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Effects of dietary lipid content on growth, body composition and pigmentation of large yellow croaker *Larimichthys croceus*

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ABSTRACT

A 60-day experiment was conducted to investigate the effects of dietary lipid content on the growth performance and pigmentation of large yellow croaker *Larimichthys croceus*. Six isonitrogenous (44% crude protein) diets containing 75 mg/kg of astaxanthin were formulated to have graded contents of lipid (2.7, 5.1, 8.6, 11.7, 14.9 and 18.1%, respectively). Each diet was fed to triplicate groups of fish (initial weight: 10.02 ± 0.02 g). The results showed that the survival rate (SR) was not significantly affected by dietary lipid contents. Fish fed with 2.7% of dietary lipid had the lowest weight gain rate (WGR). The highest value was found in fish fed diets with 11.7% of lipid. Lightness (L^*) in the ventral skin was higher than that in the dorsal skin. There were no significant differences in redness (a^*) or lightness among all treatments in both ventral and dorsal skin. Meanwhile, ventral skin yellowness was improved with increasing the dietary lipid content up to 11.7%. Carotenoid and melanin contents in the dorsal skin were not significantly affected by dietary lipid content. Carotenoids content in the ventral skin improved with increasing dietary lipid content up to 14.9%. High correlation was found between yellowness and the carotenoids content in the ventral skin. The minimum dietary lipid requirement was estimated to be 10.42% for growth. For skin pigmentation, this requirement was estimated to be 12.00% and 13.19% for ventral skin yellowness and carotenoids content, respectively.

1. Introduction

Large yellow croaker *Larimichthys croceus* is one of the most important mariculture fish species in China. The production in 2012 was 95,118 tons (China Fishery Statistical Yearbook, 2013). Normally, wild large yellow croaker has golden-yellow skin, red lips and yellow fins. In China, people like this fish species. One of the most important reasons is that the red-yellow-golden color means “fortune” and “happiness” in Chinese traditional culture. Consequently, skin coloration is one of the most important quality criteria for this species. Skin coloration also influences the consumer's impression of other quality parameters, such as freshness and health. Nevertheless, the fish has lost its natural skin coloration under intensive culture, which results in low market price and poor consumer acceptability. At present, the skin color of large yellow croaker can be improved by feeding diets with 37.5–75.0 mg/kg of astaxanthin or xanthophylls for 9 weeks (Yi et al., 2014). In addition, many factors also can affect fish pigmentation. The increasing of dietary vitamin E improved the deposition of canthaxanthin in the flesh of rainbow trout (Pozo et al., 1988). High dietary protein/carbohydrate ratio enhanced the skin melanin content of red porgy *Pagrus pagrus*, which is the main pigment responsible for the skin darkness of cultured fish (Chatzifotis et al., 2005). Rainbow trout *Oncorhyncus mykiss* raised in freshwater showed higher flesh astaxanthin concentration than those in saltwater (Storebakken and Choubert, 1991). Gouveia and Rema (2005) reported that the best water temperature range for the goldfish *Carassius auratus* to maximize skin pigmentation was 26–30 °C.

Lipid, as one of the macronutrients, plays a vital role in providing a source of concentrated energy and essential fatty acids (EFA). Adequate lipid content in the diet is important for growth performance of fish, and also for the formulation of diets and final product quality (Luo et al., 2005). Dietary lipid also plays a central role in absorption, transportation, and metabolism of lipid-soluble nutrients such as the carotenoids and fat-soluble vitamins. Torrissen et al. (1990) showed the evidence for an increased absorption of carotenoids in salmonids by increased dietary lipid level. Choubert and Baccaunau (2006) reported that rainbow trout fed with 24% lipid diet had higher astaxanthin concentration (71 mg/kg) in the fillet than those fed with 9% lipid diet (64 mg/kg). In Atlantic salmon, after 9.5 months feeding, fish fed with 39% lipid diet show higher redness (11 vs. 9.5) and yellowness (17.7 vs. 15.4) than those fed with 31% lipid diet (Bjerkeng et al., 1997). However, Einen and Roem (1997) found that dietary lipid content has positive

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effects on the deposition of carotenoid in small size Atlantic salmon (initial weight: 1 kg), while not in large size fish (initial weight: 2.5 kg). This may be caused by high level of astaxanthin (7.5 mg/kg) that was found in large fish which are close to the plateau of astaxanthin in muscle. Choubert and Luquet (1983) found that rainbow trout fed with graded level of lipid (9.4, 12.1, 17.4%) did not enhance the deposition of carotenoids in fillet. They ascribed this result to that 90% ingested astaxanthin was found in feces when shrimp meal used as pigment source. Thus, it was suggested that the positive effects of dietary lipid level on the deposition of carotenoid in fish skin or muscle could be influenced by many factors.

At present, there is no information available on the effects of dietary lipid on growth performance or skin pigmentation of the large yellow croaker. Therefore, the aim of this study was to investigate the effects of dietary lipid on the growth and skin coloration of this species.

2. Materials and methods

2.1. Experimental diets

Six isonitrogenous diets (44% crude protein) were formulated to contain graded levels of lipid (3, 6, 9, 12, 15 and 18% on a dry basis, respectively). The analyzed dietary lipid contents were 2.7, 5.1, 8.6, 11.7, 14.9 and 18.1%, respectively. The diets were named as L3, L6, L9, L12, L15 and L18. Each diet contained 75 mg/kg of astaxanthin (Carophyll® pink, astaxanthin 10%, DSM) as pigment source. Ingredients and proximate composition of the experimental diets are given in Table 1.

All dietary ingredients were finely ground into powder. Following that, astaxanthin was dissolved in fish oil and thoroughly mixed with other ingredients. Distilled water was then added (30%, v/w) to produce stiff dough. The dough was made into pellets utilizing an experimental feed mill. All diets were dried in a ventilated oven at 40 °C until the moisture content fell below 10% and were stored at −20 °C in black bags.

2.2. Experimental procedure

The feeding trial was carried out in Xihu bay of Xiangshan county, Zhejiang province, China. Large yellow croaker juveniles were purchased from a local commercial hatchery and stocked in floating sea cage (3.0 × 6.0 × 3.0 m) for 2 weeks prior to the trial. During this period, fish were fed with commercial feed without pigments. At the beginning of the feeding trial, fish of similar size (10.02 ± 0.02 g) were randomly distributed into 18 sea cages (1.5 × 1.5 × 2.0 m) at a density of 60 fish per cage. The fish were hand-fed to apparent satiation twice daily (05:00 and 17:00) for 60 days. The feed intake was recorded. During the feeding trial, the water temperature ranged from 21 to 31 °C, salinity 28 to 32‰, and the dissolved oxygen content was greater than 7 mg/L for the duration of the study.

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

Ingredients	L3	L6	L9	L12	L15	L18
Fish meal	30.00	30.00	30.00	30.00	30.00	30.00
Casein	18.00	18.00	18.00	18.00	18.00	18.00
Gelatin	4.50	4.50	4.50	4.50	4.50	4.50
Fish oil	0.20	3.20	6.20	9.20	12.20	15.20
Dextrin	40.00	33.00	26.00	19.00	12.00	5.00
Vitamin premix[a]	1.55	1.55	1.55	1.55	1.55	1.55
Mineral premix[b]	0.50	0.50	0.50	0.50	0.50	0.50
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05
Attractant[c]	0.30	0.30	0.30	0.30	0.30	0.30
Mold inhibitor[d]	0.10	0.10	0.10	0.10	0.10	0.10
Astaxanthin[e]	0.08	0.08	0.08	0.08	0.08	0.08
Microcrystalline cellulose	4.74	8.74	12.74	16.74	20.74	24.74
Proximate analyses						
Crude lipid	2.71	5.07	8.60	11.69	14.89	18.12
Crude protein	44.52	44.89	44.86	45.12	45.37	44.67
Ash	6.84	6.77	6.55	6.49	6.33	6.37
Astaxanthin (mg/kg)	71.03	71.87	70.57	72.32	71.48	71.57

[a] Vitamin premix (mg/kg 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; α-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2500 mg; ethoxyquin, 150 mg; wheat middling, 14.012 g.

[b] Mineral premix (mg/kg 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] Attractant: glycine and betaine.

[d] Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.

[e] Astaxanthin: Carophyll® pink, astaxanthin 10%, DSM.

