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Enhancing Growth Performance, Coloration, and Body Composition in Hatchery-Produced False Clownfish (*Amphiprion ocellaris*) Through Dietary Astaxanthin Supplementation Derived from Shrimp Shell Waste

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ABSTRACT

Coloration is a critical factor determining the value and marketability of marine ornamental fish, particularly in the false clownfish **(***Amphiprion ocellaris***)**. However, a significant challenge for current hatchery-produced false clownfish is their inferior coloration **(**pale, dark, less vivid**)** compared to wild-caught specimens. The present study assessed the dose-dependent impact of astaxanthin supplementation in the diet, sourced from shrimp shell waste, on growth performance, coloration, and body composition in hatchery-produced false clownfish. Six experimental groups, each containing 15 fish, received diets supplemented with astaxanthin at levels ranging from 0 **(**control**)** to 1000 mg/kg, with increments of 200mg/ kg, for 75 days, with three replicates per treatment. The findings revealed that astaxanthin supplementation notably improved growth parameters during the juvenile stage, including length-specific growth rate **(**SGR_L**)** **(**13.79–41.38%/day**)**, weight-specific growth rate **(**SGR_W**)** **(**13.33–42.67%/day**)**, and feed conversion ratio **(**FCR**)** **(**6.03–22.11%**)**. Skin coloration, measured by the a^* value and total carotenoid content, was enhanced by 93.65–163.74% and 140.78–341.20%, respectively, compared to the control group. Increasing dietary astaxanthin levels positively affected body protein composition while reducing lipid content. Based on the obtained results, a supplementation level of 600mg/ kg feed appears sufficient to improve the quality of the hatchery-produced false clownfish. These findings demonstrate the effectiveness of astaxanthin derived from shrimp shell waste in enhancing growth performance, coloration, and body composition in the hatchery-produced false clownfish, which is crucial for improving the acceptance and value of hatchery-produced fish in ornamental fish markets.

INTRODUCTION

The global demand for ornamental marine fish has increased considerably over the past twenty years, resulting in the rapid development of this industry, particularly in the Southeast Asian region **(Pouil *et al.*, 2020)**. Among the most sought-after species, the false clownfish **(***Amphiprion ocellaris***)** stands out with its vibrant colors, adaptability to captive conditions, and unique symbiotic relationship with anemone species **(Calado *et***

***al*., 2017)**. Although significant success has been achieved in the artificial breeding of false clownfish **(Calado *et al*., 2017)**, the issue of suboptimal coloration in captive-bred individuals compared to their wild counterparts remains a persistent challenge **(Ebeneezar *et al*., 2020)**. Consequently, the pressure on natural ornamental fish resources increases, leading to resource depletion and destruction of coral reef ecosystems, especially when unsustainable harvesting methods are used **(Pouil *et al*., 2019)**. Thus, finding efficient strategies to improve the coloration of the false clownfish specifically and marine ornamental fish generally is essential to enhance their value and foster the sustainable growth of marine ornamental fish farming.

Color is a critical factor determining the market value and attractiveness of ornamental fish **(Lau *et al*., 2023)**. Color changes in ornamental fish are influenced by various factors, such as pigments, genetics, endocrine control, living environment, and other behavioral and social factors **(Luo *et al*., 2021)**. To enhance the color of captive marine ornamental species, different strategies have been employed, including dietary manipulation, improvement of rearing conditions, genetic selection and breeding, and endocrine treatments **(Luo *et al*., 2021; Lau *et al*., 2023)**. Among these, supplementation of pigment nutrients, particularly carotenoids, is widely used due to its natural, effective, and straightforward approach **(Lau *et al*., 2023; Elbahnaswy & Elshopakey, 2024)**. However, selecting the appropriate type of pigment supplement that is safe for the cultured species, environmentally friendly, and determining the optimal supplementation regime, especially the dosage, remain significant challenges to be addressed.

Astaxanthin, a naturally occurring carotenoid, is widely employed in aquaculture to enhance the orange to red coloration of numerous aquatic species, including fish and crustaceans **(Lim *et al*., 2018; Elbahnaswy & Elshopakey, 2024)**. Fish cannot synthesize astaxanthin and entirely depend on dietary sources to achieve their characteristic coloration **(Sathyaruban *et al*., 2021)**. Carotenoid deficiency in the diet is the primary cause of pale and dull coloration in many fish varieties **(Lim *et al*., 2018; Lau *et al*., 2023)**. Although artificial astaxanthin is frequently utilized in aquaculture due to its availability, convenience, and affordability **(Lim *et al*., 2018)**, natural astaxanthin sources are gaining increasing attention from researchers and producers due to their multifaceted benefits and positive environmental impact **(Sathyaruban *et al*., 2021; Elbahnaswy & Elshopakey, 2024)**. Exploring and applying safe, effective, and sustainable natural astaxanthin sources is a promising approach to enhance the coloration and value of cultured marine ornamental fish while promoting environmental sustainability.

The global shrimp processing industry generates substantial quantities of by-products, including shells, cephalothorax, and wash water, comprising 50 - 60% of the total production **(Nirmal *et al*., 2020)**. According to statistics from **FAO (2024)**, the worldwide crustacean production amounted to 18.43 million metric tons in 2022. These by-products contain a significant amount of astaxanthin, ranging from 10.81 - 295.2

mg/kg, depending on the shrimp species and extraction technology **(Irna *et al*., 2017)**. Vietnam, one of the world's leading shrimp exporters with a production of 1.08 million tons in 2022, is facing a significant opportunity to utilize this astaxanthin-rich by-product resource **(Nguyen *et al*., 2022)**. The efficient use of this resource not only brings economic benefits to the shrimp processing industry but also contributes to environmental pollution mitigation **(Nirmal *et al*., 2020)**.

Based on previous studies demonstrating the potential of using natural carotenoid sources, particularly astaxanthin from crustaceans, in improving coloration and flesh quality of different fish species **(Ebeneezar *et al*., 2020; Tran *et al*., 2022)**, this research focuses on determining the most effective dosage of shrimp shell-derived astaxanthin within the range from 0 – 1000 mg/kg of feed to comprehensively improve the essential culture parameters of the false clownfish, a species with a tendency toward red coloration and may necessitate higher dosages compared to other fish species. The results from this investigation are anticipated to offer important information on effective astaxanthin supplementation methods for cultured marine ornamental fish, thereby enhancing their quality and commercial value. Furthermore, utilizing shrimp processing by-products as a natural astaxanthin source contributes to sustainable development of ornamental fish farming by minimizing waste and mitigating the environmental impact of shrimp processing in Vietnam and worldwide.

