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Shrimp shell meal in diets for large yellow croaker *Larimichthys croceus*: Effects on growth, body composition, skin coloration and anti-oxidative capacity

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ABSTRACT

A 9-week feeding trial was conducted to evaluate the effects of dietary shrimp shell meal (SSM) on the growth performance, body composition, skin coloration and anti-oxidative capacity of large yellow croaker *Larimichthys croceus*. A control diet (SM0) without SSM, a diet with 12% of SSM (SM12) and a diet with 24% of SSM (SM24) were each fed to three groups of 70-g large yellow croaker. Results showed that the specific growth rate (SGR), survival rate (SR) and protein efficiency ratio (PER) in the SM24 group were significantly lower than those in the SM12 and SM0 groups ($P < 0.05$). The whole-body lipid content in SM24 group was significantly lower than that in the other two groups ($P < 0.05$). The skin coloration and carotenoid content were significantly improved with the supplementation of SSM in the diet ($P < 0.05$). Compared to those in the SM0 group, dorsal skin melanin content and tyrosinase activity were significantly higher in SM12 group and SM24 group ($P < 0.05$). The amount of thiobarbituric acid reactive substances (TBARSs), the superoxide dismutase activity (SOD) and the concentration of reduced glutathione (GSH) in the liver were not significantly affected by dietary SSM ($P > 0.05$). However, the total anti-oxidative capacity (T-AOC) in the SM12 group was significantly higher than that in SM0. In conclusion, dietary SSM can improve the skin coloration with no negative effects on the anti-oxidative capacity of large yellow croaker. However, high dietary SSM (24%) decreased the growth performance and whole-body lipid content.

1. Introduction

Large yellow croaker *Larimichthys croceus* is one of the commercially important mariculture species in China. The yellow-golden-red skin coloration of this fish species is an important attribute to attract the consumer. Therefore, the skin color markedly affects the market price and consumer acceptability of this species. Yi et al. (2014) reported that the supplementation of astaxanthin and xanthophylls in the diet can significantly improve the skin color of large yellow croaker. Meanwhile, the previous studies also found that the skin color of red porgy *Pagrus pagrus* (Kalinowski et al., 2005) and Japanese ornamental carp *koi*, *Cyprinus carpio* L.(Sun et al., 2012) was significantly affected by dietary carotenoids.

Crustacean exoskeleton is generally rich in pigments, which is a good potential resource for the enhancement of farmed fish red and yellow skin pigmentation. By-products from shrimp and crab may contain 34–147 mg/kg carotenoids (Shahidi and Synowiecki, 1991). Latscha (1989) found that up to 98% total carotenoids extracted from various wild shrimp species were astaxanthin and its mono- and di-esters. Meanwhile, free astaxanthin accounted for 4.5–16.7% total astaxanthin. Red porgy fed with 16% of SSM for a period of 120 to 180 days achieved similar skin coloration to the wild fish (Kalinowski et al., 2007). Similarly, García-Romero et al. (2014b) showed that the supplementation of marine crab meal significantly enhanced the skin color of red porgy. Moreover, higher skin redness was found on the Atlantic cod *Gadus morhua* L. fed with the krill meal diet in contrast to a diet without krill meal (Karlsen et al., 2006). Meanwhile, pigments, which are part of this raw material, are known as antioxidants. García-Romero et al. (2014b) found that red porgy fed diet with spider marine crab *Paramola cuvieri* meal for 180 days delayed the fillet lipid oxidation during refrigerated storage, which may mainly be due to the high astaxanthin content in the crab meal. Previous studies showed that the supplementation of pigments can significantly improve the anti-oxidative capacity and stress response in large yellow croaker (Li et al., 2014), Japanese flounder *Paralichthys olivaceus* (Pham et al., 2014) and characins *Hyphessobrycon callistus* (Wang et al., 2006).