2.3. Sample collection and analysis

At the end of the feeding trial, fish were not fed for 24 h. Total number and weight of fish in each cage were determined. Six fish per cage were randomly selected and stored at −20 °C for body composition analysis. Ventral skin and left side dorsal skin of four fish per cage were sampled. The skin samples were covered with aluminum and stored at −20 °C for carotenoids and melanin content analyses. Alongside this, four fish per cage were sampled between 19:30 and 22:00 to evaluate skin color using a portable Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan) (Yi et al., 2014). The color parameters were L^*, a^* and b^* for lightness, redness and yellowness, respectively, in accordance with the recommendation of the International Commission on Illumination (CIE, 1976). Measurements were performed on the ventral skin and the left dorsal skin.

Carotenoids contents 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 of ethyl acetate:ethanol (1:1 v/v) and centrifuged (4000*g*, 5 min). The supernatant was collected, and then the pellet was extracted with 5 mL of ethyl acetate followed by 10 mL of hexane. The supernatants from the above three steps were pooled together and dried under a stream of pure nitrogen. Samples were resuspended in 4 mL of acetone with 0.02% BHT and centrifuged (10,000*g*, 5 min). Carotenoids contents were measured by spectrophotometer (UV-2401PC, Kyoto, Japan). Carotenoids content was expressed as the extinction coefficients $E_{(1\%,\ 1\ cm)} = 1900$ at 474 nm (Foss et al., 1984) for diets and $E_{(1\%,\ 1\ cm)} = 2500$ (Schiedt and Liaaen-Jensen, 1995) at 448 nm for skin.

Melanin content was measured by the method of Wilson and Dodd (1973). A sepia melanin synthetic standard was purchased from Sigma-Aldrich (M-2649, Sigma-Aldrich, USA). The proximate compositions of the experimental diets and carcasses were determined following the methods of Association of Official Analytical Chemists (AOAC, 1995).

2.4. Calculations and statistical analysis

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

$$\text{Weight gain rate} = 100 \times (\text{final mean weight} - \text{initial mean weight})/\text{initial mean weight}.$$

$$\text{Feed intake} = \text{feed consumption}/(\text{days} \times (\text{final body weight} + \text{initial body weight})/2)$$

$$\text{Carotenoids content} = 10000 * V * A/W/E_{(1\%,\ 1\ cm)}$$

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

Table 2
Effects of dietary lipid content on survival and growth of large yellow croaker *Larimichthys croceus*.

Diets	Initial weight (g)	Final weight (g)	Weight gain rate [a] (%)	Survival (%)	Feed intake [b] (g/fish/day)
L3	10.01 ± 0.03	31.40 ± 3.19^{a}	282.58 ± 14.30^{a}	91.11 ± 3.89	0.43 ± 0.01^{a}
L6	10.07 ± 0.05	34.31 ± 0.34^{ab}	340.82 ± 4.54^{ab}	93.33 ± 5.85	0.37 ± 0.01^{b}
L9	9.93 ± 0.03	41.97 ± 2.57^{ab}	409.24 ± 16.22^{bc}	100.00 ± 0.00	0.32 ± 0.01^{c}
L12	9.98 ± 0.02	46.83 ± 2.36^{b}	445.94 ± 12.61^{c}	96.66 ± 3.33	0.30 ± 0.01^{cd}
L15	10.04 ± 0.03	43.82 ± 2.33^{ab}	430.18 ± 17.98^{bc}	94.16 ± 0.84	0.29 ± 0.01^{cd}
L18	10.06 ± 0.03	45.47 ± 4.82^{ab}	437.80 ± 25.68^{c}	89.44 ± 2.42	0.27 ± 0.01^{d}

Values are means and standard errors of three replicates.
Means with different superscripts in the same column are significantly different ($P < 0.05$)
[a] Weight gain rate = 100 × (final weight - initial weight)/initial weight.
[b] Feed intake (g/fish/day) = feed consumption/(days × (final body weight + initial body weight)/2).

Data analyses were performed by SPSS 17.0 for windows. The data were analyzed by one-way ANOVA. When significant difference was detected ($P < 0.05$), Tukey multiple range test was used to rank the treatment. Regression analysis was conducted among whole body lipid, yellowness values and carotenoids contents both in dorsal skin and ventral skin. The relationship between dietary lipid content and the whole body lipid content was analyzed by the broken-line model (Robbins et al., 1979). The optimal dietary lipid content for growth and pigmentation was estimated by the broken-line analysis model using the results of specific growth rate, ventral skin yellowness and ventral skin carotenoids content.

