MINISTRY OF EDUCATION AND TRAINING
NHA TRANG UNIVERSITY

TRAN VAN DUNG

ENHANCING THE COLORATION OF CAPTIVE - BRED FALSE CLOWNFISH (*Amphiprion ocellaris* Cuvier, 1830)

Major: Aquaculture
Major code: 9620301

SUMMARY OF DOCTORAL THESIS

KHANH HOA – 2025

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KHANH HOA – 2025

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The results of this dissertation are available at the National Library of Vietnam and the Library of Nha Trang University.

INTRODUCTION

The marine ornamental fish farming industry, especially the clownfish Amphiprion ocellaris, is attracting increasing attention from farmers, researchers, and conservationists. However, a major challenge is that the color quality of cultured fish is often inferior to that of wild-caught individuals. This not only affects the value and marketability of farmed fish but also increases the pressure on wild coral reef fish resources, despite some initial successes in breeding clownfish. Studies on improving fish coloration through carotenoid supplementation and environmental manipulation have yielded positive results. However, in Vietnam, the application of natural and purified carotenoid sources is still limited. Additionally, environmental factors such as tank color and salinity have not been fully investigated. Based on these reasons, the research project "***Enhancing the coloration of captive - bred false clownfish (Amphiprion ocellaris Cuvier, 1830)***" was conducted to enhance the quality of farmed fish, reduce fishing pressure, and protect coral reef ecosystems.

Objectives: The main objective of this study is to improve the coloration of clownfish under captive conditions, achieving quality equivalent to or surpassing that of wild-caught fish, by combining optimal environmental conditions (tank color and salinity) and natural carotenoids supplementation strategies (source, dosage, and duration of carotenoid supplementation).

Research contents: To achieve the above objectives, the study focuses on three main research contents:

1. Effects of the rearing environment (tank color and salinity) on the coloration of clownfish.
2. Effects of natural carotenoid supplementation regimes (source, dosage, and duration) on the coloration of clownfish.
3. Evaluation of the combined effects of optimized rearing conditions and carotenoid supplementation to enhance the coloration of captive-bred clownfish.

Significance of the study:

The results of this study will provide an important scientific and practical basis for improving the quality and value of farmed clownfish, thereby promoting the sustainable development of the marine ornamental fish farming industry while contributing to the conservation of wild resources and coral reef ecosystems.

CHAPTER 1 – OVERVIEW

1.1. Overview of the marine ornamental fish farming industry

The marine ornamental fish farming industry has a long history and has developed rapidly in the 20th century due to advancements in aquaculture technology and techniques. At least 338 ornamental fish species from 37 families have been recorded, of which only a small portion are regularly traded in the market, and many species are facing extinction due to overfishing. The global marine ornamental fish trade reaches billions of USD annually, mainly from wild-caught fish in tropical regions. Vietnam has great potential to develop this industry but is facing challenges in seed production and high costs. However, recent progress in genetic engineering, crossbreeding, and molecular biology has opened up new opportunities. For sustainable development, priority should be given to using native species, applying good aquaculture practices, enhancing market research, and involving stakeholders.

1.2. Overview of clownfish

Clownfish (Amphiprion) are popular marine ornamental fish, comprising 30 species, including *A. ocellaris*. They live in symbiosis with sea anemones in coral reefs of tropical and subtropical regions. In Vietnam, clownfish populations are declining due to overfishing. Clownfish lay adhesive eggs and have a life cycle that includes pelagic larvae, settling juveniles, and reproducing adults. They are omnivorous, requiring a combination of various feeds and nutritional supplements in captive breeding. Clownfish are protandrous hermaphrodites, exhibit parental care, and have low fecundity. Although progress has been made in seed production, many challenges remain, particularly in the quality of juvenile fish.

1.3. Quality of ornamental fish and influencing factors

The quality of ornamental fish is evaluated based on various criteria such as appearance, coloration, health, behavior, genetics, origin, age, size, aesthetic value, and rarity. Genetic factors and selective breeding play a crucial role in creating fish strains with desirable traits, good adaptability, and high resistance. In addition, environmental factors, aquaculture techniques, nutrition, and health also have a significant impact on the quality of ornamental fish. Creating an optimal living environment, applying advanced aquaculture techniques, implementing good disease management, providing a balanced nutritional regime, and using biological products contribute to improving the quality and value of ornamental fish.

1.4. Coloration of ornamental fish and influencing factors

1.4.1. Color diversity and its role in fish

Fish coloration is highly diverse and serves three basic functions: thermoregulation, intraspecific communication, and interspecific communication. The coloration of fish is created by six types of pigment cells and the combination of biological and structural pigments. Many factors, such as genetics, environment, sex, and developmental stage, influence color changes in fish through morphological or physiological mechanisms. Morphological color changes in fish involve the increase or decrease in the number, density, shape, and distribution of pigment cells in the skin due to long-term stimuli and are controlled by neural and molecular mechanisms. Physiological color changes in fish are related to the movement, distribution, and rearrangement of pigment cells/crystals due to short-term stimuli and are under the integrated control of various physical, chemical, neural, and endocrine factors.

1.4.2. Factors influencing the coloration of ornamental fish

Feed and nutritional supplements: Under captive conditions, supplementing natural and synthetic carotenoid sources in the diet has significantly improved coloration in many fish species. However, the understanding of the mechanisms of carotenoid digestion, absorption, and metabolism in fish is limited. Vietnam has diverse carotenoid sources from plants, animals, and microorganisms. Studies on supplementing plant-based carotenoids in ornamental fish diets have shown improvements in coloration, disease resistance, and resilience. Supplementation in the form of extracts is a promising direction but requires further research.

Environment and ecology: Fish coloration reflects various physiological and behavioral aspects, including static and dynamic coloration. Fish have the ability to adapt to changes in light and environmental colors. Studies on the impact of tank color on fish have shown effects on coloration, growth, survival rate, behavior, and health. Light also influences the living activities of fish through its impact on pigment cells. Camouflage, mimicry, reproduction, sex change, and social interactions also affect the coloration of many fish species.

Genetics and selective breeding: The genetics of coloration in fish has a complex mechanism involving many functional genes. The application of omics techniques and marker-assisted selection (MAS) has helped identify the genetic basis and improve coloration in some fish species. However, to actively create desired colors, it is necessary to clarify the mechanisms of color formation, the influence of pigment sources from the diet, the theoretical basis of color improvement techniques, and the factors controlling the activity of pigment cells. In the meantime, color improvement solutions based on nutritional supplementation and environmental modification are still considered positive and effective approaches.

CHAPTER 2 – MATERIALS AND RESEARCH METHODS

2.1. Time, location, and research subjects

The research was conducted from January 2021 to December 2023. The rearing experiments were carried out at the Vinh Hoa Marine Ornamental Fish Hatchery, Nha Trang, Khanh Hoa. In-depth analyses were performed at the Laboratory and Practice Center, Nha Trang University. The research was conducted on juvenile false clownfish (*Amphiprion ocellaris*), also known as the ocellaris clownfish.

2.2. Research contents

Content 1: *Effects of rearing environment (tank color – Exp. 1; salinity – Exp. 2) on false clownfish.*

Content 2: *Effects of dietary carotenoid supplementation on false clownfish, including: sources (plant-based – Exp. 3, animal-based - Exp. 4), levels (bell pepper - Exp. 5, shrimp shell - Exp. 6), and supplementation duration (shrimp shell - Exp. 7).*

Content 3: *Evaluation of the combined effects of rearing environment and appropriate carotenoid supplementation regimes (Exp. 8) to improve the coloration of false clownfish.*

2.3. Research materials

2.3.1. Experimental fish source

False clownfish were produced at the hatchery. Fish used in the experiments had a length of 3.0 - 3.5 cm and a weight of 0.5 - 0.9 g/individual. The fish were healthy, agile, naturally colored, and of uniform size. The fish were acclimated for 3 - 5 days to adapt to the rearing tank system before starting the experimental period.

2.3.2. Water source and treatment

The water used for the experiments was natural seawater, treated according to the common and current protocol with chlorine and neutralized with sodium thiosulfate.

2.3.3. Experimental tank system

The experimental tanks were glass tanks with a volume of approximately 65 liters (55 × 35 × 38 cm). Each system consisted of 18 tanks connected to a centrally located 500-liter recirculating biological filter tank. The rearing tanks and filter tank were continuously aerated throughout the experimental period. The water recirculation rate was about 3,500 – 3,700%/tank/day. The experimental tank system was placed under a roof, with a natural lighting cycle of 12 hours light: 12 hours dark to stabilize environmental factors, combined with LED tube lighting during low light periods or when needed.

2.3.4. Preparation of carotenoid sources

Raw materials included pumpkin, bell pepper, carrot, gac fruit, chicken egg, white leg shrimp shell, copepods, and apple snail eggs. Soybean oil was used as a solvent for gac extraction, and 96% ethanol for the remaining materials. The extraction process

was performed according to Tran et al. (2022) with minor modifications, using a solvent: material ratio of 3.5 : 1.0 (v/w), homogenization by blender, microwave-assisted extraction for a total of 180 seconds, repeated for three cycles. The total carotenoid content (µg/g fresh material) obtained was: pumpkin 71.7; bell pepper 102.6; carrot 88.4; gac 463.0; chicken egg yolk 23.7; shrimp shell 52.6; copepods 113.3; and apple snail eggs 81.4. Synthetic astaxanthin (10%) was also used to compare the effectiveness with natural pigments. These carotenoid sources were incorporated into experimental diets for the fish.

2.3.5. Preparation of experimental diets

The basal diet was formulated based on the nutritional requirements for marine fish rearing, with crude protein and lipid levels of 55% and 12%, respectively (Table 2.1).

Table 2.1: Formulation and ingredients of experimental diets (g/kg)

Ingredients	**Amount (g/kg)**	**Percentage (%)**
Fish meal (Peru) (g)	425.0	42.50
Fish meal (Vietnam) (g)	136.0	13.60
Squid meal (g)	140.0	14.00
Soybean meal (g)	75.0	7.50
Corn gluten meal (g)	85.0	8.50
Wheat flour (g)	34.0	3.40
Fish oil (g)	25.7	2.57
Soybean oil (g)	30.0	3.00
Vitamin premix[1] (g)	10.0	1.00
Lysine (g)	8.0	0.80
Methionine (g)	6.0	0.60
Monocalcium phosphate (g)	6.0	0.60
Guar gum powder (g)	5.0	0.50
Sodium alginate (g)	4.3	0.43
Mineral premix[2] (g)	10.0	1.00
Carotenoids supplement (g)	0	0
Chemical and proximate composition		
Crude protein (%)	55.00	
Crude lipid (%)	12.01	
Ash (%)	11.09	
Moiture (%)	10.04	
Carotenoids (g)	0.04	

The feed production process included weighing raw ingredients, fine grinding, mixing, cooking at 90 - 95°C for 20 minutes, extruding through a 3.0 mm die, drying at 60°C for 8 hours, grinding, and sieving through a 0.8 - 1.0 mm mesh screen. The oil mixture containing carotenoids and synthetic vitamins was evenly sprayed on the surface of the feed pellets according to each experimental treatment. The supplementation levels ranged from 0 - 1,500 mg/kg feed. The feed was divided into small zip bags and stored at a temperature of ≤ 4°C. The biochemical composition and total carotenoid content of the feed were analyzed according to AOAC (2006).

2.4. Research methods

2.4.1. Effects of rearing environment on false clownfish

2.4.1.1. Effects of tank color (Exp. 1)

The experiment was arranged in a completely randomized design (CRD) with 6 tank color treatments: white, transparent (control), blue, orange, purple, and black. The tanks were prepared by attaching colored decal paper to 5 sides. Juvenile false clownfish (3.30 ± 0,02 cm and 0.65 ± 0,02 g) were stocked at a density of 15 fish/tank. The experiment lasted for 60 days with 3 replicates for each treatment. The fish were fed a commercial diet NRD.

2.4.1.2. Effects of salinity (Exp. 2)

The experiment was arranged with 6 salinity treatments: 9‰, 15‰, 21‰, 27‰, 33‰ (Control), and 39‰. Lower or higher salinity levels compared to the seawater salinity of 33‰ were created by mixing tap water or salt with an appropriate ratio. Juvenile fish (3.25 ± 0,08 cm and 0.67 ± 0,05 g) were stocked at a density of 15 fish/tank. Each treatment was replicated 3 times for 60 days.

2.4.2. Effects of dietary carotenoid supplementation on false clownfish

2.4.2.1. Effects of some plant-based carotenoid sources (Exp. 3)

The experiment was arranged with 6 treatments: pumpkin, gac fruit, bell pepper, carrot, synthetic astaxanthin, and control - no pigment supplementation. The carotenoid levels in the supplemented treatments were standardized at 250 mg/kg. Juvenile fish (3.21 ± 0,03 cm and 0.61 ± 0,02 g) were stocked at a density of 15 fish/tank. The experiment was conducted with 3 replicates for 75 days.

2.4.2.2. Effects of some animal-based carotenoid sources (Exp. 4)

The experiment was arranged with 6 treatments: chicken egg, copepods, shrimp shell, apple snail eggs, synthetic astaxanthin, and control. Juvenile fish (3.14 ± 0,02 cm and 0.54 ± 0,02 g) were stocked at a density of 15 fish/tank. The experiment was conducted with 3 replicates for 75 days.

2.4.2.3. Effects of carotenoid levels from bell pepper (Exp. 5)

The experiment was designed with 5 levels of carotenoids from bell pepper supplemented in the diet (300, 600, 900, 1,200, and 1,500 mg/kg) and a control. Juvenile fish (3.40 ± 0,04 cm and 0.72 ± 0,02 g) were stocked at a density of 15 fish/tank. Each treatment was replicated 3 times for 75 days.

2.4.2.4. Effects of carotenoid levels from shrimp shell (Exp. 6)

The experiment was designed with 5 levels of carotenoids from shrimp shell supplemented in the diet (200, 400, 600, 800, and 1,000 mg/kg) and a control. Juvenile fish (3.00 ± 0,05 cm and 0.58 ± 0,03 g) were stocked at a density of 15 fish/tank. Each treatment was replicated 3 times for 75 days.

2.4.2.5. Effects of supplementation duration of carotenoids from shrimp shell (Exp. 7)

The experiment was designed with 5 different supplementation durations of carotenoids (15, 30, 45, 60, and 75 days). The source and level of carotenoids used were the

best results from Experiments 5 and 6. Juvenile fish (3.20 ± 0,02 cm and 0.62 ± 0,04 g) were stocked at a density of 15 fish/tank. Each treatment was replicated 3 times for 75 days.

2.4.3. Evaluation of the combined effects of rearing environment and appropriate carotenoid supplementation regimes (Exp. 8) to improve the coloration of false clownfish

The experiment synthesized optimal criteria from Experiments 1-7 for fish rearing, consisting of two treatments: application (environmental adjustment and nutritional supplementation) and control (rearing under normal production conditions). Fish were cultured at a scale of 500 liters with a density of 300 individuals/tank. Each treatment was replicated 7 times, corresponding to different production cycles. At the end of the experiment, 30 fish from each tank were randomly collected to determine growth parameters, survival rate, and feed utilization efficiency. The effectiveness of color improvement in artificially produced fish was also compared with wild-caught fish through skin color indices and accumulated carotenoid content. Wild fish samples were collected 7 times per year and at the same time as the end of the experiment to increase accuracy.

2.5. Care, management, collection, and evaluation of experimental results

2.5.1. Care and management

Fish were fed 4 times/day, with the amount of feed adjusted according to the fish's appetite. Leftover feed was collected and dried to calculate feed utilization efficiency.

2.5.2. Collection and evaluation of results

Fish sampling method: At the end of the experiment, all fish were starved for 24 hours and anesthetized with 500 ppm EGME for 5-10 seconds. Measurements were applied to all surviving fish at the end of the experiment, including length, weight, and skin color indices, with specific calculation formulas as follows.

2.5.2.1. Fish skin color

Fish skin color was determined using a CR-400 Chroma Meter. Fish were measured on both sides of the body, at the position between the soft dorsal fin and the anal fin, with three measurements at each position. The meter was set to measure L^*, a^*, b^* using a D65 illuminant attached to a glass light projection tube. Measurement and setup methods followed the manufacturer's instructions. Color measurement indices used to evaluate experimental results included: L^* for measuring lightness (0-100), a^* for measuring green (a^-) to red (a^+) pigments, b^* for measuring blue (b^-) to yellow (b^+) pigments, C^*_{ab} for measuring color saturation (0-100), h^*_{ab} for measuring specific colors of the object (0-360°), ΔE^*_{ab} for measuring color differences between treatment and control groups. The values of C^*_{ab}, h^*_{ab}, and ΔE^*_{ab} were calculated based on L^*, a^*, and b^* indices. In Experiment 8, fish color was evaluated by sensory assessment using the Clownfish Exercise scale by 5 trained panelists. Evaluation was performed on 30 fish samples immediately after measuring length and weight. The average score of each group (control, application, and wild) was used for comparison.

2.5.2.2. Total carotenoid content accumulated in fish body

The total carotenoid content in the skin, muscle, whole body of fish, and feed was determined by UV-Vis spectrophotometry according to the method of Ramamoorthy and García-Romero with some modifications. Samples including skin (0.25 g), muscle (0.25 g), whole body (1-2 g), and feed (1 g) were ground in acetone containing anhydrous sodium sulfate, filtered 3 times, and centrifuged at 10,000 rpm, 4°C for 15 minutes. Absorbance was measured by a spectrophotometer, and the results were expressed in micrograms per gram (µg/g).

Total carotenoid content (µg/g) = $A \times V \times D \times 10^4 / (W \times E_{1cm}^{1\%})$

Where: A is the absorbance; V is the total volume of the extract (mL); D is the dilution ratio; W is the weight of the sample (g); and E1cm1% is the conversion factor, 2,100 (edible oil solvent = 2,100, absorption wavelength 450 nm or 480 nm, corresponding to total carotenoids or astaxanthin).

2.5.2.3. Parameters for evaluating growth, survival rate and feed utilization efficiemcy

Growth and survival rate:

+ Specific growth rate in length: SGRL (%/day) = $[(LnL_2 - LnL_1) / t] \times 100$
+ Specific growth rate in weight: SGRW (%/day) = $[(LnW_2 - LnW_1) / t] \times 100$
+ Coefficient of variation: CV (%) = SD / Mean × 100
+ Condition factor: CF (g/cm^3) = $100 \times W/L^3$
+ Survival rate: SR (%) = $[N_2 / N_1] \times 100$

Feed utilization efficiency:

+ Feed intake: FI (g/fish) = [FC - FL] / N
+ Feed conversion ratio: FCR = $FI / [W_2 - W_1]$
+ Protein efficiency ratio: PER = $[W_2 - W_1] / (FI \times P)$

2.5.2.4. Biochemical composition and activity of digestive enzymes

After anesthesia, length and weight measurements, 4-5 fish from each tank were randomly collected and stored in a freezer before grinding into a fine powder for approximate biochemical analysis according to AOAC (2006) procedures.

Similar to the biochemical analysis sampling, 4-6 fish from each tank were randomly collected. The digestive organs of the fish were separated, cleaned, frozen in liquid nitrogen, and stored at -80°C. Frozen tissue was ground into a fine powder, homogenized in cold phosphate buffer, and centrifuged to obtain the supernatant containing soluble protein. The activities of protease, lipase, and amylase enzymes were determined by the modified Anson method, the continuous pH-stat titration method, and the DNS reagent method, respectively. Specific activity was expressed as U/mg protein.

2.6. Statistical analysis

Data were calculated using Microsoft Excel 2021 software and then tested for homogeneity of variance and normal distribution. One-way ANOVA in SPSS 22.0 was used to analyze the results. Duncan's test was used for Experiments 1-7 and the Independent-Samples T-Test for Experiment 8 to determine statistically significant differences between treatments ($p < 0.05$). Results were presented as Mean ± SE.

CHAPTER 3 – RESULTS AND DISCUSSION

3.1. Effects of rearing environment on false clownfish

3.1.1. Effects of tank color

3.1.1.1. Fish skin color

Fish reared in blue and transparent tanks had the highest lightness (L^*) and redness (a^*) values and exhibited a reddish-orange color. In contrast, fish reared in black tanks had the poorest coloration. Therefore, blue and transparent colors were determined to be the most suitable for rearing false clownfish (Fig. 3.1).

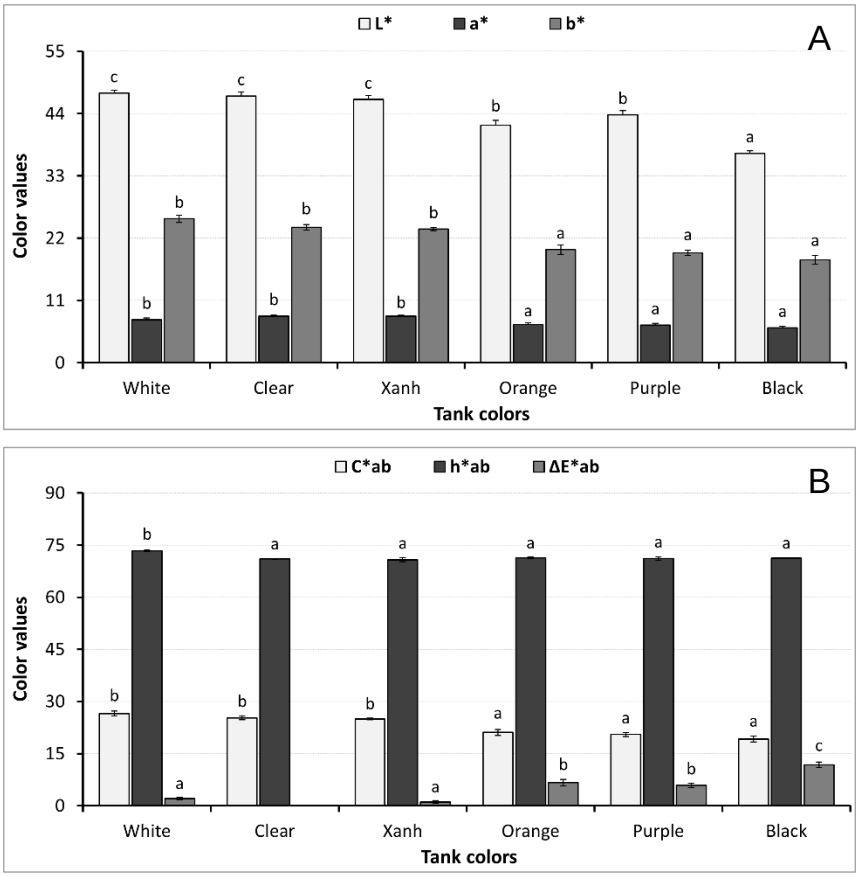

Figure 3.1: Skin color parameters of fish reared in tanks with different colors

Values are presented as mean ± standard error (n = 3). Columns with different letters indicate statistically significant differences (p < 0.05).

3.1.1.2. Total carotenoid content accumulated in fish body

Tank color significantly affected the accumulation of carotenoids in the body of false clownfish. Fish reared in blue and transparent tanks had the highest carotenoid content in the skin, muscle, and whole body, 54.6 - 63.4% higher than those in black tanks. There were no significant differences in carotenoid content between fish reared in orange and purple tanks compared to the other tanks. The results showed that blue, transparent, and white tanks were beneficial for carotenoid accumulation in the body of false clownfish (Fig. 3.2).

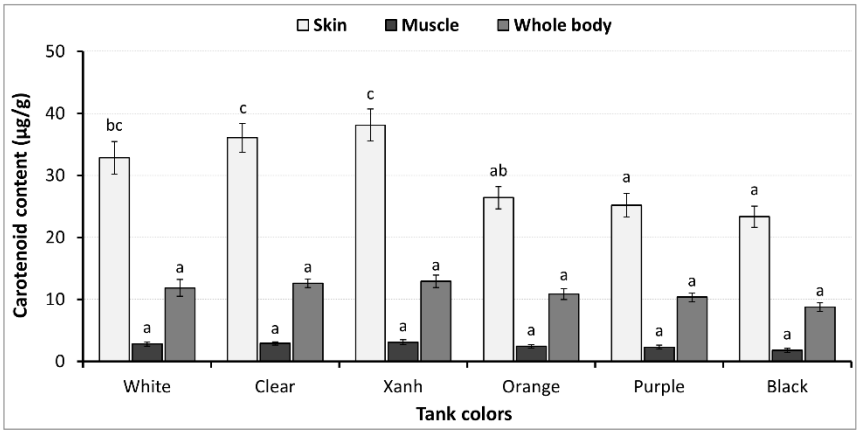

Figure 3.2: Carotenoid content accumulated in fish body (µg/g) in different tank colors

3.1.1.3. Growth parameters, survival rate and feed utilization efficiency

The results showed that fish reared in white and blue tanks achieved the highest specific growth rates in length and weight (SGR_L, SGR_W). Fish reared in blue tanks had the lowest weight variation coefficient. The highest condition factor was observed in white tanks. However, tank color did not significantly affect the length variation coefficient (CV_L) and survival rate of the fish (Table 3.1).