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Apart from its use as a pigment source, crustacean meal or by-products have become an attractive potential protein resource for aquafeeds (García-Romero et al., 2014a,b). Partial replacement of fishmeal by crustacean meal or crustacean by-product meal in diets of red porgy (García et al., 2010; García-Romero et al., 2014a,b; Kalinowski et al., 2007), Atlantic cod (Hansen et al., 2013) and Nile tilapia *Oreochromis niloticus* L. (El-Sayed, 1998), respectively, has yielded successful growth results.

China is one of the leading countries in shrimp culture with a production of 2,780,844 metric tons in 2013 (China Fishery Statistical Yearbook, 2014). Over 500,000 metric tons of shrimp shell is generated from the industrial processing of farmed shrimps (Duan et al., 2012). In the wild, the crustaceans are part of the natural diet of the large yellow croaker. Thus, the aim of the present study is to evaluate the effects of dietary SSM on the growth performance, body composition, skin coloration and anti-oxidative capacity of the large yellow croaker.

2. Materials and methods

2.1. Shrimp shell meal and the experimental diets

Fresh shrimp shells were collected from an aquatic products trading market in Qingdao, China. The shrimp shells were first dried in a ventilated oven at 35 °C for 10 h, and then ground in a mill. Ethoxyquin (0.5%) was used to protect the carotenoids in shrimp shells. Following, the resulting powder was sieved through a 160 μm mesh. The shrimp shell meal contained 47.62% crude protein, 6.12% crude lipid and 141 mg/kg astaxanthin.

Three experimental diets were designed to contain 43% crude protein and 13% crude lipid (Table 1). The control diet (SM0) without SSM supplementation consisted of 44.0% of fish meal. On the basis of the SM0, the other two diets were formulated to have 12% of SSM and 36.2% of fish meal (SM12) and 24% of SSM and 28.3% of fish meal (SM24), respectively. All finely ground dietary ingredients were mixed with water (300 g/kg) and then extruded by an experimental feeding mill into proper pellet size (4 mm × 5 mm). The diets were then dried in a ventilated oven at 40 °C for 10 h. All dried diets were stored at −20 °C. The astaxanthin content of SM0, SM12 and SM24 was 0.00 mg/kg, 15.83 mg/kg and 33.84 mg/kg, respectively (Table 1).

Table 1
Feed formulation and proximate composition of the experimental diets (% dry matter).

Ingredient	SM0	SM12	SM24
Fishmeal[1]	44.00	36.20	28.30
Soybean meal[1]	19.00	19.00	19.00
Wheat meal[1]	20.08	16.09	12.27
Shrimp shell meal[2]	0.00	12.00	24.00
Lecithin[1]	2.50	2.50	2.50
Fish oil[1]	3.61	3.34	3.00
Soybean oil[1]	3.87	3.92	3.98
Vitamin premix[3]	2.00	2.00	2.00
Mineral premix[4]	2.00	2.00	2.00
Taurine	0.50	0.50	0.50
Attractant[5]	0.30	0.30	0.30
Mold inhibitor[6]	0.10	0.10	0.10
Ethoxyquin	0.10	0.10	0.10
Proximate analyses			
Moisture (%)	5.54	4.67	4.48
Protein (%)	43.78	43.48	43.55
Lipid (%)	13.01	12.70	12.87
Ash (%)	12.81	14.32	15.59
Astaxanthin (mg/kg)	0.00	15.83	33.84

[3–6]All those ingredients were supplied by Qing Dao Master Bio-Tech, Co., Ltd. (Qingdao China).

[1] Those ingredients were supplied by Qingdao Great Seven Bio-Tech, Co., Ltd. (Qingdao China). Fishmeal: crude protein 65.6%, crude lipid 5.5%; soybean meal: crude protein 51.31%, crude lipid 1.84%; wheat meal: crude protein 15.25%, crude lipid 1.19%.

[2] Shrimp shell meal: crude protein 47.62%, crude lipid 6.12%, astaxanthin 141 mg/kg.

