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RESEARCH PAPER

Assessing Growth Performance and Flesh Quality of Silver Carp (*Hypophthalmichthys molitrix*) with Natural Carotenoids in Feeds

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Abstract

A feeding trial was conducted to evaluate the effects of natural pigment inclusion on the growth performance, carcass composition, carotenoid accumulation, coloration, and sensory quality of *Hypophthalmichthys molitrix*. Four experimental diets were prepared: a carotenoid-free control (CF) and three carotenoid-enriched diets containing 5% tomato (TEF), 5% carrot (CEF), and a mixture of 2.5% tomato and 2.5% carrot (TCEF). Fish were assigned to four treatments with triplicate groups and fed twice daily to apparent satiation for 90 days. Growth indices, including mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate, were determined using standard formulas, and proximate composition, carotenoid concentration, and palatability attributes (flavor, taste, texture) were analyzed through established procedures. The TCEF-fed fish showed significantly ($p<0.05$) superior MWG and SGR values and a reduced FCR compared with other treatments. No significant ($p>0.05$) changes were found in the proximate composition among the test groups. The highest carotenoid deposition occurred in fish fed the CEF diet, followed by TCEF and TEF. Fish receiving carotenoid-enriched diets (CEF and TCEF) exhibited significantly ($p<0.05$) enhanced lightness (L*), redness (a*), yellowness (b*), and chroma (C*) values. Palatability evaluation revealed a strong preference for TCEF, whereas CF scored the lowest. Furthermore, TCEF proved to be the most cost-efficient diet. The results demonstrate that the combined inclusion of 2.5% tomato and 2.5% carrot effectively enhances growth, coloration, and sensory quality in *H. molitrix*.

Introduction

Bangladesh remains one of the world's leading producers of fish and is experiencing a significant rise in commercial aquaculture using commercially formulated feed (Edwards and Karim, 2007). The growing demand for commercial fish feeds has greatly boosted the growth of the fish feed industry in Bangladesh, providing a wide variety of feed from several producers in the market (Ali, 2024). Unfortunately, many manufacturing companies are unable to provide quality feed due to the use of substandard raw materials, the inclusion of contaminated ingredients, and other quality-compromising factors (Khatun *et al*., 2017). Aquaculture production is likely to face major sustainability concerns since the feed sector frequently uses animal-origin protein and lipid sources. As a result, there is now a greater focus on using locally available plant-based components for producing sustainable aquafeed. Additionally, because of the increasing need for fish feed, each feasible natural resource needs to be explored as a potential feed ingredient. Moreover, consumer preferences and demand for fish and fish products are strongly affected by a number of sensory factors, such as color, flavor, and smell (Uddin *et al*., 2019). Among them, color and flavor have an important impact on purchase choices since they can cause psychological and physiological responses that influence customer acceptance and perceptions, which in turn ultimately affect the market demand for fish (Kaushik, 2011). In this regard, naturally occurring pigments/carotenoids can be considered as potential additives since they improve fish's growth and pigmentation as well as their overall quality and flavor (Chow *et al*., 2017).

Carotenoids are a group of lipophilic pigments that play a vital role in coloration, growth, reproduction, maintenance, and disease resistance in aquatic animals (de Carvalho and Caramujo, 2017). Apart from their powerful nutritional and coloring impacts, carotenoids act as antioxidants and protect against different stressors (Yeum *et al*., 2009). But like other animals, fish cannot synthesize carotenoids on their own; they must rely entirely on external food sources to obtain their required carotenoids (Gupta *et al*., 2007). Therefore, a range of carotenoids of both natural and synthetic sources, including lutein, xanthophylls, astaxanthin, cantaxanthin, α-carotene, and others, have been added to fish diets to improve their color in the captive farming system (Ninwichian *et al*., 2019). However, synthetic carotenoids are often expensive and may have adverse consequences, like environmental deterioration and carcinogenic effects (Sun *et al*., 2012; Teimouri *et al*., 2013). Additionally, consumers are now becoming more conscious of the safety of synthetic feed additives. This raises interest in using available natural carotenoid sources, such as tomato (*Solanum lycopersicum*) and carrot (*Daucus carota*), for different economically valuable fish and shrimp species. Bangladesh produces a significant number of pigmented fruits and vegetables all year long, yet one of the biggest obstacles to overcoming is post-harvest crop loss (Noman, 2021). Tomatoes and carrots often experience significant depreciation due to excessive production during peak harvesting seasons. In these situations, incorporating these crops as viable additives and carotenoid sources in aquafeed production will ensure the crops' multifunctional use, minimize losses, and benefit both sectors.

However, several studies have been carried out globally regarding the effects of carotenoid-enriched feed on improving the growth and pigmentation of a range of fish species (Laskhmi *et al*., 2015; Azab *et al*., 2016; Goda *et al*., 2018; Wagde *et al*., 2018; Abouzied *et al*., 2023; Das, 2023), but few scientific studies are available on cultured fish species in Bangladesh. In this country, *Hypophthalmichthys molitrix* is a commonly cultured exotic carp species in polyculture systems with other carps due to its rapid growth and amiable nature. Growth and flesh color of this fish can be improved through dietary natural carotenoids, which may improve its flesh quality, market demand, and price. Therefore, the research was conducted to evaluate the growth, flesh nutrient, flesh carotenoid, and palatability of *H. molitrix* with the feeds enriched with two available natural carotenoid sources (carrot and tomato).

Materials and Methods

Experimental site and period

The experiment was conducted for a duration of three months from May to July 2024, in twelve cages placed in a research pond at the University of Rajshahi, Bangladesh. Each cage, which had an overall size of 2.73m³, was built using an iron frame and a 5mm mesh monofilament nylon net. On the upper side of the cage, a narrow opening was kept for feeding and sampling of the fish more easily.

Experimental design

In the experiment, four experimental diets were prepared, each representing a distinct treatment group, viz., CF, TEF, CEF, and TCEF. Where CF was treated as the control group of fish fed a diet without carotenoid sources, and the other three were the fish groups fed with feeds incorporated with carotenoid sources at 5% tomato treated as TEF, 5% carrot treated as CEF, and a combination of 2.5% tomato and 2.5% carrot treated as TCEF. To assign the four treatments to the cages, a completely randomized design was employed, and each treatment was replicated three times.

Feed formulation and analysis

Four experimental diets were formulated using locally available ingredients. The diets included a control feed (CF), tomato-enriched feed (TEF), carrot-enriched feed (CEF), and a combined tomato and carrot-enriched feed (TCEF). The proximate composition of each ingredient was analyzed, and diet formulation was done using spreadsheet techniques. Before feed preparation, pigment sources were collected, dried in the sunlight, and processed into powder form using a grinder. To prepare a feed, each ingredient was weighed in accordance with Table 1, and the required amount of water was mixed with it to form the dough. Using a pelleting machine, the dough was formed into pellets. The dried pellets were then sealed in labeled polythene bags and kept at room temperature. The proximate compositions of the feed samples were analyzed following the methods of the Association of Official Analytical Chemists (AOAC) (2000), and the data showed no significant difference among the feeds (Table 1).

Table 1. Dietary inclusion of each ingredient and proximate compositions of the feeds

Ingredients (%)	**CF**	**TEF**	**CEF**	**TCEF**
Rice bran	20.0	15.0	15.0	15.0
Mustard oil cake	40.0	40.0	40.0	40.0
Fish meal	22.36	23.8	23.8	23.8
Wheat bran	12.64	11.2	11.2	11.2
Carrot	0.0	0.0	5.0	2.5
Tomato	0.0	5.0	0.0	2.5
Molasses	2.5	2.5	2.5	2.5
Soya-bean oil	2.0	2.0	2.0	2.0
Vitamin and mineral mix	0.5	0.5	0.5	0.5
Proximate composition (% dry basis)				
Protein	29.45 ± 0.47^a	29.72 ± 0.16^a	29.87 ± 0.08^a	29.70 ± 0.51^a
Lipid	6.36 ± 0.12^a	6.55 ± 0.34^a	6.59 ± 0.23^a	6.37 ± 0.11^a
Carbohydrate	37.81 ± 0.06^a	37.39 ± 0.56^a	37.97 ± 0.70^a	37.68 ± 0.32^a
Moisture	12.42 ± 0.27^a	12.54 ± 0.03^a	12.29 ± 0.12^a	12.12 ± 0.34^a
Ash	7.7 ± 0.22^a	7.78 ± 0.14^a	7.72 ± 0.06^a	7.55 ± 0.07^a
Fiber	5.33 ± 0.11^a	5.44 ± 0.58^a	5.65 ± 0.08^a	5.61 ± 0.15^a

**Values are expressed as mean±SD. CF = Control feed without pigments, TEF=5% Tomato-enriched feed, CEF= 5% Carrot-enriched feed, and TCEF= 2.5% Tomato + 2.5% Carrot-enriched feed. Data with the same superscripts in the same row do not differ significantly (p>0.05).*

Experimental fish releasing and feeding

From a nearby aquaculture farm, 250 juvenile *H. molitrix* were bought, and the juveniles were acclimated to the experimental setting for two weeks. Subsequently, 20 fish were stocked in each of the twelve cages. Over the course of ninety days, the fish were supplied with test diets twice a day, at a total feeding rate of 5% of their body weight, equally split between the two feedings. Accurate feeding rates were maintained by weighing the fish every two weeks and adjusting the feed quantity accordingly.

Monitoring of water quality

During the period of experiment, water quality parameters, including water temperature, pH, dissolved oxygen (DO), total alkalinity (TA), and ammonium-nitrogen (NH_3-N), were monitored periodically using a Celsius thermometer, digital pH meter (Hanna HI-98128), and HACH Kit (Model: DR/2010). The estimated parameters did not show any significant variation and remained within the suitable range for fish culture.

Analysis of growth and feed utilization

At the time of stocking, the initial weight of the fish in each cage was recorded. Thereafter, the fish were sampled at an interval of two weeks to assess weight gain. Growth performance and feed utilization were evaluated by calculating mean weight gain (MWG), specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR) using the following standard formulas (Mousavi *et al*., 2016).

- Mean weight gain =Mean final weight – Mean initial weight
- Specific growth rate (SGR % / day) = {100 (ln FBW – ln IBW)/T}

Where, FBW = final body weight (g), IBW = initial body weight (g), and T = Time period

- Survival rate (SR) = (Number of harvested fish at the end of the trial/Number of fish stocked at the beginning of the trial) × 100
- Feed conversion ratio (FCR) = