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Effects of dietary vitamin E and astaxanthin on growth, skin colour and antioxidative capacity of large yellow croaker *Larimichthys crocea*

X. Yi[1,2,*] | H. Shen[1,*] | J. Li[1] | Z. Wei[1] | J. Shentu[3] | W. Zhang[1] | K. Mai[1]

[1]The Key Laboratory of Aquaculture Nutrition and Feeds, Ministry of Agriculture, The Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao, China

[2]South China Agricultural University, Guangzhou, China

[3]Ningbo Ocean and Fisheries Research Institute, Ningbo, China

Correspondence
Wenbing Zhang, The Key Laboratory of Aquaculture Nutrition and Feeds, Ministry of Agriculture, The Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao, China.
Email: wzhang@ouc.edu.cn

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Abstract

A 10-week feeding trial was conducted to evaluate the effect of dietary vitamin E and astaxanthin on growth performance, skin colour and antioxidative capacity of large yellow croaker *Larimichthys crocea*. Six practical diets were formulated in a 2 × 3 factorial design to supplement with two levels of astaxanthin (25 and 50 mg/kg) and three levels of vitamin E (0, 120 and 800 mg/kg). The results showed that both the highest final body weight and specific growth rate were found in fish fed diets with 120 mg/kg vitamin E supplementation. No significant differences were found in survival rate, feed conversion ratio and protein efficiency ratio among all the treatments ($p > .05$). Skin lightness (L^*) was not significantly affected by dietary treatments ($p > .05$). Ventral skin redness (a^*) of fish fed diet with 25 mg/kg astaxanthin and 0 mg/kg vitamin E supplementation was significantly lower than that of fish fed with other diets. Yellowness (b^*) and carotenoid contents both in the dorsal and in the ventral skin were found to be significantly increased with increasing dietary astaxanthin or vitamin E ($p < .05$), but no significant interactions were found ($p > .05$). The vitamin E content in liver reflected the dietary vitamin E content. Level of vitamin E content in fish fed diets with 800 mg/kg vitamin E supplementation was significantly higher than that in fish fed with the other diets ($p < .05$). Liver superoxide dismutase activity and thiobarbituric acid reactive substance levels were found to be decreased with increasing dietary astaxanthin and vitamin E levels, respectively. Levels of reduced glutathione in the liver were found to be increased with increasing dietary vitamin E contents. The total antioxidative capacity in the liver was found to be decreased with increasing dietary vitamin E or astaxanthin contents. In conclusion, adequate dietary vitamin E can improve the growth of large yellow croaker, and the supplementation of astaxanthin and vitamin E benefited the skin coloration and antioxidative capacity of large yellow croaker.

KEYWORDS
antioxidation, astaxanthin, large yellow croaker, skin colour, vitamin E

1 | INTRODUCTION

Vitamin E is lipid soluble and comprises eight naturally occurring forms. Among them, α-tocopherol has the highest biopotency. Vitamin E can serve as parts of a multicomponent antioxidative defence system, which protects the cell against the adverse effects of reactive oxygen and other free radical initiators of the oxidation of polyunsaturated membrane phospholipids, critical proteins or both (NRC, 2011). Meanwhile, vitamin E is also involved in the

*These authors are contributed equally to this work.

regulation of growth, immune response, disease resistance and reproductive fitness (Galaz, Kim, & Lee, 2010; Montero, Tort, Robaina, Vergara, & Izquierdo, 2001; Niu, Jia, Hu, Meng, & Lei, 2014; Palace & Werner, 2006). Moreover, vitamin E can affect the tissue lipid composition in fish (Lebold et al., 2011; Mourente, Bell, & Tocher, 2007; Tocher et al., 2002). Fish cannot synthesize vitamin E, and their body vitamin E mainly depends on the dietary intake. Vitamin E deficiency may lead to darkened skin, lipid oxidation, anaemia, muscular and liver degeneration and lower growth performance (Chen et al., 2004; Montero et al., 2001; Niu, Jia et al., 2014; Pitaksong, Kupittayanant, & Boonanuntanasarn, 2013). Meanwhile, excessive doses of vitamin E might also exert a pro-oxidant effect and reduce growth performance (Li, Liang, et al., 2014).