MATERIALS AND METHODS

Experimental setup

A 75-day completely randomized experiment was conducted to assess the impact of astaxanthin supplementation levels in the diet from shrimp shells on the growth performance, survival, feed utilization efficiency, coloration, and body composition of juvenile *A. ocellaris*. Six astaxanthin levels (0, 200, 400, 600, 800, and 1000 mg astaxanthin per kg of feed) were tested in triplicate.

Astaxanthin preparation

Th white leg shrimp **(***Litopenaeus vannamei***)** shells were purchased from Nha Trang Seafood JSC **(**F17**)**, Khanh Hoa, Vietnam and transported on ice (< 5°C) to the Seafood Processing Technology Laboratory, Nha Trang University, Vietnam. The raw shells were preliminarily treated and kept at -20°C until further processing.

Astaxanthin extraction from shrimp shells was performed using a method similar to that outlined in our previous investigation **(Tran *et al*., 2022)**. Briefly, wet shrimp shells (100 g) were minced and mixed with 96% ethanol (VMC GROUP, Vietnam) using a solvent-to-material ratio of 3.5:1 (v/w). The mixture was subjected to microwave-assisted extraction to obtain astaxanthin, as detailed in **Tran *et al*. (2022)**. The ethanol-free extract was dissolved in 50ml of soybean oil **(**Tuong An, Vietnam**)**, purified, and quantified for astaxanthin content using UV-Vis spectrophotometry (Biochrom Ltd,

Cambridge, UK). The astaxanthin-enriched oil, containing 78.65 µg astaxanthin/g fresh shrimp shells, was used for supplementation of the experimental diets.

Experimental diets formulation

The basal control diet was formulated to contain 55% crude protein and 12% crude fat, designed to meet the nutritional needs of marine finfish juveniles **(Bowyer *et al.*, 2013)**. Table **(**1**)** presents the detailed ingredient composition and nutritional profile of the experimental diets.

Table 1. Composition and nutritional profile of the formulated experimental diets

Ingredient (g)	**Concentrations (mg/kg)**					
	Control	**200**	**400**	**600**	**800**	**1000**
Fishmeal **(**Peru**)**	425.0	425.0	425.0	425.0	425.0	425.0
Fishmeal **(**Vietnam**)**	136.0	136.0	136.0	136.0	136.0	136.0
Squid meal	140.0	140.0	140.0	140.0	140.0	140.0
Soybean meal	75.0	75.0	75.0	75.0	75.0	75.0
Corn gluten	85.0	85.0	85.0	85.0	85.0	85.0
Wheat flour	34.0	33.8	33.6	33.4	33.2	33.0
Fish oil	25.7	25.7	25.7	25.7	25.7	25.7
Soybean oil	30.0	30.0	30.0	30.0	30.0	30.0
Vitamin premix	10.0	10.0	10.0	10.0	10.0	10.0
Lysine	8.0	8.0	8.0	8.0	8.0	8.0
Methionine	6.0	6.0	6.0	6.0	6.0	6.0
Monocalcium phosphate	6.0	6.0	6.0	6.0	6.0	6.0
Guar gum powder	5.0	5.0	5.0	5.0	5.0	5.0
Sodium alginate	4.3	4.3	4.3	4.3	4.3	4.3
Mineral premix	10.0	10.0	10.0	10.0	10.0	10.0
Astaxanthin supplement	0.0	0.2	0.4	0.6	0.8	1.0
Proximate analysis (%)						
Crude protein	55.0	55.1	55.1	55.1	55.0	55.2
Crude lipid	12.0	11.9	12.1	12.2	12.1	12.1
Ash	11.3	11.5	11.4	11.7	11.1	11.6
Moisture	10.3	10.1	10.2	10.3	10.2	10.3
Carotenoids **(**g/kg**)**	0.03	0.24	0.43	0.65	0.84	1.06

The feed production followed the method described in **Tran *et al.* (2022)**. Accordingly, the feed ingredients (except astaxanthin and vitamin premix) were weighed, mixed homogeneously in a food mixer (Lifeng B20, Guangdong, China), moistened with water, and pressure cooked for 20 minutes. After allowing the mixture to cool to room temperature, the astaxanthin oil mixture **(**corresponding to each treatment**)** and the vitamin premix were incorporated and mixed thoroughly. The moist blend was subsequently extruded using a pellet mill **(**S150, Binh Quan T&M Co., Ltd., Hanoi, Vietnam**)** fitted with a 2.0-mm die. The extruded pellets were dried in an oven at 60°C for

18 hours, then crumbled and sieved to obtain pellets of 0.8- 1.0mm in size. Finally, the pellets were stored in sealed plastic boxes, covered with black plastic bags, and kept in a freezer **(**-4°C**)** throughout the experiment.

Fish rearing conditions

Fish source

Hatchery-bred clownfish juveniles from the Aquaculture Research Institute (Nha Trang University) were used for the feeding trial. Fish with uniform size and color, measuring 3.00 ± 0.05 cm in total length and weighing 0.58 ± 0.03 g, were randomly stocked at a density of 15 fish per tank or 4 L water/fish. The fish underwent a 7-day acclimation period in the experimental system prior to the commencement of the trial.

Tank system

The rearing system consisted of 18 glass tanks (60 L/tank, 55 × 35 × 38 cm). Treated natural seawater was used and supplied from the top of the tanks at a relatively uniform rate controlled by valves. Effluent water was collected and treated in a biofilter tank **(**500 L**)** located at the center of the system using filter wool and microorganisms living on the surface of bioball and coral rubble substrates. The treated water was then recirculated back to the rearing tanks. All tanks were continuously aerated throughout the experiment. The water inflow and outflow rates were maintained at approximately 1.5 L/min/tank, corresponding to a turnover rate of about 36 times/tank/day. The experimental system was placed under a roof with natural lighting conditions.

Fish management

The fish were fed to satiation by hand four times per day (7h, 10h, 13h, 16h). Uneaten feed was removed after 30min, dried (10% moisture), and used to calculate feed utilization. Fish waste was siphoned out twice daily (6h and 17h). Water was exchanged weekly (30%) and quality was monitored routinely using specialized water quality measurement devices. Specifically, pH, temperature, salinity, and dissolved oxygen were measured using a LAQUA WQ-310-K portable multiparameter water quality meter (HORIBA, Japan), and total ammonia nitrogen (TAN) was analyzed using a Hanna HI 96715 portable photometer (Hanna Instruments, USA).