3. Results

3.1. Growth performance

The effects of dietary lipid contents on growth and survival rate are shown in Table 2. No significant difference on survival rate (SR) was observed ($P > 0.05$), varying from 89.44% to 100.00% among treatments. Dietary lipid content significantly affected the weight gain rate (WGR) ($P < 0.05$), which increased with the increasing of dietary lipid content up to 11.7%. Feed intake (FI) significantly decreased with the increasing of dietary lipid contents from 2.7% to 8.6% ($P < 0.05$). Dietary lipid contents higher than 11.7% did not result in further significant decreases of FI. The minimum requirement of dietary lipid for growth was estimated to be 10.42% on the basis of weight gain rate using broken-line analysis (Fig. 1).

3.2. Body composition

Data of body composition are shown in Table 3. Whole body crude protein and ash were similar in all groups. The crude protein and ash in the whole body ranged from 16.25% to 16.96% and 3.53% to 3.93%, respectively. The whole body moisture contents were significantly decreased from 75.88% to 71.96% by dietary lipid contents. However, the whole body crude lipid contents increased with the increasing dietary lipid contents up to 11.7%. Dietary lipid contents higher than 11.7% did not result in further significant increases of body crude lipid. It was estimated at a plateau (8.75%), when the dietary lipid content was higher than 11.07% using the broken-line analysis (Fig. 2).

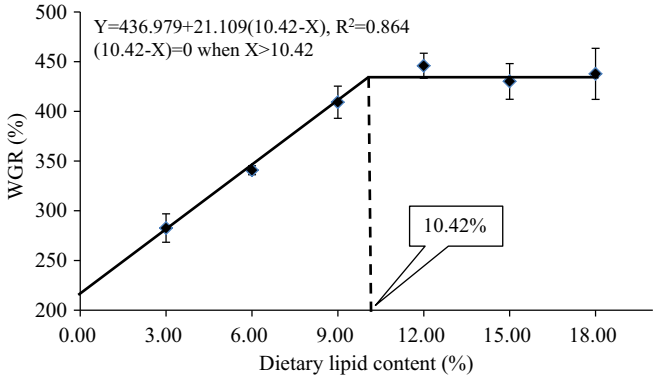

Fig. 1. Based on the broken-line analysis, relationship between dietary lipid content and the weight gain rate (WGR) indicates that the minimum requirement of dietary lipid for the growth of large yellow croaker was 10.42%. Each point represents the mean of three replicates.

3.3. Skin coloration

Data of skin color are presented in Table 4. There was little variation in dorsal skin lightness (L^*) and ventral skin lightness among all treatments ($P > 0.05$). Ventral skin lightness values were higher than those in dorsal skin. The lowest redness (a^*) values in ventral skin and dorsal skin were showed in the treatment with 2.7% of dietary lipid, though no significant differences were found ($P > 0.05$). As for dorsal skin yellowness (b^*), no significant differences were found among all treatments ($P > 0.05$). However, the ventral skin yellowness was significantly affected by dietary lipid content ($P < 0.05$) increasing with higher levels of dietary lipid up to 11.7%. Yellowness values (y) responded to the whole-body lipid content (x) both in ventral skin ($y = 38.367 + 1.803x$, $R^2 = 0.819$, $P = 0.013$) and dorsal skin ($y = 16.863 + 0.455x$, $R^2 = 0.585$, $P = 0.076$) (Table 6). The minimum requirement of dietary lipid for pigmentation was estimated to be 12.00% on the basis of ventral skin yellowness using the broken-line model analysis (Fig. 3).

3.4. Contents of carotenoids and melanin

Contents of carotenoids and melanin in skin are listed in Table 5. No significant differences were found in dorsal skin carotenoids contents and melanin contents among all treatments ($P > 0.05$). In contrast to the dorsal skin, no melanin was detected in the ventral skin. Dietary lipid content significantly affected the carotenoids content in ventral skin ($P < 0.05$). The ventral skin carotenoids content increased with the increasing dietary lipid contents up to 14.9%. A positive correlation between yellowness values (x) and carotenoids contents (y) was detected in ventral skin ($y = -171.5 + 5.228x$, $R^2 = 0.827$, $P = 0.012$).

Table 3
Effects of dietary lipid content on the body composition of large yellow croaker *Larimichthys croceus* (wet basis, %).

Diets	Moisture	Crude protein	Crude lipid	Ash
L3	75.88 ± 0.40^{a}	16.96 ± 0.32	5.55 ± 0.14^{a}	3.93 ± 0.14
L6	73.90 ± 0.49^{ab}	16.88 ± 0.37	5.78 ± 0.23^{a}	3.81 ± 0.04
L9	72.72 ± 0.42^{bc}	16.26 ± 0.05	7.91 ± 0.52^{ab}	3.60 ± 0.10
L12	72.60 ± 0.65^{bc}	16.45 ± 0.28	8.75 ± 0.60^{b}	3.53 ± 0.11
L15	71.27 ± 0.56^{c}	16.33 ± 0.10	8.89 ± 0.58^{b}	3.53 ± 0.08
L18	71.96 ± 0.53^{c}	16.25 ± 0.35	8.61 ± 0.46^{b}	3.61 ± 0.06

Values are means and standard errors of three replicates.
Means with different superscripts in the same column are significantly different ($P < 0.05$).

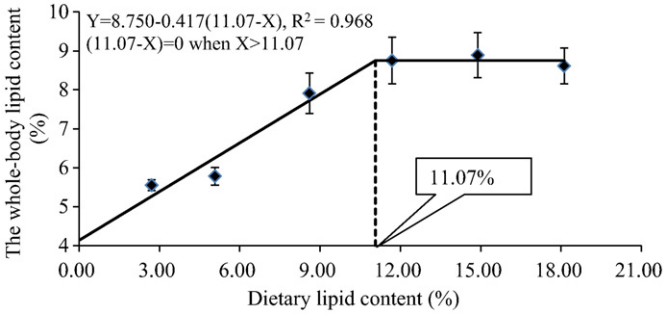

Fig. 2. Based on the broken-line analysis, relationship between dietary lipid content and whole-body lipid content indicates that the minimum dietary lipid content for the highest whole-body lipid content was 11.07%. Each point represents the mean of three replicates.

Ventral skin carotenoids content was highly related to the whole-body lipid content ($y = 15.769 + 11.184x$, $R^2 = 0.953$, $P = 0.001$). Nevertheless, dorsal skin carotenoids content presented low correlation with the whole-body lipid content ($y = 68.76 - 0.2838x$, $R^2 = 0.023$, $P = 0.776$) (Table 6). Based on the ventral skin carotenoids content, broken-line analysis showed that the minimum requirement of dietary lipid for pigmentation of large yellow croaker was estimated to be 13.19% (Fig. 4).

4. Discussion

As an important energy source, proper inclusion of dietary lipid can improve the growth of fish, especially for carnivorous species (De Silva et al., 2001; Helland and Grisdale-Helland, 1998; Hillestad and Johnsen, 1994; Lee et al., 2002; Skalli et al., 2004; Torstensen et al., 2001). When fish are fed a diet containing excessive lipid, growth could be decreased (Daniels and Robinson, 1986; Du et al., 2005; Espinós et al., 2003; López et al., 2006; Murai et al., 1985; Pei et al., 2004; Silverstein et al., 1999; Weatherup et al., 1997). In the present study, the weight gain rate increased with the increasing of dietary lipid contents up to 11.7% and kept steady thereafter. Broken-line analysis showed that the requirement of dietary lipid for large yellow croaker was estimated to be 10.42%. This requirement is higher than that observed in hybrid tilapia *Oreochromis niloticus* × *Oreochromis aureus* (5%) (Chou and Shiau, 1996) and Senegalese sole *Solea senegalensis* (8%) (Borges et al., 2009). Meanwhile, it is similar to those for grouper *Epinephelus coioides* (10%) (Luo et al., 2005) and Asian sea bass *Lates calcarifer* (10%) (Catacutan and Coloso, 1995). However, it is lower than that for meagre *Argyrosomus regius* (17%) (Chatzifotis et al., 2010). The determination of lipid requirement of fish is influenced by a variety of factors, such as dietary protein content, carbohydrate content, fish life stage, environmental temperature (NRC, 2011). Generally, carnivorous fish require higher dietary lipid than the omnivorous fish. Large yellow croaker, grouper, meagre and Asian sea bass are carnivorous species, which require higher dietary lipid than those of omnivorous fish, such as tilapia and Senegalese sole.

The correlation between dietary lipid content and the whole-body lipid content has been well studied. Increasing dietary lipid content is associated with an increase in the whole-body lipid content and a decrease in whole-body moisture content (Du et al., 2005; Lim et al., 2009; Luo et al., 2005). In the present study, the whole-body moisture contents were also inversely related to the whole-body lipid contents. This is in line with the previous studies on European sea bass *Dicentrarchus labrax* (Peres and Oliva-Teles, 1999) and rainbow trout *Salmo gairdneri* (Cho and Watanabe, 1985) where it was found that dietary lipid increased the whole-body lipid content to a certain limit, and higher dietary lipid contents did not promote further increase of the whole-body lipid deposition. Results from the present study showed that the minimum dietary lipid requirement for the highest whole-body lipid content is close to that for growth (11.07% vs. 10.42%).

In the present study, the lightness of ventral skin was higher than that of dorsal skin. This could be due to the difference of melanin content between dorsal skin and ventral skin. No melanin was detected in the ventral skin. However, high melanin content was found in the dorsal skin (Table 4). Xue et al. (2011) found that higher dorsal skin lightness of darkbarbel catfish *Pelteobagrus vachelli* was associated with lower melanin content. Similar results were also reported in the skin of Chinese longsnout catfish *Leiocassis longirostris* (Günther) (Dong et al., 2011) and red porgy (Pavlidis et al., 2008). At the same time, in the present study, there were no significant differences in melanin contents in dorsal skin among the all treatments with different dietary lipid contents. This result was in agreement with the previously mentioned study on darkbarbel catfish (Yuan et al., 2008). Many factors may influence the synthesis of melanin. For example, unsaturated fatty acid such as oleic acid and linoleic acid decreases melanin synthesis, while saturated fatty acids increase it (Ando et al., 1998). Apart from nutritional factors, environment factors also can affect melanin synthesis within the skin of fish. These factors include background color, light intensity, water temperature, and light spectrum (Kalinowski et al., 2007; Papoutsoglou et al., 2000; Pavlidis et al., 2008; Rotllant et al., 2003).

Carotenoids are lipid-soluble compounds. Dietary lipid content may influence the carotenoids' digestibility, absorption and metabolism in fish and as such could result in different pigmentation (Torrissen et al., 1990). In many studies on salmonids, better deposition of pigment and coloration of flesh were observed in fish fed with high as compared to low dietary lipid (Bjerkeng et al., 1997; Chan et al., 2002; Choubert and Baccaunau, 2006; Regost et al., 2001; Torrissen, 1985). However, some studies also showed that the deposition of carotenoids and/or coloration in flesh was not significantly affected by dietary lipid content (Einen and Skerde, 1998; Sheehan et al., 1996). In the present study, there existed ventral skin yellowness and carotenoids content plateaus when fish were fed with a certain high level of dietary lipid. In a previous study, the color saturation point was also observed in ventral skin when the large yellow croaker fed with 37.5 mg/kg or 75 mg/kg of astaxanthin for 9 weeks (Yi et al., 2014). Based on the ventral skin yellowness and carotenoids content, broken-line analysis showed that the minimum requirements of dietary lipid for large yellow croaker were estimated to be 12.00% and 13.19%, respectively (Figs. 3 and 4).

Table 4
Effects of dietary lipid content on the skin color of large yellow croaker *Larimichthys croceus*.