Fish cannot synthesize carotenoids de novo. The body pigmentation mainly depends on carotenoids from the diet (Torrissen, Hardy, Shearer, Scott, & Stone, 1990). Previous studies showed that the supplementation of carotenoids can improve the body colour of fish, such as red porgy *Pagrus pagrus* (Kalinowski, Robaina, Fernández-Palacios, Schuchardt, & Izquierdo, 2005; Tejera et al., 2007), rainbow trout *Oncorhynchus mykiss* (Bjerkeng et al., 1997; Storebakken & No, 1992), Australian snapper *Pagrus auratus* (Doolan, Booth, Allan, & Jones, 2008) and red devil *Cichlasoma citrinellum* (Pan & Chien, 2009). Apart from the pigment, carotenoids also have other important biological functions, such as antioxidation (Niu, Wen, et al., 2014; Pham, Byun, Kim, & Lee, 2014; Wang, Chien, & Pan, 2006), enhancing immunity (Amar, Kiron, Satoh, & Watanabe, 2001; Christiansen, Glette, Lie, Torrissen, & WaagbØ, 1995) and stress resistance (Pan, Chien, & Hunter, 2003).

Large yellow croaker is a commercially important mariculture fish species in China. The production in 2015 was 148 616 metric tons (China Fishery Statistical Yearbook, 2016). Skin colour is one of the most important quality criteria for commercial fish species. Under intensive culture condition, farmed large yellow croaker is susceptible to lose its natural yellow colour. Several studies have shown that the skin colour of large yellow croaker was improved by dietary carotenoids and shrimp meal supplementations (Yi, Xu et al., 2014; Yi, Zhang, Xu, Li, Zhang & Mai 2014; Yi, Li, Xu, Zhang, et al., 2015; Yi, Li, Xu, Zhou, et al., 2015; Yi et al., 2016). Previous studies showed that increasing dietary vitamin E can improve the astaxanthin deposition in Atlantic salmon *Salmo salar* L (Bjerkeng, Hamre, Hatlen, & Wathne, 1999) or the canthaxanthin deposition in rainbow trout (Pozo, Lavety, & Love, 1988). However, Jensen, Birk, Jokumsen, Skibsted, and Bertelsen (1998) did not find a similar effect in rainbow trout. Bell, McEvoy, Tocher, and Sargent (2000) reported that both vitamin E and astaxanthin have antioxidant functions in Atlantic salmon, and their antioxidative synergism was observed in vitro. At present, no study on vitamin E and its interaction with pigment was found in large yellow croaker. So, the aim of this study was to investigate the effects of dietary vitamin E and astaxanthin on the growth, body composition, skin pigmentation and antioxidative capacity of large yellow croaker.

2 | MATERIALS AND METHODS

2.1 | Experimental diets

All ingredients and nutrient contents of the experimental diets are shown in Table 1. Fish meal and soybean meal were used as the main protein sources. Fish oil was used as the main lipid source. Meanwhile, wheat meal was used as the main carbohydrate source. Six diets were formulated in a 2 × 3 factorial design to supplement with two levels of astaxanthin (25 and 50 mg/kg) and three levels of α-tocopherol (0, 120 and 800 mg/kg) in the form of DL-α-tocopherol acetate. All fine-grounded ingredients were mixed with water (300 g/kg) and then extruded by an experimental feeding mill into proper pellet size (2 mm × 3 mm). After that, the diets were dried in a ventilated oven at 40°C until the moisture below 100 g/kg. All diets were stored at −20°C.