[3] Vitamin premix (mg/kg or g/kg diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B12, 0.1 mg; vitamin K3, 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; 50% alpha-tocopheryl acetate, 240 mg; 35% ascorbic acid polyphosphate, 2000 mg; choline chloride, 2500 mg; ethoxyquin, 150 mg; wheat middling, 14.012 g.

[4] 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; $MnSO_4 \cdot H2O$, 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] Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.

2.2. Experimental procedure

The feeding trial was conducted in Ningde, Fujian Province, China. Large yellow croaker (600 fish) was obtained from the National Breeding Station of Large Yellow Croaker and reared in a floating sea cage (4.0 × 8.0 × 4.0 m) for 3 weeks to acclimate to the test environment. During the acclimation, fish were fed with the control diet. Prior to the feeding trial start, fish were fasted for 24 h and then weighed after being anesthetized with eugenol (1:10,000) (Shanghai Reagent Corp., China). Fifty fish with an initial weight of 70.24 ± 0.20 g (means ± standard error) were distributed to each of 9 sea cages (1.5 × 1.5 × 2.0 m) at a density of 0.78 kg/m^3. Fish were carefully hand fed until apparent satiation twice daily (5:00 and 17:00) for 9 weeks. During the acclimation and the feeding trial, water temperature fluctuated from 18 to 28 °C, salinity from 28 to 32‰ and the oxygen level from 6.5 to 7.9 mg/L.

2.3. Sampling and analysis

At the end of the feeding trial, fish were fasted for 24 h and then anesthetized with eugenol. After that, the number and weight of fish in each cage were collected. The body weight was obtained from bulk weighing cage by cage. Five fish per cage were randomly selected for proximate analysis of the whole-body compositions. After measuring the weight and length individually, another six fish per cage were randomly chosen for the samples of the ventral skin, left side dorsal skin and liver. All samples were stored at −20 °C. Skin coloration measurement was performed on the ventral skin and the left side dorsal skin at night (20:00–23:00) following the method of Yi et al. (2014). A portable Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan) was used. The color parameters were L^*, a^* and b^*, which represented lightness, redness and yellowness, respectively.

Carotenoid contents in the diets and skin were tested according to the method of Yi et al. (2014). They were expressed as the extinction coefficients $E_{(1\%,\ 1\ cm)} = 1900$ at 474 nm (Foss et al., 1984) for the diet and $E_{(1\%,\ 1\ cm)} = 2500$ at 448 nm (Schiedt and Liaaen-Jensen, 1995) for the skin. Crude lipid, crude protein, ash and moisture of the experimental diets and the whole fish body were determined following the methods of Association of Official Analytical Chemists (AOAC, 1995). Skin melanin content was measured by the method of Wilson and Dodd (1973). Melanin standard (Sigma-Aldrich, M-2649) was purchased from Sigma-Aldrich. Skin tyrosinase activity was determined according to the method of Chen et al. (2005).

Lipid peroxidation was measured by the amount of thiobarbituric acid reactive substances (TBARSs) in the liver. The TBARS was tested using a QuantiChrom™ TBARS Assay Kit (DTBA-100, BioAssay systems, USA) and the concentration was expressed as the malondialdehyde (MDA) equivalents (nmol/mg protein). The superoxide dismutase (SOD), reduced glutathione (GSH) and total anti-oxidative capacity (T-AOC) in the liver were measured with the commercial assay kits (Nanjing Jiancheng Bio-engineering Institute, China). One unit of SOD activity was calculated using the amount of superoxide dismutase required to inhibit the reduction of nitroblue trazolium by 50%. One unit of T-AOC was defined as the amount of enzyme that can increase the absorbance by 0 · 01 in 1 min at 37 °C. GSH content was expressed as μmol GSH per g protein.

2.4. Calculations and statistical analysis

The survival of large yellow croaker was expressed as the survival rate, growth performance as the specific growth rate, feed utilization as the protein efficiency ratio, feed conversion ratio and feed intake. Meanwhile, the condition factor was used as a parameter for body index. The calculation formulae for these parameters were as follows:

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

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

$$\text{Protein efficiency ratio} = \text{weight gain}\ (\text{g})/\text{protein intake}\ (\text{g})\ (\text{dry matter})$$

$$\text{Feed conversion ratio} = \text{dry feed fed}\ (\text{g})/(\text{final body weight} - \text{initial body weight} + \text{dead fish weight})\ (\text{g})$$

$$\text{Feed intake}\ (\%/\text{day}) = 100 \times \text{feed fed}/[\text{days} \times (\text{initial body weight} + \text{final body weight} + \text{dead fish weight})/2]$$

$$\text{Condition factor} = 100 \times \text{body weight}\ (\text{g})/(\text{body length}\ (\text{cm}))^3.$$

Results were presented as means ± S.E.M. All data were subjected to one-way ANOVA by SPSS 15.0 for windows. Regression analysis was conducted between color parameters and carotenoid content. The level of significance was chosen at $P < 0.05$, and Tukey's test was used to compare the mean values.

3. Results

3.1. Growth performance and feed utilization

Data on growth and feed utilization are shown in Table 2. The final body weight (FBW), specific growth rate (SGR), survival rate (SR), protein efficiency ratio (PER) and condition factor (CF) of fish fed with SM24 diet were significantly lower than those fed with SM12 and SM0 diets, however, the feed conversion ratio (FCR) of SM24 was significantly higher than the other two diets ($P < 0.05$). There were no significant differences in the above parameters between the SM0 group and SM12 group ($P > 0.05$). No significant difference was found in the feed intake (FI) among the three dietary treatments ($P > 0.05$).

3.2. Body composition

Data on the whole-body composition are shown in Table 3. No significant differences in the whole-body protein and moisture were found ($P > 0.05$), which ranged from 15.63% to 16.03% and from 62.13% to 67.16%, respectively. The whole-body ash contents significantly increased with the increasing of the dietary SSM supplements ($P < 0.05$) and they were positively correlated to the dietary ash contents ($y = 0.129x + 1.235$, $R^2 = 0.80$, $P = 0.001$). The whole-body lipid content in the SM24 group (13.97%) was significantly lower than that in the SM12 group (20.02%) ($P < 0.05$). The whole-body lipid contents both in the SM24 group and in the SM12 group did not significantly differ from those in the SM0 group ($P > 0.05$). The whole-body lipid content was negatively correlated to the whole-body moisture content ($y = -1.058x + 85.585$, $R^2 = 0.93$, $P = 0.000$).

Table 2
Effects of shrimp shell meal on the growth performance and feed utilization of large yellow croaker for 9 weeks.

	SM0	SM12	SM24
IBW	70.41 ± 0.32	70.10 ± 0.42	70.22 ± 0.42
FBW	135.42 ± 3.77^a	130.22 ± 1.72^a	113.63 ± 2.13^b
SR (%)	93.33 ± 1.33^a	93.33 ± 1.76^a	86.00 ± 2.00^b
SGR (%/day)	1.07 ± 0.04^a	0.98 ± 0.01^a	0.76 ± 0.02^b
CF	1.74 ± 0.01^a	1.70 ± 0.01^a	1.65 ± 0.01^b
FCR	1.38 ± 0.03^a	1.33 ± 0.07^a	1.70 ± 0.10^b
FI (%/day)	1.29 ± 0.05	1.20 ± 0.01	1.15 ± 0.03
PER	0.32 ± 0.01^a	0.26 ± 0.03^a	0.14 ± 0.02^b

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

Table 3
Effects of dietary shrimp shell meal on the body composition of large yellow croaker (wet basis, %).

Treatments	Moisture	Protein	Lipid	Ash
SM0	63.13 ± 1.56	15.97 ± 0.48	18.85 ± 1.41^{ab}	2.92 ± 0.05^a
SM12	62.47 ± 1.75	15.63 ± 0.26	20.02 ± 1.40^a	3.02 ± 0.05^b
SM24	67.16 ± 1.05	16.03 ± 0.14	13.97 ± 1.17^b	3.21 ± 0.10^c

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

3.3. Skin coloration and pigment

Data on the skin color of the experimental fish are listed in Table 4. There was no significant difference in the ventral skin lightness (L^*) or redness (a^*) among groups ($P > 0.05$). The lightness and redness of the dorsal skin decreased with the increasing supplementation of dietary SSM. Dorsal skin yellowness (b^*) of the SM24 and SM12 groups was significantly higher than that of the SM0 group ($P < 0.05$). Meanwhile, significant enhancement of ventral skin yellowness was also found with increasing levels of dietary SSM ($P < 0.05$).

Skin pigments and tyrosinase activity are presented in Table 5. Ventral skin carotenoid contents of the SM24 (66.93 μg/g) and SM12 groups (62.88 μg/g) were significantly higher than that of the SM0 group (29.58 μg/g) ($P < 0.05$). Similarly, dorsal skin carotenoid contents markedly increased from 10.88 μg/g to 18.00 μg/g when dietary SSM content increased from 0% to 24% ($P < 0.05$). No melanin and tyrosinase activity were detected in the ventral skin. However, high melanin content and tyrosinase activity were found in the dorsal skin. Dorsal skin melanin content increased from 89.02 μg/g to 107.64 μg/g when dietary SSM content increased from 0% to 24%. Dorsal skin tyrosinase activities of the SM12 and SM24 groups were significantly higher than that of the SM0 group ($P < 0.05$).

The relationship between carotenoid concentration and CIE values is presented in Table 6. Redness presented low correlation with the carotenoid contents both in the dorsal skin ($y = -3.124x + 10.077$, $R^2 = 0.277$, $P = 0.145$) and ventral skin ($y = -2.329x + 54.955$, $R^2 = 0.007$, $P = 0.829$). However, yellowness values were significantly linear related to the carotenoid contents in the dorsal skin ($y = 1.634x - 12.982$, $R^2 = 0.514$, $P = 0.030$) and ventral skin ($y = 2.385x - 83.883$, $R^2 = 0.465$, $P = 0.043$). As to the lightness, low correlation between lightness and carotenoid content was found both in the dorsal skin ($y = -1.577x + 130.074$, $R^2 = 0.365$, $P = 0.084$) and ventral skin ($y = 3.03x - 184.802$, $R^2 = 0.022$, $P = 0.703$). In contrast, lightness of dorsal skin was significantly linear related to the dorsal skin melanin ($y = 0.149x + 59.561$, $R^2 = 0.513$, $P = 0.030$).

3.4. Anti-oxidative responses in the liver

Data on the anti-oxidative responses in the liver of large yellow croaker are shown in Table 7. No significant differences were detected in MDA, GSH and SOD activities among treatments ($P > 0.05$). The T-AOC in the SM12 group was significantly higher than that in the SM0 group ($P < 0.05$). No significant differences in the T-AOC were shown between the SM0 and SM24 groups or between the SM12 and SM24 groups ($P > 0.05$).

Table 4
Effects of dietary shrimp shell meal on the skin color of large yellow croaker.

Treatments	Dorsal skin			Ventral skin		
	L^*	a^*	b^*	L^*	a^*	b^*
SM0	74.93 ± 0.32^a	-1.07 ± 0.19^a	15.11 ± 0.48^a	77.38 ± 0.76	2.15 ± 0.45	49.85 ± 1.34^a
SM12	72.44 ± 0.47^b	-1.15 ± 0.19^{ab}	17.02 ± 0.37^b	77.49 ± 0.61	2.13 ± 0.60	57.33 ± 0.35^b
SM24	72.21 ± 0.32^b	-2.14 ± 0.30^b	18.55 ± 0.45^b	77.61 ± 0.28	2.14 ± 0.07	61.16 ± 0.48^c

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

4. Discussion

4.1. Growth performance

All diets were well accepted by large yellow croaker during the 9-week feeding trial. This was shown by the FI values in the present study. Although the FI decreased with the increasing of dietary SSM supplementation, no significant differences in FI were found among all the treatments (Table 2). This was in agreement with previous studies on red porgy (García et al., 2010; Kalinowski et al., 2007). Meanwhile, García-Romero et al. (2014b) found that the supplementation of marine crab meal in the diets seems to induce the appetite response of red porgy reflected with a higher feed intake in fish fed with marine crab meal diets in contrast to those fed a control diet. Cejas et al. (2003) revealed that there were no negative effects on the growth performance and feed utilization of red porgy fed with the combination diet of 88% commercial pellets and 12% defrosted shrimp. This was in line with the study of Kalinowski et al. (2005), in which it was also found that there were no significant differences in growth performance and feed conversion ratio between red porgy fed with 8% of dietary SSM and 16% of dietary SSM for 105 days. Moreover, Kalinowski et al. (2007) reported that the red porgy fed diet with 16% of SSM for 180 days had improved growth performance and protein utilization. Shahidi and Synowiecki (1992) reported that shrimp shell proteins were well balanced in their amino acid composition and, as such, they may serve as an excellent component in aquafeed. However, high level of chitin (17–23%) and ash content (28–34%) was also found in shrimp shell (Ferrer et al., 1996; RØdde et al., 2008), which has been shown to cause low in vitro protein digestibility (60–67%) (Ibrahim et al., 1999) and low in vivo protein (66.7%) and energy (41.4%) apparent digestibility coefficients in Atlantic cod (Tibbetts et al., 2006). In the present study, the poor growth and feed utilization of SM24 group may be due to the low digestibility and utilization of SSM, because significant lower PER and higher FCR were observed in the SM24 group compared to those in the SM0 and SM12 groups (Table 2). Moreover, high supplementation of SSM and low inclusion of fish meal may reduce the feed palatability. Usually, the reduction in diet palatability results in a decrease in feed intake, which could in turn cause reduced growth (Aragão et al., 2003; Kissil et al., 2000). In this study, the feed intake decreased from 1.29%/day to 1.15%/day with the increasing of SSM from 0% to 24% and the decreasing of fishmeal from 44.00% to 28.30% in the diets, though no significant difference was found in feed intake among groups (Table 2). The exact reason for the poor growth and feed utilization in the group with 24% of dietary SSM is not clear yet. However, the present study indicated that the supplementation of 12% SSM in the diet had no significantly negative effects on survival, growth and feed utilization of large yellow croaker, while the dietary fishmeal content decreased from 44.00% in the control group to 36.20% in the SM12 group. Further study is needed to find the optimal content of SSM in diets for large yellow croaker.

4.2. Body composition

In the present study, the whole-body lipid in the SM24 group was lower than those in the SM12 and SM0 groups. This was in line with the reports on red porgy (Cejas et al., 2003; García et al., 2010) and Atlantic cod (Hansen et al., 2013). However, significantly higher whole-body lipid content was found in red porgy fed with 20% marine crab *Chaceon affinis* meal diet compared to the 10% marine crab meal diet (García-Romero et al., 2014a). Alternatively, García-Romero et al. (2014b) reported that the supplementation of 20% marine spider crab meal in the diet had no significant effect on the whole-body lipid content of red porgy. The differences in these results could be due to the differences in the crustacean species used in the diet and/or the supplementation level. A higher proportion of chitin, chitosan and ash in the diets may reduce the total digestible nutrients intake, which could be the explanation as to the whole-body lipid reduction that appears in fish fed with a higher supplementation of SSM. Chitin and chitosan can impede absorption of dietary lipids and lipid soluble vitamins by forming gels in the intestinal tract (Koide, 1998; Liao et al., 2007).

4.3. Skin coloration and pigment

Crustaceans are rich in carotenoids with most existing as the esterified astaxanthin (Shahidi and Synowiecki, 1991). Choubert and Luquet (1983) found that 88% astaxanthin in the Norwegian shrimp meal was diester astaxanthin with the remaining existing as the monoester form. The supplementation of crustacean meal in the diet can significantly improve the skin color of red porgy (Cejas et al., 2003; García et al., 2010; García-Romero et al., 2014b; Kalinowski et al., 2005; Kalinowski et al., 2007; Tejera et al., 2007). Similarly, the addition of crustacean meal in feed can also increase the fillet color and pigment content in salmonids (Choubert and Luquet, 1983; Saito and Regier, 1971). Compared to the fishmeal diet, more skin redness and muscle

Table 5
Effects of dietary shrimp shell meal on the skin pigments and tyrosinase activity of large yellow croaker.

	Skin carotenoid concentration (μg/g)		Skin melanin (μg/g)		Skin tyrosinase (U/g)	
	Ventral skin	Dorsal skin	Ventral skin	Dorsal skin	Ventral skin	Dorsal skin
SM0	29.58 ± 2.38^a	10.88 ± 1.00^a	ND	89.02 ± 4.13^a	ND	10.98 ± 0.46^a
SM12	62.88 ± 3.79^b	14.98 ± 1.48^{ab}	ND	92.27 ± 2.89^{ab}	ND	14.24 ± 0.66^b
SM24	66.93 ± 1.66^b	18.00 ± 1.56^b	ND	107.64 ± 5.13^b	ND	13.83 ± 0.34^b

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.

Table 6
Relationship between carotenoid concentration (y) and CIE (L^*, a^*, b^*) values (x) both in dorsal skin and ventral skin (n = 9).

	CIE	Equation	R^2	*P*
Dorsal skin	L^*	y = −1.577x + 130.074	0.365	0.084
	a^*	y = −3.124x + 10.077	0.277	0.145
	b^*	y = 1.634x − 12.982	0.514	0.030
Ventral skin	L^*	y = 3.03x − 184.802	0.022	0.703
	a^*	y = −2.329x + 54.955	0.007	0.829
	b^*	y = 2.385x − 83.883	0.465	0.043

whiteness and yellowness were found in Atlantic cod fed with krill meal (Karlsen et al., 2006). In the present study, large yellow croaker fed with SSM diets had higher carotenoid concentrations and yellowness both in the dorsal and ventral skin (Tables 4 and 5). Compared to the result of Yi et al. (2014), the present study showed higher ventral skin carotenoid concentration (66.93 mg/kg vs 46.07 mg/kg) with lower dietary astaxanthin content (33.84 mg/kg vs 37.5 mg/kg). Moreover, no significant differences were found in skin carotenoid concentration between fish fed with SM12 diet (15.83 mg/kg astaxanthin) and SM24 diet (33.84 mg/kg astaxanthin) in the present study. The large yellow croaker used in the present study and the previous study (Yi et al., 2014) had similar size and feeding period (9 weeks). These results suggested that the esterified astaxanthin from SSM in the present study is more efficient than the unesterified synthetic astaxanthin used in Yi et al. (2014) for the skin pigmentation of large yellow croaker. This is in agreement with reports on red porgy (Tejera et al., 2007) and sea bream *Pagrus major* (Lorenz, 1998). However, for rainbow trout and Atlantic salmon, esterified astaxanthin is less efficient than free astaxanthin for flesh pigmentation (Foss et al., 1987). Carotenoids deposited in the flesh of salmons are in the free, unesterified form (Shahidi and Brown, 1998; Storebakken and No, 1992), while the carotenoids in fish skin, such as red porgy and sea bream, are in the esterified form (Ibrahim et al., 1984; Tejera et al., 2007). Differences in utilization of alternate forms of carotenoids between species may be due to species-specific differences in the lipolytic enzyme selectivity towards fatty acids of the esterified carotenoids and the hydrolysis rate in substrate efficiency. They may also be due to the differences of the carotenoid uptake rates from the gastrointestinal tract and the subsequent pigmentation of peripheral tissues (Tejera et al., 2007).