Diets	Dorsal skin			Ventral skin		
	L^{*a}	a^{*a}	b^{*a}	L^*	a^*	b^*
L3	72.01 ± 0.99	−3.30 ± 0.28	19.36 ± 0.40	80.16 ± 0.42	0.63 ± 0.03	48.37 ± 0.74^{a}
L6	72.16 ± 0.78	−2.80 ± 0.16	19.66 ± 1.46	79.75 ± 0.45	1.88 ± 0.13	49.18 ± 1.08^{a}
L9	72.60 ± 0.11	−2.44 ± 0.37	19.82 ± 0.19	79.32 ± 0.93	0.92 ± 0.07	50.86 ± 0.95^{ab}
L12	70.69 ± 0.93	−2.71 ± 0.21	20.37 ± 0.97	79.28 ± 0.32	2.46 ± 0.39	55.58 ± 0.54^{c}
L15	70.60 ± 1.18	−2.58 ± 0.11	20.88 ± 1.00	79.47 ± 0.36	1.77 ± 0.56	53.15 ± 0.44^{bc}
L18	74.14 ± 0.50	−2.55 ± 0.27	21.80 ± 0.67	80.15 ± 0.33	2.30 ± 0.46	55.09 ± 0.34^{c}

Values are means and standard errors of three replicates.
Means with different superscripts in the same column are significantly different ($P < 0.05$).
[a] L^*, a^* and b^* represent lightness, redness and yellowness, respectively.

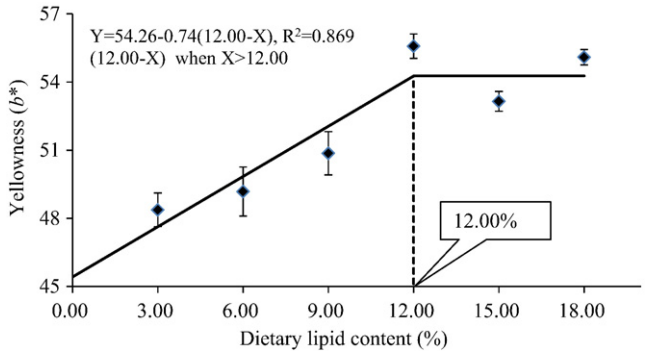

Fig. 3. Based on the broken-line analysis, relationship between dietary lipid content and ventral skin yellowness (b^*) indicates that the minimum requirement of dietary lipid for the pigmentation of large yellow croaker was 12.00%. Each point represents the mean of three replicates.

It is suggested that the minimum requirement of dietary lipid for skin pigmentation of large yellow croaker was higher than that for growth (10.42%). This was in line with the previous study on Atlantic salmon. In that study, fish fed diet with 30.8% of crude lipid got the highest growth. However, the highest astaxanthin deposition in fillet was found in fish fed diet with 38.9% of crude lipid (Einen and Roem, 1997).

In the present study, significantly positive correlations between skin yellowness and the whole-body lipid content as well as carotenoids content and the whole-body lipid content were found in the ventral skin (Table 6). This result is in agreement with a previous study on rainbow trout (Abdul-Malak et al., 1975), in which a positive correlation between canthaxanthin deposition and lipid contents in fillet was found. Christiansen and Wallace (1988) also reported a similar positive correlation between fillet canthaxanthin contents and lipid contents in small (1+) Arctic charr *Salvelinus alpinus* L.

Although the lowest redness values were found in fish fed with 2.7% of dietary lipid, no significant differences in redness were found in ventral skin or dorsal skin among the treatments. It is suggested that the dietary lipid had no significant effect on skin redness of large yellow croaker. However, in salmonids, dietary lipid contents significantly improved the fillet redness (Choubert and Baccaunau, 2006; Regost et al., 2001). The discrepancy in these results is likely due to species-specific differences in pigment deposition. Salmonids would prefer to deposit red pigments in the body resulting in reddened flesh and skin coloration. However, large yellow croaker would prefer to deposit pigments in the skin resulting in gold-yellow skin. Similarly, gilthead sea bream *Sparus aurata* can convert astaxanthin to lutein esters and epilutein esters to exhibit golden forefront (Gomes et al., 2002). Channel catfish *Ictalurus punctatus* can use red pigments and yellow pigments resulting in yellow skin and muscle (Li et al., 2007).

Table 5
Effects of dietary lipid content on the contents of carotenoid and melanin in dorsal skin and ventral skin of large yellow croaker *Larimichthys croceus*.

