Effect of Dietary Carotenoids on Survival, Growth, and Carotenoid Accumulation in Mandarinfish, *Synchiropus splendidus* (Herre, 1927)

Amonrat Kanokrung[1], Khakhanang Ratananikom[2] and Rawiwan Watanadilok[1*]

ABSTRACT

The color of ornamental fish is crucial in determining their value, and it is always compromised when reared in captivity. This study aimed to examine the effects of dietary carotenoids from *Artemia* enriched with four microalgae on growth, survival, and carotenoid accumulation in mandarinfish, *Synchiropus splendidus*. *Artemia* nauplii were enriched with one of the following microalgae: *Tetraselmis gracilis*, *Isochrysis galbana*, *Nannochloropsis oculata* and *Dunaliella salina*. Subsequently, the enriched *Artemia* nauplii were fed to 60 days old mandarinfish for a period of 60 days. At the end of the feeding period, survival was 100% for all dietary treatments. The growth performance varied significantly ($p<0.05$) for the various diets. The highest weight gain, length gain and specific growth rate (SGR) were found in the mandarinfish fed with the diet enriched with *T. gracilis* (weight gain = 0.80±0.58 g; length gain = 0.27±0.02 cm; SGR = 2.98±0.03% day^{-1}). The highest total carotenoid content of the mandarinfish was observed in the fish fed with *Artemia* enriched with *T. gracilis* and *I. galbana* at 1.79±0.32 μg·g^{-1}. The results showed that mandarinfish accumulated the highest amount of beta-carotene when fed with *Artemia* enriched with *T. gracilis* (0.39 μg·g^{-1}of wet weight). In addition, all of the treatment groups accumulated the carotenoid pigments, beta-carotene, echinenone, canthaxanthin, and zeaxanthin but in different amounts.

Keywords: *Artemia*, Carotenoid, Mandarinfish, Microalgae enrichment

INTRODUCTION

Mandarinfish, *Synchiropus splendidus* (Herre, 1927), is an ornamental fish that is popular in aquarium culture. It possesses physical features similar to those of goby, yet it is classified in the Callionymidae Family and is also known as a dragonet. Its natural habitat is the coral reef (Rhyne *et al*., 2012). The mandarinfish generally reach about 30–60 mm in total length and has distinct characteristics, such as characteristically outward-set eyes. The coloration of mandarinfish displays diversity, with vivid shades including orange, red, green, and blue observed both in the wild and in exhibits. The types of carotenoids found in mandarinfish in nature include beta-carotene and astaxanthin (Kanokrung and Watanadilok, 2016). Furthermore, feeding mixed microalgae-enriched *Artemia* to mandarinfish had been shown to have a positive effect on reproduction and spawning (Pratoomyot *et al*., 2016). While entrepreneurs in the ornamental marine fish trade have shown interest in mandarinfish, they often face challenges in culturing them to exhibit the vibrant coloration seen in wild specimens.

The colors displayed by ornamental fish are a result of pigments called carotenoids, which include beta-carotene, lutein, astaxanthin, zeaxanthin, and canthaxanthin. Both synthetic and natural

[1]Institute of Marine Science, Burapha University, Chonburi, Thailand
[2]Faculty of Science and Health Technology, Kalasin University, Kalasin, Thailand
[*]Corresponding author. E-mail address: rawiwan@buu.ac.th
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sources of carotenoids, such as yeast, bacteria, higher plants, and algae, have been used as dietary supplements to enhance skin color of fishes and crustaceans (Yesilayer and Erdem, 2011; Hekimoğlu *et al*., 2017; Goda *et al*., 2018; Wongphonprateep and Kasirak, 2023). Mandarinfish are among the ornamental fish that accumulate carotenoids only through feeding, so their diet plays a crucial role in the intensity of their coloration (Choubert *et al*., 2006). Zooplanktons like copepods, *Artemia* and rotifers are natural food sources for mandarinfish in nature, with microalgae being a key food source for these zooplanktons due to their high nutritional value (Costard *et al*., 2012). Carotenoid content accumulation of zooplanktons provided by microalgae plays a significant role in the coloration of fish. Microalgae can pass on carotenoids to *Artemia*. Gui *et al*. (2022) reported that total carotenoid content found in *Artemia* eggs and nauplii fed with *Dunaliella salina* or *Isochrysis galbana* was higher than those fed with other diets and that the *Artemia* which was enriched with beta-carotene from *D. salina* and fed to platyfish, *Xiphophorus maculatus* significantly enhanced total carotenoid content. Furthermore, platyfish fed beta-carotene-enriched *Artemia* improved their mucosal immune responses and skin carotenoids (Abdollahi *et al*., 2019). It was also found that supplementing beta carotene in the feed of rainbow trout gave rise to significantly greater weight gain, specific growth rate and survival rate than the control (Keleştemur and Çoban, 2016). Overall, microalgae as a source of carotenoids from nature has been widely used to supplement the diets of various marine species for promoting growth parameters and pigmentation. It was reported that supplementing the diet of *Amphiprion frenatus* with two types of ground microalgae (*Nannochloropsis oculata* and *Porphyridium cruentum*), as a source of natural pigmentation, resulted in greater weight gain and specific growth rate compared to the control. The fish fed with a diet enriched by *N. oculata* had the highest total carotenoid content (Hekimoğlu *et al*., 2017). In another study, koi carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) juveniles fed with a diet supplemented with biomass of *Chlorella vulgaris*, as a source of astaxanthin, had the highest total carotenoid content but with similar growth and feed efficiency to the groups fed with *Haematococcus pluvialis*, and *Arthrospira maxima* (spirulina) supplement diet (Gouveia *et al*., 2003). However, growth enhancement was observed in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) fed a diet supplemented with vitamin A or beta-carotene, with beta-carotene to vitamin A conversion ratio of 9:1 (Hu *et al*., 2006). Despite of the previously mentioned reports, to our knowledge, the effects of carotenoids on growth and carotenoid accumulation in mandarinfish have not been reported. Therefore, the present study investigated the effects of dietary carotenoids enriched by four species of microalgae, *T. gracilis, N. oculatae, D. salina,* and *I. galbana* on the growth, survival and carotenoid accumulation in mandarinfish.

MATERIALS AND METHODS

Preparation of live feeds

Seawater was adjusted to salinities of 30 and 25 psu and then sterilized in an autoclave at 15 psi and 121 °C for 15 min. *Isochrysis galbana, Tetraselmis gracilis,* and *Dunaliella salina* were cultured at 30 psu, while *Nannochloropsis oculata* was cultured at 25 psu. Guillard "f/2" medium with varying levels of nitrogen and phosphorus was used. *I. galbana* was cultured with this medium at 1.760 mmol $N \cdot L^{-1}$, and 0.082 mmol $P \cdot L^{-1}$ while *T. gracilis, D. salina,* and *N. oculata* were cultured with the same medium at 2.64 mmol $N \cdot L^{-1}$, and 0.041 mmol $P \cdot L^{-1}$. The algal culture conditions included a temperature of 25 °C, light intensity of 3,500–4,000 lux with alternate 12 h lighting and 12 h dark cycle. Microalgae were harvested during the stationary phase after five days for feeding newly hatched *Artemia* in each experiment.

Preparation of Artemia nauplii

Artemia cysts were incubated at temperature between 25–27 °C. One gram of *Artemia* eggs was placed in a 10-L glass bottle filled with clean seawater. Vigorous aeration was provided for a 24 h period using an air stone. After hatching, the newly hatched *Artemia* nauplii (Instar I) were harvested by ceasing aeration, which allowed the Instar I nauplii to settle and the eggshells to float to the surface. After a settling period of 5–10 min, the nauplii were

gently siphoned into another glass bottle containing brine water with a salinity of 30 psu. This bottle was then aerated with an air stone, promoting the development of the Instar-I nauplii to the Instar II stage (Lavens and Sorgeloos, 1996).

At the Instar-II stage, where their nutritional values is subject to enhancement, the nauplii were fed with one of four different microalgae: *T. gracilis, I. galbana, N. oculata* or *D. salina*. For this purpose, Instar II *Artemia* were distributed into containers, each containing dilution at a density of ten *Artemia* per milliliter. The experimental design included four treatments based on the species and density of algae: *T. gracilis* at 5×10^5 cells·mL^{-1} (Treatment 1), *I. galbana* at 8×10^5 cells·mL^{-1} (Treatment 2), *N. oculata* at 10×10^5 cells·mL^{-1} (Treatment 3), *D. salina* at 8×10^5 cells·mL^{-1} (Treatment 4), alongside a control group that did not receive any nutritional enhancement.

Experimental set up

The mandarinfish were acquired from a commercial dealer and placed in glass containers with recirculating water at the Institute of Marine Science, Burapha University. They were then transferred to a triplicate of experimental rectangular glass tanks (30×46×30 cm), equipped with sediment filters and air pumps, and filled with 20 L of 32 psu seawater. Twelve mandarinfish fry (60 days old; an initial mean weight of 0.55±0.18 g and initial mean length of 2.02±0.03 cm) were reared in each glass tank (1 larva·2 L^{-1} of seawater). The fry were fed twice a day at 9.00 a.m. and 3.00 p.m. with the enriched or un-enriched *Artemia* naupii as described above, at a density of 8 nauplii·mL^{-1}. The fry were cultured for two months and measured once a month for the standard length, total length, and weight. At the end of the experimental period, the fry were sampled for the determination of pigment content, following the method of Schüep and Schierle (1995).

Growth and survival rate

The growth parameters investigated in this study include: specific growth rate (SGR, % day^{-1}), weight gain (g), length gain (cm) and survival rate (%). They were calculated according to Berchielli-Morais *et al*. (2016) as follows:

$$\text{Weight gain} = \text{final weight (g)} - \text{initial weight (g)}$$

$$\text{Length gain} = \text{final length (cm)} - \text{initial length (cm)}$$

$$\text{Survival rate} = (\text{number of survivors/total number of stocked fish}) \times 100$$

$$\text{SGR} = [\ln(\text{final weight (g)}) - \ln(\text{initial weight (g)})/\text{number of rearing days}] \times 100$$

Measurement of pigments in mandarinfish

A 5-gram sample (whole body fish) was cut into small pieces and grinding with mortar and pestle for a few minutes. Then, 20 mL of acetone was added to the sample and gently mixed with a stirring rod for ten minutes. The samples were then further mixed by ultrasonic vibration for 10 min, and subsequently centrifuged at 2,500×g at 20 °C. The supernatant was carefully removed and this step was repeated until the supernatant was colorless. The combined supernatant was transferred into a separatory funnel containing a mixture of hexane and chloroform (1:1, v/v with 0.1% of NaCl). Deionized water was added in equal volume to dehydrate the supernatant, and gently shaken. After complete separate, the upper hexane-chloroform layer was collected. This process was repeated until the hexane-chloroform solution became colorless. Then, Na_2SO_4 was added to dehydrate the sample (Schiedt and Liaaen-Jensen, 1995), and the solvent was removed using a rotary vacuum evaporator, followed by drying with nitrogen. The carotenoid sample was stored at -40 °C before total carotenoid content measurement and analysis by HPLC.

Total carotenoids content

The extract was mixed with petroleum ether until reaching a volume of 1 mL. The total carotenoid content of the fish was determined at absorption values of 450 and 470 nm using a spectrophotometer (SPEKOL 2000, Analytik Jena,

Germany). The total carotenoid concentration was calculated by using the following formula.

$$\text{Total carotenoids } (\mu g \cdot g^{-1}) = \frac{A \times \text{Dilution volume (mL)} \times 1{,}000}{E^{1\%}_{1\,cm} \times \text{sample weight (g)}}$$

where A is the absorbance value at max (450 and 470 nm), and $E^{1\%}_{1\,cm}$ is the extinction coefficient of the carotenoids in petroleum ether at 450 nm (2590) and 470 nm (2200) (Carvalho *et al*., 2012).

Analysis of carotenoid contents by HPLC

The carotenoid solution of 1 mL from the previous step was filtrated through a 0.22 mm membrane filter. A 20 µL volume of the sample solution was injected into an HPLC (Agilent 1200 Series Quaternary Pump)equipped with UV-Vis photodiode array detector. The sample and standard solutions were analyzed at wavelengths of 450 nm and 470 nm using a Venusil XBP Silica column (Bonna-Agela Technologies Inc., 150×4.6 mm Ø). The mobile phase employed a gradient elution of hexane (I) and acetone (II), in which the gradient elution system started at 100% (I) and gradually changing to 5% (II) in 5 min, then to 10% (II) in 15 min, 20% (II) in 20 min, and finally returning to 95% (I) in 25 min, all at a flow rate of 0.8 mL·min^{-1}. The system detected carotenoids in the range of 380–600 nm. The types and quantities of carotenoid in the samples were identified and quantified by retention times and peak areas compared with those of standard solutions (beta-carotene, echinenone, canthaxanthin, zeaxanthin and astaxanthin) (Yasir and Qin, 2010).

Preparation of carotenoid standard solutions

Each of the standard solutions (beta-carotene, echinenone, canthaxanthin, zeaxanthin and astaxanthin) was prepared at five concentrations 5, 10, 20, 40, and 50 ppm. Beta-carotene and canthaxanthin were dissolved in petroleum ether, while zeaxanthin, echinenone, and astaxanthin were dissolved in ethanol. All solvents were stored in screw-topped brown bottles and purged nitrogen before being stored at -20 ºC (Schiedt and Liaaen-Jensen, 1995).

Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA) followed by Duncan's multiple-range test. All tests were considered significant at $p<0.05$. The analyses were facilitated by the software SPSS for windows version 20.0.

RESULTS

Growth and survival rate of mandarinfish

After 2 months of the experiment, the mandarinfish fed with diets enriched with four different microalgae showed significant differences in final weight, weight gain, length gain, and SGR as compared to the control group ($p<0.05$) (Table 1).

After one month, it was found that the mandarinfish had the highest weight gain, length gain and SGR when they were fed *Artemia* nauplii enriched with *Tetraselmis gracilis*, followed by *Isochrysis galbana* (Table 1). Moreover, the experiment revealed that the mandarinfish had the lowest weight gain, length gain and SGR when they were fed *Artemia* nauplii without enrichment (control group). The treatment groups and the control group were significantly different ($p<0.05$). At the end of the experimental period, the highest weight gain, length gain and SGR were obtained from the mandarinfish fed with *Artemia* nauplii enriched with *T. gracilis*, followed by *I. galbana*, compared to the control group. The weight, length, and SGR of the treatment groups were significantly ($p<0.05$) higher than the control group. The survival of the mandarinfish in all treatments and the control was 100% throughout the trials, as shown in Table 1.

Total carotenoid content in Artemia nauplii and mandarinfish

Artemia enriched with four different algae showed an increase in total carotenoid values compared to the control group ($p<0.05$) (Table 2). However, *Artemia* fed with *N. oculata*, *T. gracilis* and *I. galbana* did not exhibit differences in total carotenoid content, but they were significantly higher than those fed with *Dunaliella salina*. The total quantity of carotenoids in *Artemia* fed with *T. gracilis* and *I. galbana* did not differ from the other diets ($p>0.05$). At the end of the experiment, the total carotenoid contents in the mandarinfish fed with *Artemia* enriched with the 4 species of microalgae were not significantly different ($p>0.05$), although the groups fed with diets of *T. gracilis* and *I. galbana* tended to have higher total carotenoid values (1.79±0.32 and 1.78±0.31 μg·g^{-1}, respectively) than the rest (Table 2).

Further analysis revealed that all treatment groups accumulated varying amounts of beta-carotene, echinenone, canthaxanthin, and zeaxanthin (Table 3). The only significant ($p<0.05$) difference in beta carotene content was observed between T1 (*Artemia* fed with *T. gracilis*; 0.39± 0.06 μg·g^{-1}) and T4 (*Artemia* fed with *D. salina*; 0.35±0.07 μg·g^{-1}), which had higher levels compared to the control (0.023±0.04 μg·g^{-1}). However, T4 itself was not significantly different from T2 and T3 in terms of beta-carotene content, and showed similar ($p>0.05$) beta-carotene content with the control. The accumulation of beta-carotenoid in the mandarinfish skin after two months of feeding with different microalgae did not show a significant difference from the control group as shown in Figure 1.

Table 1. Growth performance of mandarinfish (*Synchiropus splendidus*) fed with *Artemia nauplii* enriched with 4 different planktons for 2 months.

Treatment	Final weight (g)		Weight gain (g)		Length gain (cm)		SGR (% day^{-1})		Survival rate (%)	
	1 month	2 months	1 month	2 months	1 month	2 months	1 month	2 months	1 month	2 months
T	0.78±0.01^{d}	1.18±0.02^{b}	0.23±0.01^{c}	0.63±0.02^{c}	0.11±0.01^{c}	0.33±0.02^{d}	1.18±0.06^{c}	2.55±0.04^{c}	100	100
T1	0.95±0.01^{a}	1.35±0.03^{a}	0.40±0.01^{a}	0.80±0.03^{a}	0.27±0.03^{a}	0.61±0.02^{a}	1.83±0.04^{a}	2.98±0.06^{a}	100	100
T2	0.90±0.02^{ab}	1.31±0.02^{ab}	0.35±0.02^{b}	0.76±0.02^{ab}	0.24±0.02^{ab}	0.58±0.03^{ab}	1.67±0.08^{b}	2.90±0.06^{b}	100	100
T3	0.82±0.00^{cd}	1.27±0.03^{ab}	0.27±0.00^{d}	0.72±0.03^{b}	0.20±0.02^{b}	0. 51±0.02^{c}	1.33±0.00^{d}	2.80±0.09^{b}	100	100
T4	0.85±0.02^{bc}	1.29±0.02^{ab}	0.30±0.02^{c}	0.74±0.02^{b}	0.21±0.04^{b}	0.56±0.02^{b}	1.46±0.10^{c}	2.84±0.05^{b}	100	100

Note: T: control (*Artemia* without enrichment); T1: *Artemia* fed with *Tetraselmis gracilis*; T2: *Artemia* fed with *Isochrysis galbana*; T3: *Artemia* fed with *Nannochloropsis oculata*; T4: *Artemia* fed with *Dunaliella salina*; Densities of planktons used as indicated in materials and methods; Mean±SD in the same column superscripted with different lowercase letters are significantly ($p<0.05$) different.

Table 2. Total carotenoid contents of diets (*Artemia* nauplii enriched with different microalgae) and mandarinfish fed with the diets.

	Total carotenoids (μg·g^{-1})				
	Control	*Tetraselmis gracilis*	*Isochrysis galbana*	*Nannochloropsis oculata*	*Dunaliella salina*
Artemia nauplii	1.79±0.14^{c}	3.18±0.13^{ab}	3.30±0.15^{ab}	3.44±0.04^{a}	3.13±0.19^{b}
Mandarinfish	1.36±0.09^{a}	1.79±0.32^{a}	1.78±0.31^{a}	1.58±0.25^{a}	1.49±0.24^{a}

Note: Mean±SD in the same row superscripted with different lowercase letters are significantly ($p<0.05$) different.

Table 3. Carotenoid accumulation in mandarinfish fed with *Artemia* nauplii enriched with various algal species for 2 months.

Treatment	Contents of carotenoid (μg·g^{-1})			
	Beta-carotene	Echinenone	Canthaxanthin	Zeaxanthin
T	0.23±0.04^{c}	0.21±0.03	0.20±0.06	0.11±0.01
T1	0.39±0.06^{a}	0.29±0.03	0.26±0.06	0.13±0.02
T2	0.29±0.01^{bc}	0.28±0.04	0.27±0.08	0.12±0.02
T3	0.30±0.06^{abc}	0.25±0.03	0.25±0.05	0.11±0.01
T4	0.35±0.07^{ab}	0.29±0.09	0.18±0.06	0.17±0.12

Note: T: control (*Artemia* without enrichment); T1: *Artemia* fed with *Tetraselmis gracilis*; T2: *Artemia* fed with *I. galbana*; T3: *Artemia* fed with *Nannochloropsis oculata*; T4: *Artemia* fed with *Dunaliella salina*; Mean±SD in the same column superscripted with different lowercase letters are significantly ($p<0.05$) different, whereas mean values without superscripts denote non-significantly ($p>0.05$) different.

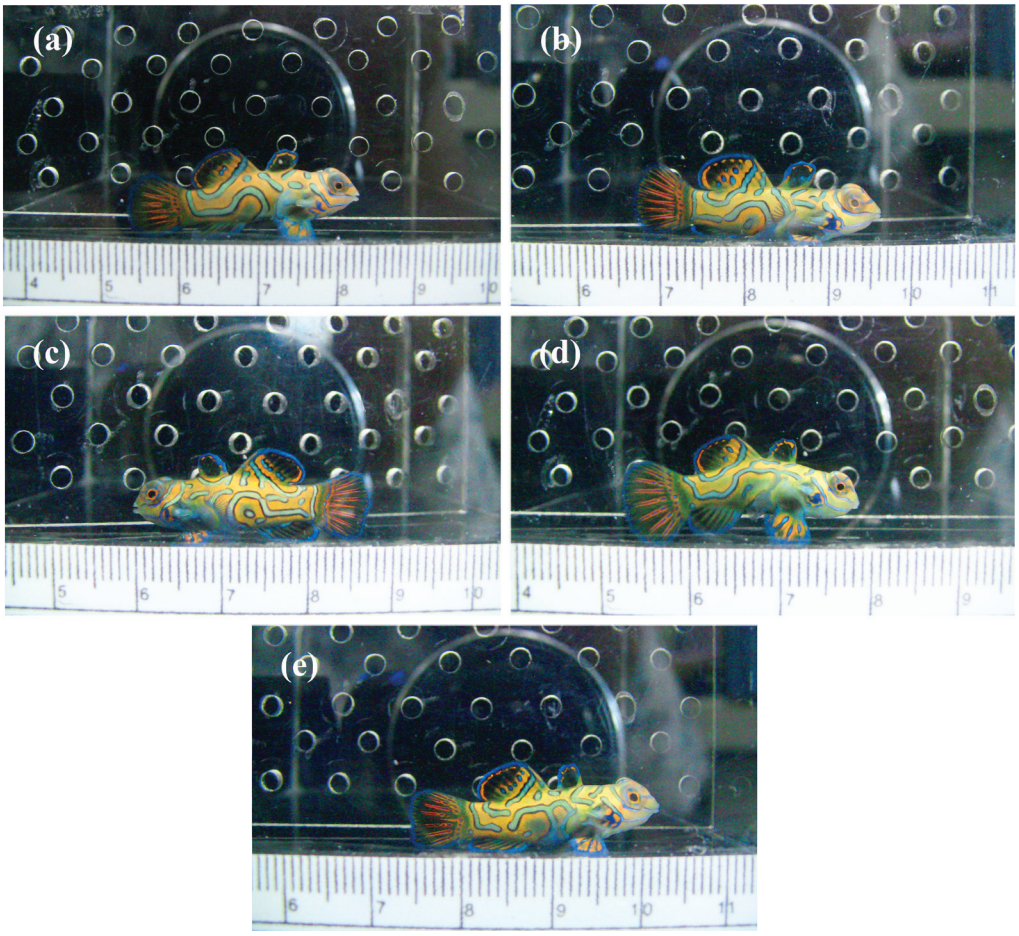

Figure 1. Comparison of body color in mandarinfish after 2 months of feeding with different diet treatments: (a) T1 (*Artemia* fed with *Tetraselmis gracilis*); (b) T2 (*Artemia* fed with *Isochrysis galbana*); (c) T3 (*Artemia* fed with *Nannochloropsis oculata*); (d) T4 (*Artemia* fed with *Dunaliella salina*); (e) control (*Artemia* without enrichment).

DISCUSSION

It is a known fact that neither fish nor other aquatic animals can produce pigments by themselves, but they can only have gained through feeding (Kanokrung *et al*., 2013). In this study, *Artemia* Instar II accumulated carotenoids, especially beta-carotene, by being fed with different types of microalgae including *Tetraselmis gracilis, Isochrysis galbana, Nannochloropsis oculata,* and *Dunaliella salina*. The study found that *Artemia* fed on different microalgae contained higher total carotenoid contents than that of the control (Table 2).

It is noteworthy that the ability of *Artemia* to carry dietary carotenoids is influenced by the type of microalgae they were fed, resulting in varying total carotenoid content. Huang and Hui (2022) have stated that carotenoids could accumulate in *Artemia* 30 min after feeding, with certain microalgae like *D. salina* and *H. pulvialis* being effective in promoting carotenoid accumulation. However, *Artemia* fed with tomato and marigold flower showed no carotenoids at 5 hours post-feeding.

Among the enriched Artemia, quantities of total carotenoids were slightly different, with the group fed *N. oculata* having the highest carotenoid content and the group fed *D. salina* having the lowest. Different species of microalgae produce different carotenoids through a varying biosynthesis process. Common carotenoids found in microalgae include beta-carotene, zeaxanthin, lutein, and fucoxanthin (Sathyaruban *et al*., 2021).

