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Research

Enhancement of Growth Performance and Body Coloration Through The Inclusion of Torch Ginger Powder in The Feed of Red Tilapia (*Oreochromis* sp.)

Norfazreena Mohd Faudzi[1]*, David Dexter Baru[1], Fui Fui Ching[1] and Shigeharu Senoo[1,2]

1. Higher Institution Centre of Excellence (HICoE), Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400, Kota Kinabalu, Sabah, Malaysia
2. UMS-Kindai Aquaculture Development Centre, Kindai University, Shirahama, Wakayama, 649-2211, Japan
*Corresponding author: fazreenafaudzi@ums.edu.my

ABSTRACT

Red tilapia (*Oreochromis* sp.) is a highly produced aquaculture fish among freshwater species. Coloration is an important factor in determining consumer preferences. Therefore, an 8-week experiment was conducted to enhance the growth performance and body coloration through the inclusion of torch ginger in the feed of red tilapia. Five experimental feeds were prepared from commercial feed (crude protein: 34%, crude lipid: 5%) containing torch ginger powder at 2.5, 5.0, 7.5, and 10% (T_1, T_2, T_3, & T_4) inclusion levels while feeding without torch ginger act as a control (T_0). Red tilapia (Initial body weight: 0.4±0.1g; Initial total length: 4.0±0.1cm) were distributed at stocking number 20 fish/tank into fiberglass tanks with a capacity of 80 L and triplicates for each treatment. The fish were fed with experimental feeds until satiation. The growth of fish was significantly higher (P<0.05) when the fish fed with T_1, T_2, and T_3 compared to control treatment T_0. Moreover, the feed intake was significantly increased (P<0.05) when the torch ginger powder was added to the experimental feeds. In addition, the FCR was significantly better (P<0.05) when the fish fed on T_1, T_2, and T_3 compared to T_0. The inclusion of torch ginger powder in the feed did not significantly affect (P<0.05) the body protein and lipids of the experimental fish. The increment of redness was significantly higher (P<0.05) when the fish fed with T_1, T_2, T_3, and T_4 compared to T_0. However, it showed a declining trend of redness when the inclusion of torch ginger increased to 5.0% and above. Thus, the present study suggested that the inclusion of torch ginger powder improved the performance and enhanced the body coloration of juvenile red tilapia. However, a further increase of torch ginger powder by more than 2.5% did not help in the enhancement of the body coloration.

Key words: *Oreochromis* sp., torch ginger powder, aquafeed, growth performances, body coloration

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INTRODUCTION

The aquaculture industry is rapidly rising because of the need to feed a growing global population. Aquaculture is also the sector of animal production for food that is expanding the quickest when compared to other industries (FAO, 2016). In industrialized and developing nations, at least 7.8 billion people will get at least 17% of their daily average animal protein from fish in the year 2020 (FAO, 2022). Red tilapia (*Oreochromis* sp.) is an easy species to breed and affordable for daily human consumption. It can be considered one of the top-produced species in Malaysia, which is produced at a rate of 44.7%, followed by catfish (36.7%), and carps (10.08%) (FAO, 2022). It also reported that red tilapia produces the largest yield in terms of value, with a value of USD 27 million (FAO, 2022).

Recently, appearance such as coloring is an essential feature that can affect the perception of quality and selling price. Poor handling, severe stress, or a lack of natural carotenoid sources are commonly associated with the loss of a fish's body pigmentation. In other ways, vivid color effects raise anticipation for superior quality, nutrient-

dense, and healthy food (Amiruddin *et al*., 2021). In captivity, the inclusion of carotenoids in the feed is one of the easy and less stressful approaches that may enhance the coloration of the fish (Das & Biswas, 2016). Two types of carotenoids are being used in the aquaculture industry which are synthetic carotenoids and natural carotenoids. Even though the use of synthetic carotenoids is easier than natural carotenoids, the price is high and difficult to obtain in the market (Natália & Sandra, 2016).