Water quality parameters were maintained in optimal ranges throughout the study, including temperature: 28–31°C, salinity: 31–34‰, pH: 7.9–8.3, dissolved oxygen (DO): > 5.0 mg/L, and TAN: < 1.0 mg/L. Fish behavior and mortality were recorded daily.

Evaluation parameters and analytical methods

On day 75, all surviving fish were collected for evaluation. Fish were starved for 24 hours and anesthetized using 0.05% Ethylene Glycol Monophenyl Ether for 15-20 seconds prior to sampling. Length, weight, and color were measured after blotting fish

dry. The samples were then stored at -80°C for subsequent astaxanthin and biochemical analyses.

Growth parameters and survival rate

Fish growth was assessed using length (L) and weight (W), measured with a plastic-coated grid paper (1 mm precision) and an electronic analytical balance (Model PA4102, OHAUS, USA). Growth performance parameters, including length-specific growth rate (SGR_L), weight-specific growth rate (SGR_W), condition factor (CF), and survival rate (SR), were calculated using equations described in **Tran *et al.* (2022)**:

$SGR_L = [(LnL_2 - LnL_1) / t] \times 100$

$SGR_W = [(LnW_2 - LnW_1) / t] \times 100$

$CF = 100 \times W / L^3$

$SR (\%) = (N_2 / N_1) \times 100$

Where L_1, L_2 are the initial and final total lengths (mm), W_1, W_2 are the initial and final weights (g), t is the experiment duration (days), and N_1, N_2 are the initial and final fish numbers.

Feed utilization parameters

Feed utilization parameters were evaluated using feed intake (FI), residue, remaining feed, and weight gain. Equations for feeding rate (FR), feed conversion ratio (FCR), protein efficiency ratio (PER), and lipid efficiency ratio (LER) were also adapted from **Tran *et al.* (2022)**:

$FR (\%BW/day) = 100 \times FI / [(W_1 + W_2) / 2] / t$

$FCR = FI / (W_2 – W_1)$

$PER = (W_2 – W_1) / (FI \times P)$

$LER = (W_2 – W_1) / (FI \times L)$

Where P and L represent the protein (55%) and lipid (12%) content in the feed, respectively.

Skin color parameters

Upon completion of the experiment, fish skin color was assessed using a CR-400 Chroma Meter (Japan) on both lateral sides, located between the soft dorsal and anal fins, and adjacent to the middle white stripe. Three measurements were taken at each position to determine the average lightness (L^*), redness (a^*), and yellowness (b^*) values for each fish, following the manufacturer's guidelines. The CIE (International Commission on Illumination) color measurement system was used **(Hunter & Harold, 1987)**.

To assess the impact of dietary astaxanthin supplementation levels on skin color, the average color values (L^*, a^*, b^*) of the treatment groups were compared with those of the unsupplemented control group using the color discrepancy equation $\Delta E^*_{ab} = [(L^*_1 – L^*_2)^2 + (a^*_1 – a^*_2)_2 + (b^*_1 – b^*_2)^2]^{1/2}$. Additionally, the hue angle (hab) and chroma (C^*_{ab}) were determined for each fish using the equations $h^*_{ab} = \arctan (b^*/a^*)$ and $C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$, respectively **(Pathare *et al.*, 2013)**, to further assess skin color differences

between treatments.

Total accumulated carotenoid content

The total carotenoid contents in the skin, muscle, whole body, and feed were determined using a UV-Vis spectrophotometer, based on the methodology previously reported by the authors **(Tran *et al*., 2022)**. In brief, samples of skin (0.25 g), muscle (0.25 g), whole body (1.0 g), and feed (1.0 g) were homogenized in acetone containing anhydrous sodium sulfate using an Ultra-turrax® homogenizer. The homogenized samples were filtered and centrifuged to obtain the supernatant, which was stored at 4°C. The absorbance of the carotenoid extract was measured spectrophotometrically (Libra S22, Biochrom Ltd, Cambridge, UK).

Total carotenoid content (TC) (µg/g) was calculated using the equation:

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

where A is the absorbance at 450nm, V is the extract volume (mL), D is the dilution factor (if any), W is the sample weight (g), and ${}^{1\%}E_{1cm}$ is the specific extinction coefficient of total carotenoids in acetone at 450nm (2,100) **(Schiedt & Liaaen-Jensen, 1995)**.

Proximate biochemical composition

Proximate composition analyses, including moisture, crude protein, crude lipid, and ash content, were conducted on the experimental diets and whole fish samples following the standard methods described by **AOAC (2006)**. Crude protein content was determined using the Kjeldahl method, which involved multiplying the total nitrogen content by a conversion factor of 6.25. Crude lipid content was measured by performing Soxhlet extraction using hexane as the solvent. Ash content was determined by incinerating the samples at 600°C for 6 hours. Moisture content was assessed by drying the samples in an oven at 105°C until a constant weight was achieved.

Statistical analysis

The experimental data were expressed as mean ± standard deviation (SD) and subjected to one-way analysis of variance (ANOVA) using SPSS 26.0 statistical software. Prior to analysis, the data were checked for normality and homogeneity of variance to ensure the assumptions of ANOVA were met. When significant differences were detected among treatments, Duncan's multiple range test was applied to identify specific differences between means at a significance level of $P< 0.05$. The final results were reported as mean ± standard error (SE).

RESULTS

Growth performance and survival rate

Table (2) presents the growth performance parameters and survival rates of *A. ocellaris* fed diets containing varying levels of astaxanthin. The results demonstrated that the fish receiving diets supplemented with 600 and 800 mg astaxanthin per kg of feed achieved the highest final length (L_2), length-specific growth rate (SGR_L), final weight (W_2), and weight-specific growth rate (SGR_W), with increases ranging from 8.29 – 41.38% and 25.49 – 42.67% compared to the unsupplemented control group, respectively ($P<0.05$). No significant differences were observed in growth performance among the astaxanthin supplementation levels from 600 to 1000 mg/kg ($P> 0.05$).

Table 2. Growth and survival rates of *A. ocellaris* in experimental treatments

Parameter	Dietary astaxanthin levels (mg/kg)					
	Control	**200**	**400**	**600**	**800**	**1000**
L_1 (cm)	3.00±0.05	3.00±0.05	3.00±0.05	3.00±0.05	3.00±0.05	3.00±0.05
W_1 (g)	0.58±0.03	0.58±0.03	0.58±0.03	0.58±0.03	0.58±0.03	0.58±0.03
L_2 (cm)	3.74±0.02^{a}	3.85±0.01^{b}	3.95±0.01^{c}	4.05±0.02^{d}	4.07±0.03^{d}	4.03±0.04^{cd}
W_2 (g)	1.02±0.02^{a}	1.10±0.01^{b}	1.20±0.02^{c}	1.28±0.02^{d}	1.29±0.02^{d}	1.27±0.04^{cd}
SGR_L (%/day**)**	0.29±0.01^{a}	0.33±0.01^{b}	0.37±0.01^{c}	0.40±0.01^{d}	0.41±0.01^{d}	0.39±0.02^{cd}
SGR_W (%/day**)**	0.75±0.03^{a}	0.85±0.02^{b}	0.97±0.03^{c}	1.05±0.02^{cd}	1.07±0.02^{d}	1.04±0.04^{cd}
CV_L (%)	11.33±0.67^{b}	10.33±0.33^{ab}	9.33±0.88^{ab}	8.33±0.88^{a}	9.33±0.88^{ab}	10.67±0.88^{ab}
CV_W (%)	35.33±1.76^{b}	33.00±1.16^{b}	30.33±2.91^{ab}	25.67±0.33^{a}	27.00±0.58^{a}	34.33±2.33^{b}
CF (g/cm^3)	1.95±0.01	1.91±0.03	1.95±0.02	1.92±0.01	1.92±0.01	1.94±0.01
SR (%)	91.11±2.22	95.55±2.22	95.55±2.22	97.78±2.22	97.78±2.22	95.55±2.22

Mean values ± standard errors (n = 3) are presented. Superscript letters denote significant differences ($P<0.05$) among treatments within the same row.

Furthermore, dietary astaxanthin supplementation also positively affected the coefficient of variation in weight (CV_W), as evidenced by the higher uniformity in the 600 mg astaxanthin per kg group compared to the control ($P< 0.05$). However, other parameters, including coefficient of variation in length (CV_L), condition factor (CF), and survival rate (SR), were not significantly influenced by the dietary astaxanthin supplementation levels under the experimental conditions. These findings suggest that dietary astaxanthin supplementation significantly improves growth performance in *A. ocellaris*, with the optimum levels ranging from 600 to 800 mg/kg.

Feed utilization parameters

The impact of astaxanthin supplementation on feed utilization parameters in *A. ocellaris* is presented in Table (3). Although the feeding rate (FR) did not differ significantly among the treatment groups ($P> 0.05$), the feed utilization efficiency parameters, such as feed conversion ratio (FCR), protein efficiency ratio (PER), and lipid efficiency ratio (LER), exhibited significant impacts of astaxanthin. The best FCR was

achieved in the 600mg astaxanthin per kg treatment, with a 22.11% reduction compared to the unsupplemented control group ($P< 0.05$). Similarly, PER and LER reached the highest values at 600mg/ kg, increasing by 27.17 and 27.55%, respectively, when compared to the control group ($P< 0.05$). Notably, there were no significant differences found in FCR, PER, and LER among the astaxanthin supplementation levels from 600 to 1000mg/ kg ($P> 0.05$), indicating that 600mg/ kg is the most suitable level to enhance feed utilization efficiency in *A. ocellaris* under the experimental conditions.

Table 3. Feed utilization efficiency of *A. ocellaris* in experimental treatments

Parameter	Dietary astaxanthin levels (mg/kg)					
	Control	**200**	**400**	**600**	**800**	**1000**
FR (%BW/day**)**	1.45±0.04	1.54±0.10	1.68±0.11	1.56±0.06	1.67±0.08	1.65±0.09
FCR	1.99±0.08^{c}	1.87±0.09^{c}	1.81±0.08^{bc}	1.55±0.04^{a}	1.65±0.05^{ab}	1.66±0.03^{ab}
PER	0.92±0.04^{a}	0.98±0.05^{ab}	1.01±0.05^{abc}	1.17±0.03^{d}	1.10±0.04^{cd}	1.09±0.02^{bcd}
LER	4.21±0.17^{a}	4.47±0.21^{a}	4.62±0.21^{ab}	5.37±0.15^{c}	5.06±0.17^{bc}	5.02±0.09^{bc}

Mean values ± standard errors (n = 3) are presented. Superscript letters denote significant differences *(P<0.*05) among treatments within the same row.

Skin color

The skin color of *A. ocellaris* was evaluated using the colorimetric parameters lightness (L^*), redness (a^*), yellowness (b^*), hue angle (h^*_{ab}), chroma (C^*_{ab}), and total color difference (ΔE^*_{ab}) (Table 4). The results demonstrated a significant enhancement in redness (a^*) in the astaxanthin-supplemented groups compared to the unsupplemented control ($P< 0.05$). The highest redness (a^*) value (21.60) was observed in the 800mg/ kg treatment, representing a 163.74% increase compared to the control, while the suitable range for color enhancement was 600– 1000mg/ kg. The yellowness (b^*) value was the highest in the 600 and 800mg/ kg treatments, increasing by 68.88 and 76.75%, respectively, compared to the control. Increasing the astaxanthin level to 1000mg/ kg did not result in further significant improvement ($P> 0.05$). The lightness (L^*) value decreased by 5.73 and 8.06% in the 800– 1000mg/ kg treatments ($P< 0.05$), indicating that high astaxanthin levels (≥ 800mg/ kg) may darken the fish skin.

Table 4. Colorimetric parameters of *A. ocellaris* in experimental treatments

Parameter	Dietary astaxanthin levels (mg/kg)					
	Control	**200**	**400**	**600**	**800**	**1000**
L^*	49.75± 0.48^{d}	49.02±0.14^{cd}	48.41±0.34^{c}	47.74±0.53^{bc}	46.90±0.38^{ab}	45.74±0.47^{a}
a^*	8.19±0.54^{a}	15.86±0.28^{b}	17.95±0.31^{c}	20.85±0.49^{de}	21.60±0.39^{e}	20.11±0.37^{d}
b^*	16.00±0.65^{a}	22.72±0.42^{b}	23.91±0.36^{bc}	27.02±1.22^{de}	28.28±0.80^{e}	25.29±0.57^{cd}
h^*_{ab}	62.95±0.62^{c}	55.06±0.83^{b}	53.10±0.82^{ab}	52.28±1.79^{ab}	52.61±0.50^{ab}	51.50±0.32^{a}
C^*_{ab}	17.97±0.83^{a}	27.71±0.30^{b}	29.90±0.21^{c}	34.16±0.77^{de}	35.59±0.84^{e}	32.31±0.66^{d}
ΔE^*_{ab}		10.24±0.26^{a}	12.66 ±0.21^{b}	17.00±0.58^{cd}	18.42±0.83^{d}	15.66±0.58^{c}

Mean values ± standard errors (n=3) are presented. Superscript letters denote significant differences (P<0.05) among treatments within the same row.