A study by Schüler *et al*. (2020) revealed that the microalgae *Tetraselmis* sp. CTP4 could produce carotenoids such as violaxanthin, lutein, and beta-carotene. Additionally, *Artemia* enriched with *Spirulina* and synthetic canthaxanthin significantly increased the efficiency of producing beta-carotene, leading to improved immunity of goldfish (Elshafey *et al*., 2023).

This study examined the effects of feeding mandarinfish with different types of microalgae on their growth. It was found that feeding *Artemia* with microalgae improved the fish's growth, with significant increase in weight gain and specific growth rate compared to the control group. The highest weight gain and specific growth rate (SGR) were observed in fish fed with *T. gracilis*. Carotenoids were found to play a crucial role in metabolic process of fish, thus enhancing growth (Amar *et al*., 2001). Hekimoğlu *et al*. (2017) reported the result of supplementing the diet with *N. oculata* for feeding tomato clownfish and found weight gain and SGR were greater than the control. Upon the completion of the experiment of the present study, death of the fish was not observed throughout the experiment, indicating that the survival rate of the mandarinfish was not influenced by carotenoids from the *Artemia* fed with the four species of microalgae. This result is consistent with the study of Ghotbi and Takami (2011) who found that carotene-enriched diet did not impact the survival rate of rainbow trout.

The present study also revealed that mandarinfish fed enriched *Artemia* accumulated different types of carotenoids in varying amounts across the treatment groups. While the quantities of other carotenoid types (echinenone, canthaxanthin and zeaxanthin) did not show significant differences among treatments, beta-carotene levels in fish fed *Artemia* enriched with *T. gracilis* and *D. salina* were significantly higher than those fed non-enriched *Artemia*. This finding is consistent with previous research by Sales *et al*. (2021), who found that supplementing aquafeed with *Nannochloropsis gaditana* increased carotenoid contents in muscle of juvenile gilthead seabream, *Sparus aurata*, after 39 days of feeding, with beta-carotene being the highest. Similarly, Keleştemur and Çoban (2016) observed enhanced growth of rainbow trout fed beta-carotene-supplemented diets, while Hu *et al*. (2006) reported a direct relationship between beta-carotene and fish growth performance. In our study, we found that the growth performance of mandarinfish was influenced by dietary *Artemia* nauplii enriched with *T. gracilis*, with beta-carotene identified as the major pigment responsible for stimulating growth in mandarinfish.

Carotenoids are recognized as sources of skin coloration in fish (Johnson *et al*., 1991), and *Artemia* enriched with microalgae as carotenoid sources have been utilized to enhance the skin coloration of ornamental fishes (Aziz *et al*., 2022). However, in the present study, no discernible difference in skin coloration was observed among the mandarinfish fed the enriched diets compared to the control group, despite the increased quantity of beta-carotene in the fish. This might be due to the fact that the experimental period was too short, thus resulting in insufficient accumulation of carotenoids, for the change to take place. A report by Yasir and Qin (2010) discovered that the use of carotenoids to enhance coloration of fish varied with fish variety, pigment and duration of application. The present study revealed that *Artemia* enrichment resulted in benefiting the growth and survival rate of mandarinfish. It was also found that *Artemia* enrichment with microalgae highly accumulated carotenoids, particularly in mandarinfish fed with *Artemia* enriched with *T. gracilis* which highly accumulated beta-carotene (0.39 ± 0.06 $\mu g \cdot g^{-1}$ wet weight). These results are consistent with the study of Hata and Hata (1969), who studied the transformational change process in *Artemia salina* fed with microalgae in order to investigate the change of carotenoid in *Artemia*.

CONCLUSIONS

The mandarinfish that were fed with *Artemia* nauplii enriched with four species of microalgae *Tetraselmis gracilis, Isochrysis galbana, Nannochloropsis oculata,* and *Dunaliella salina* exhibited varying levels of carotenoid accumulation. Beta-carotene was identified as the predominant in the mandarinfish fed on this enriched diet. Notably, the highest beta-carotene accumulation occured in fish receiving a diet enriched with *T. gracilis*, which also corresponded to superior growth performances in the mandarinfish. Consequently, a diet supplemented with *T. gracilis* can be considered a growth promoter for mandarinfish.

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