Current research has focused on using natural carotenoids in fish feed. Many species that have been evaluated have shown improved body color when plant materials such as red paprika, green water meal, spirulina meal, and banana peel have been utilized (Shapawi *et al*., 2017; Coswatte *et al*., 2020; Amiruddin *et al*., 2021; Elshafey *et al*., 2022). On the other hand, fish fed with commercial feed without the inclusion of carotenoids showed less coloration on the body (Coswatte *et al*., 2020). Other than that, the inclusion of natural carotenoids not only enhanced body coloration but also improved the growth performance of the fish (Maiti, 2017; Naeem *et al*., 2021). It has been reported that carotenoids can be a good growth promoter for several fish species (Goda *et al*., 2018; Tiewsoh *et al*., 2019).

Torch ginger, *Etlingera elatior* which is shaped like a torch and has an appealing color of red that grows in tropical regions such as Malaysia and Indonesia (Ravindran, 2017). Commonly, this plant is essential and frequently utilized in the daily life of Indonesians for food ingredients or traditional medicine (Maruzy, 2016). Torch ginger is mainly used for consumption and ornamental decorations however, this plant also contains secondary metabolites such as flavonoids, glycosides, tannins, steroids, terpenoids, and phenols, indicating that the plant is bioactive (Chan *et al*., 2011). This indicates that the plant has a wide range of pharmacological activities, such as anti-microbial, anti-inflammatory, antioxidant, anti-hyperglycemic, and anti-hypouricemic (Juwita *et al*., 2018). Other than that, it was reported that the extracted oil from torch ginger contains properties of therapeutic agents in treating sore throats and dizziness (Norddin & Tien, 2021). In addition, it was discovered that torch ginger contains anthocyanin, a red pigment that is both water-soluble and cytotoxic and can be used as a natural carotenoid source. However, it is unclear whether torch ginger has any specific effect on fish, especially on growth and body coloration. In the present study, the torch ginger powder was derived from the flower itself as the petals of the flowers contain high total carotenoid content compared to the stems of a plant. Therefore, the present study was conducted to enhance growth performance and body coloration through the inclusion of torch ginger powder in the feed of red tilapia.

MATERIALS AND METHODS

Preparation of torch ginger powder

The fresh torch ginger used to produce the powder for the experiment was procured from local farmers in Tawau and Kota Kinabalu, Sabah. The flowers were washed and separated from the stem and bud. The flowers were then finely chopped, and oven dried for 17 hr at a temperature of 60 °C. Finally, the dried flowers were ground and sieved to obtain fine powder for further use.

Preparation of experimental feeds

Five experimental feeds were prepared for the experiment. The base of the experimental feeds was a commercial Tilapia Starter (Leong Hup Aqua 5912) containing 34% crude protein and 5% crude lipid. The feed was ground, and the torch ginger powder (crude protein: 12.47±0.55%, crude lipid: 3.82±0.31%, moisture: 12.57±0.59%, & ash: 11.75±0.13%) was included in the feed at 2.5 (T_1), 5.0 (T_2), 7.5 (T_3) and 10.0% (T_4) (Table 1) (Amiruddin *et al*., 2021). The feed without torch ginger was served as a control (T_0). The feeds were then re-pelleted and dried in an oven for 7 hr at a temperature of 60 °C. Dried feeds were kept in airtight containers and refrigerated for further use.

Table 1. An experimental feed with different inclusion of torch ginger powder

	Experimental Feed				
	T_0	T_1	T_2	T_3	T_4
Tilapia Starter (g)	100.0	97.5	95.0	92.5	90.0
Torch Ginger Powder (g)	0.0	2.5	5.0	7.5	10.0

Rearing procedure

Red tilapia juveniles (Initial body weight: 0.4±0.1 g; Initial total length: 4.0±0.1 cm) were acclimatized to experimental conditions for a week and fed with commercial Tilapia Starter feed before starting the feeding experiment. The fish (*n*=20) were randomly distributed into a square fiberglass tank with a

capacity of 80 L and 3 replicates for each treatment. The tanks were provided with aeration and the treatments were randomly arranged. During the experiment, bottom cleaning and 50% water exchange were done for each tank every day. The water quality such as temperature, dissolved oxygen, and pH were maintained at 24.7 to 26.5 °C, 6.0 to 7.5 mg/L, and 6.75 to 7.28, respectively. The fish were fed with respective feeds at 0830 and 1430 until satiated for 8 weeks. Measurements of total length and body weight were taken before and after the experiment. Bulk measurements for each tank were done once every two weeks. The experimental fish was anesthetized before measurement. All data collected was calculated as follows.

$$\text{Weight gain (\%)} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100$$

$$\text{SGR (\% day}^{-1}\text{)} = \frac{\text{Ln Final body weight (g)} - \text{Ln Initial body weight (g)}}{\text{Time (days)}} \times 100$$

$$\text{Survival} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

$$\text{Feed intake (g)} = \text{Total feed intake for 12 weeks}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Wet weight gain (g)}}$$

Biochemical analysis

The total carotenoid concentration of torch ginger powder and experimental feeds was determined using the method described by Arredondo-Figueroa *et al*. (2003). The experimental feeds and fish were analyzed for proximate composition. Using the Kjeltec-Protein Analyzer (KjeltecTM 2300, Foss, Sweden) and Soxtec-Lipid Analyzer (SoxtecTM 2043, Foss, Sweden), the crude protein and lipid were determined, respectively (AOAC, 1999). A semi-automatic fiber analysis instrument (FibertecTM System, Foss Analytical, Sweden) was used to assess the fiber content of the torch ginger and experimental feeds (AOAC, 1999). Following oven drying at 105 °C for 24 hr and incineration in a muffle furnace at 550 °C for 6 hr, respectively, the moisture and ash levels were assessed (AOAC, 1999).

Coloration analysis

The body pigmentation of experimental fish was analyzed using the method by Mat Nawang *et al*. (2019). The experimental fish (n=3) were anesthetized and photographed using a digital microscope Dino-lite microscope (Model AM4113T, Dino-Lite brand, Taiwan). All digital images were saved as Tagged Image File Format (TIFF) files and analyzed using ImageJ software version 1.53. The redness (%) of the experimental fish bodies was calculated using the formula as follows;

$$\text{Redness (\%)} = \frac{R}{R+G+B} \times 100$$

Where,

R = red

G = green

B = blue

Statistical analysis

All quantitative data were analyzed using one-way analysis of variance (ANOVA) through Statistical Package for Social Science (SPSS version 28) software. The significant differences between all treatments were compared using Tukey's multiple range test at a value of 0.05.

RESULTS

Results on the proximate composition of torch ginger powder and experimental feed are shown in Table 2. The torch ginger powder contained 12.47% of crude protein, 3.82% of crude lipid, 12.57% of moisture, 11.75% of ash, 2.43% of fiber, and 20.41 µg/g of total carotenoid. However, the inclusion of torch ginger powder in the experimental feeds did not significantly affect (P>0.05) crude protein, crude lipid, moisture, ash, and fiber content for each treatment. Meanwhile, the inclusion of torch ginger powder significantly increases (P<0.05) the total carotenoid in the feeds.

Table 2. Proximate composition and total carotenoid content of torch ginger and experimental feeds used in the experiment

Attributes	Torch Ginger	Treatment				
		T_0	T_1	T_2	T_3	T_4
Crude protein (%)	12.47± 0.55	34.83± 0.44	33.88± 0.39	33.56± 0.07	33.91± 1.19	33.08± 1.50
Crude lipid (%)	3.82± 0.31	5.08± 0.94	5.90± 0.26	4.87± 0.83	4.73± 0.45	4.38± 0.14
Moisture (%)	12.57± 0.59	4.53± 0.05	5.28± 0.10	5.18± 0.64	4.86± 0.15	5.16± 0.21
Ash (%)	11.75± 0.13	10.45± 0.17	10.20± 0.04	10.22± 0.02	10.19± 0.03	9.98± 0.62
Fiber (%)	2.43± 0.49	1.59± 0.11	1.18± 0.06	1.15± 0.34	1.04± 0.03	1.14± 0.22
Total carotenoid content (µg/g)	20.41± 0.66	9.45± 0.38^{a}	10.09± 0.38^{a}	11.64± 0.38^{b}	12.09± 0.38^{bc}	12.75± 0.38^{c}