Table 3.1: Growth and survival rate of fish reared in tanks with different colors

Parameters	Tank colors					
	White	Clear	Blue	Orange	Purple	Black
L_1 (cm)	3.30 ± 0.02	3.30 ± 0.02	3.30 ± 0.02	3.30 ± 0.02	3.30 ± 0.02	3.30 ± 0.02
W_1 (g)	0.65 ± 0.02	0.65 ± 0.02	0.65 ± 0.02	0.65 ± 0.02	0.65 ± 0.02	0.65 ± 0.02
L_2 (cm)	3.94 ± 0.02^{b}	3.77 ± 0.02^{a}	3.93 ± 0.02^{b}	3.84 ± 0.04^{a}	3.85 ± 0.03^{a}	3.81 ± 0.03^{a}
W_2 (g)	1.25 ± 0.01^{c}	1.04 ± 0.01^{a}	1.23 ± 0.02^{c}	1.13 ± 0.04^{b}	1.16 ± 0.01^{b}	1.10 ± 0.03^{ab}
SGR_L (%/day)	0.29 ± 0.01^{b}	0.22 ± 0.01^{a}	0.29 ± 0.01^{b}	0.25 ± 0.02^{a}	0.25 ± 0.01^{a}	0.24 ± 0.01^{a}
SGR_W (%/day)	1.09 ± 0.02^{c}	0.79 ± 0.02^{a}	1.07 ± 0.03^{c}	0.92 ± 0.05^{b}	0.96 ± 0.02^{b}	0.87 ± 0.04^{ab}
CV_L (%)	8.11 ± 0.43	7.02 ± 0.19	7.12 ± 0.37	8.67 ± 0.47	8.60 ± 0.51	6.78 ± 0.89
CV_W (%)	23.3 ± 0.99^{bc}	21.2 ± 0.83^{ab}	19.0 ± 0.79^{a}	25.8 ± 1.94^{c}	25.6 ± 1.64^{c}	20.9 ± 1.12^{ab}
CF (g/cm^3)	2.05 ± 0.02^{c}	1.96 ± 0.01^{a}	2.03 ± 0.01^{bc}	1.99 ± 0.02^{ab}	2.03 ± 0.03^{bc}	1.99 ± 0.01^{ab}
SR (%)	95.5 ± 2.23	97.8 ± 2.23	95.5 ± 2.23	91.1 ± 2.20	95.5 ± 2.23	95.5 ± 2.23

Data are presented as Mean ± SE (n = 3). Mean values bearing different superscript letters within the same row indicate statistically significant differences (p < 0.05).

Fish reared in white and blue tanks achieved the best results, with FCR decreasing by 17.4 – 19.4% and PER increasing by 19.8 – 23.1%. The poorest results were observed in transparent tanks (Table 3.2).

Table 3.2: Feed utilization efficiency of fish reared in tanks with different colors

Parameters	Tank colors					
	White	Clear	Blue	Orange	Purple	Black
FI (g/fish)	0.98 ± 0.03^{b}	0.79 ± 0.03^{a}	0.97 ± 0.02^{b}	0.90 ± 0.05^{ab}	0.95 ± 0.04^{b}	0.89 ± 0.04^{ab}
FCR	1.62 ± 0.02^{a}	2.01 ± 0.02^{c}	1.66 ± 0.05^{a}	1.89 ± 0.04^{b}	1.88 ± 0.02^{b}	1.99 ± 0.04^{c}
PER	1.12 ± 0.02^{c}	0.91 ± 0.01^{a}	1.09 ± 0.03^{c}	0.96 ± 0.01^{ab}	0.97 ± 0.01^{b}	0.92 ± 0.02^{ab}

3.1.2. Effects of salinity on false clownfish

3.1.2.1. Fish skin color

The redness (a^*) was highest at 33‰ and lowest at 9‰, with a difference of 49.1%. The yellowness (b^*) and chroma (C^*_{ab}) were highest at 39‰ and lowest at 9‰. The lightness (L^*) and hue angle (h^*_{ab}) were highest at 9‰ and lowest at 33‰. The color difference compared to the control at 33‰ (ΔE^*_{ab}) increased with decreasing salinity, being highest at 9‰. There was no difference in skin color between 33‰ and 39‰. The salinity range of 33-39‰ helped the fish achieve optimal skin color (dark orange, vibrant, with depth) compared to low salinities (bright but pale yellow). The salinity range of 33-39‰ was suitable for false clownfish (Fig. 3.3).

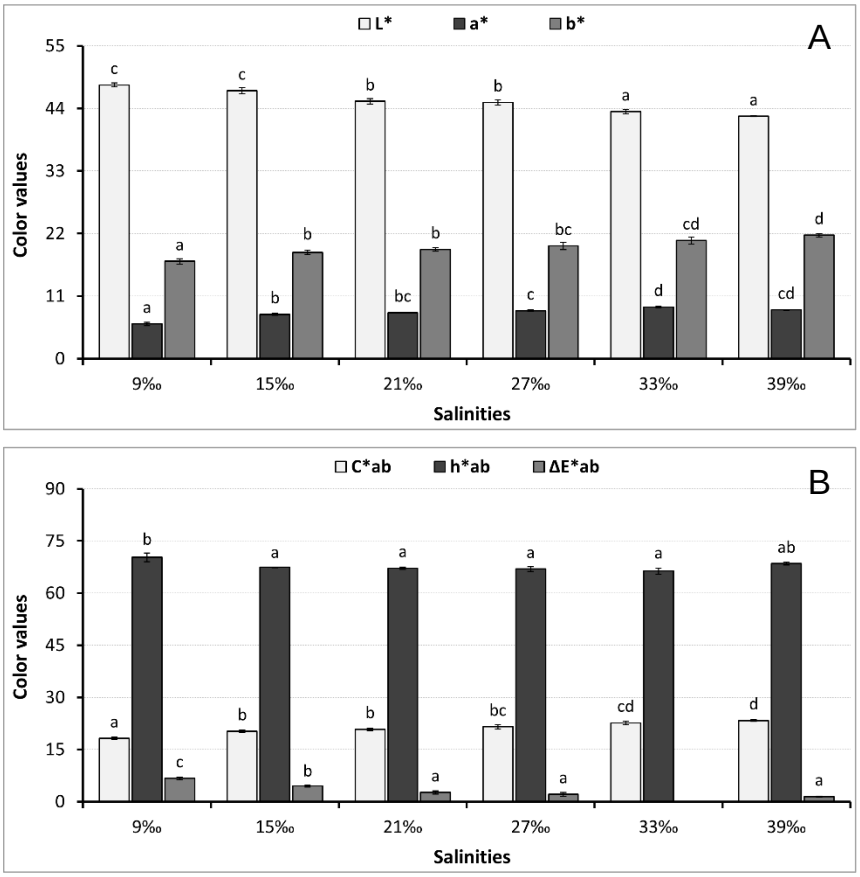

Figure 3.3: Skin color parameters of fish reared at different salinity levels

3.1.2.2. Total carotenoid content accumulated in fish body

From 9-33‰, the carotenoid content accumulated in the skin, muscle, and whole body of the fish increased linearly with salinity. At 33‰, the accumulated carotenoid content was highest, increasing by 87.6% in the skin, 80.5% in the muscle, and 53.6% in the whole body compared to 9‰. The salinity range of 33-39‰ was determined to be suitable for carotenoid accumulation in the body of false clownfish (Fig. 3.4).

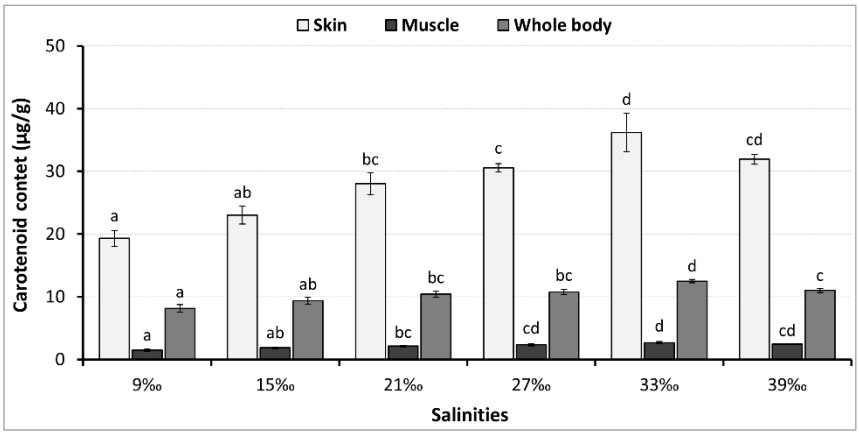

Figure 3.4: Carotenoid content accumulated in fish body (µg/g) at different salinities

3.1.2.3. Growth parameters, survival rate and feed utilization efficiency

Fish reared at 33‰ salinity achieved the highest SGR_L and SGR_W, which were 50% and 80% higher than those at 9‰ salinity, respectively. There were no significant differences in SGR_L between 33 and 39‰, and in SGR_W among 27, 33, and 39‰. The CV_L was lowest at 39‰ and highest at 15-33‰. CF and SR were better at higher salinities (15-39‰) compared to 9‰. The optimal salinity was 33‰, and fish grew well within the range of 27-39‰ (Table 3.3).

Table 3.3: Growth and survival rate of fish reared at different salinity levels

Parameters	Salinity					
	9‰	**15‰**	**21‰**	**27‰**	**33‰**	**39‰**
L_1 (cm)	3.25 ± 0.08	3.25 ± 0.08	3.25 ± 0.08	3.25 ± 0.08	3.25 ± 0.08	3.25 ± 0.08
W_1 (g)	0.67 ± 0.05	0.67 ± 0.05	0.67 ± 0.05	0.67 ± 0.05	0.67 ± 0.05	0.67 ± 0.05
L_2 (cm)	3.71 ± 0.02^{a}	3.79 ± 0.01^{b}	3.83 ± 0.02^{b}	3.90 ± 0.02^{c}	3.97 ± 0.01^{d}	3.93 ± 0.03^{cd}
W_2 (g)	0.93 ± 0.02^{a}	1.05 ± 0.02^{b}	1.07 ± 0.03^{b}	1.13 ± 0.02^{bc}	1.22 ± 0.03^{c}	1.16 ± 0.04^{c}
SGR_L (%/day)	0.22 ± 0.01^{a}	0.26 ± 0.01^{b}	0.27 ± 0.01^{b}	0.30 ± 0.01^{c}	0.33 ± 0.01^{d}	0.32 ± 0.01^{cd}
SGR_W (%/day)	0.55 ± 0.04^{a}	0.75 ± 0.03^{b}	0.78 ± 0.04^{b}	0.87 ± 0.02^{bc}	0.99 ± 0.05^{c}	0.92 ± 0.06^{c}
CV_L (%)	5.19 ± 0.46^{b}	7.31 ± 0.75^{c}	7.21 ± 0.05^{c}	6.84 ± 0.38^{c}	7.49 ± 0.66^{c}	3.67 ± 0.02^{a}
CV_W (%)	19.1 ± 1.62	25.0 ± 3.00	23.5 ± 1.33	22.5 ± 1.67	24.4 ± 1.75	21.4 ± 1.20
CF (g/cm^3)	1.83 ± 0.02^{a}	1.92 ± 0.01^{b}	1.90 ± 0.03^{b}	1.91 ± 0.02^{b}	1.95 ± 0.03^{b}	1.92 ± 0.03^{b}
SR (%)	75.1 ± 6.24^{a}	93.3 ± 3.84^{b}	97.8 ± 2.23^{b}	97.8 ± 2.23^{b}	100 ± 0.00^{b}	97.8 ± 2.23^{b}

The best FCR and PER were observed at a salinity of 33‰, while the poorest values were at 9‰: FCR decreased by 16.3%, while PER increased by 63.3% compared to 9‰ salinity. In terms of feed utilization efficiency, the salinity range of 27-39‰ was determined to be suitable for the fish, especially at 33‰ (Table 3.4).

Table 3.4: Feed utilization efficiency of fish reared at different salinity levels

Parameters	Salinity					
	9‰	**15‰**	**21‰**	**27‰**	**33‰**	**39‰**
FI (g/fish)	0.95 ± 0.05^{a}	1.09 ± 0.02^{b}	1.15 ± 0.03^{bc}	1.17 ± 0.03^{bc}	1.25 ± 0.05^{c}	1.27 ± 0.04^{c}
FCR	2.09 ± 0.08^{d}	2.00 ± 0.02^{cd}	1.97 ± 0.04^{bcd}	1.81 ± 0.02^{ab}	1.75 ± 0.08^{a}	1.87 ± 0.04^{abc}
PER	0.49 ± 0.03^{a}	0.63 ± 0.02^{b}	0.64 ± 0.03^{b}	0.71 ± 0.02^{bc}	0.80 ± 0.05^{c}	0.71 ± 0.04^{bc}

3.2. Effects of dietary carotenoid supplementation on false clownfish

3.2.1. Effects of some plant carotenoid sources

3.2.1.1. Fish skin color

The redness (a^*) and yellowness (b^*) of the fish skin in the groups supplemented with astaxanthin, bell pepper, and gac fruit were the highest, while the control group had the lowest values. Similarly, the chroma (C^*_{ab}) and color difference (ΔE^*_{ab}) indices also showed the superiority of astaxanthin, bell pepper, and gac fruit supplementation compared to the other groups and the control (Fig. 3.5).

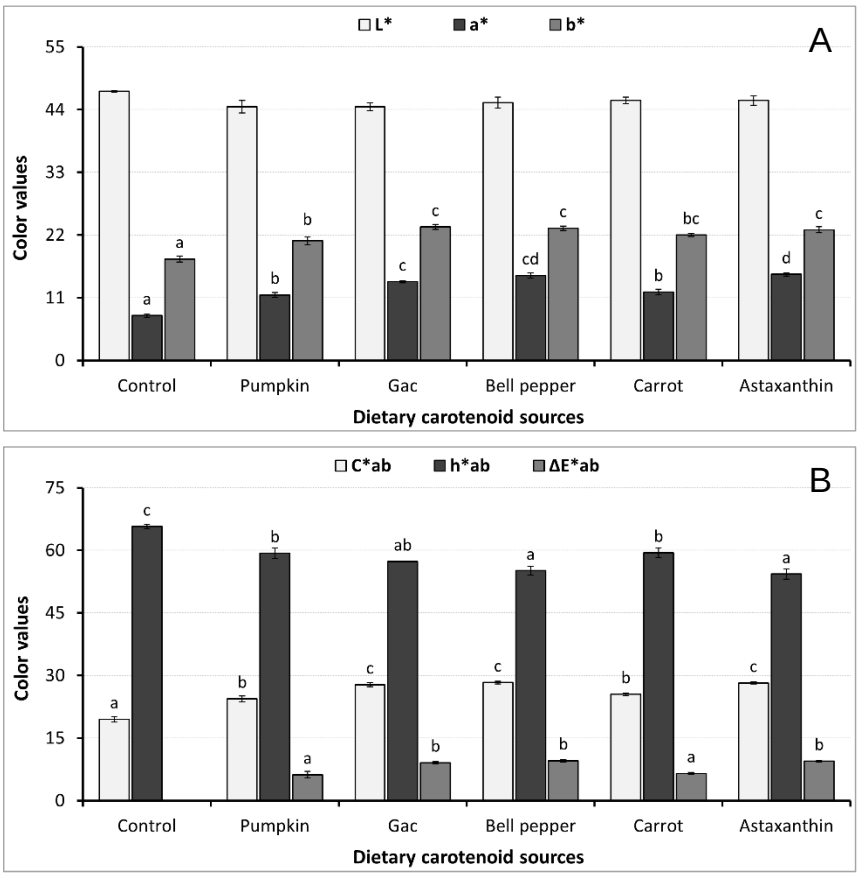

Figure 3.5: Skin color parameters of fish fed diets with plant carotenoid sources

3.2.1.2. Total carotenoid content accumulated in fish body

Supplementing the diet with carotenoid sources significantly increased the total carotenoid content accumulated in the fish body, including the skin, muscle, and whole body, compared to the control group. There was no difference in the accumulated carotenoid content in the fish body among the groups supplemented with bell pepper, gac fruit, and astaxanthin, indicating that these three sources were effective in enhancing color in false clownfish (Fig. 3.6).

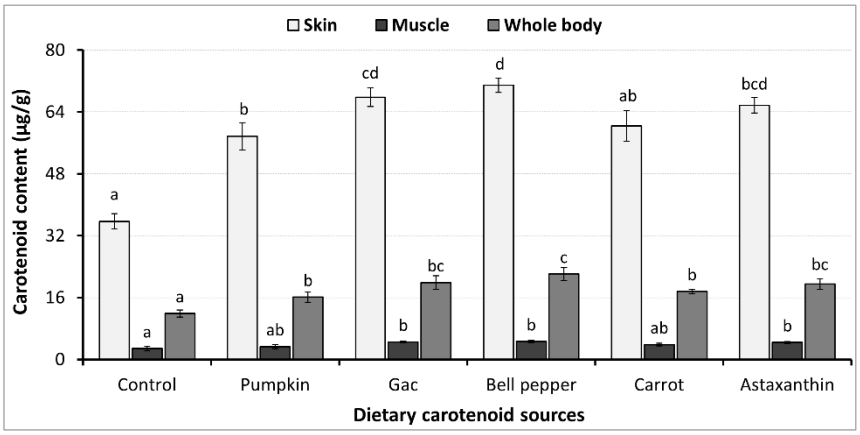

Figure 3.6: Carotenoid content accumulated in fish body (µg/g) fed diets with plant carotenoid sources

3.2.1.3. Growth parameters, survival rate and feed utilization efficiency

Supplementing the diet with carotenoids from gac fruit and bell pepper significantly improved the SGR_L and SGR_W of the fish, with increases of 34.5 – 41.4% and 39.0 – 42.7%, respectively, compared to the control. Moreover, fish in these supplemented groups had more uniform sizes, as evidenced by lower weight variation coefficients compared to the other groups (Table 3.5).

Table 3.5: Growth and SR of fish fed diets with plant carotenoid sources

Parameters	Supplementary carotenoid sources					
	Control	Pumpkin	Gac	Bell pepper	Carrot	Astaxanthin
L_1 (cm)	3.21 ± 0.03	3.21 ± 0.03	3.21 ± 0.03	3.21 ± 0.03	3.21 ± 0.03	3.21 ± 0.03
W_1 (g)	0.61 ± 0.02	0.61 ± 0.02	0.61 ± 0.02	0.61 ± 0.02	0.61 ± 0.02	0.61 ± 0.02
L_2 (cm)	4.00 ± 0.02^{a}	4.17 ± 0.02^{b}	4.35 ± 0.03^{c}	4.30 ± 0.03^{c}	4.20 ± 0.04^{b}	4.12 ± 0.04^{b}
W_2 (g)	1.13 ± 0.04^{a}	1.31 ± 0.05^{bc}	1.47 ± 0.03^{d}	1.44 ± 0.02^{cd}	1.33 ± 0.05^{bc}	1.27 ± 0.04^{b}
SGR_L (%/day)	0.29 ± 0.01^{a}	0.35 ± 0.01^{b}	0.41 ± 0.01^{c}	0.39 ± 0.01^{c}	0.36 ± 0.01^{b}	0.33 ± 0.01^{b}
SGR_W (%/day)	0.82 ± 0.05^{a}	1.02 ± 0.05^{bc}	1.17 ± 0.03^{d}	1.14 ± 0.03^{cd}	1.04 ± 0.05^{bc}	0.98 ± 0.04^{b}
CV_L (%)	12.4 ± 0.54	11.3 ± 0.42	9.91 ± 0.73	10.0 ± 0.76	11.3 ± 0.54	11.5 ± 0.90
CV_W (%)	40.3 ± 0.63^{b}	34.5 ± 1.44^{ab}	29.0 ± 1.91^{a}	29.2 ± 2.58^{a}	35.4 ± 1.93^{b}	35.2 ± 1.83^{b}
CF (g/cm^3)	1.76 ± 0.03	1.80 ± 0.04	1.78 ± 0.01	1.81 ± 0.01	1.80 ± 0.01	1.81 ± 0.02
SR (%)	93.3 ± 3.85	95.6 ± 2.22	97.8 ± 2.22	97.8 ± 2.22	95.6 ± 2.22	95.6 ± 2.22

Supplementation of carotenoids from gac fruit and bell pepper significantly improved the feed utilization efficiency of the fish. The best values for FCR and PER were achieved in the gac fruit supplemented group (Table 3.6).

Table 3.6: Feed utilization efficiency of fish fed diets with plant carotenoid sources

Parameters	Supplementary carotenoid sources					
	Control	Pumpkin	Gac	Bell pepper	Carrot	Astaxanthin
FI (g/fish)	1.09 ± 0.07	1.23 ± 0.06	1.29 ± 0.09	1.40 ± 0.05	1.30 ± 0.11	1.22 ± 0.06
FCR	2.11 ± 0.05^{c}	1.78 ± 0.09^{b}	1.50 ± 0.07^{a}	1.70 ± 0.06^{ab}	1.80 ± 0.06^{b}	1.86 ± 0.06^{b}
PER	0.86 ± 0.02^{a}	1.03 ± 0.05^{b}	1.22 ± 0.06^{c}	1.07 ± 0.04^{b}	1.01 ± 0.04^{b}	0.98 ± 0.03^{ab}

3.2.2. Effects of some animal carotenoid sources

3.2.2.1. Fish skin color

Supplementation of carotenoids from synthetic astaxanthin, shrimp shells and Copepoda reduced lightness (L^*), increased redness (a^*), yellowness (b^*), chroma (C^*_{ab}) and total color difference (ΔE^*_{ab}), while decreasing hue (h^*_{ab}) of fish skin compared to the control group. These results demonstrate the effectiveness of carotenoid supplementation in enhancing coloration, making the fish skin redder and more vivid (Fig. 3.7).

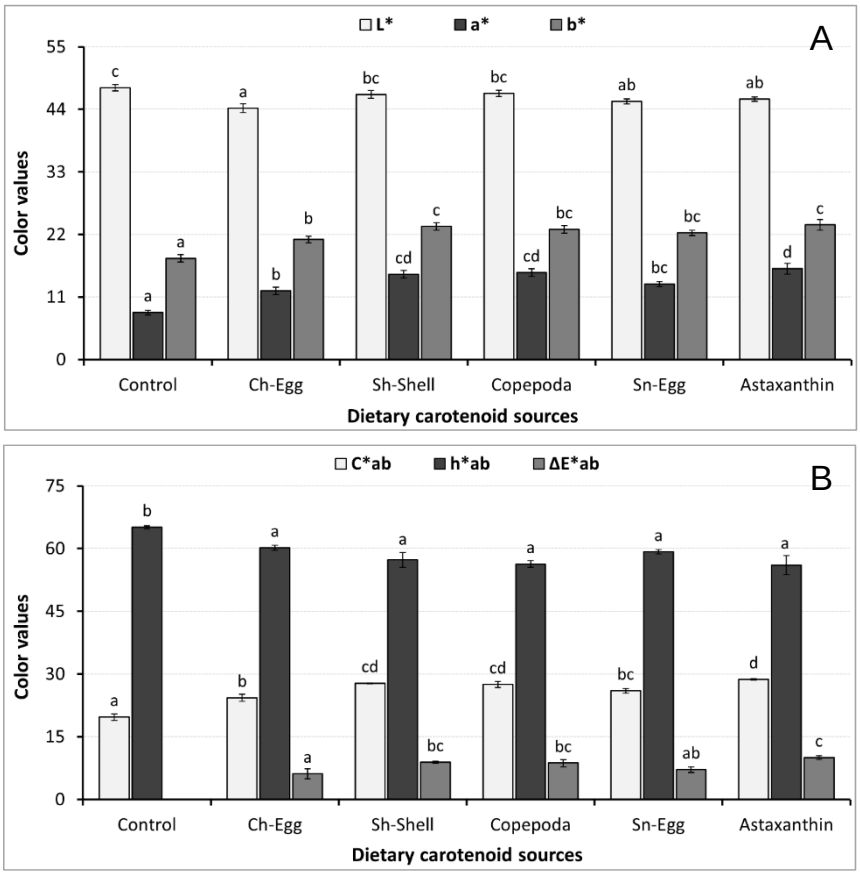

Figure 3.7: Skin color parameters of fish fed with animal carotenoid sources

3.2.2.2. Total carotenoid content accumulated in fish body

The total carotenoid content accumulated in the skin, muscle and whole body of fish significantly increased in the carotenoid supplemented groups, especially from Copepoda, shrimp shells and synthetic astaxanthin, compared to the control group. These results are consistent with the enhanced color parameters observed on the fish skin, indicating the strong accumulation effect of carotenoids in the fish body when supplemented in the diet (Fig. 3.8).

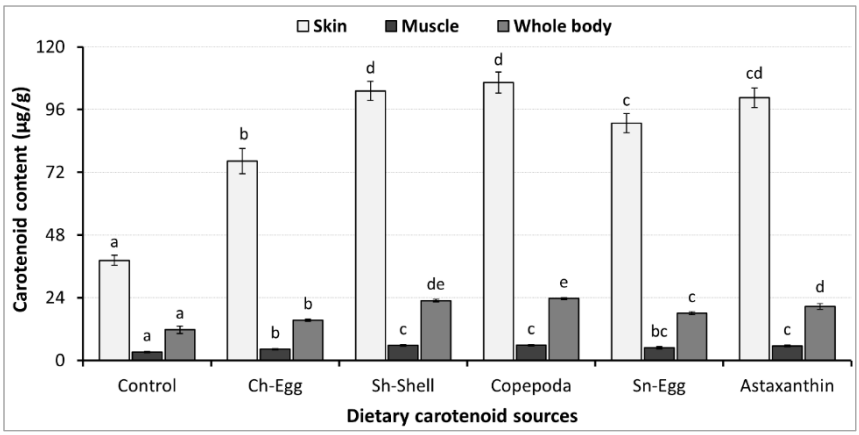

Figure 3.8: Carotenoid content accumulated in fish body (µg/g) fed with animal carotenoid sources

3.2.2.3. Growth parameters, survival rate and feed utilization efficiency

Supplementation of carotenoids, especially from shrimp shells and Copepoda, into the diet significantly improved SGR_W and SGR_L of fish compared to the control group and the group supplemented with synthetic astaxanthin. However, there were no differences in CF and SR among treatments (Table 3.7).

Table 3.7: Growth and survival rate of fish fed with animal carotenoid sources

Parameters	Supplementary carotenoid sources					
	Control	Ch-Egg	Sh-Shell	Copepoda	Sn-Egg	Astaxanthin
L_1 (cm)	3.14 ± 0.02	3.14 ± 0.02	3.14 ± 0.02	3.14 ± 0.02	3.14 ± 0.02	3.14 ± 0.02
W_1 (g)	0.54 ± 0.02	0.54 ± 0.02	0.54 ± 0.02	0.54 ± 0.02	0.54 ± 0.02	0.54 ± 0.02
L_2 (cm)	3.92 ± 0.03^{a}	4.07 ± 0.02^{b}	4.23 ± 0.02^{cd}	4.27 ± 0.01^{d}	4.17 ± 0.03^{c}	4.04 ± 0.04^{b}
W_2 (g)	1.08 ± 0.02^{a}	1.20 ± 0.03^{bc}	1.33 ± 0.03^{d}	1.36 ± 0.01^{d}	1.25 ± 0.02^{c}	1.17 ± 0.02^{b}
SGR_L (%/day)	0.30 ± 0.01^{a}	0.35 ± 0.01^{b}	0.40 ± 0.01^{cd}	0.41 ± 0.01^{d}	0.38 ± 0.01^{c}	0.33 ± 0.01^{b}
SGR_W (%/day)	0.92 ± 0.03^{a}	1.06 ± 0.03^{bc}	1.20 ± 0.03^{d}	1.24 ± 0.01^{d}	1.12 ± 0.02^{c}	1.03 ± 0.02^{b}
CV_L (%)	12.7 ± 0.48	11.1 ± 0.59	9.46 ± 1.03	9.73 ± 0.64	11.2 ± 0.55	11.4 ± 1.12
CV_W (%)	39.9 ± 0.33^{b}	34.4 ± 1.40^{ab}	29.0 ± 1.94^{a}	29.2 ± 2.44^{a}	35.3 ± 1.89^{b}	35.1 ± 1.75^{b}
CF (g/cm^3)	1.78 ± 0.01	1.78 ± 0.02	1.76 ± 0.02	1.76 ± 0.01	1.73 ± 0.01	1.78 ± 0.03
SR (%)	95.6 ± 2.22	95.6 ± 2.22	97.8 ± 2.22	97.8 ± 2.22	95.6 ± 2.22	95.6 ± 2.22

Although there was no difference in FR among treatments, fish fed diets supplemented with carotenoids from shrimp shells and Copepoda achieved the lowest FCR, and highest PER, while the control and synthetic astaxanthin groups had the lowest results (Table 3.8).

Table 3.8: Feed utilization efficiency of fish fed with animal carotenoid sources

Parameters	Supplementary carotenoid sources					
	Control	Ch-Egg	Sh-Shell	Copepoda	Sn-Egg	Astaxanthin
FI (g/fish)	1.05 ± 0.05^{a}	1.17 ± 0.05^{ab}	1.20 ± 0.05^{abc}	1.32 ± 0.08^{bc}	1.36 ± 0.03^{c}	1.21 ± 0.03^{abc}
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}
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}

3.2.3. Effects of carotenoid levels from bell pepper

3.2.3.1. Fish skin color

Redness (a^*), yellowness (b^*) and chroma (C^*_{ab}) increased, while lightness (L^*) and hue (h^*_{ab}) decreased at the supplementation level of 900 mg/kg diet. The overall color difference of fish skin compared to the control (ΔE^*_{ab}) was also more pronounced in the 900 mg/kg supplemented group. Increasing the supplementation level above 900 mg/kg did not provide additional benefits, indicating that this level is most suitable for the requirements of false clownfish (Fig. 3.9).

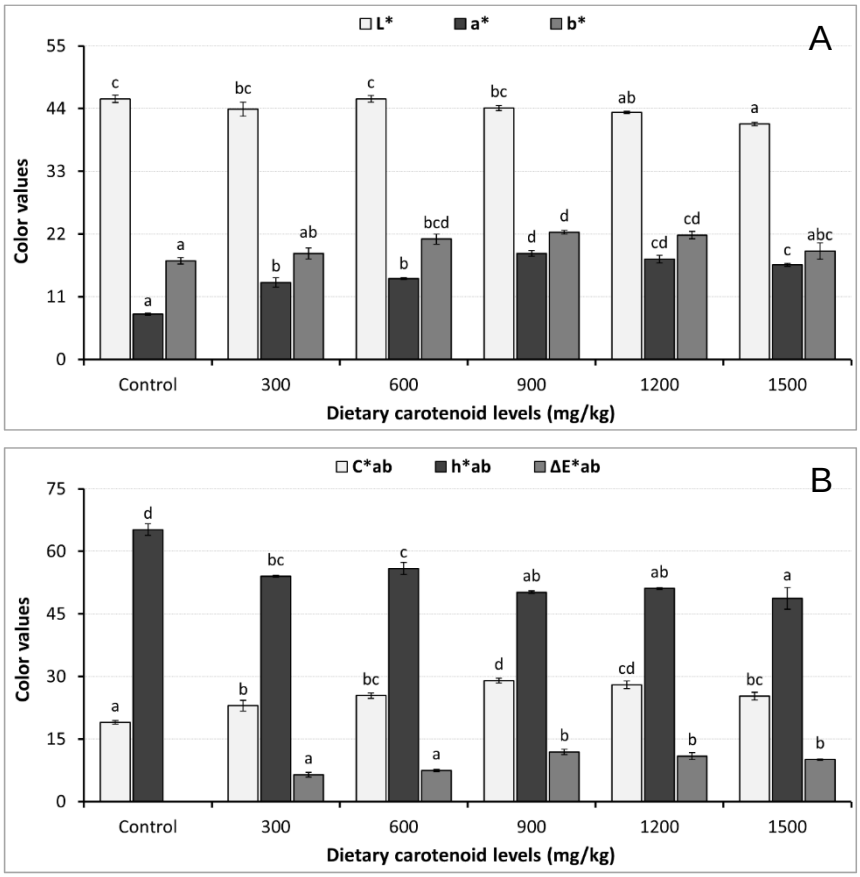

Figure 3.9: Skin color parameters of fish fed with different carotenoid levels from bell pepper

3.2.3.2. Total carotenoid content accumulated in fish body

Supplementation of carotenoids from bell pepper into the diet significantly increased the accumulation of total carotenoids in the skin, muscle and whole body of false clownfish compared to the control. The level of carotenoid accumulation increased linearly with the supplemented carotenoid levels, reaching the highest values at 900 - 1,500 mg/kg diet. However, supplementation of carotenoids at levels > 900 mg/kg did not further improve carotenoid accumulation in the fish body, indicating that the supplementation level of 900 mg/kg diet is suitable for the requirements of this fish species (Fig. 3.10).

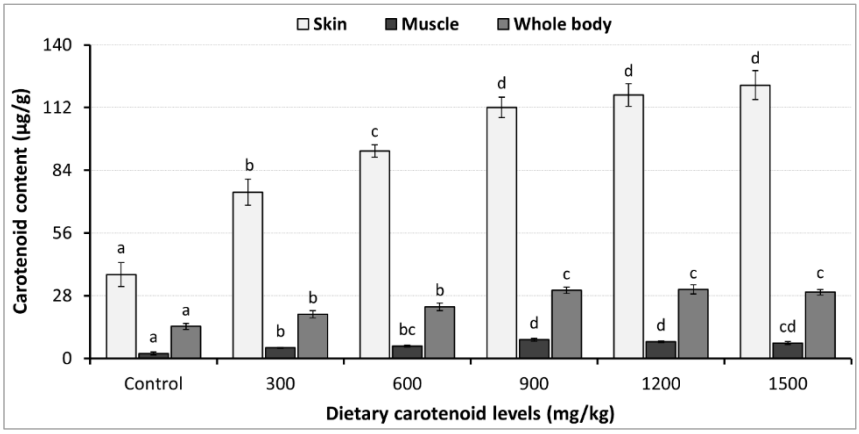

Figure 3.10: Carotenoid content accumulated in the body (µg/g) of fish fed with different supplemented carotenoid levels from bell pepper

3.2.3.3. Growth parameters, survival rate and feed utilization efficiency

Supplementation of carotenoids from bell pepper into the diet at levels ≥ 900 mg/kg significantly improved SGR_L and SGR_W of fish compared to lower supplementation levels and the control. The supplementation level of 900 mg/kg diet was determined to be suitable for false clownfish (Table 3.9).

Table 3.9: Growth and SR of fish fed with different carotenoid levels from bell pepper

Parameters	Supplementary carotenoid levels (mg/kg)					
	Control	300	600	900	1.200	1.500
L_1 (cm)	3.40 ± 0.04	3.40 ± 0.04	3.40 ± 0.04	3.40 ± 0.04	3.40 ± 0.04	3.40 ± 0.04
W_1 (g)	0.72 ± 0.02	0.72 ± 0.02	0.72 ± 0.02	0.72 ± 0.02	0.72 ± 0.02	0.72 ± 0.02
L_2 (cm)	4.02 ± 0.04^{a}	4.05 ± 0.02^{a}	4.07 ± 0.06^{a}	4.26 ± 0.03^{b}	4.21 ± 0.02^{b}	4.31 ± 0.06^{b}
W_2 (g)	1.22 ± 0.02^{a}	1.23 ± 0.01^{a}	1.27 ± 0.03^{a}	1.44 ± 0.03^{b}	1.41 ± 0.05^{b}	1.46 ± 0.06^{b}
SGR_L (%/day)	0.22 ± 0.01^{a}	0.23 ± 0.01^{a}	0.24 ± 0.02^{ab}	0.30 ± 0.01^{c}	0.28 ± 0.01^{bc}	0.31 ± 0.02^{c}
SGR_W (%/day)	0.70 ± 0.02^{a}	0.72 ± 0.02^{a}	0.76 ± 0.03^{a}	0.93 ± 0.03^{b}	0.89 ± 0.04^{b}	0.94 ± 0.06^{b}
CV_L(%)	12.2 ± 0.96	8.86 ± 0.87	9.66 ± 0.23	9.53 ± 0.22	9.88 ± 1.14	10.4 ± 0.90
CV_W(%)	31.7 ± 1.8	25.7 ± 1.75	26.3 ± 1.03	27.3 ± 0.57	30.7 ± 3.16	32.1 ± 1.66
CF (g/cm^3)	1.88 ± 0.03	1.90 ± 0.01	1.90 ± 0.04	1.88 ± 0.02	1.87 ± 0.03	1.81 ± 0.01
SR (%)	95.3 ± 2.33	95.3 ± 2.33	97.7 ± 2.33	97.7 ± 2.33	95.3 ± 2.33	97.7 ± 2.33

Supplementation of carotenoids from bell pepper into the diet at levels ≥ 900 mg/kg enhanced feed intake (FI and FR) and feed utilization efficiency (FCR, FER and PER) of fish. The supplementation level of 900 mg/kg was determined to be optimal (Table 3.10).

Table 3.10: Feed utilization efficiency of fish fed with different carotenoid levels from bell pepper

Parameters	Supplementary carotenoid levels (mg/kg)					
	Control	300	600	900	1.200	1.500
FI (g/fish)	1.01 ± 0.03^{a}	0.97 ± 0.01^{a}	1.07 ± 0.04^{a}	1.24 ± 0.07^{b}	1.26 ± 0.06^{b}	1.32 ± 0.08^{b}
FCR	2.02 ± 0.03^{d}	1.88 ± 0.03^{bc}	1.96 ± 0.04^{cd}	1.72 ± 0.02^{a}	1.85 ± 0.05^{bc}	1.79 ± 0.05^{ab}
PER	0.90 ± 0.01^{a}	0.97 ± 0.02^{bc}	0.93 ± 0.02^{ab}	1.06 ± 0.01^{d}	0.98 ± 0.03^{bc}	1.02 ± 0.03^{cd}

3.2.3.4. Body biochemical composition and digestive enzyme activities in fish

The supplemented carotenoid levels from bell pepper into the diet significantly affected the protein and lipid composition in the body of clownfish, while not impacting moisture and ash content. Fish supplemented with carotenoids from 600 mg/kg diet and above had higher protein content (17.7 – 18.5%) compared to the control group (16.2%), while the lipid content was lower in the groups supplemented with 900 and 1,500 mg/kg diet (5.28 – 5.40%) compared to the control and 300 mg/kg supplemented groups (6.51 – 6.62%). The protein and lipid contents in the fish body varied inversely, with protein increasing and lipid decreasing as the supplemented carotenoid levels increased (Table 3.11).

Table 3.11: Body biochemical composition of fish (%ww) fed with different supplemented carotenoid levels from bell pepper

Parameters	Supplementary carotenoid levels (mg/kg)					
	0 (ĐC)	**300**	**600**	**900**	**1.200**	**1.500**
Moiture (%)	72.7 ± 0.30	72.3 ± 0.40	71.9 ± 0.20	71.9 ± 0.48	71.8 ± 0.25	71.9 ± 0.44
Protein (%)	16.2 ± 0.64^{a}	16.9 ± 0.33^{ab}	17.7 ± 0.46^{bc}	18.4 ± 0.33^{c}	18.1 ± 0.29^{bc}	18.5 ± 0.29^{c}
Lipid (%)	6.62 ± 0.29^{b}	6.51 ± 0.31^{b}	6.10 ± 0.33^{ab}	5.40 ± 0.33^{a}	5.73 ± 0.23^{ab}	5.28 ± 0.26^{a}
Tro (%)	4.13 ± 0.19	4.14 ± 0.21	4.17 ± 0.18	3.95 ± 0.32	4.11 ± 0.13	4.09 ± 0.20

Supplementation of carotenoids from bell pepper into the diet improved the activity of digestive enzymes in the digestive organs of false clownfish. The activity of protease and lipase enzymes increased linearly with the supplemented carotenoid levels, with supplementation levels from 900 mg/kg diet and above resulting in significantly higher protease activity (2.70 – 2.82 U/mg) compared to 300 mg/kg and control (1.57 – 1.82 U/mg), while lipase activity was higher at supplementation levels from 600 mg/kg compared to 0 - 300 mg/kg diet. Although amylase activity also increased linearly with the supplemented carotenoid levels, there were no significant differences among groups (Table 3.12).

Table 3.12: Activity of digestive enzymes of fish (U/mg) fed with different supplemented carotenoid levels from bell pepper

Parameters	Supplementary carotenoid levels (mg/kg)					
	0 (ĐC)	**300**	**600**	**900**	**1.200**	**1.500**
Amylase	9.82 ± 1.73	11.5 ± 1.61	12.0 ± 1.91	13.5 ± 1.74	13.0 ± 1.89	13.6 ± 1.17
Protease	1.57 ± 0.31^{a}	1.82 ± 0.15^{ab}	2.50 ± 0.27^{bc}	2.76 ± 0.22^{c}	2.70 ± 0.26^{c}	2.82 ± 0.25^{c}
Lipase	2.96 ± 0.62^{a}	3.55 ± 0.33^{a}	4.98 ± 0.43^{b}	5.40 ± 0.36^{b}	5.22 ± 0.37^{b}	5.59 ± 0.36^{b}

3.2.4. Effects of carotenoids content from shrimp shells

3.2.4.1. Fish skin color

Carotenoids supplementation in feed significantly improved fish skin color. Supplementation levels of 600-800 mg/kg provided the best results for the a^*, b^*, C^*_{ab} and ΔE^*_{ab} indices. However, excessively high carotenoids content (800-1,000 mg/kg) may darken the fish skin (Fig. 3.11).

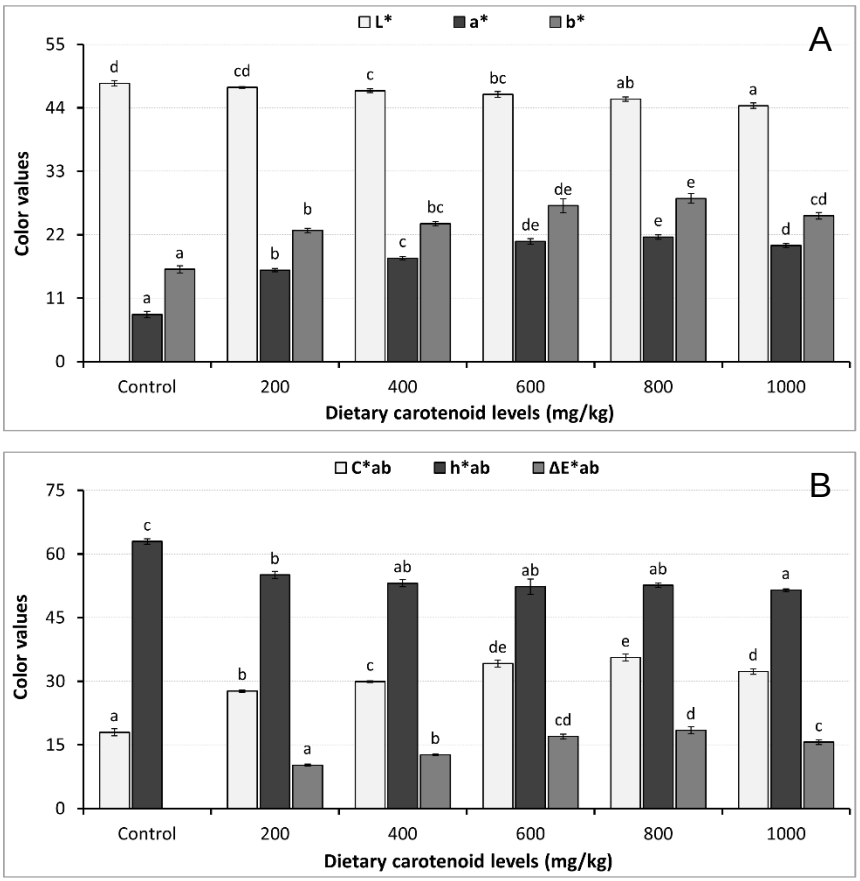

Figure 3.11: Skin color indices of fish fed with different carotenoids levels from shrimp shells

3.2.4.2. Total carotenoid content accumulated in fish body

The total carotenoid content accumulated in the false clownfish body increased linearly with the amount of carotenoids supplementation, reaching the highest at 600-1,000 mg/kg feed. The results showed that the supplementation level of 600 mg/kg feed is appropriate to enhance the total carotenoid content in the false clownfish body (Fig. 3.12).

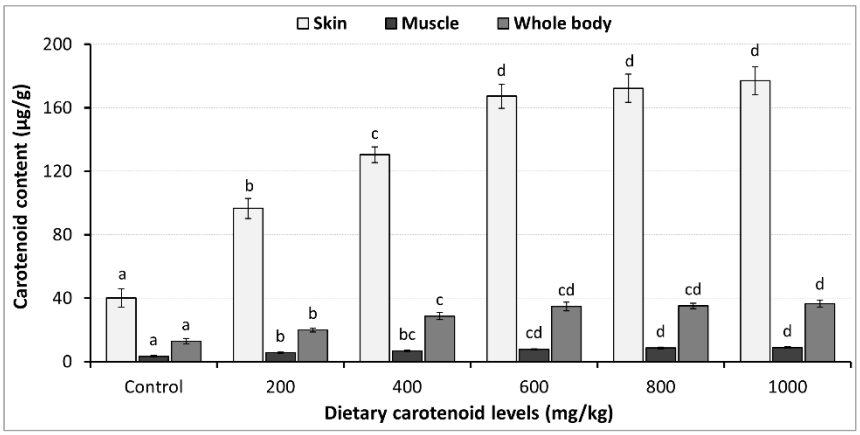

Figure 3.12: Carotenoid content accumulated in fish body (µg/g) fed with different carotenoids levels from shrimp shells

3.2.4.3. Growth parameters, survival rate and feed utilization efficiency

Fish fed with carotenoids supplementation at 600 and 800 mg/kg achieved the highest SGR_L and SGR_W, with an increase of 37.9 - 42.7% compared to the control. There was no significant difference in growth rate between supplementation levels of 600 - 1000 mg/kg feed. The supplementation level of 600 - 800 mg/kg feed was determined to be appropriate (Table 3.13).

Table 3.13: Growth and survival rate of fish fed with different carotenoids levels from shrimp shells

Parameters	Supplementary carotenoids levels (mg/kg)					
	Control	**200**	**400**	**600**	**800**	**1.000**
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 (%)	11.3 ± 0.67	10.3 ± 0.33	9.33 ± 0.88	8.33 ± 0.88	9.33 ± 0.88	10.7 ± 0.88
CV_W (%)	35.3 ± 1.76^{b}	33.0 ± 1.16^{b}	30.3 ± 2.91^{ab}	25.7 ± 0.33^{a}	27.0 ± 0.58^{a}	34.3 ± 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 (%)	91.1 ± 2.22	95.6 ± 2.22	95.6 ± 2.22	97.8 ± 2.22	97.8 ± 2.22	95.6 ± 2.22

Fish fed with carotenoids supplementation at 600 mg/kg achieved the best results, with FCR reduced by 21.2% and PER increased by 27.2% compared to the control. There was no significant difference in these parameters between supplementation levels of 600 - 1,000 mg/kg feed, indicating that the 600 mg/kg level is suitable for false clownfish (Table 3.14).

Table 3.14: Feed utilization efficiency of fish fed with different carotenoids levels from shrimp shells

Parameters	Supplementary carotenoids levels (mg/kg)					
	Control	**200**	**400**	**600**	**800**	**1000**
FI (g/fish)	0.87 ± 0.03	0.97 ± 0.07	1.12 ± 0.09	1.09 ± 0.05	1.17 ± 0.07	1.14 ± 0.08
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}

3.2.4.4. Body biochemical composition and digestive enzyme activities in fish

The carotenoids supplementation level affected the crude protein and lipid contents in the false clownfish body but did not impact the ash and moisture contents. The crude protein content increased linearly with the increase of carotenoids supplementation, reaching the highest at levels ≥ 600 mg/kg feed (19.7 - 19.9%) and lowest in the control treatment (16.4%). Conversely, the crude lipid content decreased linearly with the increase of carotenoids supplementation, highest in the control treatment (7.88%) and lowest at the 1,000 mg/kg feed level (5.13%). However, there was no significant difference in crude protein and lipid contents at supplementation levels of 600 - 1,000 mg/kg feed (Table 3.15).

Table 3.15: Fish body biochemical composition (% wet weight) fed with different carotenoids levels from shrimp shells

Parameters	Supplementary carotenoid levels (mg/kg)					
	Control	200	400	600	800	1.000
Moiture (%)	69.9 ± 0.85	69.2 ± 0.42	69.2 ± 0.57	68.4 ± 0.37	68.6 ± 0.67	68.8 ± 0.20
Protein (%)	16.4 ± 0.46^{a}	17.5 ± 0.30^{b}	18.4 ± 0.20^{b}	19.7 ± 0.24^{c}	19.7 ± 0.31^{c}	19.9 ± 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}
Tro (%)	5.84 ± 0.28	6.32 ± 0.34	6.34 ± 0.27	6.40 ± 0.21	6.28 ± 0.20	6.14 ± 0.16

Carotenoids supplementation in feed positively improved the activity of protease and lipase digestive enzymes in the digestive organs of false clownfish, with an increasing trend proportional to the carotenoids supplementation level. The activities of protease and lipase at supplementation levels of 600 - 1,000 mg/kg feed were higher than at 200 mg/kg and the control, 2.31 - 2.43 U/mg compared to 1.31 - 1.79 U/mg (protease) and 6.51 - 6.72 U/mg compared to 2.95 - 4.55 U/mg (lipase), respectively. There was no significant difference in the activities of these two enzymes at supplementation levels of 400 - 1,000 mg/kg feed, indicating that the 400 mg/kg level may be suitable to enhance digestive enzyme activity in false clownfish. However, amylase enzyme activity did not differ between treatments (Table 3.16).

Table 3.16: Digestive enzyme activity of fish (U/mg) fed with different carotenoids levels from shrimp shells

Parameters	Supplementary carotenoid levels (mg/kg)					
	Control	200	400	600	800	1.000
Amylase	10.4 ± 1.12	11.4 ± 0.39	12.5 ± 1.00	13.7 ± 0.39	13.9 ± 0.69	12.9 ± 0.89
Protease	1.31 ± 0.16^{a}	1.79 ± 0.12^{b}	2.19 ± 0.09^{bc}	2.43 ± 0.13^{c}	2.51 ± 0.20^{c}	2.31 ± 0.10^{c}
Lipase	2.95 ± 0.27^{a}	4.55 ± 0.58^{b}	5.87 ± 0.41^{c}	6.70 ± 0.24^{c}	6.72 ± 0.27^{c}	6.51 ± 0.54^{c}

3.2.5. Effects of carotenoids supplementation duration from shrimp shells

3.2.5.1. Fish skin color

The redness (a^*), yellowness (b^*), and color saturation (C^*ab) values were highest in the treatments supplemented for 45-75 days. However, the skin color brightness tended to decrease linearly with prolonged supplementation time. There was no difference in the effectiveness of color improvement between the 45-75 day groups, indicating that a supplementation period of 45 days is suitable (Fig. 3.13).

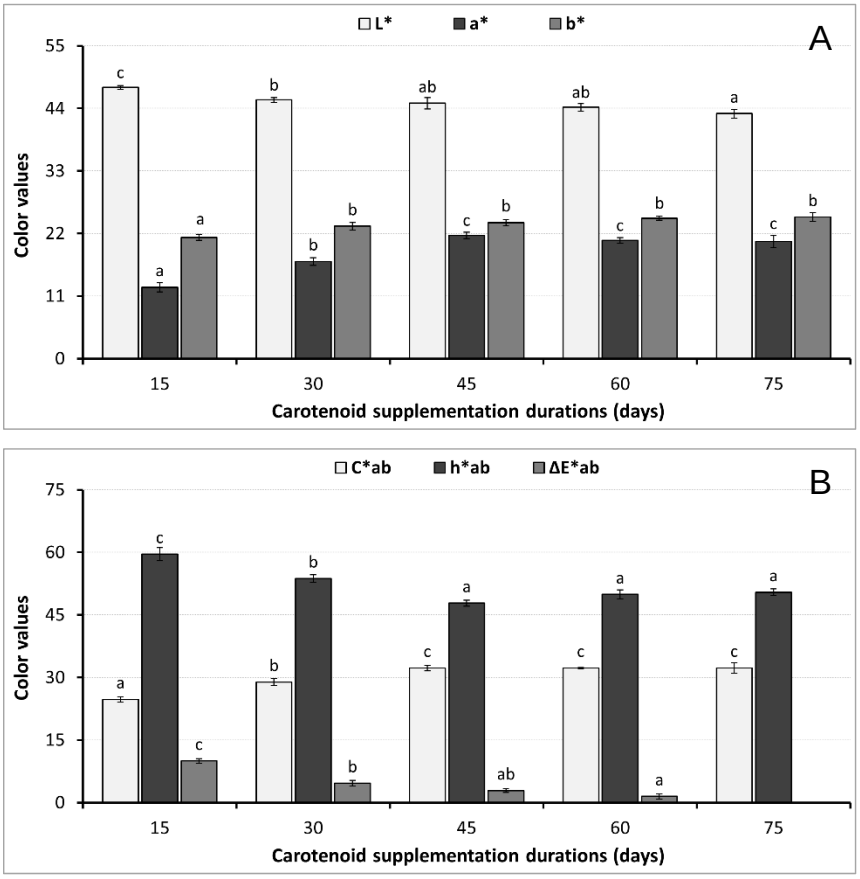

Figure 3.13: Skin color indices of fish fed with different carotenoids supplementation times

3.2.5.2. Total carotenoid content accumulated in fish body

The total carotenoid content accumulated in the skin, muscle, and whole body of fish increased linearly with the duration of carotenoids supplementation. The 75-day supplementation regime achieved the highest results, increasing by 78.2-109.2% compared to the 15-day group. There was no difference in the carotenoid content accumulated in the skin, muscle, and whole body of fish between the 45-, 60-, and 75-day supplementation regimes. Increasing the carotenoids supplementation time beyond 45 days did not lead to further improvement in the accumulated pigment content. The carotenoids supplementation time of 45 days was determined to be appropriate (Fig. 3.14).

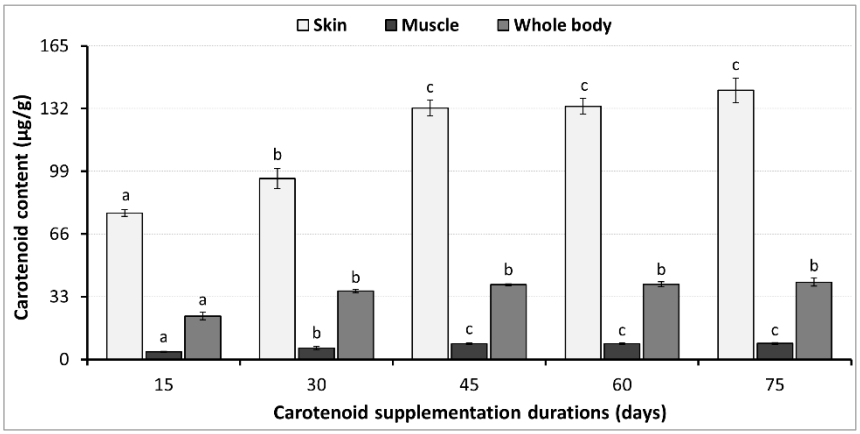

Figure 3.14: Carotenoid content accumulated in fish body (µg/g) fed with different carotenoids supplementation times

3.2.5.3. Growth parameters, survival rate and feed utilization efficiency

Fish fed with carotenoids supplementation for 60 days achieved higher SGR_W compared to the 15-day supplementation group. There was no difference in this parameter among the groups supplemented for 45-75 days. The carotenoids supplementation duration of 45 days was determined to be suitable for clownfish (Table 3.17).

Table 3.17: Growth and survival rate of fish fed with different carotenoids supplementation durations

Parameters	Supplementation duration (days)				
	15	30	45	60	75
L_1 (cm)	3.20 ± 0.02	3.20 ± 0.02	3.20 ± 0.02	3.20 ± 0.02	3.20 ± 0.02
W_1 (g)	0.62 ± 0.04	0.62 ± 0.04	0.62 ± 0.04	0.62 ± 0.04	0.62 ± 0.04
L_2 (cm)	4.15 ± 0.03	4.21 ± 0.03	4.22 ± 0.01	4.27 ± 0.03	4.23 ± 0.02
W_2 (g)	1.31 ± 0.02	1.37 ± 0.03	1.39 ± 0.01	1.41 ± 0.02	1.38 ± 0.02
SGR_L (%/day)	0.35 ± 0.01	0.37 ± 0.01	0.37 ± 0.01	0.38 ± 0.01	0.37 ± 0.01
SGR_W (%/day)	1.00 ± 0.02^{a}	1.06 ± 0.03^{ab}	1.07 ± 0.01^{b}	1.10 ± 0.02^{b}	1.07 ± 0.02^{b}
CV_L (%)	19.5 ± 0.33^{d}	11.9 ± 0.43^{b}	8.93 ± 0.44^{a}	14.3 ± 0.54^{c}	21.7 ± 0.41^{e}
CV_W (%)	35.3 ± 1.55	30.6 ± 0.60	28.2 ± 0.70	31.7 ± 1.27	32.1 ± 2.30
CF (g/cm^3)	1.84 ± 0.01	1.84 ± 0.03	1.84 ± 0.01	1.82 ± 0.01	1.83 ± 0.01
SR (%)	97.8 ± 2.22	97.8 ± 2.22	95.6 ± 2.22	93.3 ± 3.85	95.6 ± 4.44

Fish fed with the supplementation regimes of 45 days or longer achieved better FCR and PER values compared to the 15-30 day supplementation groups. There was no difference in these two parameters between the 45, 60, and 75 day supplementation regimes. Therefore, the 45-day supplementation regime was determined to be appropriate in terms of feed utilization efficiency (Table 3.18).

Table 3.18: Feed utilization efficiency of fish fed with different carotenoids supplementation durations

Parameters	Supplementation duration (days)				
	15	30	45	60	75
FI (g/fish)	1.35 ± 0.03	1.41 ± 0.05	1.24 ± 0.06	1.28 ± 0.07	1.25 ± 0.04
FCR	1.95 ± 0.06^{b}	1.88 ± 0.03^{b}	1.62 ± 0.08^{a}	1.60 ± 0.04^{a}	1.64 ± 0.03^{a}
PER	0.94 ± 0.03^{a}	0.97 ± 0.02^{a}	1.13 ± 0.06^{b}	1.14 ± 0.03^{b}	1.11 ± 0.02^{b}

3.3. Evaluation of the combined effects of rearing environment and appropriate carotenoid supplementation regimes to improve the coloration of false clownfish

3.3.1. Fish skin color

The results showed that fish in the applied treatment had the highest skin redness (a^*) and chroma (C^*_{ab}), the lowest hue angle (h^*_{ab}), as well as higher scores on the Clownfish Color Exercise scale compared to the other two treatments, indicating a deeper, more vivid, and intense red color. In contrast, fish in the control treatment tended to be yellow/pale, while fish in the wild-caught treatment had a color that leaned towards orange-yellow and was darker. The overall color difference index (ΔE^*_{ab}) of fish in the applied treatment was also significantly higher than that of fish in the wild-caught treatment ($p < 0.05$) (Fig. 3.15, 3.16, and 3.17).

