addition of canthaxanthin in diet improved the redness of skin. Thus, red pigments are also needed in the diet for the pigmentation of large yellow croaker.

Colour parameters and carotenoid content of fish were related. Several different mathematical models can be used to describe the relationship between them (Bjerkeng 2000). Some studies showed that colour parameters and carotenoid content were linearly dependent (Skrede & Storebakken 1986a,b; Bjerkeng *et al*. 1997; Hatlen *et al*. 1998; Baker *et al*. 2002; Teimouri & Amirkolaie 2015). However, several researches demonstrated that higher relationship can be achieved using nonlinear model (King 1996; Kalinowski *et al*. 2007). In the present study, both yellowness and lightness had a significantly linear relationship with the carotenoid content either in dorsal skin or in ventral skin. However, redness had no significantly linear relationship with carotenoid content both in ventral skin and in dorsal skin. This was in agreement with the previous study on *Pethia conchonius* (Hamilton, 1822) (Teimouri & Amirkolaie 2015), in which it was found that lightness and carotenoid content in fillet were not significantly related. In the present study, yellowness, redness and carotenoid content were linearly related to the proportion of dietary lutein or canthaxanthin. Results from the present study showed a measurable benefit of dietary lutein or canthaxanthin in terms of skin redness, yellowness and carotenoid content in large yellow croaker.

In conclusion, the present study showed that both lutein and canthaxanthin are needed in the diet for large yellow croaker for pigmentation. Lutein provided higher yellowness and carotenoid content in skin than canthaxanthin. However, canthaxanthin offered higher redness in skin, which was more likely to the wild fish. Thus, higher proportion of canthaxanthin in the combination regiment of canthaxanthin and lutein can be better.

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