2.2 | Experimental procedure

This study was performed in strict accordance with the Standard Operation Procedures (SOPs) of the Guide for the Use of Experimental Animals of Ocean University of China. All animal care and use procedures were approved by the Institutional Animal Care and Use Committee of Ocean University of China (Permit Number: 20,001,001). The feeding trial was conducted in Ningde, Fujian Province, China. Large yellow croaker (1800 fish) were obtained from the National Breeding Station of Large Yellow Croaker and reared in a floating sea cage (4.0 × 4.0 × 4.0 m) for 2 weeks to acclimate the environment. During the acclimation, fish were fed with the commercial feed for large yellow croaker (Haid Group, Guangzhou, China). Prior to the feeding trial, fish were fasted for 24 hr and then weighed after being anaesthetized with eugenol (1:10,000) (Shanghai Reagent Corp., China). Fish (1260) with the initial weight of 3.00 ± 0.01 g were distributed into 18 sea cages (1.5 × 1.5 × 2.0 m) at a density of 70 fish per cage. Fish were carefully handfed until apparent satiation twice daily (5:00 and 17:00 hrs) for 10 weeks. During the acclimation and the feeding trial, water temperature fluctuated from 18 to 26°C (average 22.8°C), salinity from 28‰ to 32 ‰ and the dissolved oxygen from 6.8 to 7.9 mg/L.

2.3 | Sampling and analysis

At the end of the feeding trial, fish were fasted for 24 hr and then anaesthetized with eugenol before sampling. Six fish per cage were randomly selected for body composition analysis. Ventral skin, left-sided dorsal skin and liver of the other six fish per cage were sampled. All samples were stored at −20°C. Skin coloration measurement was taken on ventral skin and left-sided dorsal skin at night (20:00-23:00) following the method of Yi, Xu, et al. (2014). A portable Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan) was used. The colour parameters were L^*, a^* and b^*, which represented lightness, redness and yellowness, respectively.

TABLE 1 Ingredients and approximate composition of the experimental diets (g/kg)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Ingredients[a]	V0/A25	V0/A50	V120/A25	V120/A50	V800/A25	V800/A50
Fish meal[b]	440.0	440.0	440.0	440.0	440.0	440.0
Soybean meal[b]	190.0	190.0	190.0	190.0	190.0	190.0
Wheat meal[b]	214.5	214.2	214.5	214.2	212.9	212.6
Beer yeast[b]	20.0	20.0	20.0	20.0	20.0	20.0
Soy lecithin	15.0	15.0	15.0	15.0	15.0	15.0
Fish oil	75.3	75.3	75.3	75.3	75.3	75.3
Vitamin premix[c]	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin E[d]	0.00	0.00	0.24	0.24	1.60	1.60
Mineral premix[e]	20.0	20.0	20.0	20.0	20.0	20.0
Attractant[f]	3.0	3.0	3.0	3.0	3.0	3.0
Mould inhibitor[g]	1.0	1.0	1.0	1.0	1.0	1.0
Ethoxyquin	1.0	1.0	1.0	1.0	1.0	1.0
Astaxanthin[h]	0.25	0.50	0.25	0.50	0.25	0.50
Chemical analysis						
Moisture	39.9	38.5	39.2	46.1	38.8	38.0
Protein	437.9	437.6	433.7	439.4	436.6	434.1
Lipid	125.4	123.7	127.8	125.6	132.4	124.5
Vitamin E (mg/kg)	93.91	88.19	266.99	248.26	875.93	867.79
Astaxanthin (mg/kg)	24.66	51.12	25.33	50.75	24.89	50.06

[a]All ingredients were supplied by Qingdao Great Seven Bio-Tech, Co., Ltd. (Qingdao China).
[b]Fish meal: crude protein 656.0 g/kg, crude lipid 54.6 g/kg; soybean meal: crude protein 513.1 g/kg, crude lipid 18.4 g/kg; wheat meal: crude protein 152.5 g/kg, crude lipid 11.9 g/kg; beer yeast: crude protein 478.6 g/kg, crude lipid 15.1 g/kg.
[c]Vitamin premix (mg or g/kg diet) without DL-α-tocopherol acetate: 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; ascorbic acid, 2000 mg; choline chloride, 2500 mg; ethoxyquin, 150 mg; wheat middling, 14.012 g.
[d]Vitamin E: 50% alpha-tocopheryl acetate.
[e]Mineral premix (mg or g/kg diet): NaF, 2 mg; KI, 0.8 mg; $CoCl_2$·6H2O (10 g/kg), 50 mg; $CuSO_4$·$5H_2O$, 10 mg; $FeSO_4$·H_2O, 80 mg; $ZnSO_4$·H_2O, 50 mg; $MnSO_4$·H_2O, 60 mg; $MgSO_4$·$7H_2O$, 1200 mg; Ca (H_2PO_4)2·H_2O, 3000 mg; NaCl, 100 mg; zeolite, 15.447 g.
[f]Attractant: glycine and betaine.
[g]Mould inhibitor: 500 g/kg calcium propionic acid and 500 g/kg fumaric acid.
[h]Astaxanthin: Carophyll® pink, astaxanthin 100 g/kg, DSM.

