



Comparative impact of synthetic and natural animal-derived carotenoids on growth, feed utilization, and pigment enhancement in *Amphiprion ocellaris*

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Abstract

Coloration significantly influences the perceived value and consumer acceptance of ornamental fish within the aquarist community. Carotenoids, pigments essential for coloration, are primarily responsible for the vibrant hues observed in ornamental fish. This investigation assessed the effects of supplementing natural carotenoids (extracted from chicken eggs, shrimp shells, copepods, and golden apple snail eggs) versus synthetic astaxanthin (Carophyll Pink, 10%) on the growth, feed utilization, and color enhancement in juvenile clownfish (*Amphiprion ocellaris*). Juvenile specimens (3.14 ± 0.02 cm in total length and 0.54 ± 0.02 g in body weight) were subjected to five carotenoid-enriched diets, each supplemented with an identical dosage of 250 mg/kg of feed, in addition to a control group without supplementation. The fish were cultivated in glass tanks (60 liters per tank), each housing 15 individuals, with triplicate tanks per dietary treatment over a period of 75 days. Findings revealed that supplementation with both natural and synthetic carotenoids significantly improved growth and coloration over the control ($p < 0.05$). Notably, carotenoids derived from shrimp shells and copepods outperformed synthetic astaxanthin in enhancing growth and feed efficiency. Additionally, the skin redness intensity and the accumulated carotenoid concentration were highest in these two natural treatments, with no significant difference compared to the synthetic astaxanthin group ($p > 0.05$). This research highlights that supplementing with natural carotenoids from shrimp shells and copepods is a viable and effective method to augment growth and coloration in clownfish, suggesting a potential alternative to synthetic astaxanthin to elevate the quality and commercial value of farmed marine ornamental fish.

Keywords: Natural carotenoid, Synthetic astaxanthin, False clownfish, Growth, Coloration

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Introduction

The false clownfish (*Amphiprion ocellaris*) is a popular marine ornamental fish, highly valued for its vibrant colors and good adaptability in captive conditions (da Silva et al., 2022). Despite significant progress in breeding this species, challenges remain related to the loss of pigmentation in captive fish, which reduces their commercial value and increases the pressure on natural populations (Calado et al., 2017; Nhan et al., 2019). This loss of pigmentation is thought to be caused by various factors such as stress, water quality, rearing systems, and particularly the content of carotenoid pigments in the diet (Bolker & Hill, 2000; Vissio et al., 2021; Yasir & Qin, 2010). To improve the coloration of cultured fish, techniques such as nutritional supplementation, environmental control, and genetic manipulation have been developed (Lau et al., 2023).

Color plays a crucial role in the survival, reproduction, and commercial value of many ornamental fish species (Gupta et al., 2007; Lau et al., 2023; Sköld et al., 2016). Color is determined by genetic factors, the rearing environment, and pigments, mainly carotenoids, in the diet (Luo et al., 2021). Due to their inability to synthesize carotenoids *de novo*, fish must obtain them from their diet to develop their characteristic colors (Gupta et al., 2007). Carotenoids also play important roles in fish health, growth, reproduction, and immune function (Elbahnaswy & Elshopakey, 2024; Lim et al., 2023). Among them, astaxanthin stands out for its availability, color-enhancing efficiency, antioxidant properties, and overall health benefits (Lim et al., 2023, 2018). To meet the demand for color enhancement, natural and synthetic pigment supplements have been widely used in aquaculture (Elbahnaswy & Elshopakey, 2024; Lau et al., 2023; Sathyaruban et al., 2021). However, synthetic carotenoids have some drawbacks, such as limited color range, low bioavailability, weak antioxidant capacity, poor color retention, and concerns about consumer acceptance (Gupta et al., 2007; Pereira da Costa & Campos Miranda-Filho, 2020; Trichet & Amaya, 2022). Therefore, the demand for alternative natural carotenoid sources is increasing (Elbahnaswy & Elshopakey, 2024; Sathyaruban et al., 2021). Many studies have demonstrated the positive impact of supplementing natural carotenoids in the diet of aquatic species (Ebenezar et al., 2020; Nhan et al., 2019; Pham et al., 2014). However, supplementation in the form of raw materials at high rates (15%–30%) can reduce feed quality, digestibility, nutrient absorption, and water quality (García-Chavarría & Lara-Flores, 2013; Nhan et al., 2019; Tran et al., 2022). Therefore, the use of

carotenoids in the form of extracts offers many benefits, such as reducing the supplementation rate (0.01%–2.0%), increasing digestibility, absorption, and ease of storage (Ebenezar et al., 2020; Tran et al., 2022). In particular, animal-derived carotenoids, which primarily exist in the free form of astaxanthin and canthaxanthin, generally have higher bioavailability compared to those from plant sources (Schweiggert & Carle, 2017). Many animal carotenoid sources such as copepods, shrimp, poultry eggs, and mollusks have abundant carotenoid content (0.14–17,200 mg/kg) (Gupta et al., 2007; Schweiggert & Carle, 2017; Tran et al., 2022; Vilgrain et al., 2023). Although widely used in terrestrial animal husbandry, their application in aquaculture, especially for ornamental fish, remains limited. This study aims to evaluate the impact of supplementing natural carotenoids from chicken eggs, shrimp shells, copepod, golden apple snail eggs, and synthetic astaxanthin on the growth, feed utilization efficiency, and coloration of false clownfish. The results will provide valuable information on the potential of animal-derived natural carotenoids in improving the quality of artificially produced ornamental fish.

Materials and Methods

Ethics statement

The procedures are conducted in accordance with the national regulations on the use of animals in research in Vietnam, including the Law on Livestock Breeding 2018 and Decree No. 26/2019/ND-CP of the Government, detailing a number of articles and measures implementing the Law on Fisheries, including regulations on the management, protection, and exploitation of endangered, precious, and rare aquatic species. Experiments on marine fish were exempted from ethical approval requirements. However, throughout the study, efforts were made to minimize stress to the fish by providing optimal rearing, care, handling, and sampling conditions.