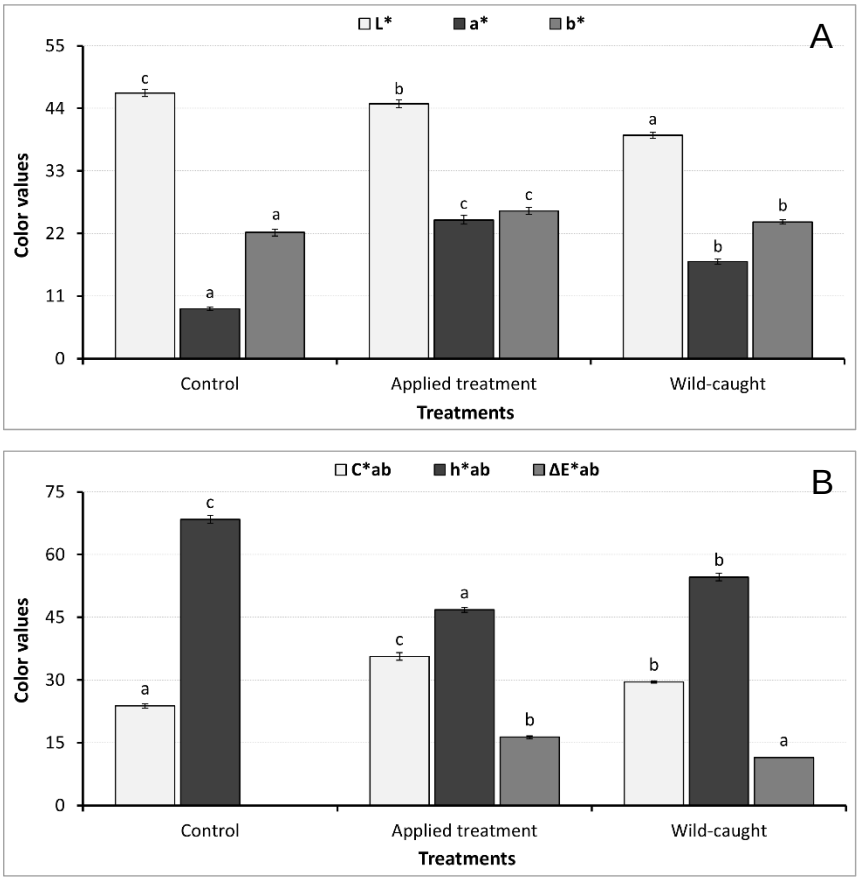

Fig. 3.15: Skin color indices of clownfish in the control, applied, and wild-caught treatments: (A) L^*, a^*, b^*, and (B) C^*_{ab}, h^*_{ab}, ΔE^*_{ab}

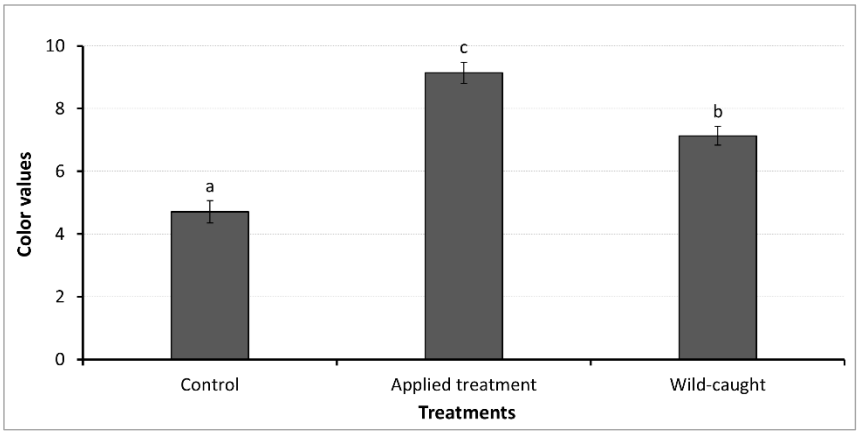

Fig. 3.16: Skin color scores based on the Clownfish Color Exercise scale of clownfish in the control, applied, and wild-caught treatments

Fig. 3.17: Morphology of clownfish in the control (middle), applied (left), and wild-caught (right) treatments

3.3.2. Total carotenoid content accumulated in fish body

The total carotenoid content accumulated in the skin, muscle, and whole body of fish was highest in the wild-caught treatment, followed by the applied treatment, and lowest in the control treatment ($p < 0.05$). Specifically, the carotenoid content accumulated in the skin of fish in the wild-caught treatment was 11.2% and 275.7% higher than that in the applied and control treatments, respectively (Fig. 3.18).

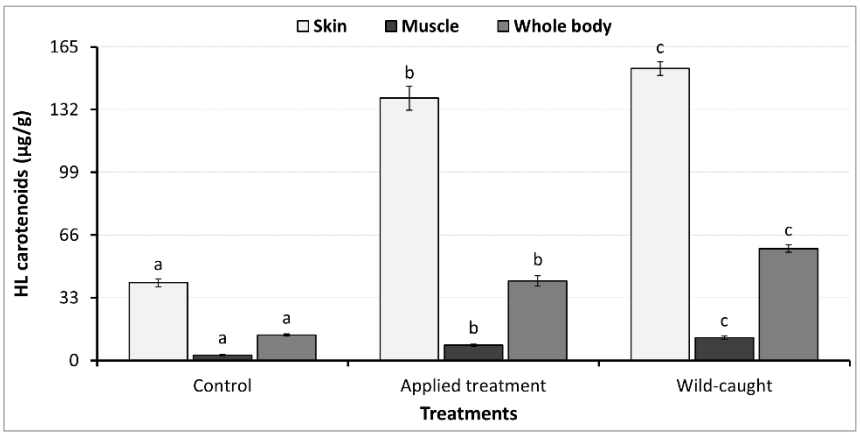

Fig. 3.18: Total accumulated carotenoid content in the body of clownfish (µg/g) in the control, applied, and wild-caught treatments

3.3.3. Growth parameters, survival rate and feed utilization efficiency

The application of optimal parameters in fish culture significantly improved the specific growth rate in length (SGR_L) and weight (SGR_W) of fish compared to the control, with increases of 22.9% and 25.7%, respectively. On the other hand, parameters such as CV_L, CV_W, CF, and SR did not differ between the two experimental groups, indicating that the application of optimal parameters did not negatively affect the experimental fish (Table 3.19).

Table 3.19: Growth and survival rate of fish in the application and control groups

Parameters	Control	Application	F; Sig.	Increase (%)
L_1 (cm)	3.17 ± 0.02	3.17 ± 0.02	-	
W_1 (g)	0.59 ± 0.03	0.59 ± 0.03	-	
L_2 (cm)	3.71 ± 0.01^a	3.85 ± 0.02^b	3.37; 0.00	3.77
W_2 (g)	0.95 ± 0.01^a	1.07 ± 0.01^b	4.41; 0.00	12.6
SGR_L (%/day)	0.35 ± 0.01^a	0.43 ± 0.01^b	2.75; 0.00	22.9
SGR_W (%/day)	1.05 ± 0.04^a	1.32 ± 0.02^b	6.03; 0.00	25.7
CV_L (%)	11.5 ± 0.41	10.4 ± 1.04	0.16; 0.79	-
CV_W (%)	28.8 ± 1.19	26.4 ± 1.64	1.36; 0.27	-
CF (g/cm^3)	1.85 ± 0.03	1.88 ± 0.01	9.64; 0.22	-
SR (%)	97.1 ± 1.35	99.0 ± 0.96	5.33; 0.27	-

The feed intake (FI) of fish in the application group were higher than those in the control group, with an increases of 16.2%. Feed utilization efficiency indicators such as FCR and PER were also better in the application group compared to the control group, with improvements of 14.6% and 16.8%, respectively. The application of research results in production improved the feed utilization efficiency of fish (Table 3.20).

Table 3.20: Feed utilization efficiency of fish in the application and control groups

Parameters	Control	Application	F; Sig.	Increase (%)
FI (g/fish)	0.68 ± 0.03^a	0.79 ± 0.02^b	0.98; 0.00	16.2
FCR	1.92 ± 0.03^b	1.64 ± 0.01^a	5.78; 0.00	-14.6$^\#$
PER	0.95 ± 0.02^a	1.11 ± 0.01^b	2.84; 0.00	16.8

CHAPTER 4 – CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusion

Rearing environment (tank color and salinity) significantly influenced the coloration and growth of clownfish. The best coloration was achieved when fish were reared in blue and transparent tanks with a salinity of 33‰. In contrast, black tanks and low salinity (9‰) diminished the color quality of the fish, resulting in dark, dull, and pale skin appearance.

Supplementation of carotenoids from plant sources (gac fruit, bell pepper) and animal sources (shrimp shell, copepods) considerably improved the coloration, growth, and feed utilization efficiency of clownfish. Bell pepper and shrimp shell were identified as the best supplementary sources, with recommended supplementation levels of 900 mg/kg feed and 600 mg/kg feed, respectively. A carotenoids supplementation period of 45 days from shrimp shell, instead of 75 days, was determined to be sufficiently effective in enhancing the coloration of clownfish.

When applying the combination of environmental parameters (blue tanks, salinity of 33‰) and dietary carotenoids supplementation regime (from shrimp shell, 600 mg/kg feed, for 45 days) in practical production, the fish exhibited superior results in terms of skin color indices, accumulated pigment content, growth parameters, and feed utilization efficiency compared to the control group. Compared to wild-caught fish, although the accumulated carotenoids content in captive-bred fish was significantly lower, the skin color evaluation indices demonstrated superiority, with the captive-bred fish exhibiting deeper, more vivid, and distinct red skin coloration. This highlights the effectiveness of applying the integrated solution in practical production to enhance the color quality of captive-bred clownfish.

The findings obtained in this study play a substantial role and hold significant importance for the ornamental fish industry in general and clownfish farming in particular, supporting the improvement of productivity, quality, and commercial value of the products, while contributing to reducing the fishing pressure on natural coral reef fish resources.

4.2. Recommendations

Further studies should focus on elucidating the roles and mechanisms of action of carotenoids, especially specific components, in the body of clownfish, including bioavailability, bioaccessibility, absorption, metabolism, and bioaccumulation. This will help explain the results obtained in the current study and optimize the supplementation effectiveness of this pigment source in the diet of clownfish.

Furthermore, deeper investigations into the complex interactions among environmental factors and supplementary nutrition, rather than single factors as in the current study, should also be conducted to provide a more comprehensive and holistic understanding, thereby aiding in the development of effective and sustainable strategies for improving the coloration of clownfish.

KEY FINDINGS

Dissertation title: Enhancing the coloration of captive-bred false clownfish (*Amphiprion ocellaris* Cuvier, 1830).

Major: Aquaculture

Major code: 9620301

Ph.D. Student: Tran Van Dung

Course: 2020

Supervisors: 1. Prof. Dr. Pham Quoc Hung
2. Assoc. Prof. Dr. Hua Thai Nhan

Institution: Nha Trang University

Key findings:

The doctoral dissertation has made significant new contributions to science and practice in the field of ornamental fish farming in general and clownfish in particular, specifically:

1. Demonstrating that blue tank color and 33‰ salinity are ideal conditions for vastly improving fish coloration while enhancing growth and feed utilization efficiency.
2. Determining that supplementing 600 mg/kg of shrimp shell for 45 days yields the best fish coloration, while simultaneously improving growth, feed utilization efficiency, body biochemical composition, and digestive enzyme activities.
3. Successfully applying optimized technical solutions to production practices. Results show that the commercially produced clownfish exhibit superior coloration compared to control fish and wild-caught fish, characterized by deep, vivid, bright, well-defined, and harmonious orange-red hues. This enhances the commercial value and competitiveness of the product in the market.
4. Making important contributions to improving the quality of captive-bred clownfish, meeting customer preferences, fostering sustainable development of the farming industry, and reducing pressure on wild populations.

The research findings, particularly those on coloration, have profound scientific value and practical significance. These are novel contributions, first published in Vietnam, providing additional invaluable scientific evidence for global research and contributing to the refinement of high-quality clownfish production processes.

Supervisors **Ph.D. Student**



Prof. Pham Quoc Hung & Assoc. Prof. Hua Thai Nhan **Tran Van Dung**

LIST OF AUTHOR'S PUBLISHED WORKS

1. **Tran Van Dung**, Nguyen Huu Khang, Tran Thi Le Trang, Hua Thai Nhan, Pham Quoc Hung, 2023. Effects of tank color on growth, survival rate, and feed utilization efficiency of clownfish (*Amphiprion ocellaris* Cuvier, 1830*). Journal of Science and Technology, Thai Nguyen University*, 228(13): 95-103.

2. **Tran Van Dung**, Nguyen Huu Khang, Luong Thi Hau, Hua Thai Nhan, Pham Quoc Hung, 2023. Effects of tank color on skin coloration and carotenoid accumulation in clownfish (*Amphiprion ocellaris* Cuvier, 1830*). Journal of Fisheries Science and Technology, Nha Trang University*, 3/2023: 101-112.

3. **Dung V. Tran**, Thanh T. Dang, Hau T. Luong, Nhan. T. Hua, Hung Q. Pham, 2024. Natural carotenoids extracted from red bell pepper for enhancement of growth and coloration of false clownfish, *Amphiprion ocellaris*. *Aquaculture, Aquarium, Conservation & Legislation - Bioflux*, 17(2): 542-554.

4. **Dung Van Tran**, Hau Thi Luong, Khanh Thi Pham, Thanh Trung Dang, Nhan Thai Hua, Hung Quoc Pham, 2024. Plant-based carotenoid supplementation: Growth, feed utilization efficiency, and coloration in false clownfish (*Amphiprion ocellaris*). *Israeli Journal of Aquaculture – Bamidgeh*, 76(1): 1 - 12.

5. **Dung Van Tran**, Trang Le Thi Tran, Nam Xuan Doan, Thanh Trung Dang, Nhan Thai Hua, Hung Quoc Pham, 2025. Comparative impact of synthetic and natural animal-derived carotenoids on growth, feed utilization, and pigment enhancement in *Amphiprion ocellaris*. *Fisheries and Aquatic Sciences*, 28(1): 10 – 20.