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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.