Preparation of carotenoid sources

Animal source materials, including chicken eggs (*Gallus gallus domesticus*), whiteleg shrimp shells (*Penaeus vannamei*), copepods (*Pseudodiaptomus annandalei*), and golden apple snail eggs (*Pomacea canaliculata*), were collected from different locations in Nha Trang City, Khanh Hoa Province, Vietnam. Ethanol 96% was used as a solvent to extract carotenoids following the method described in Tran et al (2022). Fresh materials (except for chicken eggs and snail eggs, which were boiled and

only the yolks were used) (50 g) were mixed with the solvent at a solvent-to-material ratio of 3:1 (mL/g) and homogenized using a blender (Philips HR2118, 600 W, Philips, Amsterdam, the Netherlands). The homogenates were placed in 250 mL glass beakers and subjected to carotenoid extraction using a microwave oven (Sharp microwave, Sakai, Japan) for 180 s. After extraction, the samples were filtered through a filter cloth to separate the extracts from the residues. The residues were further extracted twice (for a total of three extractions) to maximize the recovery of carotenoids from the source materials. The extracts from the three extractions were combined, and the carotenoid content in the samples was determined by colorimetry using a UV-Vis spectrophotometer (Biochrom, Cambridge, UK). The results showed that the total carotenoid contents (TC) in chicken eggs, shrimp shells, copepods, and apple snail eggs were 23.7 µg/g, 52.6 µg/g, 113.3 µg/g, and 81.4 µg/g fresh weight, respectively. The synthetic astaxanthin source used was Carophyll Pink CWS (DSM Nutritional Products), containing 10% astaxanthin. These carotenoid sources were incorporated into the diets of clownfish according to different experimental treatments.

Experimental diets

The basal diet (55% crude protein and 12% crude lipid) was formulated to meet the nutritional requirements of marine fish larvae according to their specific needs. The detailed feed composition is presented in Table 1. Four natural carotenoid sources, including chicken eggs (Ch-gg), shrimp shells (Sh-shell), copepods (Cope), and snail eggs (Sn-egg), and a synthetic carotenoid source, astaxanthin (Astax), were supplemented to the basal diet at a level of 250 mg/kg diet. In addition, a control diet (Control) without carotenoid supplementation was tested.

The experimental diets were manufactured following the standard procedures described in detail in Tran et al. (2022), which ensured the quality and consistency of the diets. The main steps of the procedure included weighing the ingredients, grinding, mixing, cooking, cooling, adding carotenoids and vitamins, pelleting, and oven-drying to 10% moisture content. The final feed pellets were 0.8–1.0 mm in size and were stored at -4°C until use.

Rearing conditions

Juvenile clownfish with an initial size of 3.14 ± 0.02 cm and 0.54 ± 0.02 g were acclimatized in 60-L glass tanks (55 × 35 ×

Table 1. Formulation and proximate chemical composition of the experimental diets (g/kg)

Ingredient	Treatments					
	Control	Ch-egg	Sh-shell	Cope	Sn-egg	Astax
Fishmeal (Peru) (g)	470	470	470	470	470	470
Fishmeal (Vietnam) (g)	180	180	180	180	180	180
Squid meal (g)	170	170	170	170	170	170
Corn gluten meal (g)	115.90	115.65	115.65	115.65	115.65	115.65
Soybean oil (g)	45.6	45.6	45.6	45.6	45.6	45.6
Vitamin premix (g) ¹⁾	12.8	12.8	12.8	12.8	12.8	12.8
Lysine (g)	0.5	0.5	0.5	0.5	0.5	0.5
Methionine (g)	0.2	0.2	0.2	0.2	0.2	0.2
Mineral premix (g) ²⁾	5.0	5.0	5.0	5.0	5.0	5.0
Carotenoids supplement (g)	0	0.25	0.25	0.25	0.25	0.25
Chemical and proximate composition						
Crude protein (%)	55.00	55.16	55.07	55.21	55.10	55.19
Crude lipid (%)	12.01	12.09	11.96	12.12	11.97	12.16
Ash (%)	11.09	11.11	10.92	11.21	11.14	11.16
Moisture (%)	10.04	10.11	10.01	10.17	10.09	9.92
Carotenoids (g)	0.04	0.27	0.28	0.25	0.26	0.28

¹⁾ Vitamin premix (mg/kg diet): Vitamin A, 1,000,000 IU; Vitamin D3, 300,000 IU; Vitamin C monophosphate, 10,000 mg; Pantothenic acid, 2,500 mg; Vitamin E, 2,000 mg; Vitamin B3, 2,000 mg; Vitamin K3, 500 mg; Vitamin B1, 500 mg; Vitamin B6, 500 mg; Vitamin B2, 320 mg; Folic acid, 200 mg; Biotin, 20 mg; Vitamin B12, 5 mg; Inositol, 10 mg; Choline chloride, 5 mg.

²⁾ Mineral premix (mg/kg diet): Zn (ZnO), 4,750 mg; Mn (MnSO₄ · H₂O), 1,900 mg; Mg (MgO), 1,050 mg; Co (CoCO₃), 47.5 mg; Se (Na₂SeO₃), 47.5 mg; I (Ca(IO₃)₂ · H₂O), 19 mg; P (CaHPO₄ · 2H₂O), 0.7%; Ca (CaHPO₄ · 2H₂O), 0.8%; Moisture, 10%; Ash, 2%; Ethoxyquin, 240 mg; Carrier (Dextrose), 86%. Provimi Vietnam, Bien Hoa city, Dong Nai province, Vietnam.

38 cm) at a stocking density of 15 fish per tank for one week. The tanks were part of a recirculating system with a stable water flow rate of 1.5 L/min under natural light conditions. Fish were fed four times a day (7:00, 10:00, 13:00, and 16:00 h). Uneaten feed was collected, dried, and kept separately for feed efficiency evaluation. Tank maintenance included siphoning every two days to remove waste and a weekly 30% water exchange. Optimum water quality was maintained with temperature ranging from 27 °C to 31 °C, salinity from 32‰ to 34‰, pH from 7.8 to 8.2, dissolved oxygen concentration above 5.0 mg/L, and Total Ammonia Nitrogen (TAN) maintained below 1.5 mg/L. Daily observations were conducted to monitor fish health and environmental conditions.