Diets	Carotenoid (mg/kg)		Melanin (mg/kg)	
	Dorsal skin	Ventral skin	Dorsal skin	Ventral skin [a]
L3	66.85 ± 2.51	76.77 ± 2.74[a]	68.36 ± 1.80	ND
L6	66.74 ± 4.29	83.54 ± 4.56[ab]	67.43 ± 2.44	ND
L9	69.52 ± 4.55	97.87 ± 0.56[bc]	77.29 ± 4.24	ND
L12	61.30 ± 4.20	112.98 ± 3.97[cd]	69.89 ± 3.69	ND
L15	68.74 ± 7.71	119.56 ± 0.63[d]	76.16 ± 3.94	ND
L18	66.50 ± 1.76	112.64 ± 1.06[cd]	72.32 ± 3.30	ND

Values are means and standard errors of three replicates.
Means with different superscripts in the same column are significantly different ($P < 0.05$).
ND: not detectable.
[a] No melanin can be detected in ventral skin.

Table 6
Regression analysis between the whole body lipid content (x) and the color parameters (y) in dorsal skin and ventral skin.

Sample area	Color parameters	Equation	R^2	P
Ventral skin	Yellowness	$y = 38.367 + 1.803x$	0.819	0.013
	Carotenoids content	$y = 15.769 + 11.184x$	0.953	0.001
Dorsal skin	Yellowness	$y = 16.863 + 0.455x$	0.585	0.076
	Carotenoids content	$y = 68.76 - 0.2838x$	0.023	0.776

Some studies reported that color parameters and carotenoids content in fish were linearly related (Baker et al., 2002; Ingle de la Mora et al., 2006). However, several researchers have shown that a better relationship between color parameters and carotenoids content may be achieved by non-linear relationships (Christiansen et al., 1995; King, 1996). In the present study, the carotenoids content in ventral skin had a highly linear relationship with yellowness ($Y = -171.5 + 5.228X$, $R^2 = 0.827$, $P = 0.012$). However, there was no significantly linear relationship between yellowness and carotenoids content in dorsal skin. This may be due to the deposition of carotenoids being lower in the dorsal skin compared to the ventral skin, and no significant differences were found between groups fed with different dietary lipid contents. Meanwhile, no significantly linear relationship was found between the lightness and carotenoids content or the redness and carotenoids content. This is in agreement with a previous study on rainbow trout (Ingle de la Mora et al., 2006), in which it was found that lightness was not significantly correlated to the carotenoids content, while redness and yellowness were well linear related. The discrepancy in these results is likely due to the species-specific differences in pigment deposition and the corresponding skin and/or fillet coloration. Generally, for salmonids, redness exhibits the best correlation to the increasing carotenoids contents (Bjerkeng, 2000). As a fish species characterized with golden-yellow skin, it is the yellowness parameter which shows the highest correlation to carotenoids content in the skin of large yellow croaker.

In conclusion, under the present experimental conditions, dietary lipid content significantly influenced the growth, body composition and pigmentation of juvenile large yellow croaker. The minimum dietary lipid requirement was estimated to be 10.42% based on the WGR. The whole-body lipid content was estimated to reach a plateau (8.75%), when the dietary lipid content was higher than 11.07%. For pigmentation, the minimum dietary lipid requirement was estimated to be 12.00% and 13.19% regarding the ventral skin yellowness and carotenoids content, respectively.

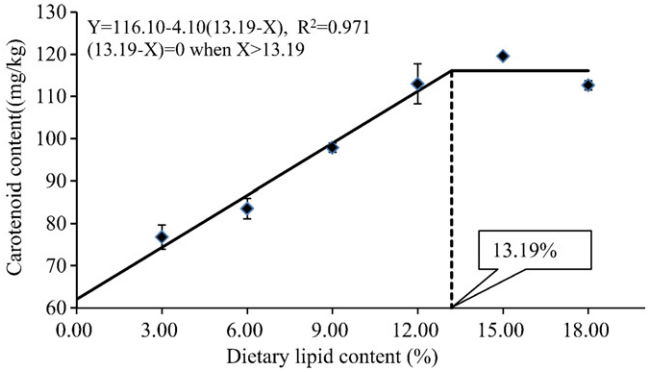

Fig. 4. Based on the broken-line analysis, relationship between dietary lipid content and carotenoids content in ventral skin indicates that the minimum requirement of dietary lipid for the pigmentation of large yellow croaker was 13.19%. Each point represents the mean of three replicates.

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