Carotenoid contents in the diet and skin were tested according to the method of Yi, Xu, et al. (2014) and calculated using the extinction coefficients $E_{(1\%,\ 1\ cm)}$ = 1900 at 474 nm for diet and $E_{(1\%,\ 1\ cm)}$ = 2500 at 448 nm for skin. Crude protein was determined by digestion using the Kjeldahl method (Kjeltec FOSS 2300; Tecator) and estimated by multiplying *N* by 6.25. Crude lipid was measured by ether extraction using the Soxhlet method (Soxhlet Extraction System B-811). Ash was determined by muffle furnace at 550°C for 4 hr. DL-α-Tocopherol acetate contents in the diets and liver were determined according to the HPLC method described by Salo-Väänänen et al. (2000). Standard DL-α-tocopherol was purchased from Sigma-Aldrich (T3251, Sigma-Aldrich).

Lipid peroxidation was measured by the amount of thiobarbituric acid reactive substances (TBARS) in liver. TBARS was tested using a QuantiChrom™ TBARS assay kit (DTBA-100, BioAssay systems, USA), and the concentration was expressed as the malondialdehyde (MDA) production (nM/mg protein). Superoxide dismutase (SOD), reduced glutathione (GSH) and the total antioxidative capacity (T-AOC) in liver were measured with the superoxide dismutase assay kit (WST-1 method), reduced glutathione assay kit and total antioxidative capacity assay kit (Nanjing Jiancheng Bio-engineering Institute, China), respectively. The results of these enzymatic assays were given in units of enzyme activity per milligram of protein. One unit of SOD was defined as the amount of enzyme causing 50% inhibition in the NBT reduction rate. 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. The GSH content was expressed as μM/g protein.

2.4 | Calculations and statistical analysis

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

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

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

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

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

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

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.

The data on vitamin E and astaxanthin were subjected to two-way ANOVA by SPSS 15.0 for windows (SPSS Statistics Inc, Chicago, USA), testing the main effects of vitamin E and astaxanthin, and their full factorial interaction. Tukey's test was conducted for individual means only if there was a significant interaction ($p < .05$). Regression analysis was conducted between dietary vitamin E content and liver vitamin E content.

3 | RESULTS

Data on the growth performance and feed utilization are listed in Table 2. There were no significant differences in survival rate (SR), feed conversion ratio (FCR) and protein efficiency ratio (PER) among all the treatments ($p > .05$). The final body weight (FBW) and specific growth rate (SGR) were significantly affected by dietary vitamin E ($p < .05$), not by dietary astaxanthin and its interaction with dietary vitamin E. Fish fed diets with 120 mg/kg vitamin E supplementation had significantly higher FBW and SGR than those fed diets with 0 or 800 mg/kg vitamin E supplementation ($p < .05$). Crude protein, crude lipid, ash and moisture in the whole body were not significantly affected by dietary vitamin E and astaxanthin as well as their interactions (Table 3).

Data on the skin colour and carotenoid content are presented in Table 4. No significant differences were shown on the lightness (L^*) both in the dorsal and in the ventral skin among all the treatments ($p > .05$). Ventral skin redness (a^*) of fish fed diet with 25 mg/kg astaxanthin and 0 mg/kg vitamin E supplementation was significantly lower than that of fish fed with other diets. However, dorsal skin redness was not affected by dietary astaxanthin and vitamin E supplementation. Yellowness (b^*) of the dorsal and ventral skin was significantly influenced by dietary astaxanthin or vitamin E ($p < .05$), but not their interactions. Yellowness increased with increasing dietary astaxanthin or vitamin E both in the dorsal and in ventral skin. Similar to the variation in skin yellowness, carotenoid contents both in the dorsal and in the ventral skin were significantly influenced by dietary astaxanthin or vitamin E ($p < .05$), but not their interactions.

TABLE 2 Effects of dietary vitamin E and astaxanthin on the growth performance and feed utilization of large yellow croaker[†]

Astaxanthin level(mg/kg)	Vit E level (mg/kg)	IBW (g)	FBW (g)	SGR	SR (%)	FCR	PER
Individual treatment means							
25	0	3.01	21.11	2.78	93.33	1.19	1.96
50	0	3.02	22.28	2.86	96.19	1.16	1.97
25	120	3.02	24.61	3.00	95.71	1.03	2.25
50	120	2.98	23.15	2.93	91.90	1.21	1.89
25	800	3.01	20.03	2.71	94.76	1.25	1.86
50	800	3.02	21.28	2.79	95.71	1.13	2.05
Pooled S.E.M.		0.01	0.42	0.03	1.04	0.03	0.06
Means of main effect							
25		3.01	21.95	2.83	94.60	1.16	2.02
50		3.01	22.24	2.86	94.60	1.16	1.97
	0	3.01	21.69[a]	2.82[a]	94.76	1.17	1.96
	120	3.00	23.88[b]	2.96[b]	93.81	1.12	2.07
	800	3.02	20.65[a]	2.75[a]	95.24	1.19	1.96
ANOVA: *p*-values							
Astaxanthin		.664	.526	.319	1.000	.897	.665
Vit E		.531	.001	.000	.877	.681	.693
Astaxanthin × Vit E		.251	.073	.083	.498	.228	.211

FBW, final body weight; FCR, feed conversion ratio; IBW, initial body weight; PER, protein efficiency ratio; SGR, specific growth rate; SR, survival rate.

[†]Treatment means represent the average values for three tanks per treatment. Superscripts denote significant differences between diet treatments, and the absence of superscripts denotes no significant differences. Means followed by the same letter are not significantly different.

TABLE 3 Effects of dietary vitamin E and astaxanthin on body composition of large yellow croaker[†] (g/kg wet basis)

Astaxanthin level(mg/kg)	Vit E level (mg/kg)	Moisture	Protein	Lipid	Ash
Individual treatment means					
25	0	738.9	157.7	73.7	38.0
50	0	747.3	157.7	79.0	38.2
25	120	734.2	156.8	81.6	36.5
50	120	745.1	159.9	69.2	37.4
25	800	735.8	156.9	79.0	36.8
50	800	731.6	160.0	81.1	37.6
Pooled S.E.M.		2.5	1.1	2.7	0.4
Means of main effect					
25		736.3	157.1	78.1	37.0
50		741.4	159.2	76.4	37.7
	0	743.1	157.7	76.3	38.1
	120	739.7	158.3	75.4	37.0
	800	733.7	158.4	80.0	37.2
ANOVA: *p*-values					
Astaxanthin		.334	.199	.725	.292
Vit E		.340	.914	.675	.273
Astaxanthin × Vit E		.445	.651	.283	.873

[†]Treatment means represent the average values for three tanks per treatment.

Fish fed with higher dietary astaxanthin had higher skin carotenoid content. Carotenoid contents of fish fed diets with 0 mg/kg vitamin E supplementation were significantly lower than those of fish fed diets with 120 or 800 mg/kg vitamin E supplementation in the two sample areas ($p < .05$).

Data on antioxidative capacity indexes in liver are given in Table 5. The vitamin E content and total antioxidative capacity (T-AOC) of liver were significantly affected by dietary astaxanthin and vitamin E as well as their interactions ($p < .05$). Liver vitamin E content was positively related to the dietary vitamin E content ($y = 0.385x - 36.252$, $R^2 = .956$, $p = .001$). When the diets supplemented with 800 mg/kg vitamin E, significantly higher vitamin E content in liver was found in the group with 50 mg/kg astaxanthin compared with the group with 25 mg/kg astaxanthin ($p < .05$). However, the liver vitamin E content was not influenced by dietary astaxanthin both in the groups with 0 and 120 mg/kg vitamin E supplementation. As to the T-AOC, the highest value was found in the group with 0 mg/kg vitamin E and 25 mg/kg astaxanthin supplementation among all the treatments. Meanwhile, fish fed with high dietary astaxanthin had significantly lower T-AOC value than that of the fish fed with low dietary astaxanthin when the diets were supplemented with 800 mg/kg vitamin E ($p < .05$). Liver SOD was significantly affected by dietary astaxanthin ($p < .05$), not by dietary vitamin E and its interaction with dietary astaxanthin. Liver SOD decreased with increasing dietary astaxanthin. In contrast to liver SOD, liver MDA content was significantly influenced by dietary vitamin E ($p < .05$), not by dietary astaxanthin and its interaction with dietary vitamin E. Fish fed diets with 0 mg/kg vitamin E supplementation had significantly higher MDA content in liver than fish fed diets with 120 mg/kg or 800 mg/kg vitamin E supplementation ($p < .05$). Liver GSH concentration was significantly affected by dietary vitamin E and its interaction with dietary astaxanthin ($p < .05$), not by dietary astaxanthin. Liver GSH concentration was found to be increased with increasing dietary vitamin E.

4 | DISCUSSION

Both vitamin E and carotenoid are lipid soluble and involved in the regulation of antioxidation, enhancing immunity and stress resistance (Galaz et al., 2010; Montero et al., 2001; Niu, Wen, et al., 2014; Wang et al., 2006). Adequate vitamin E and carotenoid in the diet can improve the growth performance of fish. In the present study, dietary vitamin E significantly affected the FBW and SGR. Niu, Jia, et al., 2014 found that turbot *Scophthalmus maximus* fed lower (0, 120, 240 mg/kg) vitamin E diets had significantly lower FBW and SGR compared to those fed with the optimal (480 mg/kg) vitamin E diet. Similar results were reported in cobia (Zhou et al., 2013), grouper *Epinephelus malabaricus* (Lin & Shiau, 2005), rohu (Sau, Paul, Mohanta, & Mohanty, 2004) and hybrid striped bass (Kocabas & GatlinIII, 1999). Nevertheless, there were no significant effects of vitamin E-deficient diets on the growth performance in gilthead sea bream *Sparus aurata* (Montero et al., 2001), golden shiner *Notemigonus crysoleucas* (Chen et al., 2004), turbot and halibut *Hippoglossus hippoglossus* L. (Tocher et al., 2002). The differences in those studies could attribute to the fish size, experimental durations, cultivation environments and other feed components such as astaxanthin, vitamin C, selenium

and HUFA contents (Hamre, 2011). In addition, a negative effect was also observed on growth when fish fed with excess doses of vitamin E, in the present study. Similar studies were reported in grass carp *Ctenopharyngodon idellus*, cobia, parrot fish *Oplegnathus fasciatus* (Galaz et al., 2010; Li, Liang, et al., 2014; Zhou et al., 2013). This could be because of the accumulation of vitamin E radicals, which may act as a pro-oxidant (Hamre, 2011).

In the present study, the whole-body composition of large yellow croaker was not significantly affected by dietary treatments. This is in agreement with previous studies on grass carp (Li, Liang, et al., 2014), soft-shelled turtle (Huang & Lin, 2004) and mrigal *Cirrhinus mrigala* (Paul, Sarkar, & Mohanty, 2004). However, in golden shiner, lower whole-body crude protein, crude lipid and dry matter were found in fish fed with vitamin E-deficient diet compared to vitamin E-supplemented diets, which may be associated with severity of muscular atrophy (Chen et al., 2004). Meanwhile, in Atlantic salmon, the whole-body lipid content was significantly higher in fish fed diets with astaxanthin supplementation compared to the unsupplemented diet (Christiansen, Lie, & Torrissen, 1995; Christiansen & Torrissen, 1996; Christiansen, Glette, et al., 1995).

Skin colour reflects the quality parameters of fish such as freshness and health, which can influence the market price of fish. So it is one of the most important quality criteria for fish species (Olsen & Baker, 2006). Yi, Xu, et al. (2014) reported that astaxanthin can significantly improve the skin colour and carotenoid content of large yellow croaker. In the present study, the carotenoid content and yellowness increased with increasing dietary astaxanthin both in the dorsal and in the ventral skin (Table 4). Apart from pigment, many factors can also affect fish pigmentation, such as protein/carbohydrate ratio, water temperature, light spectrum and intensity (Chatzifotis et al., 2005; Doolan, Allan, Booth, & Jones, 2008; Gouveia & Rema, 2005). Previous studies showed that one of the vitamin E deficiency signs was the dyspigmentation or darker skin (Chen et al., 2004; Kocabas & GatlinIII, 1999). Meanwhile, Pozo et al. (1988) reported that dietary vitamin E enhanced the deposition of canthaxanthin in the flesh of rainbow trout. Bjerkeng et al. (1999) also found that the fillet astaxanthin deposition and redness of Atlantic salmon were improved by vitamin E supplementation. This could be due to the fact that both the carotenoid and vitamin E are lipid-soluble antioxidants, adequate vitamin E can save carotenoid as the antioxidant and more carotenoid can be deposited in the body of fish. In the present study, significantly lower yellowness values and carotenoid contents were found in fish fed diets without vitamin E supplementation (Table 4). The only synergism effect of dietary vitamin E and astaxanthin on pigmentation was observed in the ventral skin redness. Similarly, Bell et al. (2000) demonstrated that an interactive sparing of astaxanthin supplementation on tissue vitamin E concentration was only observed in brain of Atlantic salmon.

TABLE 4 Effects of dietary vitamin E and astaxanthin on skin colour and carotenoid content (CC)of large yellow croaker[†]

Astaxanthin level(mg/kg)	Vit E level (mg/kg)	Ventral skin				Dorsal skin			
		$L^{*‡}$	$a^{*‡}$	$b^{*‡}$	CC (μg g^{-1})	L^*	a^*	b^*	CC (μg g^{-1})
Individual treatment means									
25	0	77.15	-0.47^{a}	50.35	48.49	75.84	−2.16	16.42	6.12
50	0	76.35	2.39^{b}	54.51	62.57	75.41	−3.14	16.80	11.12
25	120	78.76	2.06^{b}	54.05	71.79	74.04	−2.71	16.98	10.94
50	120	77.16	2.52^{b}	55.80	100.14	76.77	−2.19	18.23	12.07
25	800	77.67	1.74^{b}	53.73	81.62	76.21	−2.40	16.70	12.01
50	800	78.70	2.09^{b}	57.12	88.91	75.71	−2.86	19.08	14.00
Pooled S.E.M.		0.32	0.29	0.60	4.53	0.31	0.13	0.28	0.51
Means of main effect									
25		77.86	1.11	52.71^{a}	67.30^{a}	75.36	−2.42	16.70^{a}	9.69^{a}
50		77.40	2.34	55.81^{b}	83.88^{b}	75.96	−2.73	18.02^{b}	12.40^{b}
	0	76.75	0.96	52.43^{x}	55.53^{x}	75.63	−2.65	16.58^{x}	8.62^{x}
	120	77.96	2.29	54.93^{y}	85.97^{y}	75.40	−2.45	16.92^{xy}	11.51^{y}
	800	78.19	1.92	55.42^{y}	85.26^{y}	75.96	−2.63	17.21^{y}	13.01^{y}
ANOVA: *p*-values									
Astaxanthin		.124	.030	.001	.014	.299	.198	.003	.016
Vit E		.429	.005	.010	.002	.716	.736	.033	.011
Astaxanthin × Vit E		.191	.025	.387	.318	.055	.055	.118	.283

[†]Treatment means represent the average values for three tanks per treatment. Tukey's test was conducted for individual means only if there was a significant interaction (ANOVA: $p < .05$). Superscripts denote significant differences between diet treatments, and the absence of superscripts denotes no significant differences. Means followed by the same letter are not significantly different.

[‡]L^*, a^* and b^* represent lightness, redness and yellowness, respectively.