Experimental design

The study used a completely randomized design to examine the effects of dietary supplementation of carotenoids from different sources on growth, feed utilization, and coloration in juvenile clownfish. Five carotenoid-supplemented diets and a non-supplemented control group were formulated (Table 1), each with three replicates and a duration of 75 days.

At the end of the experiment, all surviving fish were harvested to measure indices of length, weight, survival rate (SR), feed efficiency, and skin color parameters (L^* , a^* , b^* , C^* , h^* , and Delta E), and carotenoid content in tissues (skin, muscle, and whole body). Fish were starved for 24 h and lightly anesthetized with 0.05% Ethylene Glycol Monophenyl Ether (Merck KGaA, Germany, Merck, Darmstadt, Germany) before sampling to ensure accurate measurements. Fish were then blotted dry for measurements. Six individuals were randomly selected from each tank and stored at -80 °C for laboratory carotenoid analysis.

Methods for determining some indicators

Determining growth parameters, survival rate, and feed efficiency

Growth rate assessment parameters:

$$\text{Specific growth rate in length: } \text{SGR}_L (\%/day) = [(\ln L_2 - \ln L_1) / t] \times 100$$

$$\text{Specific growth rate in weight: } \text{SGR}_W (\%/day) = [(\ln W_2 - \ln W_1) / t] \times 100$$

$$\text{Coefficient of length variation: } \text{CV}_L (\%) = \text{SD}_L / \text{Mean}_L \times 100$$

$$\text{Coefficient of weight variation: } \text{CV}_W (\%) = \text{SD}_W / \text{Mean}_W \times 100$$

$$\text{Condition factor: } \text{CF} (\text{g}/\text{cm}^3) = 100 \times W / L^3$$

Survival rate:

$$\text{Survival rate: } \text{SR} (\%) = (N_2 / N_1) \times 100$$

Feed utilization efficiency:

$$\text{Feeding rate: } \text{FR} (\% \text{BW}/\text{day}) = 100 \times \text{FI} / [(W_1 + W_2) / 2] / t$$

$$\text{Feed conversion ratio: } \text{FCR} = \text{FI} / (W_2 - W_1)$$

$$\text{Feed efficiency ratio: } \text{FER} (\%) = (W_2 - W_1) / \text{FI} \times 100$$

$$\text{Protein efficiency ratio: } \text{PER} = (W_2 - W_1) \times \text{PI}$$

$$\text{Lipid efficiency ratio: } \text{LER} = (W_2 - W_1) \times \text{LI}$$

where: L_1 and L_2 are the lengths of fish at the start and end of the experiment (mm); W_1 and W_2 are the corresponding weights of fish (g); t is the duration of the experiment (days); Standard Deviation of Length (SD_L) and Standard Deviation of Weight (SD_W) are the standard deviations of fish length (mm) and weight (g); BW is the body weight of fish (g); FI is the amount of feed intake (g); PI is the amount of protein intake (g, 55%); LI is the amount of lipid intake (g, 12%); N_1 and N_2 are the number of fish at the start and end of the experiment (individuals).

Skin color intensity and accumulated carotenoid content

Skin color intensity of fish

Live fish skin color was measured using a CR-400 colorimeter (KONICA Minolta, Tyoko, Japan) at the midpoint between the dorsal and anal fins, twice on each side of the body. Measurements were taken on all surviving fish at the end of the experiment. The colorimeter is based on the principle of spectrophotometry, using optical filters to simulate the CIE 1931 color matching function, providing information in the CIELAB and CIELCH color spaces. The lightness (L^*), green-red coordinate (a^*), and blue-yellow coordinate (b^*), chroma (C^*), and hue angle (h^*) were recorded (Hunter & Harold, 1987). Color change (ΔE^*) between astaxanthin-supplemented clownfish (L_2^* , a_2^* , b_2^*) and the control group (L_1^* , a_1^* , b_1^*) was calculated using the formula $\Delta E^* = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$ (Tomasevic et al., 2019).

Accumulated carotenoid content in fish

Total carotenoid content was determined using the UV-Vis spectrophotometry method as described in Tran et al. (2022). Skin and muscle samples (0.25 g/sample), whole body, and feed (1.0 g/sample) were homogenized with acetone and Na_2SO_4 , and then filtered and centrifuged to obtain the extract. The absorbance of the extract was measured to determine the carotenoid content, and the results were expressed as $\mu\text{g/g}$ sample.

The formula for calculating TC is as follows:

$$\text{TC} (\mu\text{g}/\text{g}) = (A \times V \times D \times 10^4) / (W \times E_{1\text{cm}}1\%)$$

where A is the absorbance; V is the volume of the extract (mL); D is the dilution factor; W is the sample weight (g); and $E_{1\text{cm}}^{1\%}$ is the conversion factor, which is set at 2.100 for edible oil with an absorption wavelength of 485 nm.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 22.0 (Armonk, NY, USA). The assumptions of normality and homogeneity of variances were tested prior to applying one-way ANOVA to assess the differences among groups. In the event of a statistically significant difference, Duncan's Multiple Range Test was conducted to determine the specific differences between groups at a significance level of 5% ($p < 0.05$). Results are reported as mean values \pm SE.

Results

Growth performance and survival rate

The addition of carotenoids to the diet significantly improved the growth performance of clownfish compared to the control (Table 2). Fish fed diets supplemented with natural carotenoids, especially from copepods and shrimp shells, achieved significantly higher specific growth rates in length and weight of 21.2%–24.2% and 30.4%–34.8%, respectively, compared to the control ($p < 0.05$). The coefficient of variation in weight (CV_w) of fish in the two aforementioned treatments was also

lower, indicating greater uniformity than the control ($p < 0.05$). Notably, the growth performance and CV_w of fish in the two treatments supplemented with carotenoids from copepods and shrimp shells were similar and significantly higher than in the treatment supplemented with synthetic astaxanthin ($p < 0.05$). However, the coefficient of variation in length (CV_l) and other indices such as SR and condition factor (CF) did not differ significantly among the experimental treatments ($p > 0.05$).