Melanin has many biological functions, such as camouflage, sexual display purposes, anti-oxidation and protection against UV radiation (Hill, 1992; Jacobson et al., 1995). The critical step in melanin biogenesis is the oxidation of tyrosine by tyrosinase, which is active only in specialized organelles called melanophores (Riley, 1997). Many factors can influence the generation and death of skin pigment cells and melanin biosynthetic (Dong et al., 2011; Pavlidis et al., 2008). In the present study, lightness in the ventral skin was not affected by the supplementation of dietary SSM. This result was in agreement with previous studies on red porgy (García et al., 2010; García-Romero et al., 2014b; Kalinowski et al., 2007) and Atlantic cod (Karlsen et al., 2006) fed with crab meal or shrimp meal. This may be due to the fact that no melanin was detected in the ventral skin (Table 5). However, in the dorsal skin, the lightness decreased with increasing dietary SSM. This may be ascribed to the increase of tyrosinase activity with SSM supplementation, and consequently the increase of melanin content in the dorsal skin (Table 5).

In this study, yellowness significantly correlated with the carotenoid content both in the dorsal skin and ventral skin. This was in agreement with previous studies (Bjerkeng et al., 1997; Nickell and Bromage, 1998). In contrast to the yellowness, lightness and redness demonstrated low correlation with the carotenoid content in the skin. Similarly, Ingle de la Mora et al. (2006) reported that lightness was not correlated with the carotenoid content. However, in this study, the lightness was linearly related with the melanin in the dorsal skin.

4.4. Anti-oxidative activities

Beyond its role as a pigment source, astaxanthin also has various biological functions (Niu et al., 2014), such as improving anti-oxidative activities (Martin et al., 1999), elevation of stress tolerance (Pan et al., 2010) and enhancement of disease resistance (Amar et al., 2001). Li et al. (2014) reported that the supplementation of astaxanthin in the diet can significantly improve the anti-oxidative capacity and immune response of large yellow croaker. However, in the present study, astaxanthin from the SSM showed no significant improvement on the anti-oxidative capacity of large yellow croaker. The positive effects of astaxanthin may be affected by many factors. Chitosan, rich in the exoskeletons of crustaceans, is a potentially noxious molecule for some fish at certain levels (Dautremepuits et al., 2004). On the other hand, low digestibility and absorption of astaxanthin within shrimp shell meal could be a partial explanation. In a study by Choubert and Luquet (1983) almost 90% of ingested pigments were found in the feces when rainbow trout were fed with a Norwegian shrimp meal diet. Further study is needed to clarify it in large yellow croaker.

Table 7
Effects of dietary shrimp shell meal on the antioxidant activities in the liver of large yellow croaker.

	T-AOC[1] (U/mg protein)	MDA[2] (nmol/mg protein)	GSH[3] (μmol/g protein)	SOD[4] (U/mg protein)
SM0	2.94 ± 0.09[a]	5.81 ± 0.29	41.66 ± 6.23	356.06 ± 16.44
SM12	3.77 ± 0.03[b]	6.76 ± 1.05	27.62 ± 4.63	353.61 ± 21.37
SM24	3.18 ± 0.21[ab]	4.54 ± 0.69	31.07 ± 2.13	391.41 ± 4.01

Values are means and standard errors of three replicates.
Means with different superscripts in the same column are significantly different ($P < 0.05$).
[1] T-AOC: total anti-oxidative capacity.
[2] MDA: malondialdehyde.
[3] GSH: reduced glutathione.
[4] SOD: superoxide dismutase.

5. Conclusion

In the present study, the supplementation of SSM in the diet significantly improved the skin coloration and skin carotenoid content in large yellow croaker. Low supplementation of SSM (12%) had no significantly negative effects on the growth performance and feed utilization during the 9 week feeding trial. However, relative higher inclusion of shrimp shell meal (24%) significantly reduced the growth and the whole-body lipid content. The anti-oxidative capacity of large yellow croaker was not significantly affected by the supplementation of SSM in the diet. In conclusion, it was recommended that the supplementation of 12% of dietary SSM was good to the skin color improvement of large yellow croaker.

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