Table 3 shows the results on growth performance, survival, and feed utilization. Fish fed with T_1, T_2, and T_3 are significantly higher (P<0.05) growth compared to control treatment T_0. Feeding red tilapia with TG_4 did not show a significant difference with all treatments. The survival of fish ranged between 55-66% and no significant difference (P>0.05) was observed. Fish fed with T_2, T_3, and T_4 showed significantly higher (P<0.05) consumption compared to T_1 and T_0. Meanwhile, the feed conversion ratio (FCR) for fish fed with T_1, T_2, and T_3 showed significantly better (P<0.05) FCR compared to control treatment T_0. FCR for fish fed with T_4 showed no significant difference (P>0.05) with all treatments.

Table 3. Growth performances, survival, and feed utilization of red tilapia fed with different inclusion of torch ginger in the feed

Attributes	Treatment				
	T_0	T_1	T_2	T_3	T_4
Final body weight (g)	5.21± 0.73^{b}	6.13± 0.56a	6.50± 0.23^{a}	6.53± 0.22a	5.82± 0.47^{ab}
Body weight gain (%)	202.98± 42.36^{b}	256.33± 32.44a	277.46± 13.30^{a}	279.64± 12.51a	238.46± 27.27^{ab}
SGR (%/day)	1.30± 0.27	1.55± 0.18	1.38± 0.22	1.38± 0.07	1.25± 0.14
Survival (%)	66.00± 2.89	60.00± 10.00	55.00± 5.00	60.00± 5.00	56.67± 7.64
Daily feed intake (g/fish/day)	0.23± 0.00^{b}	0.23± 0.01b	0.25± 0.01^{a}	0.24± 0.00^{a}	0.24± 0.00^{a}
Total feed intake (g/fish)	13.68± 0.26b	14.00± 0.30^{b}	14.75± 0.43a	14.63± 0.25a	14.55± 0.18a
Feed conversion ratio (FCR)	1.38± 0.24^{a}	1.10± 0.11^{b}	1.06± 0.03^{b}	1.05± 0.06^{b}	1.23± 0.15^{ab}

Mean (±SD) values (n=3) with different superscripts within the row are significantly different (P< 0.05).

Results on whole-body proximate composition are shown in Table 4. The inclusion of torch ginger powder in the feed did not significantly affect (P>0.05) the body protein. However, T_4 showed significantly higher (P<0.05) ash content in the fish body than in other treatments. In addition, T_4 also showed significantly higher (P<0.05) moisture in the fish body compared to T_1.

Table 4. Whole-body proximate composition of red tilapia fed with different inclusion of torch ginger in the feed

Attributes	Treatment				
	T_0	T_1	T_2	T_3	T_4
Moisture (%)	21.13± 1.31^{ab}	20.50± 2.70^{b}	22.23± 1.42^{ab}	21.48± 1.56^{ab}	24.34± 1.2^{1}a
Ash (%)	20.28± 2.42^{b}	22.15± 2.42^{b}	22.92± 1.96^{b}	22.25± 1.01^{b}	26.53± 1.26^{a}
Crude protein (%)	16.15± 0.82	16.05± 0.45	15.77± 0.40	15.38± 1.33	16.04± 1.30
Crude lipid (%)	15.86± 1.21	15.65 ± 0.74	15.35± 0.28	15.36± 1.03	15.97± 0.23

Mean (±SD) values (*n*=3) with different superscripts within the row are significantly different (*P*< 0.05).

The body coloration of red tilapia fed with different inclusions of torch ginger in the feed is shown in Figure 1. The inclusion of torch ginger powder in T_1, T_2, T_3, and T_4 significantly affected (*P*<0.05) the body pigmentation of experimental fish compared to control treatment T_0 (Table 5). However, the inclusion of torch ginger powder more than 2.5% did not further increase the body pigmentation.