The hue angle (h^*_{ab}), which represents the actual color, decreased by 16.43% with increasing astaxanthin supplementation levels from 0 to 800mg/ kg, indicating a shift towards a more reddish hue. Simultaneously, the chroma (C^*_{ab}), which measures color saturation or intensity, increased by 98.05% within the same supplementation range, suggesting an enhancement in color vividness. The total color difference (ΔE^*_{ab}), a comprehensive parameter that quantifies the overall color difference, demonstrated the profound influence of astaxanthin levels on color improvement. The largest ΔE^*_{ab} value (18.42) was achieved at 800mg/ kg, which was not statistically different from the value observed at 600mg/ kg (P> 0.05). These findings underscore the efficacy of dietary astaxanthin supplementation at 600– 800mg/ kg in enhancing the skin color of *A. ocellaris*. The vibrant coloration not only improves the aesthetic appeal of the fish but also significantly contributes to their overall quality and market value in the ornamental fish trade.

Total accumulated carotenoid content

The study findings revealed that the levels of carotenoids accumulated in the skin, muscle, and whole body of *A. ocellaris* were significantly elevated in the astaxanthin-supplemented groups compared to the unsupplemented control (P< 0.05), and the increase was correlated with the dietary astaxanthin concentrations (Table 5). The skin was the primary site of carotenoid accumulation, with the highest concentration reaching 177.01µg/ g in the 1000mg/ kg treatment, representing a substantial increase of 341.20% compared to the control group (40.12 µg/g). Correspondingly, at the same supplementation level, the carotenoid concentrations in the muscle and whole body were elevated by 161.06 and 183.13%, respectively, compared to the control (P< 0.05). It is worth noting that, there were no significant differences in the carotenoids accumulated in the skin, muscle, and whole body among the supplementation groups ranging from 600 to 1000mg/ kg (P> 0.05), suggesting that a dietary inclusion level of 600mg/ kg is sufficient for effective enhancement of carotenoid content in *A. ocellaris*.

Table 5. Total carotenoid content accumulated (TC) in tissues of *A. ocellaris* in experimental treatments

TC (µg/g)	Dietary astaxanthin levels (mg/kg)					
	Control	**200**	**400**	**600**	**800**	**1000**
Whole body	12.92±1.81^{a}	20.03±1.29^{b}	28.73±2.25^{c}	34.76±2.68^{cd}	35.19±1.68^{cd}	36.58±2.20^{d}
Skin	40.12±5.96^{a}	96.60±6.31^{b}	130.41±5.00^{c}	167.28±7.58^{d}	172.24±8.82^{d}	177.01±8.33^{d}
Muscle	3.39±0.52^{a}	5.64±0.39^{b}	6.80±0.54^{bc}	7.86±0.26^{cd}	8.64±0.46^{d}	8.85±0.52^{d}

Mean values ± standard errors **(**n=3**)** are presented. Superscript letters denote significant differences *(P*<0.05) among treatments within the same row.

In conclusion, the present investigation underscores the crucial importance of incorporating astaxanthin into the diet to effectively boost the accumulated carotenoid content in *A. ocellaris*. Although the highest efficacy was observed at the 1000mg/ kg inclusion level, the 600mg/ kg concentration proved to be the most advantageous, as it

resulted in a substantial enhancement of carotenoid content without necessitating additional increases in astaxanthin supplementation. This finding has significant implications for optimizing the cost-effectiveness of rearing this popular ornamental fish species in aquaculture settings.

Proximate body composition analysis

Table (6) presents the results of the proximate analysis of the whole body composition of the fish. The levels of astaxanthin supplementation had a significant impact on the protein and lipid content of the fish ($P< 0.05$). Protein content exhibited an upward trend with increasing astaxanthin supplementation, with the highest values observed in the 600– 1000mg/ kg treatment groups, representing a substantial increase of 20.28– 21.44% relative to the control group. In contrast, lipid content displayed a downward trend with increasing astaxanthin levels, with the 1000mg/ kg treatment group showing the lowest value, a marked reduction of 34.90% compared to the control. Astaxanthin supplementation did not exert a significant influence on the ash and moisture content, which ranged from 5.84– 6.34% and 68.43 – 69.86%, respectively ($P> 0.05$). The present findings underscore the promising potential of dietary astaxanthin supplementation in enhancing the body composition of *A. ocellaris*, notably by elevating protein content and reducing lipid content. This finding may have significant ramifications for promoting the overall health and quality of the cultured fish in aquaculture settings.

Table 6. Whole body proximate composition of *A. ocellaris* in experimental treatments

Parameter	Dietary astaxanthin levels (mg/kg)					
	Control	200	400	600	800	1000
Protein (%)	16.37±0.46^{a}	17.50±0.30^{b}	18.38±0.20^{b}	19.69±0.24^{c}	19.70±0.31^{c}	19.88±0.32^{c}
Lipid (%)	7.88±0.36^{d}	6.70±0.16^{c}	5.86±0.20^{b}	5.45±0.13^{ab}	5.33±0.10^{ab}	5.13±0.12^{a}
Ash (%)	5.84±0.28	6.32±0.34	6.34±0.27	6.40±0.21	6.28±0.20	6.14±0.16
Moiture (%)	69.86±0.85	69.16±0.42	69.22±0.57	68.43±0.37	68.56±0.67	68.81±0.19

Mean values ± standard errors **(**n=3**)** are presented. Superscript letters denote significant differences **(***P*<0.05**)** among treatments within the same row.

