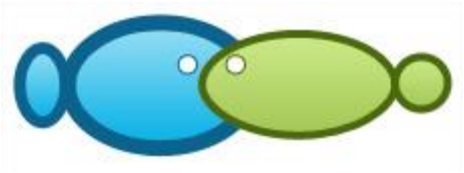

Dietary *Artemia*-derived carotenoids modulate growth and coloration performance in *Amphiprion frenatus* reared in a recirculating aquaculture system

Dung V. Tran, Trang L. T. Tran, Duy Q. Tran, Duong K. D. Nguyen, Nam X. Doan, Dai T. Vu

Department of Aquaculture, School of Fisheries and Life Sciences, Nha Trang University, 650000 Nha Trang, Vietnam. Corresponding author: D. T. Vu, daivt@ntu.edu.vn

Abstract. Captive-bred tomato clownfish (*Amphiprion frenatus*) typically exhibit inferior coloration compared to wild specimens, reducing their market value and maintaining pressure on wild populations. The primary cause is that commercially available marine ornamental feeds, predominantly formulated for food fish production, lack sufficient carotenoids for optimal pigmentation. This study evaluated the effects of Artemia biomass-derived carotenoid supplementation (0, 100, 200, 300, 400, 500 mg kg^{-1}) on growth performance and coloration of juvenile fish (initial size: 3.10±0.04 cm; 0.52±0.05 g) reared in recirculating systems for 56 days. Results demonstrated that dietary carotenoid supplementation significantly improved both growth and coloration in a dose-dependent manner. The 400 mg kg^{-1} treatment achieved optimal performance with specific growth rates increasing by 20.7% (length) and 16.3% (weight), feed conversion ratio improving by 12.3%, and color quality markedly enhanced with redness (a^*) and chroma (C^*_{ab}) values increasing by 59.0% and 51.4%, respectively, at the optimal dose compared to controls (all $p < 0.05$). Carotenoid accumulation in skin increased by 130.5% and in whole body by 92.1% at the highest dose (500 mg kg^{-1}); however, growth and coloration parameters showed no significant improvement beyond 400 mg kg^{-1} ($p > 0.05$), indicating saturation of absorption and/or deposition capacity. These findings identify an optimal dietary carotenoid supplementation strategy using a natural pigment source to enhance cultured fish quality, meeting premium ornamental market demands while reducing pressure on wild stocks.

Keywords: astaxanthin, clownfish, feed enrichment, marine ornamental fish, pigmentation.

Introduction. The global ornamental fish trade has expanded significantly over the past two decades, with marine species commanding premium prices due to their vibrant coloration and distinctive behaviors (Calado et al 2017; Merilaita & Kelley 2018). The tomato clownfish (*Amphiprion frenatus*) is particularly prized for its distinctive intense red-orange pigmentation, unique symbiotic association with sea anemones, and remarkable adaptability to captive conditions (Madhu & Madhu 2010). However, commercial clownfish production confronts persistent challenges of suboptimal growth rates and inferior coloration compared to wild-caught specimens (Tran 2025). Notably, cultured juveniles beyond 3-4 cm frequently exhibit pronounced body darkening - a natural characteristic intensified by suboptimal environmental and nutritional conditions - substantially diminishing market value (Salis et al 2018; Nguyen et al 2025).

The coloration disparity between wild and cultured fish has been attributed to environmental conditions, stress responses, and critically, nutritional deficiencies in commercial feeds (Pegu & Singh 2025). Unlike wild fish consuming diverse prey assemblages, captive clownfish typically receive diets formulated for food fish larviculture that prioritize growth over pigmentation, as species-specific ornamental feeds remain commercially unavailable (Calado et al 2017; de Carvalho & Caramujo 2017). Carotenoids have been identified as essential for both physiological function and color expression in fish. These lipophilic pigments serve dual roles: functioning as potent antioxidants and serving as precursors for skin and scale coloration (Nakano & Wiegertjes 2020). Fish cannot

synthesize carotenoids de novo and must acquire them entirely through dietary sources (de Carvalho & Caramujo 2017), thereby rendering dietary carotenoid supplementation an indispensable strategy for enhancing cultured fish coloration.

Current aquaculture practices predominantly rely on synthetic carotenoids such as astaxanthin and canthaxanthin (Pereira da Costa & Campos Miranda-Filho 2020). While efficacious in salmon and shrimp production, these compounds present significant limitations: variable bioavailability, differential tissue accumulation, growing consumer preference for natural alternatives, and emerging biosafety concerns (Sathyaruban et al 2021). These constraints have driven interest in identifying cost-effective natural carotenoid sources.

Artemia represents a promising natural carotenoid source with established credibility in aquaculture applications (Asemenova & Khajibayev 2025). Live *Artemia* has long been recognized as a superior larval feed for marine fish due to its exceptional nutritional profile, including significant carotenoid content (Gilchrist & Green 1960). Biomass-cultured *Artemia* accumulates diverse carotenoids - including canthaxanthin, echinenone, β-carotene, and astaxanthin - at concentrations modulable through culture conditions (Nelis et al 1988; Elshafey et al 2023). Unlike single-compound synthetic supplements, *Artemia*-derived carotenoids provide a complex matrix of pigments that may exhibit synergistic antioxidant effects and enhanced bioavailability (Gilchrist & Green 1960; Elshafey et al 2023). Recent advances in *Artemia* biomass production technology have improved commercial feasibility by reducing production costs (Bengtson et al 1991; Van Stappen et al 2020; Nguyen et al 2024). Despite these technical advances and the theoretical advantages of natural carotenoid matrices, systematic investigation of *Artemia*-sourced carotenoid supplementation in marine ornamental fish nutrition remains limited.

Previous research on carotenoid nutrition in clownfish has primarily focused on synthetic astaxanthin and beta-carotene, with reported supplementation levels ranging from 50 mg kg^{-1} for highly bioavailable forms to 20,000 mg kg^{-1} for less bioavailable natural sources (Lau et al 2023), but results have been inconsistent due to differences in pigment source, chemical form, and exposure duration. Critically, no studies have comprehensively evaluated natural carotenoid sources from *Artemia* biomass across multiple dosage levels while simultaneously assessing both production performance and color quality in tomato clownfish at the commercially critical pre-market juvenile stage (3-5 cm), when coloration directly affects market value. This gap is significant because this size class represents a critical production bottleneck in commercial operations (Nguyen et al 2025; Tran 2025).

Recirculating aquaculture systems (RAS) have gained prominence in ornamental fish production due to their capacity for maintaining stable water quality and enabling year-round production (Gupta et al 2024). However, the controlled environment and reduced natural productivity in RAS may exacerbate nutritional deficiencies (Aich et al 2020). Comprehensive evaluation necessitates integrated analysis across multiple dimensions - coloration, growth, feed efficiency, survival, body composition, and carotenoid deposition - to establish optimal supplementation levels.

The current study investigates how *Artemia* biomass-derived carotenoids across a concentration gradient (0-500 mg kg^{-1}) modulate growth performance and pigmentation in pre-market stage tomato clownfish under RAS conditions. We hypothesize that: (1) *Artemia*-sourced carotenoids will enhance both growth and coloration in a dose-dependent manner; (2) an optimal dosage will exist beyond which benefits plateau; and (3) natural carotenoid supplementation will improve feed utilization efficiency and body composition while promoting physiologically significant carotenoid accumulation.

Material and Method

Experimental design. A completely randomized design with six dietary carotenoid supplementation levels (0, 100, 200, 300, 400, and 500 mg kg^{-1} of *Artemia* biomass-derived carotenoids) was used, with three replicate tanks per treatment. Each 60-L tank stocked 20 fish (stocking density: 1 fish per 3 L). The experimental period was 56 days. The feeding trial was conducted at the Marine Ornamental Fish Hatchery, Faculty of Aquaculture, Nha Trang University, Vietnam, with the entire study - including the

acclimation period, the 56-day feeding trial, and subsequent sample analyses - carried out from May to August 2025.

Juvenile tomato clownfish (initial total length 3.10±0.04 cm; body weight 0.52± 0.05 g; mean±SD, n = 360) were produced at the Marine Ornamental Fish Hatchery, Faculty of Aquaculture, Nha Trang University, Vietnam. Fish were selected based on good health status and uniform size, then acclimated to experimental conditions for 7 days prior to trial initiation.

Culture system and management. A RAS comprising 18 glass tanks (55 × 35 × 38 cm, 60 L) connected to a central biological filter tank (800 L containing coral gravel and plastic bioball media) was configured following Tran et al (2022). Water was circulated at 1.4-1.6 L min^{-1} using submersible pumps (Lifetech AP5300, 80 W). A 12L:12D photoperiod was maintained using natural light supplemented with LED lighting (combined intensity approximately 5,000-8,000 lux at water surface).

Water quality parameters were maintained as follows: temperature 27-31°C, salinity 30-35‰, pH 7.8-8.2, dissolved oxygen 4-6 mg L^{-1}, total ammonia nitrogen < 1.0 mg L^{-1}. Tanks were cleaned twice daily and water was exchanged at 30-50% weekly.

Preparation of experimental diets. Carotenoids were extracted from freshly harvested *Artemia franciscana* biomass (cultured at Khanh Hoa province, initial carotenoid content 40.9±1.96 µg g^{-1} wet weight, n = 3 batches) following the microwave-assisted extraction method of Tran et al (2022). Briefly, biomass was extracted three times with 96% (v/v) ethanol (3.5:1 v/w ratio, ethanol volume to biomass weight), extracts were pooled, concentrated under vacuum at 40°C, and dissolved in food-grade soybean oil (1:5 w/v ratio) to produce a solution containing 5.8±0.2% total carotenoids (w/v), which was stored at 4°C protected from light.

A commercial extruded pellet diet (NRD G8, particle size 0.8 mm, INVE Aquaculture, Thailand) formulated for marine fish juveniles was used as the basal feed. According to manufacturer specifications, the proximate composition included crude protein > 55%, crude lipid > 9%, crude fiber < 1.9%, and moisture ~8%. The carotenoid-oil extract was sprayed onto pellet surfaces using a coating technique with continuous mixing for 20 minutes to create six experimental treatments: 0 (control), 100, 200, 300, 400, and 500 mg total carotenoid kg^{-1} feed. The control treatment received an equivalent volume of pure soybean oil to balance lipid content across treatments. After coating, pellets were dried at 60°C to approximately 10% moisture, vacuum-packed in aluminum foil bags, and stored at 4°C in darkness until use.

Actual carotenoid concentrations in experimental feeds were verified by UV-Vis spectrophotometry at 450 nm following the acetone extraction method detailed in Tran et al (2022). Analyzed values were 6±2 (background from basal feed), 97±5, 196±7, 294±10, 403±12, and 508±14 mg kg^{-1} for the six respective treatments, corresponding to a mean coating efficiency of 97.2±2.4% for supplemented treatments and confirming close adherence to nominal supplementation levels.

Fish rearing and sampling. Fish were fed to apparent satiation four times daily (07:00, 10:00, 13:00, 16:00) following Tran et al (2022). Feed consumption was determined by collecting uneaten feed after 30 minutes, drying at 60°C for 24 hours, and weighing. Survival was recorded daily.

After 56 days, fish were fasted for 24 hours, anesthetized with 2-phenoxyethanol (Ethylene Glycol Monophenyl Ether, 500 mg L^{-1}) until loss of equilibrium (5-10 seconds), counted to determine survival, and individually measured and weighed while maintained under anesthesia. Skin coloration was immediately measured using a chromameter (Minolta CR-400) on all individuals following the method described in Doan et al (2025). Eight fish per tank (24 fish per treatment) were randomly selected, euthanized by prolonged anesthesia (2-phenoxyethanol, 1,000 mg L^{-1}), and stored at -20°C for carotenoid analysis (skin, muscle, whole body) and biochemical composition.

Evaluation parameters

Growth performance and feed utilization efficiency. Total length (TL) and body weight (BW) were measured using a ruler (±1.0 mm) and electronic balance (VNS LED-A, ±0.01 g). Growth parameters including specific growth rate for length (SGR_L) and weight (SGR_W), condition factor (CF), coefficient of variation for length (CV_L) and weight (CV_W), and survival rate (SR) were calculated. Feed utilization indices including feeding rate (FR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were determined according to standard formulae described in Doan et al (2025).

Skin coloration. Coloration was measured using a CR-400 Chroma Meter (Konica Minolta, Japan) in the CIE 1976 $L^*a^*b^*$ color space with D65 illuminant. The measurement area was bounded by the soft dorsal fin (dorsally), anal fin (ventrally), posterior to the longitudinal white stripe in the head region (anteriorly), and caudal peduncle (posteriorly), avoiding white stripe regions and fins to ensure measurements on the characteristic red-orange pigmented skin area of the species. Each fish was measured on both body sides, three replicates per side.

Color indices included: L^* (lightness, 0-100), a^* (red-green axis), b^* (yellow-blue axis), chroma $C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$, hue angle $h^*_{ab} = \arctan(b^*/a^*)$, and total color difference $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ relative to control (Tomasevic et al 2019).

Total carotenoid content. Carotenoid content in skin (0.25 g from both sides), muscle (0.25 g), whole body (0.50 g), and feed (1 g) was determined according to Tran et al (2022). Samples were homogenized in 20 mL acetone per gram of sample with 1.5 g anhydrous Na_2SO_4 using an Ultra-Turrax homogenizer (T10, IKA, 10,000 rpm, 2 min), filtered through Whatman No. 1 filter paper and re-extracted 2-3 times until filtrate was colorless, then centrifuged at 10,000 rpm, 4°C for 15 minutes. Absorbance was measured at 450 nm (Biochrom Ltd, UK).

Carotenoid content (μg g^{-1}) was calculated as:

$$\text{Carotenoid} = A \times V \times D \times 10^4 / (W \times 2100)$$

where: A = absorbance; V = extraction volume (mL); D = dilution factor; W = sample weight (g); 2100 = specific absorption coefficient of β-carotene in acetone at 450 nm ($E_{1cm}^{1\%}$).

Biochemical composition. Four fish per tank (12 fish per treatment) were homogenized (T10 Ultra-Turrax, IKA) and stored at -80°C. Biochemical composition was analyzed according to the Association of Official Analytical Chemists (AOAC 2006): crude protein (Kjeldahl, N × 6.25), crude lipid (Soxhlet, petroleum ether for 6 hours), ash (550°C for 12 hours), and moisture (105°C to constant weight). Each sample was analyzed in triplicate. Results were presented as % wet weight.

Statistical analysis. Data were analyzed using SPSS 26.0 after verification of normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). Survival data were arcsine-transformed prior to analysis but presented as percentages. One-way ANOVA was used to compare treatments, with Duncan's multiple range test identifying significant differences among treatments ($p < 0.05$). Results were presented as mean±SE.

Results

Environmental conditions. Environmental parameters in the recirculating system were maintained stably throughout the 56-day experimental period: temperature 27.92-30.41°C, salinity 33.34±0.87‰, pH 8.01±0.15, dissolved oxygen 5.33±0.19 mg L^{-1}, and total ammonia nitrogen 0.52±0.13 mg L^{-1} (Table 1). These values were all within suitable ranges for clownfish *Amphiprion* spp. (Ha 2005; Anil et al 2022; Tran 2025), confirming that carotenoid effects on fish were the primary factor evaluated in this study.

Table 1

Water quality parameters during the 56-day experimental period

Value	Parameters					
	Temperature (°C)		Salinity (‰)	pH	DO (mg L^{-1})	TAN (mg L^{-1})
	Morning	Afternoon				
Mean±SD	27.92±1.16	30.41±0.63	33.34±0.87	8.01±0.15	5.33±0.19	0.52±0.13
Min-Max	26.00-30.00	29.00-32.00	32.00-35.00	7.80-8.30	5.00-5.70	0.40-0.90

Note: DO = dissolved oxygen; TAN = total ammonia nitrogen.

Growth performance, survival, and feed utilization efficiency. Dietary supplementation with *Artemia* biomass-derived carotenoids significantly affected growth performance of tomato clownfish (Table 2). The 400 mg kg^{-1} treatment achieved the highest SGR_L and SGR_W, which were 20.7% and 16.3% higher, respectively, than the control ($p < 0.05$). Increasing the supplementation level to 500 mg kg^{-1} did not further improve growth ($p > 0.05$). Coefficients of variation (CV_L and CV_W) were lowest in the 400 mg kg^{-1} treatment, indicating greater uniformity ($p < 0.05$). Condition factor (CF, 1.92-2.00 g cm^{-3}) and survival rate (95.53-100%) did not significantly differ among treatments ($p > 0.05$).

Table 2

Growth performance and survival of tomato clownfish fed diets supplemented with *Artemia* biomass-derived carotenoids at different levels

Parameter	Carotenoid supplementation level (mg kg^{-1})					
	Control	100	200	300	400	500
L_1 (cm)	3.10±0.04	3.10±0.04	3.10±0.04	3.10±0.04	3.10±0.04	3.10±0.04
W_1 (g)	0.52±0.05	0.52±0.05	0.52±0.05	0.52±0.05	0.52±0.05	0.52±0.05
L_2 (cm)	3.65±0.02^{a}	3.66±0.03^{a}	3.69±0.02^{ab}	3.70±0.02^{ab}	3.76±0.02^{b}	3.72±0.03^{ab}
W_2 (g)	0.93±0.01^{a}	0.95±0.02^{ab}	0.98±0.02^{abc}	1.00±0.01^{bc}	1.02±0.02^{c}	1.01±0.02^{bc}
SGR_L (% day^{-1})	0.29±0.01^{a}	0.30±0.01^{ab}	0.31±0.01^{ab}	0.31±0.01^{ab}	0.35±0.01^{c}	0.32±0.01^{bc}
SGR_W (% day^{-1})	1.04±0.02^{a}	1.08±0.04^{ab}	1.13±0.03^{abc}	1.17±0.03^{bc}	1.21±0.04^{c}	1.18±0.03^{bc}
CV_L (%)	6.06±0.08^{b}	6.41±0.19^{b}	8.60±0.56^{c}	8.25±0.10^{c}	4.50±0.40^{a}	7.11±0.53^{b}
CV_W (%)	19.13±0.36^{b}	19.50±0.31^{b}	29.67±3.65^{c}	28.64±1.03^{c}	13.05±1.51^{a}	22.19±0.80^{b}
CF (g cm^{-3})	1.92±0.01	1.94±0.01	1.97±0.04	2.00±0.01	1.92±0.02	1.92±0.01
SR (%)	95.57±4.43	97.77±2.23	95.53±2.23	100	100	100

Note: L_1, W_1 = initial length and weight (day 0); L_2, W_2 = final length and weight (day 56). Values are presented as mean±SE (n = 3). Means with different superscript letters within the same row indicate significant differences ($p < 0.05$).

Feed utilization efficiency was markedly improved in groups supplemented with 200-500 mg kg^{-1} (Table 3). The lowest FCR and highest PER were observed in the 500 mg kg^{-1} group ($p < 0.05$), though not significantly different from the 400 mg kg^{-1} group ($p > 0.05$). Feeding rate (FR) ranged from 2.13 to 2.34% BW day^{-1} and showed no significant differences among treatments ($p > 0.05$).

Table 3

Feed utilization efficiency of tomato clownfish fed diets supplemented with *Artemia* biomass-derived carotenoids at different levels

Parameter	Carotenoid supplementation level (mg kg^{-1})					
	Control	100	200	300	400	500
FR (%BW day^{-1})	2.23±0.05	2.34±0.05	2.16±0.08	2.21±0.02	2.25±0.04	2.13±0.06
FCR	2.20±0.03^{b}	2.23±0.07^{b}	1.99±0.05^{a}	1.96±0.02^{a}	1.93±0.03^{a}	1.87±0.03^{a}
PER	0.82±0.01^{a}	0.81±0.02^{a}	0.91±0.02^{b}	0.93±0.01^{bc}	0.94±0.01^{bc}	0.97±0.01^{c}

Values are presented as mean±SE (n = 3). Means with different superscript letters within the same row indicate significant differences ($p < 0.05$).

Skin coloration. Skin color indices improved with increasing carotenoid supplementation (Table 4, Figure 1). Lightness (L^*), redness (a^*), yellowness (b^*), and chroma (C^*_{ab}) all increased significantly with supplementation ($p < 0.05$). At the optimal 400 mg kg^{-1} level, a^* increased by 59.0% and C^*_{ab} by 51.4% compared to the control, while L^* and b^* increased by 23.8% and 48.1%, respectively. The highest a^* value (64.2% increase) was observed at 500 mg kg^{-1}; however, no significant differences in color parameters were found between the 400 and 500 mg kg^{-1} treatments ($p > 0.05$). Hue angle (h^*_{ab}) decreased numerically from 57.30 in the control to 53.87 in the 500 mg kg^{-1} treatment but showed no significant differences among treatments ($p > 0.05$).

Table 4

Skin color indices of tomato clownfish fed diets supplemented with *Artemia* biomass-derived carotenoids at different levels

Parameter	*Carotenoid supplementation level (mg kg^{-1})*					
	Control	*100*	*200*	*300*	*400*	*500*
L^*	36.04±0.21^{a}	40.76±1.04^{b}	42.58±0.52^{bc}	43.77±1.59^{bc}	44.61±0.42^{c}	45.97±1.48^{c}
a^*	12.20±0.10^{a}	12.94±1.04^{ab}	15.55±0.92^{bc}	17.15±1.57^{cd}	19.40±0.90^{d}	20.03±0.44^{d}
b^*	19.02±0.52^{a}	20.59±0.77^{a}	23.89±0.52^{b}	25.18±1.25^{b}	28.16±0.29^{c}	27.43±0.16^{c}
C^*_{ab}	22.60±0.50^{a}	24.33±1.20^{a}	28.51±0.94^{b}	30.48±1.91^{b}	34.21±0.75^{c}	33.97±0.13^{c}
h^*_{ab}	57.30±0.49	57.96±1.17	57.00±0.98	55.88±1.13	55.48±0.97	53.87±0.76
ΔE^*_{ab}		5.54±1.29^{a}	9.10±1.00^{ab}	11.32±2.49^{bc}	14.71±0.36^{c}	15.48±1.11^{c}

Values are presented as mean±SE (n = 3). Means with different superscript letters within the same row indicate significant differences ($p < 0.05$).

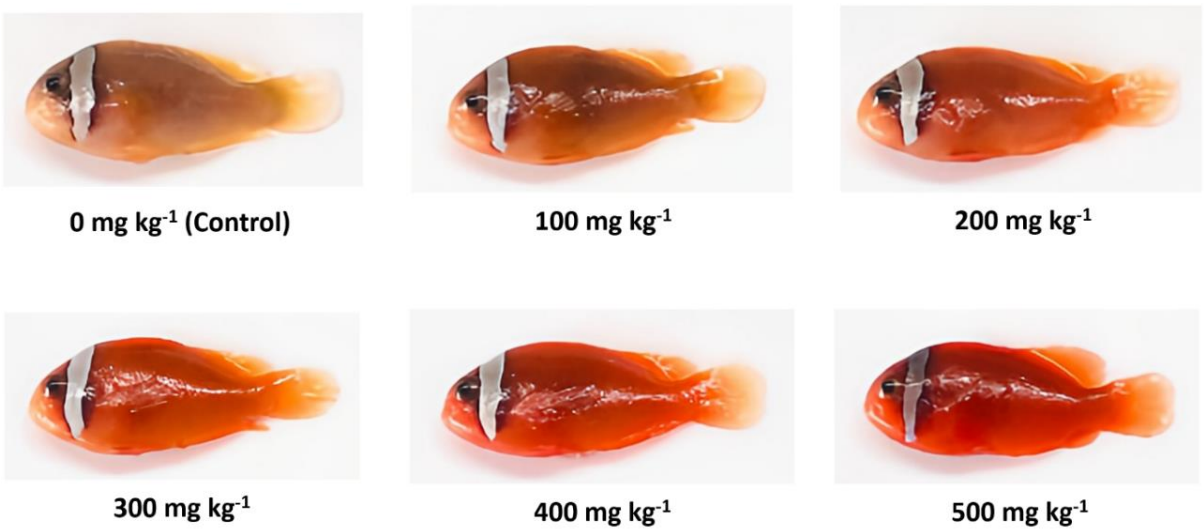

Figure 1. Representative body coloration of tomato clownfish fed diets supplemented with *Artemia* biomass-derived carotenoids at different levels for 56 days.

Total carotenoid accumulation. Carotenoid accumulation in tissues increased proportionally with dietary supplementation levels (Table 5). Skin was the primary accumulation site, with concentrations in the 500 mg kg^{-1} treatment 130.5% higher than the control ($p < 0.05$). Similar trends were observed in whole body (+92.1% increase) and muscle (+87.8% increase) compared to the control ($p < 0.05$). No significant differences in carotenoid concentrations were found between the 400 and 500 mg kg^{-1} treatments across all three tissue types ($p > 0.05$).

Table 5

Total carotenoid accumulation (μg g^{-1} wet weight) in tissues of tomato clownfish fed diets supplemented with *Artemia* biomass-derived carotenoids at different levels

Parameter	*Carotenoid supplementation level (mg kg^{-1})*					
	Control	*100*	*200*	*300*	*400*	*500*
Skin	33.88±1.41^{a}	40.67±1.91^{a}	57.61±2.17^{b}	63.14±3.20^{b}	75.39±2.11^{c}	78.11±2.17^{c}
Whole body	10.72±0.66^{a}	14.01±0.15^{b}	16.27±0.64^{c}	18.37±0.37^{d}	19.39±0.36^{de}	20.59±0.42^{e}
Muscle	1.89±0.17^{a}	2.01±0.10^{a}	2.26±0.19^{a}	2.59±0.31^{ab}	3.21±0.17^{bc}	3.55±0.38^{c}

Values are presented as mean±SE (n = 3). Means with different superscript letters within the same row indicate significant differences ($p < 0.05$).

Body biochemical composition. Carotenoid supplementation significantly affected body protein content, which reached its maximum in the 400 mg kg^{-1} treatment (10.6% higher than the control, $p < 0.05$; Table 6). Other body composition parameters including lipid (5.62-6.70%), ash (5.58-5.86%), and moisture (70.07-70.90%) did not significantly differ among treatments ($p > 0.05$).

Table 6

Body biochemical composition (% wet weight) of tomato clownfish fed diets supplemented with *Artemia* biomass-derived carotenoids at different levels

Parameter	*Carotenoid supplementation level (mg kg^{-1})*					
	Control	*100*	*200*	*300*	*400*	*500*
Moisture (%)	70.90±0.35	70.71±0.26	70.26±0.75	70.17±0.42	70.07±0.66	70.29±0.22
Protein (%)	16.73±0.25^{a}	16.94±0.42^{a}	17.48±0.36^{ab}	17.66±0.48^{ab}	18.50±0.14^{b}	18.20±0.19^{b}
Lipid (%)	6.70±0.21	6.50±0.30	6.55±0.37	6.22±0.32	5.62±0.36	5.85±0.26
Ash (%)	5.58±0.33	5.79±0.21	5.66±0.40	5.86±0.09	5.75±0.25	5.58±0.22

Values are presented as mean±SE (n = 3). Means with different superscript letters within the same row indicate significant differences ($p < 0.05$).

Discussion. This study demonstrates that dietary supplementation with *Artemia* biomass-derived carotenoids at 400 mg kg^{-1} simultaneously improves color quality, growth performance, and feed utilization efficiency of tomato clownfish after 56 days of rearing in a recirculating system. Compared to the control group, fish receiving 400 mg kg^{-1} supplementation achieved 20.7% and 16.3% higher specific growth rates for length and weight, respectively, 59.0% increase in skin redness (a^{*}), 51.4% increase in chroma (C^{*}_{ab}), and 122.5% increase in skin carotenoid accumulation ($p < 0.05$). Increasing the dose to 500 mg kg^{-1} provided no significant further improvement ($p > 0.05$), reflecting physiological limits in carotenoid absorption and storage capacity. These findings demonstrate a dual role for carotenoids - enhancing both pigmentation and growth - and expand understanding of carotenoid physiological functions in marine ornamental fish, thereby providing a scientific basis for optimizing production of high-quality commercial fish.

The significant improvement in growth performance (20.7% for length and 16.3% for weight) and body protein accumulation (+10.6% compared to the control) observed at 400 mg kg^{-1} demonstrates multifunctional roles of carotenoids beyond pigmentation effects. Three primary mechanisms may explain these effects. First, the antioxidant activity of carotenoids protects membrane lipids and DNA from reactive oxygen species (ROS) - induced damage, particularly important under intensive culture conditions with chronic stress; reduced oxidative stress allows fish to allocate energy toward growth rather than cellular repair (Nakano & Wiegertjes 2020). Second, *Artemia* biomass contains β-carotene (a provitamin A carotenoid) supporting cell division and differentiation, along with astaxanthin and canthaxanthin - carotenoids with high antioxidant activity - synergistically contributing to growth improvement (Elshafey et al 2023). Third, carotenoids may modulate immune function, reducing negative impacts of subclinical infections on growth (Lim et al 2023).

The improvement in FCR and PER while maintaining stable FR (Table 3), coupled with increased body protein content (Table 6), suggests that carotenoids may enhance intestinal health and nutrient absorption efficiency, or regulate protein metabolism pathways through nuclear receptors such as retinoic acid receptors (Elshafey et al 2023; Liao et al 2025). The lack of further improvement at 500 mg kg^{-1} reflects a balance between metabolic benefits and energetic costs of absorbing and transporting excess carotenoids, a phenomenon described in studies of physiological trade-offs (Koch & Hill 2018).

The observed effects reflect the complex carotenoid metabolism pathway from consumption to physiological expression. After release from the feed matrix by lipase, carotenoids are absorbed into intestinal cells via micelle-dependent mechanisms and transported to target tissues through lipoproteins (Liao et al 2025). A portion of carotenoids undergoes biotransformation into other carotenoid forms or is cleaved to produce vitamin A (β-carotene → retinal → retinol), resulting in allocation between color display functions and other physiological roles (Pegu & Singh 2025). This dual role explains why the 400 mg kg^{-1} dose represents an optimal level: in dermal cells, carotenoids accumulate as free forms or bound to proteins (carotenoproteins) to optimize optical properties, while in other tissues they perform metabolic functions such as antioxidant defense and vitamin A precursor roles (Liao et al 2025).

The marked improvement in skin color indices reflects the essential role of carotenoids in tomato clownfish pigment expression, consistent with the inability of vertebrates to synthesize carotenoids de novo (de Carvalho & Caramujo 2017). The magnitude of improvement in redness (+59.0% at optimal 400 mg kg^{-1}) and chroma (+51.4%) observed in this study exceeds results reported for endemic dwarf chameleon fish (*Badis badis*) (+10.4-47.4%; Biswas et al 2024) but is lower than that in discus fish (*Symphysodon* spp.) (+214.7-319.0%) supplemented with astaxanthin (Song et al 2017), indicating that tomato clownfish exhibit good responsiveness to dietary carotenoids. The simultaneous increase in a^* (redness), b^* (yellowness), and L^* (lightness) indicates that carotenoids not only enhance red pigmentation but also increase overall brightness and saturation, resulting in more vibrant and visually appealing coloration.

Notably, the substantial increase in ΔE^*_{ab} values (14.71-15.48) in the 400-500 mg kg^{-1} groups confirms readily observable color improvement - a critical factor for commercial value as brightly colored fish command premium prices in the marine ornamental trade (Calado et al 2017). The plateau in color improvement at 400 mg kg^{-1} reflects maximum storage capacity in dermal pigment cells (chromatophores), particularly erythrophores and xanthophores responsible for the characteristic red-orange coloration of this species (Sathyaruban et al 2021).

The preferential accumulation pattern of skin (+122.5%) > whole body (+80.9%) > muscle (+69.8%) at the optimal 400 mg kg^{-1} dose relative to the control reflects a carotenoid allocation strategy based on ecological function. Skin is the primary display organ for conspecific communication and territorial defense in clownfish, thus receiving priority - an example of allocation trade-offs in evolutionary biology (Merilaita & Kelley 2018). This pattern reflects differential selection pressures in marine ornamental fish, where coloration plays essential roles in biological communication, compared to other fish species with different carotenoid allocation strategies (Tripathy et al 2025). The absence of significant differences between 400 and 500 mg kg^{-1} ($p > 0.05$) indicates that intestinal absorption capacity and tissue storage capacity have reached saturation, consistent with studies on physiological limits of carotenoid absorption in fish (Choubert et al 1995; Song et al 2017).

The optimal dose of 400 mg kg^{-1} carotenoids represents a biological optimum for growth and pigmentation. While this dose appears to balance effectiveness against ingredient cost, detailed economic analysis under practical production conditions is needed to determine specific profitability. The improvement in color indices can increase commercial value; however, comprehensive cost-benefit analysis is required to establish economic viability.

Several aspects warrant further investigation to expand practical applications of *Artemia* biomass-derived carotenoids in clownfish aquaculture. First, elucidating the specific carotenoid composition through high-performance liquid chromatography (HPLC)

would clarify which compounds - whether astaxanthin, canthaxanthin, or β-carotene-drive the observed improvements in pigmentation and metabolic performance (Liao et al 2025). Second, optimizing supplementation strategies beyond the current 56-day protocol deserves attention; investigating dose-duration interactions could identify economically efficient alternatives, such as pulse feeding with higher concentrations for shorter periods or lower maintenance doses following initial loading (Doan et al 2025). Understanding color stability after supplementation withdrawal would further refine feeding protocols (Brown et al 2016). Third, the physiological mechanisms underlying the observed metabolic improvements require validation through stress and disease challenge studies to confirm the immunomodulatory potential suggested by our antioxidant findings (Lim et al 2023). Finally, translating these results to commercial production necessitates comprehensive economic evaluation comparing *Artemia* biomass against alternative sources including synthetic astaxanthin and microalgal extracts, with particular emphasis on bioavailability, cost-effectiveness, and sustainability considerations for feed formulation optimization.

Conclusions. Based on growth, coloration, and feed efficiency outcomes, dietary supplementation with *Artemia* biomass-derived carotenoids at 400 mg kg^{-1} represents the optimal dose for enhancing both color quality and growth performance of tomato clownfish in recirculating systems. Higher doses (500 mg kg^{-1}) provide no additional benefits due to physiological limits in carotenoid absorption and storage. This finding provides a scientific basis for developing specialized feeds for marine ornamental fish, contributing to enhanced commercial value and sustainable development of the industry.

Future research should focus on: (1) characterizing specific carotenoid profiles (astaxanthin, canthaxanthin, β-carotene) through HPLC analysis and evaluating post-supplementation color retention to optimize feeding duration; (2) validating physiological benefits through stress and disease challenge trials to confirm immunomodulatory effects; (3) comparing cost-effectiveness and bioavailability of *Artemia* biomass versus synthetic and microalgal carotenoid sources to guide commercial-scale implementation.

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Conflicts of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Authors:
Dung Van Tran (DVT), Department of Aquaculture, School of Fisheries and Life Sciences, Nha Trang University, 02 Nguyen Dinh Chieu Street, North Nha Trang Ward, Khanh Hoa Province, Vietnam, e-mail: dungtv@ntu.edu.vn
Trang Le Thi Tran (TLTT), Department of Aquaculture, School of Fisheries and Life Sciences, Nha Trang University, 02 Nguyen Dinh Chieu Street, North Nha Trang Ward, Khanh Hoa Province, Vietnam, e-mail: letrang@ntu.edu.vn
Duy Quoc Tran (DQT), Department of Aquaculture, School of Fisheries and Life Sciences, Nha Trang University, 02 Nguyen Dinh Chieu Street, North Nha Trang Ward, Khanh Hoa Province, Vietnam, e-mail: duy.tq.66ntts@ntu.edu.vn
Duong Khanh Duc Nguyen (DKDN), Department of Aquaculture, School of Fisheries and Life Sciences, Nha Trang University, 02 Nguyen Dinh Chieu Street, North Nha Trang Ward, Khanh Hoa Province, Vietnam, e-mail: duongndk.63ntts@ntu.edu.vn
Nam Xuan Doan (NXD), Department of Aquaculture, School of Fisheries and Life Sciences, Nha Trang University, 02 Nguyen Dinh Chieu Street, North Nha Trang Ward, Khanh Hoa Province, Vietnam, e-mail: namdx@ntu.edu.vn
Dai Trong Vu (DTV), Department of Aquaculture, School of Fisheries and Life Sciences, Nha Trang University, 02 Nguyen Dinh Chieu Street, North Nha Trang Ward, Khanh Hoa Province, Vietnam, e-mail: daivt@ntu.edu.vn

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