Feed utilization efficiency

Although the daily feeding rate (FR) did not differ significantly, clownfish fed diets supplemented with natural carotenoids from shrimp shells and copepods had significantly lower FCR than the other treatments, ranging from 1.51–1.60 compared to 1.91–1.95 ($p < 0.05$). Similarly, the other feed utilization efficiency indices, including FER, PER, and LER, were also better in the diets supplemented with carotenoids from shrimp shells and copepods (Table 3).

Skin color intensity

The results of measuring the skin color parameters of clownfish after supplementation with different carotenoid sources are shown in Fig. 1. Specifically, lightness (L^*) decreased significantly in the groups supplemented with carotenoids from chicken eggs, snail eggs, and synthetic astaxanthin compared to the control ($p < 0.05$; Fig. 1A). In contrast, skin redness (a^* in-

Table 2. Growth performance and survival rate of *Amphiprion ocellaris* fed diets supplemented with different carotenoid sources

Parameters	Treatments					
	Control	Ch-egg	Sh-shell	Cope	Sn-egg	Astax
L_1 (cm)	3.14 \pm 0.02	3.14 \pm 0.02	3.14 \pm 0.02	3.14 \pm 0.02	3.14 \pm 0.02	3.14 \pm 0.02
W_1 (g)	0.54 \pm 0.02	0.54 \pm 0.02	0.54 \pm 0.02	0.54 \pm 0.02	0.54 \pm 0.02	0.54 \pm 0.02
L_2 (cm)	3.92 \pm 0.03 ^a	4.07 \pm 0.02 ^b	4.23 \pm 0.02 ^{cd}	4.27 \pm 0.01 ^d	4.17 \pm 0.03 ^c	4.04 \pm 0.04 ^b
W_2 (g)	1.08 \pm 0.02 ^a	1.20 \pm 0.03 ^{bc}	1.33 \pm 0.03 ^d	1.36 \pm 0.01 ^d	1.25 \pm 0.02 ^c	1.17 \pm 0.02 ^b
SGR_l (%/d)	0.30 \pm 0.01 ^a	0.35 \pm 0.01 ^b	0.40 \pm 0.01 ^{cd}	0.41 \pm 0.01 ^d	0.38 \pm 0.01 ^c	0.33 \pm 0.01 ^b
SGR_w (%/d)	0.92 \pm 0.03 ^a	1.06 \pm 0.03 ^{bc}	1.20 \pm 0.03 ^d	1.24 \pm 0.01 ^d	1.12 \pm 0.02 ^c	1.03 \pm 0.02 ^b
CV_l (%)	12.65 \pm 0.48	11.06 \pm 0.59	9.46 \pm 1.03	9.73 \pm 0.64	11.24 \pm 0.55	11.39 \pm 1.12
CV_w (%)	39.92 \pm 0.33 ^b	34.39 \pm 1.40 ^{ab}	28.97 \pm 1.94 ^a	29.15 \pm 2.44 ^a	35.33 \pm 1.89 ^b	35.10 \pm 1.75 ^b
CF (g/cm ³)	1.78 \pm 0.01	1.78 \pm 0.02	1.76 \pm 0.02	1.76 \pm 0.01	1.73 \pm 0.01	1.78 \pm 0.03
SR (%)	95.55 \pm 2.22	95.55 \pm 2.22	97.78 \pm 2.22	97.78 \pm 2.22	95.55 \pm 2.22	95.55 \pm 2.22

Data are expressed as mean \pm SE (n = 3).

Means with different superscript letters within the same row are significantly different ($p < 0.05$).

L_1 , Initial total length; W_1 , initial body weight; L_2 , final total length; W_2 , final body weight; SGR_l , specific growth rate in length; SGR_w , specific growth rate in weight; CV_l , coefficient of variation of length; CV_w , coefficient of variation of weight; CF, condition factor; SR, survival rate.

Table 3. Feed utilization efficiency in *Amphiprion ocellaris* fed diets supplemented with different carotenoid sources

Parameters	Treatments					
	Control	Ch-egg	Sh-shell	Cope	Sn-egg	Astax
FR (%BW/day)	1.73 ± 0.07	1.80 ± 0.10	1.71 ± 0.04	1.84 ± 0.10	2.01 ± 0.03	1.90 ± 0.07
FCR	1.95 ± 0.08 ^c	1.80 ± 0.15 ^{bc}	1.51 ± 0.01 ^a	1.60 ± 0.08 ^{ab}	1.91 ± 0.01 ^c	1.93 ± 0.10 ^c
FER (%)	51.4 ± 2.32 ^a	56.5 ± 4.89 ^{ab}	66.1 ± 0.18 ^c	63.0 ± 3.03 ^{bc}	52.5 ± 0.17 ^a	52.1 ± 2.73 ^a
PER	0.94 ± 0.04 ^a	1.03 ± 0.09 ^{ab}	1.20 ± 0.01 ^c	1.14 ± 0.05 ^{bc}	0.96 ± 0.01 ^a	0.95 ± 0.05 ^a
LER	4.28 ± 0.19 ^a	4.71 ± 0.41 ^{ab}	5.50 ± 0.01 ^c	5.25 ± 0.25 ^{bc}	4.38 ± 0.01 ^a	4.34 ± 0.23 ^a

Data are expressed as mean ± SE (n = 3).

Means with different superscript letters within the same row are significantly different ($p < 0.05$).

FR, feeding rate; FCR, feed conversion ratio; FER, feed efficiency ratio; PER, protein efficiency ratio; LER, lipid efficiency ratio.