TABLE 5 Effects of dietary vitamin E and astaxanthin on liver antioxidative capacity of large yellow croaker[†]

Astaxanthin level(mg/kg)	Vit E level (mg/kg)	Vit E (μg/g)	T-AOC (U/mg prot)	SOD (U/mg prot)	GSH (μM/L)	MDA (nM/mg prot)
Individual treatment means						
25	0	16.75^{a}	8.73^{a}	182.77	42.61^{ab}	2.32
50	0	16.78^{a}	6.99^{c}	147.88	37.84^{b}	2.49
25	120	34.15^{ab}	7.39^{c}	177.34	46.58^{a}	1.66
50	120	46.51^{b}	7.33^{c}	148.35	37.84^{b}	1.90
25	800	268.74^{c}	8.00^{b}	182.95	41.71^{ab}	1.53
50	800	347.31^{d}	6.55^{d}	159.93	48.69^{a}	1.33
Pooled S.E.M.		30.38	0.17	4.12	1.16	0.11
Means of main effect						
25		106.55	8.04	181.01^{a}	43.64	1.84
50		136.87	6.96	152.05^{b}	41.46	1.91
	0	16.77	7.86	165.33	40.24	2.41^{a}
	120	40.33	7.36	162.84	42.21	1.78^{b}
	800	308.02	7.27	171.44	45.20	1.43^{b}
ANOVA: *p*-values						
Astaxanthin		.000	.000	.004	.159	.528
Vit E		.000	.000	.689	.048	.000
Astaxanthin × Vit E		.000	.000	.843	.002	.239

GSH, reduced glutathione; MDA, malondialdehyde; SOD, superoxide dismutase; T-AOC, total antioxidative capacity.
[†]Treatment means represent the average values for three tanks per treatment. Tukey's test was conducted for individual means only if there was a significant interaction (ANOVA: $p < .05$). Superscripts denote significant differences between diet treatments, and the absence of superscripts denotes no significant differences. Means followed by the same letter are not significantly different.

Reactive oxygen species (ROS) are produced by normal physiological processes, and they are essential to the cellular functions at low concentration (Bell et al., 2000; Matés, 2000). Cells possess a multilevel antioxidant defence system, which includes a number of radical- and peroxide-scavenging enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase) and low molecular weight molecules (e.g., glutathione, vitamin E, vitamin C and carotenoids) (Yu, 1994). Fish, especially marine fish, contain high level of PUFA, which are vulnerable to oxidative stress. Therefore, an increase in antioxidant nutrients in aquafeed is essential for fish (Betancor et al., 2012). Both vitamin E and carotenoids are lipid-soluble antioxidant nutrients (Machlin & Bendich, 1987). Previous studies showed that the supplementation of vitamin E and/or carotenoids can significantly enhance the antioxidative capacity of fish and shrimp, thereby increasing their stress resistance and health (Bell et al., 2000; Li, Wu, Zhou, Xie, Zhou & Mai, 2014; Niu, Wen, et al., 2014; Pham et al., 2014). In the present study, the highest and lowest T-AOC values were found in fish fed diet 1 (V0/A25) and diet 6 (V800/A50), respectively. The T-AOC includes enzymatic and non-enzymatic antioxidative activities (Wang et al., 2006). The change in liver T-AOC, in the present study, could be attributed to the deficiency of vitamin E and astaxanthin, which increased the activities of other enzymatic and non-enzymatic antioxidants, and the higher supplementation of astaxanthin and vitamin E exerted a pro-oxidant effect. This was supported by high amount of vitamin E and skin carotenoid contents found in fish fed with higher dietary vitamin E and astaxanthin (Tables 4 and 5). Meanwhile, the content of MDA in liver reduced with increasing dietary vitamin E. In addition, the liver SOD activity was also significantly lower in fish fed with higher dietary astaxanthin than in those fed with lower ones. Similar results were found in olive flounder (Pham et al., 2014) and characin (Wang et al., 2006), in which fish fed the carotenoid diets had lower SOD activities compared to that fed the control diet. Moreover, liver GSH content increased with increasing dietary vitamin E and astaxanthin, in the present study. Sila et al. (2015) reported that astaxanthin can reduce the oxidative stress by elevating GSH levels and other antioxidant enzyme activities in the diabetic rats. Meanwhile, vitamin E can also improve the GSH levels in fish and crustaceans (Dandapat, Chainy, & Rao, 2000; Huang & Huang, 2004).

5 | CONCLUSION

In this study, supplementation of dietary vitamin E can improve the growth of large yellow croaker. No significant interactions between dietary vitamin E and astaxanthin were found in the growth performance and feed utilization. However, the synergistic effect of dietary vitamin E and astaxanthin significantly improved the skin coloration and liver antioxidative capacity.

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