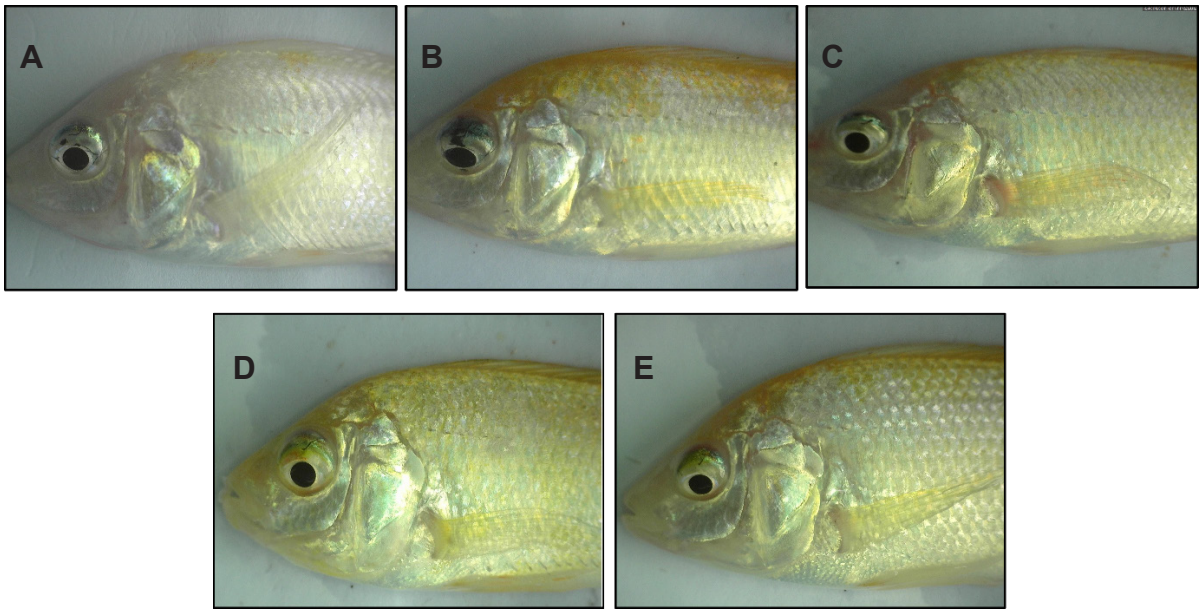

Fig. 1. Body coloration of red tilapia fed with different inclusion of torch ginger in the feed; (A) Red tilapia fed T_0; (B) Red tilapia fed T_1; (C) Red tilapia fed T_2; (D) Red tilapia fed T_3; (E) Red tilapia fed T_4

Table 5. Redness of red tilapia fed with different inclusion of torch ginger in the feed

Attributes	Treatment				
	T_0	T_1	T_2	T_3	T_4
Initial Redness (%)	0.298±0.020				
Final Redness (%)	0.317± 0.020^{b}	0.374± 0.036^{a}	0.368± 0.01^{a}	0.386± 0.04^{a}	0.360± 0.00^{a}
Increment of redness (%)	2.57± 1.42^{b}	31.90± 5.45^{a}	25.20± 0.24^{a}	22.68± 0.95^{a}	21.17± 0.24^{a}

Mean (±SD) values (*n*=3) with different superscripts within the row are significantly different (*P*< 0.05).