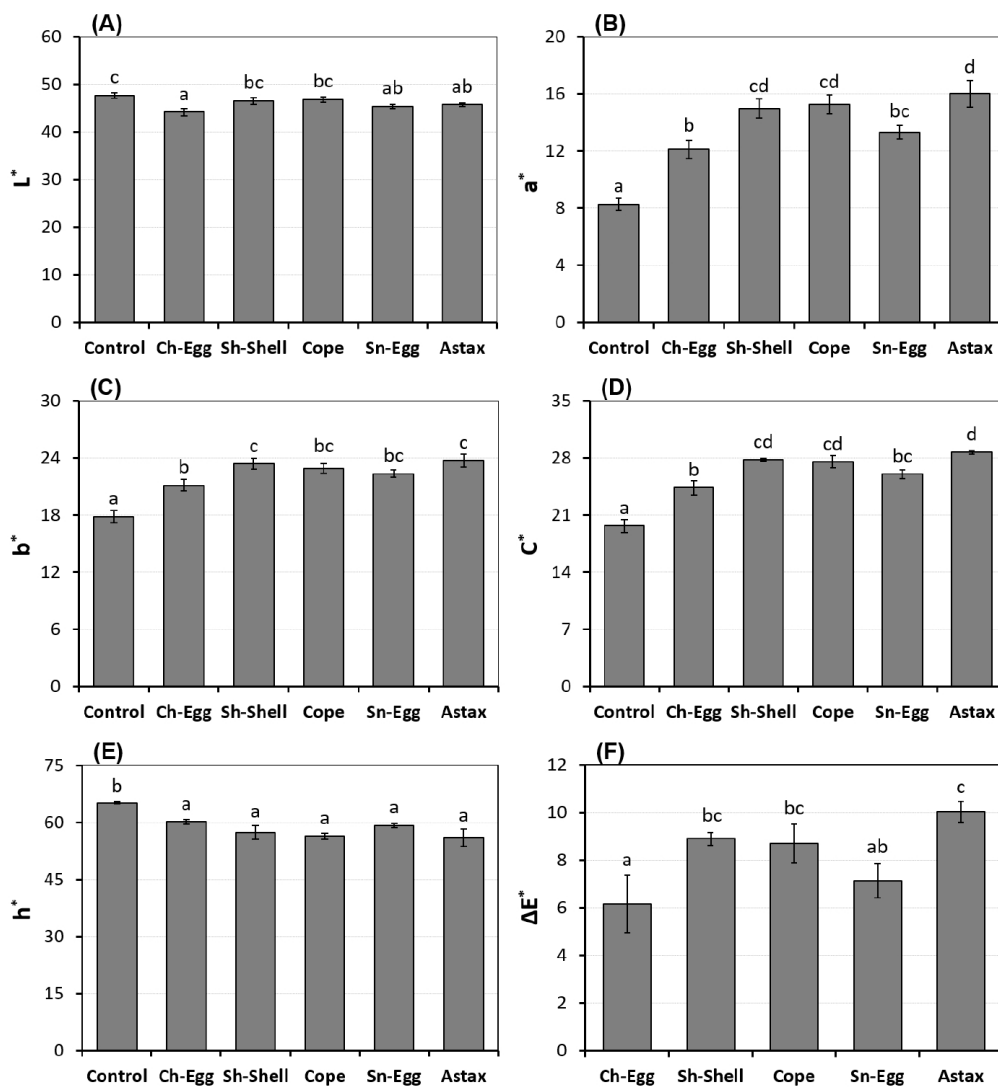


Fig. 1. Skin pigmentation metrics in *Amphiprion ocellaris* following supplementation with different carotenoid sources: luminance (L*, A), red - green intensity (a*, B), yellow - blue intensity (b*, C), chroma (C*, D), hue angle (h*, E), and color difference (ΔE*, F). Different letters on the bars indicate statistically significant differences ($p < 0.05$).

dex) was higher in the carotenoid-supplemented groups than in the control, with the highest values achieved in the treatments supplemented with carotenoid sources from synthetic astaxanthin, copepods, and shrimp shells (Fig. 1B). The increment in skin redness ranged from 80.9% to 93.5% as compared to the control. A similar trend was observed in the measurements of other color indices, including skin yellowness (b^*), color saturation (C^*), and hue angle (h^*) (Fig. 1C–1E). Interestingly, the contrasting trend of high C^* and low h^* values in the shrimp shell, copepod, and synthetic astaxanthin treatments compared to the other treatments suggests that the skin color of fish in these treatments was more vivid and saturated, and closer to the desired red color in clownfish. The overall color difference (ΔE^*) compared to the control was also more pronounced in the group of three aforementioned supplemented treatments than in the other treatments (Fig. 1F).

Accumulation of carotenoids

The TC accumulated in the skin, muscle, and whole body of fish is shown in Fig. 2. Specifically, the carotenoid content in the skin increased significantly in the carotenoid-supple-

mented groups compared to the control group. Among them, the groups supplemented with carotenoids from copepods, shrimp shells, and synthetic astaxanthin achieved the highest results, with an estimated increase of 162%–177% compared to the control (Fig. 2A). Similarly, the carotenoid content in the muscle and whole body of fish also increased significantly in the experimental groups compared to the control (Fig. 2B and 2C). The results demonstrate that supplementing the diet with carotenoid sources, particularly copepods, shrimp shells, and synthetic astaxanthin, effectively enhances the accumulation of carotenoids in fish tissues. This indicates the effectiveness of carotenoid supplementation in the diet to enhance the color quality of ornamental fish in general.

Discussion

Growth and coloration are two of the most important traits of interest in ornamental aquaculture, especially for slow-growing species such as clownfish (*Amphiprion* spp.). To reach the size of juveniles for commercial farming (3.5–4.5 cm), they require 5–6 months depending on the farming conditions, while this period

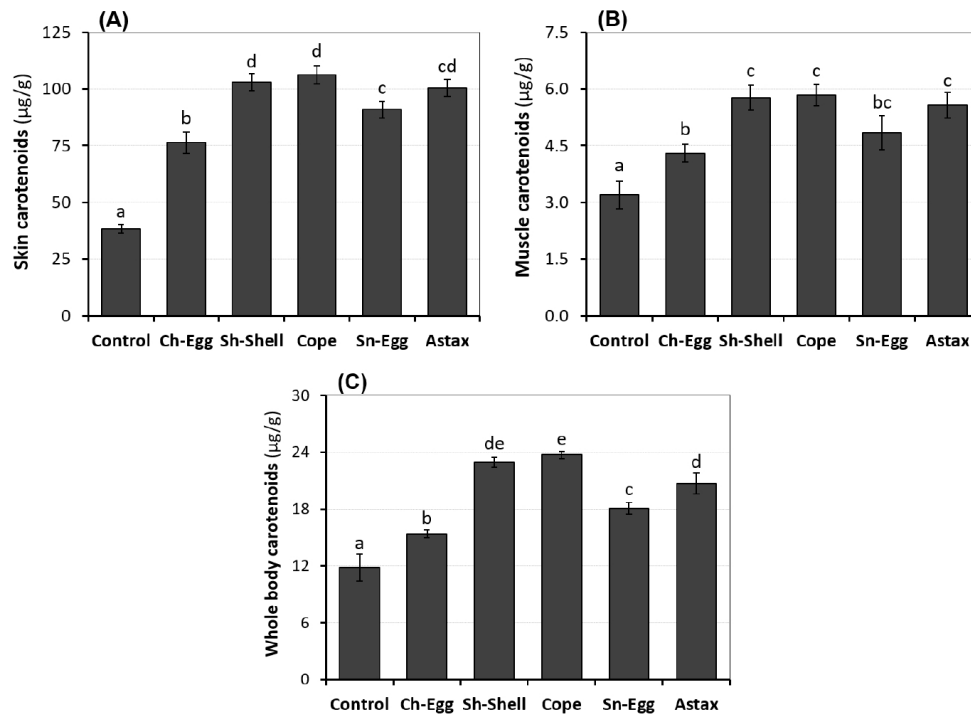


Fig. 2. Total carotenoid accumulation in tissues of *Amphiprion ocellaris* fed diets supplemented with different carotenoid sources: skin (A), muscle (B), and whole Body (C). Different letters on the bars indicate statistically significant differences ($p < 0.05$).

is only 1.0–1.5 months for the majority of marine fish species farmed for food (Calado et al., 2017; Madhu et al., 2012). Therefore, enhancing growth would shorten the farming time and increase the economic efficiency of the production process. The present study demonstrated that the supplementation of carotenoids from copepods and shrimp shells significantly improved the growth performance and feed utilization efficiency of clownfish, with increases of 21.2%–34.8% and 15.2%–22.6%, respectively, compared to the control (Table 3). This result confirmed the role of carotenoids, especially those extracted from copepods and shrimp shells, in the farming outcomes of fish in general and clownfish in particular.

Recent studies have emphasized the crucial role of carotenoids, which are bioactive substances, in the development, feed conversion efficiency, and overall health of aquatic species (Elbahnaswy & Elshopakey, 2024; Lim et al., 2023). The superior growth in fish supplemented with carotenoids can be explained through several biological mechanisms: for example, they stimulate the activity of digestive enzymes, thereby enhancing nutrient metabolism (Amar et al., 2001; Zhu et al., 2022). In addition, carotenoids also act as antioxidants, reducing oxidative stress and modulating the expression of genes involved in various physiological processes, supporting the efficient use of energy for growth (Fang et al., 2021; Lim et al., 2023). Furthermore, studies by James et al. (2006) and Zhao et al. (2022) suggested that carotenoids have a positive impact on gut structure by improving the characteristics of villi and enhancing the balance of the gut microbiota, which contributes to more efficient digestion and nutrient absorption (James et al., 2006; Zhao et al., 2022).

The findings of the present study are consistent with numerous previous studies investigating the effects of dietary carotenoids in marine fish species such as *Pagrus pagrus* (Kalinowski et al., 2011), *Pseudosciaena crocea* (Li et al., 2014), *Trachinotus ovatus* (Xie et al., 2017), *Dicentrarchus labrax* (Saleh et al., 2018), and *Epinephelus akaara* (Song et al., 2021). However, some other studies failed to demonstrate significant growth enhancements from carotenoid supplementation, such as in the cases of *P. pagrus* (Tejera et al., 2007), *Paralichthys olivaceus* (Pham et al., 2014), *Plectropomus leopardus* (Zhu et al., 2022), and *Amphiprion percula* (Tran et al., 2022). This indicates the complexity and inconsistency in the effects of carotenoids on fish growth. Specifically, the varying results could stem from the variations in factors such as fish species, life stage, diet, source and dosage of carotenoids, as well as different rearing conditions

(Elbahnaswy & Elshopakey, 2024; Kalinowski et al., 2011; Lau et al., 2023; Lim et al., 2018). In contrast, the current findings, together with Ebenezzar et al. (2020), showed the benefits of carotenoid supplementation on the growth of *A. ocellaris*, which was not observed in the works of Díaz-Jiménez et al. (2021), Nhan et al. (2019), and Seyedi et al. (2013). This inconsistency indicates that there are some factors that have not yet been elucidated that affect the impact of carotenoids, and highlights the need for further research on this topic, such as the influence of environmental factors, nutrient interactions, and genetic factors. Although the present study did not include cytotoxicity assays and histological examinations, the data suggest that carotenoid supplementation did not cause any overt negative effects on the CF and SR of the fish (> 95%). This implies that *A. ocellaris* can adapt to dietary carotenoid supplementation under the experimental conditions. However, further studies, including cytotoxicity assays and histological examinations, are necessary to comprehensively evaluate the safety and benefits of carotenoid supplementation in this fish species.

The current study demonstrated that supplementing carotenoids from various dietary sources significantly improved the coloration of *A. ocellaris*, addressing the challenges of discoloration and pigmentation depletion that occur in captive-bred clownfish, which reduce their market value (Nhan et al., 2019). Clownfish tend to lose their brightening color in captivity due to lack of appropriate dietary supply, as well as other factors such as captive stress, water quality, and dietary level or feed consumption (Ebenezzar et al., 2020; Nhan et al., 2019). Previous efforts to enhance and maintain the coloration of cultured ornamental fish through dietary supplementation have been reported (Díaz-Jiménez et al., 2021; Ebenezzar et al., 2020; Pham et al., 2014; Tran et al., 2022; Yasir & Qin, 2010). In the present study, the supplementation of carotenoids from copepods, shrimp shells, and synthetic astaxanthin significantly enhanced the coloration of clownfish, including both skin color indices and accumulated carotenoid content, compared to the control. In the two most important measured parameters, skin redness (a^*) and skin accumulated carotenoid content, the degree of color improvement was estimated to increase by 80.9%–93.5% and 162.4%–177.6%, respectively, in the pigment-supplemented groups compared to the control (Figs. 1 and 2). This confirmed the effectiveness of dietary copepod-, shrimp shell-, and synthetic astaxanthin-derived carotenoids on the coloration of clownfish, which is consistent with previous reports noting similar trends in the effects on this fish species when using different sources of carotenoids,