DISCUSSION

Growth performance, survival rate, and feed utilization efficiency

The present study demonstrated that the dietary inclusion of astaxanthin derived from shrimp shells significantly enhanced the growth performance and feed utilization efficiency of the false clownfish, particularly at supplementation levels ranging from 600 to 800mg/ kg feed. This finding aligns with numerous prior investigations that have highlighted the beneficial role of astaxanthin in improving fish production efficiency **(Lim *et al*., 2018; Lau *et al*., 2023; Elbahnaswy & Elshopakey, 2024)**. The positive effects of astaxanthin may be attributed to a combination of various mechanisms such as

(i) enhancing nutrient utilization efficiency through modulating digestive enzyme activities **(Hassaan *et al*., 2021; Meng *et al*., 2024)**. They may also be ascribed to **(**ii**)** the indirect effects such as antioxidation, stress reduction, regulation of gene expression and endocrine hormones **(**growth hormone**)** related to growth and health **(Saleh *et al*., 2018)** in addition to **(**iii**)** promoting the activities of gut microbiota, especially beneficial bacteria, optimizing metabolic processes **(Wang *et al*., 2024)**. Among these causes, **(**iv**)** improving intestinal structure, enhancing nutrient absorption capacity **(Fang *et al*., 2021; Zhao *et al*., 2022; Meng *et al*., 2024; Wang *et al*., 2024)** is identified. Notably, astaxanthin supplementation did not exert any significant impact on the survival rate of fish, which is consistent with the findings reported in several previous studies on the false clownfish **(Díaz-Jiménez *et al*., 2021; Tran *et al*., 2022)**. The high survival rate **(**> 9z0%**)** in all treatments indicated that the false clownfish has good adaptability to the culture conditions, and the inclusion of dietary astaxanthin does not appear to be a critical determinant of survival in this fish species.

However, the effect of dietary astaxanthin supplementation on fish growth remains ambiguous and controversial. While several studies reported positive impacts on various fish species **(Kalinowski *et al*., 2011; Li *et al*., 2014; Saleh *et al*., 2018; Fang *et al*., 2021; Zhao *et al*., 2022)**, others did not record any significant improvement **(Pham *et al*., 2014; Tran *et al*., 2022; Zhu *et al*., 2022)**. Even for the same false clownfish species, the results were relatively different among studies **(Nhan *et al*., 2019; Ebeneezar *et al*., 2020; Díaz-Jiménez *et al*., 2021)**. This suggests that the effectiveness of dietary astaxanthin supplementation depends on many variables, including fish species, developmental stage, feed type, source and level of astaxanthin, feeding duration, as well as culture conditions **(Lim *et al*., 2018; Elbahnaswy & Elshopakey, 2024)**. Notably, increasing the astaxanthin level to 1000mg/ kg feed did not bring additional benefits but even negatively affected the growth of the false clownfish, similar to previous reports on some other fish species **(Song *et al*., 2016; Yu *et al*., 2021)**. The reason is possibly due to the excess of astaxanthin that may reduce the digestibility and absorption of nutrients in the digestive tract **(Song *et al*., 2016; Yu *et al*., 2021)**, however further in-depth investigations are necessary to clarify this mechanism. In conclusion, supplementation of 600mg astaxanthin/kg feed, extracted from shrimp shells, had positive impacts on the growth performance and feed utilization efficiency of the false clownfish. The findings from this study contribute to elucidating the importance of astaxanthin in ornamental fish culture.

Skin coloration and total carotenoids accumulation

In this study, astaxanthin supplementation significantly improved the skin color parameters (redness a^*, yellowness b^*, hue angle h^*_{ab}, and chroma C^*_{ab}) and the total carotenoids accumulation in the body of the false clownfish. The best values of both these groups of indices were generally achieved at the supplementation levels of 600- 1000mg/ kg feed, and no significant differences were found among them (P> 0.05). Therefore, an

astaxanthin supplementation level of 600mg/ kg was determined to be most appropriate for the false clownfish in order to save costs while still ensuring color enhancement effectiveness. Previous studies also recorded the positive effects of astaxanthin supplementation, both natural and synthetic, on skin coloration and carotenoids accumulation in the false clownfish **(Nhan *et al*., 2019; Ebeneezar *et al*., 2020; Díaz-Jiménez *et al*., 2021)**. However, the effectiveness of color improvement in this fish species varied depending on the sources of carotenoids used, supplementation levels and duration, fish size as well as other husbandry and management conditions **(Yasir & Qin, 2010; Lau *et al*., 2023)**.

The findings of this study suggested that an astaxanthin supplementation level of 600mg/ kg feed was suitable for the goal of improving skin coloration and enhancing carotenoids accumulation in false clownfish. Increasing the astaxanthin level to 1000mg/ kg did not bring significant improvements in skin color indices and accumulated carotenoids content. Notably, the redness (a^*) and yellowness (b^*) values even tended to slightly decrease at the 1000mg/ kg level, while the accumulated carotenoids content only increased insignificantly compared to the 600mg/ kg level. This observation aligns with the previous report of **Díaz-Jiménez *et al*. (2021)**, possibly due to the excess of astaxanthin in the diet that negatively affected the metabolism, leading to reduced pigment accumulation in the skin and increased excretion of the unabsorbed amount into the environment **(Page & Davies 2006)**. Further studies should determine the apparent digestibility coefficients **(**ADCs**)** of astaxanthin at various supplementation levels, especially from 600- 1000mg/ kg feed, to clarify the digestibility, absorption and accumulation of astaxanthin in the false clownfish.

In addition to increasing the red and yellow color on the skin of the false clownfish, the deposition of astaxanthin reduced skin brightness, reflected in the decrease of the lightness **(**L^***)** value with increasing astaxanthin supplementation levels. This result has also been previously reported in the red parrotfish, angelfish, red porgy, and rainbow trout **(Kalinowski *et al*., 2011; Song *et al*., 2016; Micah *et al*., 2022; Zhao *et al*., 2022)**. However, there are still many issues that need to be clarified regarding the effect of dietary astaxanthin supplementation on the false clownfish, especially the metabolic mechanisms, absorption of astaxanthin into intermediate products such as zeaxanthin and canthaxanthin accumulated in the body of this fish species, as well as the correlation between displayed color, the total carotenoids content accumulated in the skin, and the geographical origin of the fish **(Yasir & Qin, 2010; Díaz-Jiménez *et al*., 2021)**.

Biochemical composition of fish

The findings of the current investigation revealed that the dietary astaxanthin significantly influenced the protein and lipid content of the false clownfish, while it exerted no impact on moisture and ash content. Notably, the protein content increased, and the lipid content exhibited a decreasing trend as the levels of astaxanthin supplementation increased. This finding aligns with previous investigations on the