DISCUSSION

The present study shows that the inclusion of torch ginger powder promotes better growth for red tilapia. Fish fed with T_1, T_2, and T_3 had significantly higher final body weight and weight gain compared to fish fed with control feed T_0. No significant difference was observed when the fish fed with T_4. It has been reported that carotenoids are known to have a positive role in the metabolism, thus improving the growth of fish (Wade *et al*., 2015; Goda *et al*., 2018). Similarly, the addition of astaxanthin from

green algae, *Haematococcus pluvialis* in the feed can enhance the weight gain of red tilapia and puffer fish (Cheng *et al*., 2017; Tuan Harith *et al*., 2022). Other than that, the inclusion of marigold petals in the feed improved the growth of European seabass, *Dicentrarchus labrax* (Goda *et al*., 2018). Judan Cruz *et al*. (2021) found that feeding juvenile red tilapia with the inclusion of *Daucus carota* peel and *Ipomoea aquatica* leaves in the feed improved the growth performance. On the other hand, the addition of rounded brittle fern weed, *Laurencia obtuse* as a natural carotenoid in the feed for rainbow trout, *Oncorhynchus mykiss* resulted in lower growth compared to the control treatment (Baki *et al*., 2018). The plant-derived raw materials that participated in the feed are one of the factors that contributed to the growth pattern (Baki *et al*., 2018).

The results of growth performance were reflected in to increased trend of feed intake when the fish fed feed with torch ginger powder. The inclusion of torch ginger allowed the fish to better utilize the feed compared to the control treatment. The feed conversion ratio (FCR) value was significantly better in treatments T_1, T_2, and T_3 compared to T_0. The results could be related to the potassium content of torch ginger, which promotes the digestibility and palatability of the feed (Andra, 2023). Other than that, a previous study stated that supplementation of carotenoids in the feed shows the efficiency of feed utilization in fish (Hien *et al*., 2022). Survival rate was found to be between 55% to 56% throughout the experiment period and no significant difference was observed.

Within two months duration, the redness of fish significantly increased in fish fed with the inclusion of torch ginger powder compared to the control treatment. It is proved that supplementation of natural carotenoids from torch ginger enhances the coloration of red tilapia body. However, a further increase in inclusion level above 2.5% did not improve the body coloration of the fish. Thus, indicates that 2.5% of torch ginger powder contributes to the maximum deposited carotenoid in the fish skin. This is the first study reported on the use of torch ginger to enhance coloration on fish. Feeds that contain natural carotenoid sources could enhance body or flesh color for a certain period after consumption (Amiruddin *et al*., 2021). In the previous study, it was reported that the addition of 10% *Spirulina platensis* in the feed for rainbow trout, *Oncorhynchus mykiss* has resulted in maximum deposition of carotenoid in the skin and fillet tissue (Teimouri *et al*., 2013). Other than that, Amiruddin *et al*. (2021) stated that the inclusion of paprika powder 2 to 8% significantly enhances the body coloration of red tilapia. In another study by Arous *et al*. (2014), the incorporation of green algae, *Dunaliella salina* extract significantly improved the body coloration of red tilapia, making the fish color brighter and more attractive. Feeding carp, *Cyprinus carpio* using marigold petals and banana peels also showed an improvement in body coloration (Coswatte *et al*., 2020). Artemia enriched with spirulina and canthaxanthin also resulted in the enhancement of the body coloration of goldfish, *Carassius auratus* compared to the control treatment (Elshafey *et al*., 2022).

The inclusion of torch ginger powder did not have a significant effect on the protein and lipid content of whole-body composition on red tilapia. This could be reflected in the proximate composition of the experimental feeds. Different inclusion levels of torch ginger powder did not affect the crude protein, crude lipid, moisture, ash, and fiber of experimental feeds. Other than that, the fiber content in the feed is suitable and did not affect the fish digestibility, thus, revealing that the nutrients from the feeds were able to be digested by the fish.

CONCLUSION

The findings in the present study demonstrate that torch ginger can be a potential enhancer of fish body coloration without affecting fish growth and feed intake. The finding also suggested that a further increase of torch ginger powder by more than 2.5% did not help in the increased redness of red tilapia.

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ETHICAL STATEMENT

The Malaysian Code of Practice (MYCODE) for the care and use of animals for scientific purposes was followed when handling and caring for the experimental fish throughout the experimental period. The Code comprehensively covered all aspects of animal care and utilization in the research including scientific activities as well as the well-being and environmental needs of the animals.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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