including both natural and synthetic ones (Díaz-Jiménez et al., 2021; Ebenezzar et al., 2020; Nhan et al., 2019; Seyedi et al., 2013). Compared to the plant-based carotenoid sources that we tested previously (Tran et al., 2024), the color-enhancing efficiency of animal-based carotenoid sources (copepods and shrimp shells) was significantly higher, with increases in a* index and skin accumulated carotenoid content in these two groups compared to the control of 80.9%–93.5% and 162.4%–177.6%, respectively. In contrast, the plant-based carotenoid sources (bell pepper and gac fruit) only achieved increases of 75.7%–89.2% and 89.6%–98.4%, respectively. This result further supports the argument that the color-enhancing efficiency in a particular fish species varies depending on the characteristics of the carotenoid source used and the method of supplementation (Díaz-Jiménez et al., 2021; Seyedi et al., 2013; Tran et al., 2022; Yasir & Qin, 2010). Future research should focus on identifying the specific factors that influence this variation, such as the chemical structure of the carotenoid, dosage, duration of supplementation, and the absorption capacity of the fish.

In conclusion, supplementation with carotenoids, both natural and synthetic, improved growth, feed utilization efficiency, and coloration in clownfish. Natural carotenoids from copepods and shrimp shells improved growth and feed utilization efficiency better than synthetic astaxanthin, but the color-enhancing effects were similar. Synthetic astaxanthin remains an effective solution for enhancing ornamental fish coloration due to its availability and convenience in use. However, natural carotenoids would be a more sustainable solution for long-term aquaculture, especially considering the issues related to safety, multidimensional effects on the health of the cultured species, and environmental friendliness (Elbahnaswy & Elsho-pakey, 2024; Trichet & Amaya, 2022). This study supports the use of natural carotenoids to improve the quality and value of cultured ornamental fish, thereby contributing to reducing the pressure on wild reef fish stocks and protecting coral reef ecosystems. However, our study still has several limitations. Firstly, we focused on evaluating the PER and LER based on the protein and lipid content in the feed intake, rather than determining the actual accumulation of protein and lipid in the fish body. Secondly, the optimal dosage and duration of carotenoid supplementation from copepods and shrimp shells need to be further investigated. Thirdly, the mechanisms of carotenoid metabolism, absorption, and accumulation in fish body remain to be elucidated. Lastly, the effects of carotenoid supplementation from copepods and shrimp shells on various health parameters,

such as antioxidant capacity, immunity, and stress resistance, warrant further research. Future studies addressing these aspects would provide a more comprehensive understanding of the benefits and limitations of natural carotenoid supplementation in ornamental fish nutrition.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

This study conformed to the guidance of animal ethical treatment for the care and use of experimental animals.

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References

Amar EC, Kiron V, Satoh S, Watanabe T. Influence of various dietary synthetic carotenoids on bio-defence mechanisms in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac Res.* 2001;32:162-73.

- Bolker JA, Hill CR. Pigmentation development in hatchery-reared flatfishes. *J Fish Biol.* 2000;56:1029-52.
- Calado R, Olivotto I, Oliver MP, Holt GJ. Marine ornamental species aquaculture. Hoboken, NJ: John Wiley & Sons; 2017.
- da Silva CRB, Hoepner CM, Mercader M, Laudet V, da Silva KB. The impact of popular film on the conservation of iconic species: anemonefishes in the aquarium trade. In: Laudet V, Ravasi T, editors. Evolution, development and ecology of anemonefishes: model organisms for marine science. Boca Raton, FL: CRC Press; 2022. p. 223.
- Díaz-Jiménez L, Hernández-Vergara MP, Pérez-Rostro CI, Olvera-Novoa MÁ. The effect of two carotenoid sources, background colour and light spectrum on the body pigmentation of the clownfish *Amphiprion ocellaris*. *Aquac Res.* 2021;52:3052-61.
- Ebenezeer S, Prabu DL, Chandrasekar S, Tejpal CS, Madhu K, Sayooj P, et al. Evaluation of dietary oleoresins on the enhancement of skin coloration and growth in the marine ornamental clown fish, *Amphiprion ocellaris* (Cuvier, 1830). *Aquaculture.* 2020;529:735728.
- Elbahnaswy S, Elshopakey GE. Recent progress in practical applications of a potential carotenoid astaxanthin in aquaculture industry: a review. *Fish Physiol Biochem.* 2024;50:97-126.
- Fang H, Xie J, Zhao W, Liu Z, Liu Y, Tian L, et al. Study supplementation of astaxanthin in high-fat diet on growth performance, antioxidant ability, anti-inflammation, non-specific immunity and intestinal structure of juvenile *Trachinotus ovatus*. *Aquac Nutr.* 2021;27:2575-86.
- García-Chavarría M, Lara-Flores M. The use of carotenoid in aquaculture. *Res J Fish Hydrobiol.* 2013;8:38-49.
- Gupta SK, Jha AK, Pal AK, Venkateshwarlu G. Use of natural carotenoids for pigmentation in fishes. Pretoria: Council of Scientific and Industrial Research [CSIR]; 2007.
- Hunter RS, Harold RW. The measurement of appearance. Hoboken, NJ: John Wiley & Sons; 1987.
- James R, Sampath K, Thangarathinam R, Vasudevan I. Effect of dietary spirulina level on growth, fertility, coloration and leucocyte count in red swordtail, *Xiphophorus helleri*. *Isr J Aquac Bamid.* 2006;58.
- Kalinowski CT, Robaina LE, Izquierdo MS. Effect of dietary astaxanthin on the growth performance, lipid composition and post-mortem skin colouration of red porgy *Pagrus pagrus*. *Aquac Int.* 2011;19:811-23.
- Lau CC, Mohd Nor SA, Tan MP, Yeong YS, Wong LL, Van de Peer Y, et al. Pigmentation enhancement techniques during ornamental fish production. *Rev Fish Biol Fish.* 2023;33:1027-48.
- Li M, Wu W, Zhou P, Xie F, Zhou Q, Mai K. Comparison effect of dietary astaxanthin and *Haematococcus pluvialis* on growth performance, antioxidant status and immune response of large yellow croaker *Pseudosciaena crocea*. *Aquaculture.* 2014;434:227-32.
- Lim KC, Yusoff FM, Karim M, Natrah FMI. Carotenoids modulate stress tolerance and immune responses in aquatic animals. *Rev Aquac.* 2023;15:872-94.
- Lim KC, Yusoff FM, Shariff M, Kamarudin MS. Astaxanthin as feed supplement in aquatic animals. *Rev Aquac.* 2018;10:738-73.
- Luo M, Lu G, Yin H, Wang L, Atuganile M, Dong Z. Fish pigmentation and coloration: molecular mechanisms and aquaculture perspectives. *Rev Aquac.* 2021;13:2395-412.
- Madhu R, Madhu K, Rethesh T. Life history pathways in false clown *Amphiprion ocellaris* Cuvier, 1830: a journey from egg to adult under captive condition. *J Mar Biol Assoc India.* 2012;54:77-90.
- Nhan HT, Minh TX, Liew HJ, Hien TTT, Jha R. Effects of natural dietary carotenoids on skin coloration of false clownfish (*Amphiprion ocellaris* Cuvier, 1830). *Aquac Nutr.* 2019;25:662-8.
- Pereira da Costa D, Campos Miranda-Filho K. The use of carotenoid pigments as food additives for aquatic organisms and their functional roles. *Rev Aquac.* 2020;12:1567-78.
- Pham MA, Byun HG, Kim KD, Lee SM. Effects of dietary carotenoid source and level on growth, skin pigmentation, antioxidant activity and chemical composition of juvenile olive flounder *Paralichthys olivaceus*. *Aquaculture.* 2014;431:65-72.
- Saleh N, Wassef EA, Shalaby SM. The role of dietary astaxanthin in European sea bass (*Dicentrarchus labrax*) growth, immunity, antioxidant competence and stress tolerance. *Egypt J Aquat Biol Fish.* 2018;22:189-200.
- Sathyaruban S, Uluwaduge DI, Yohi S, Kuganathan S. Potential natural carotenoid sources for the colouration of ornamental fish: a review. *Aquac Int.* 2021;29:1507-28.
- Schweiggert RM, Carle R. Carotenoid deposition in plant and animal foods and its impact on bioavailability. *Crit Rev Food Sci Nutr.* 2017;57:1807-30.
- Seyedi SM, Sharifpour I, Ramin M, Jamili S. Effect of dietary

- astaxanthin on survival, growth, pigmentation clownfish, *Amphiprion ocellaris*, Cuvier. Indian J Fundam Appl Life Sci. 2013;3:391-5.
- Sköld HN, Aspengren S, Cheney KL, Wallin M. Chapter four - fish chromatophores—from molecular motors to animal behavior. Int Rev Cell Mol Biol. 2016;321:171-219.
- Song JH, Cho YS, Park JY, Kim GD, Lim HK. Effects of astaxanthin produced by *Paracoccus haeundaensis* on growth and body color in *Epinephelus akaara*. Isr J Aquac Bamid. 2021;73:1-12.
- Tejera N, Cejas JR, Rodríguez C, Bjerkeng B, Jerez S, Bolaños A, et al. Pigmentation, carotenoids, lipid peroxides and lipid composition of skin of red porgy (*Pagrus pagrus*) fed diets supplemented with different astaxanthin sources. Aquaculture. 2007;270:218-30.
- Tomasevic I, Tomovic V, Milovanovic B, Lorenzo J, Đorđević V, Karabasil N, et al. Comparison of a computer vision system vs. traditional colorimeter for color evaluation of meat products with various physical properties. Meat Sci. 2019;148:5-12.
- Tran DV, Dang TT, Cao TTT, Hua NT, Pham HQ. Natural astaxanthin extracted from shrimp waste for pigment improvement in the orange clownfish, *Amphiprion percula*. Aquac Res. 2022;53:4190-8.
- Tran DV, Luong HT, Pham KT, Dang TT, Hua NT, Pham HQ. Plant-based carotenoid supplementation: growth, feed utilization efficiency, and coloration in false clownfish (*Amphiprion ocellaris*). Isr J Aquac Bamid. 2024;76.
- Trichet VV, Amaya E. Astaxanthin use as carotenoid source and its benefits in feeds. In: Allen Davis D, editor. Feed and feeding practices in aquaculture. Cambridge: Woodhead; 2022. p. 309-35.
- Vilgrain L, Maps F, Basedow S, Trudnowska E, Madoui MA, Niehoff B, et al. Copepods' true colors: astaxanthin pigmentation as an indicator of fitness. Ecosphere. 2023;14:e4489.
- Vissio PG, Darias MJ, Di Yorio MP, Sirkin DIP, Delgadin TH. Fish skin pigmentation in aquaculture: the influence of rearing conditions and its neuroendocrine regulation. Gen Comp Endocrinol. 2021;301:113662.
- Xie J, Chen X, Niu J, Wang J, Wang Y, Liu Q. Effects of astaxanthin on antioxidant capacity of golden pompano (*Trachinotus ovatus*) *in vivo* and *in vitro*. Fish Aquat Sci. 2017;20:6.
- Yasir I, Qin JG. Effect of dietary carotenoids on skin color and pigments of false clownfish, *Amphiprion ocellaris*, Cuvier. J World Aquac Soc. 2010;41:308-18.
- Zhao W, Guo YC, Huai MY, Li L, Man C, Pelletier W, et al. Comparison of the retention rates of synthetic and natural astaxanthin in feeds and their effects on pigmentation, growth, and health in rainbow trout (*Oncorhynchus mykiss*). Antioxidants. 2022;11:2473.
- Zhu X, Hao R, Zhang J, Tian C, Hong Y, Zhu C, et al. Dietary astaxanthin improves the antioxidant capacity, immunity and disease resistance of coral trout (*Plectropomus leopardus*). Fish Shellfish Immunol. 2022;122:38-47.