rainbow trout and red porgy **(Kalinowski *et al*., 2011; Besharat *et al*., 2021; Zhao *et al*., 2022)**. Astaxanthin, acting as an antioxidant, not only protects lipids from oxidation but also regulates lipid metabolism, including β-oxidation of fatty acids, reduction, and elongation of carbon chains **(Bell *et al*., 2000; Kalinowski *et al*., 2011)**. Astaxanthin may inhibit the production of acetyl CoA in the liver, an important intermediate in lipid and ATP synthesis, while stimulating the utilization of fatty acids for energy production and maintaining homeostasis, leading to reduced lipid accumulation in fish **(Kalinowski *et al*., 2023)**. Simultaneously, the enhanced utilization of lipids helps spare protein for energy metabolism and promotes protein deposition in fish **(Zhao *et al*., 2022; Kalinowski *et al*., 2023)**. These findings explain the improved growth performance of the false clownfish in the astaxanthin-supplemented treatments, especially at 600mg/ kg diet, compared to lower supplementation levels and the control in the present study. However, the impact of dietary astaxanthin supplementation on the biochemical composition of fish is variable and contradictory in previous studies **(Ebeneezar *et al*., 2020; Besharat *et al*., 2021; Micah *et al*., 2022; Kalinowski *et al*., 2023; Wang *et al*., 2024)**. This discrepancy may be due to the combined effects of various variables, such as species, developmental stage, carotenoid source, dose, duration of supplementation, as well as environmental and rearing conditions **(Lim *et al*., 2018; Elbahnaswy & Elshopakey, 2024)**. Therefore, future studies should focus on elucidating the mechanisms of astaxanthin action on lipid metabolism and other nutritional components in the false clownfish.

CONCLUSION

This study demonstrated the potential of utilizing astaxanthin derived from shrimp waste to enhance the growth performance and coloration of the false clownfish. Supplementing 600mg astaxanthin/kg feed was the most effective level, resulting in the best growth performance and coloration after 75 days of rearing while being cost-effective compared to higher supplementation levels. This finding confirms that astaxanthin from shrimp shells is a promising natural pigment source for marine ornamental fish production.

The study provides an effective and sustainable solution for utilizing fishery by-products, enhancing the value of cultured false clownfish, contributing to reducing fishing pressure on wild stocks, and promoting sustainable marine ornamental fish farming. Nevertheless, additional investigations are required to elucidate the mechanisms underlying astaxanthin absorption, metabolism, and deposition in false clownfish and assess the effects of astaxanthin supplementation on immune response, antioxidant status, and stress tolerance. Additionally, optimizing the relationship between astaxanthin dose and duration of supplementation to shorten the production cycle and improve economic efficiency should be considered.

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REFERENCES

AOAC. (2006). Official Methods of Analysis, 18th edition. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Bell, J.G.; McEvoy, J.; Tocher, D.R. and Sargent, J.R. (2000). Depletion of α-tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. The Journal of Nutrition, 130(7): 1800-1808.

Besharat, M.; Islami, H.R.; Soltani, M. and Mousavi, S.A. (2021). Effect of different levels of nanoliposome-coated astasxanthin on growth performance, body proximate composition, liver enzyme activity and pigmentation of rainbow trout (*Oncorhynchus mykiss*). Aquaculture Research, 52(10): 5069-5077.

Bowyer, J.N.; Qin, J.G. and Stone, D.A. (2013). Protein, lipid and energy requirements of cultured marine fish in cold, temperate and warm water. Reviews in Aquaculture, 5(1): 10-32.

Calado, R.; Olivotto, I.; Oliver, M.P. and Holt, G.J. (2017). Marine ornamental species aquaculture. Wiley Blackwell, Hoboken, New Jersey, USA.

Díaz-Jiménez, L.; Hernández-Vergara, M.P.; Pérez-Rostro, C.I. and Olvera-Novoa, M.Á. (2021). The effect of two carotenoid sources, background colour and light spectrum on the body pigmentation of the clownfish *Amphiprion ocellaris*. Aquaculture Research, 52(7): 3052-3061.

Ebeneezar, S.; Prabu, D.L.; Chandrasekar, S.; Tejpal, C.; Madhu, K.; Sayooj, P. and Vijayagopal, P. (2020). Evaluation of dietary oleoresins on the enhancement of skin coloration and growth in the marine ornamental clown fish, *Amphiprion ocellaris* (Cuvier, 1830). Aquaculture, 529, 735728.

Elbahnaswy, S. and Elshopakey, G.E. (2024). Recent progress in practical applications of a potential carotenoid astaxanthin in aquaculture industry: a review. Fish Physiology and Biochemistry, 50(1): 97-126.

Fang, H.; Xie, J.; Zhao, W.; Liu, Z.; Liu, Y.; Tian, L. and Niu, J. (2021). 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*. Aquaculture Nutrition, 27: 2575–2586.

FAO. (2024). The State of World Fisheries and Aquaculture 2024. Blue Transformation in Action. Rome.

Hassaan, M.S.; Mohammady, E.Y.; Soaudy, M.R.; Sabae, S.A.; Mahmoud, A.M. and El-Haroun, E.R. (2021). Comparative study on the effect of dietary β-carotene and phycocyanin extracted from *Spirulina platensis* on immune-oxidative stress biomarkers, genes expression and intestinal enzymes, serum biochemical in Nile tilapia, *Oreochromis niloticus*. Fish & Shellfish Immunology, 108: 63-72.

Hunter, R.S. and Harold, R.W. (1987). The Measurement of Appearance, second edition. John Wiley and Sons. New York.

Irna, C.; Jaswir, I.; Othman, R. and Jimat, D.N. (2018). Comparison between high-pressure processing and chemical extraction: Astaxanthin yield from six species of shrimp carapace. Journal of Dietary Supplements, 15**(**6**)**: 805-813.

Kalinowski, C.T.; Robaina, L.E. and Izquierdo, M.S. (2011). Effect of dietary astaxanthin on the growth performance, lipid composition and post-mortem skin colouration of red porgy *Pagrus pagrus*. Aquaculture International, 19**(**5**)**: 811–823.

Kalinowski, C.T.; Betancor, M.B.; Torrecillas, S.; Sprague, M.; Larroquet, L.; Véron, V.; Panserat, S.; Izquierdo, M.S.; Kaushik, S.J. and Fontagné-Dicharry, S. (2023). More than an antioxidant: role of dietary astaxanthin on lipid and glucose metabolism in the liver of rainbow trout **(***Oncorhynchus mykiss***)**. Antioxidants, 12**(**10, 136.

Lau, C.C.; Nor, S.A.M.; Tan, M.P.; Yeong, Y.S.; Wong, L.L.; de Peer, Y.V.; Sorgeloos, P. and Danish-Daniel, M. (2023). Pigmentation enhancement techniques during ornamental fish production. Reviews in Fish Biology and Fisheries, 33**(**4**)**: 1027-1048.

Li, M.; Wu, W.; Zhou, P.; Xie, F.; Zhou, Q. and Mai, K. (2014). Comparison effect of dietary astaxanthin and *Haematococcus pluvialis* on growth performance, antioxidant status and immune response of large yellow croaker *Pseudosciaena crocea*. Aquaculture, 434: 227–232.

Lim, K.C.; Yusoff, F.M.; Shariff, M. and Kamarudin, M.S. (2018). Astaxanthin as feed supplement in aquatic animals. Reviews in Aquaculture, 10: 738–773.

Luo, M.; Lu, G.; Yin, H.; Wang, L.; Atuganile, M. and Dong, Z. (2021). Fish pigmentation and coloration: Molecular mechanisms and aquaculture perspectives. Reviews in Aquaculture, 13**(**4**)**: 2395-2412.

Meng, X.; Yang, F.; Zhu, L.; Zhan, L.; Numasawa, T. and Deng, J. (2024). Effects of dietary astaxanthin supplementation on growth performance, antioxidant status, immune response, and intestinal health of rainbow trout **(***Oncorhynchus mykiss***)**. Animal Nutrition, 17: 387-396.

Micah, A.D.; Wen, B.; Wang, Q.; Zhang, Y.; Yusuf, A.; Thierry, N.N.B.; Tokpanou, O.S.; Onimisi, M.M.; Adeyemi, S.O.; Gao, J. and Chen, Z. (2022). Effect of dietary astaxanthin on growth, body color, biochemical parameters and

transcriptome profiling of juvenile blood parrotfish **(***Vieja melanurus* ♀× *Amphilophus citrinellus* ♂**)**. Aquaculture Reports, 24, 101142.

Nguyen, M.T.; Nguyen, T.B.; Dang, K.K.; Luu, T.; Thach, P.H.; Nguyen, K.L.P. and Nguyen, H.Q. (2022). Current and potential uses of agricultural by-products and waste in main food sectors in Vietnam - A circular economy perspective. In Circular Economy and Waste Valorisation: Theory and Practice from an International Perspective **(**pp. 131-151**)**. Cham: Springer International Publishing.

Nhan, H.T.; Minh, T.X.; Liew, H.J.; Hien, T.T.T. and Jha, R. (2019). Effects of natural dietary carotenoids on skin coloration of false clownfish **(***Amphiprion ocellaris* Cuvier, 1830**)**. Aquaculture Nutrition, 25**(**3**)**: 662-668.

Nirmal, N.P.; Santivarangkna, C.; Rajput, M.S. and Benjakul, S. (2020). Trends in shrimp processing waste utilization: An industrial prospective. Trends in Food Science and Technology, 103: 20–35.

Page, G.I. and Davies, S.J. (2006). Tissue astaxanthin and canthaxanthin distribution in rainbow trout **(***Oncorhynchus mykiss***)** and Atlantic salmon **(***Salmo salar***)**. Comparative Biochemistry and Physiology, 143A: 125–132.

Pathare, P.B.; Opara, U.L. and Al-Said, F.A.J. (2013). Colour measurement and analysis in fresh and processed foods: A review. Food and Bioprocess Technology, 6: 36-60.

Pham, M.A.; Byun H.G.; Kim, K.D. and Lee, S.M. (2014). Effects of dietary carotenoid source and level on growth, skin pigmentation, antioxidant activity and chemical composition of juvenile olive flounder *Paralichthys olivaceus*. Aquaculture 431: 65–72.

Pouil, S.; Tlusty, M.F.; Rhyne, A.L. and Metian, M. (2020). Aquaculture of marine ornamental fish: overview of the production trends and the role of academia in research progress. Reviews in Aquaculture, 12**(**2**)**: 1217-1230.

Saleh, N.E.; Wassef, E.A. and Shalaby, S.M. (2018). The role of dietary astaxanthin in European sea bass **(***Dicentrarchus labrax***)** growth, immunity, antioxidant competence and stress tolerance. Egyptian Journal of Aquatic Biology & Fisheries, 22**(**5**)**: 189 - 200.

Sathyaruban, S.; Uluwaduge, D.I.; Yohi, S. and Kuganathan, S. (2021). Potential natural carotenoid sources for the colouration of ornamental fish: a review. Aquaculture International, 22: 1507-1528.

Schiedt, K. and Liaaen-Jensen, S. (1995). Isolation and analysis. In: Britton, G.; Liaaen-Jensen, S. and Pfander, H. **(**Eds.**)**, Carotenoids: Isolation and analysis **(**vol. 1A, pp. 81-108**)**. Birkhäuser Verlag, Basel.

Song, X.; Wang, L.; Li, X.; Chen, Z.Z.; Liang, G. and Leng, X. (2016). Dietary astaxanthin improved the body pigmentation and antioxidant function but not the growth of discus fish **(***Symphysodon* spp.**)**. Aquaculture Research, 48**(**4**)**: 1359-1367.

Tran, V.D.; Dang, T.T.; Cao, T.T.T.; Hua, T.N. and Pham, Q.H. (2022). Natural astaxanthin extracted from shrimp waste for pigment improvement in the orange clownfish, *Amphiprion percula*. Aquaculture Research, 53**(**11**)**: 4190-4198.

Wang, C.; Qin, S.; Sun, F.; Shao, Y.; Du, R.; Gao, Z. and Cui, Y. (2024). Effects of feeding *Haematococcus pluvialis* powder on astaxanthin accumulation, biochemical compositions, antioxidant activity, and gut microbial community in juvenile crucian carp **(***Carassius auratus* L.**)**. Aquaculture Reports, 36, 102109.

Yasir, I. and Qin, J.G. (2010). Effect of dietary carotenoids on skin color and pigments of false clownfish, *Amphiprion ocellaris*, Cuvier. Journal of the World Aquaculture Society, 41**(**3**)**: 308-318.

Yu, W.; Lin, H.; Yang, Y.; Zhou, Q.; Chen, H.; Huang, X.; Zhou, C.; Huang, Z. and Li, T. (2021). Effects of supplemental dietary *Haematococcus pluvialis* on growth performance, antioxidant capacity, immune responses and resistance to *Vibrio harveyi* challenge of spotted sea bass *Lateolabrax maculatus*. Aquaculture Nutrition, 27**(**2**)**: 355-365.

Zhao, W.; Guo, Y.C.; Huai, M.Y.; Li, L.; Man, C.; Pelletier, W.; Wei, H.L.; Yao, R. and Niu, J. (2022). 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, 11, 2473.

Zhu, X.; Hao, R.; Zhang, J.; Tian, C.; Hong, Y.; Zhu, C. and Li, G. (2022). Dietary astaxanthin improves the antioxidant capacity, immunity and disease resistance of coral trout **(***Plectropomus leopardus***)**. Fish & Shellfish Immunology, 122: 38-47.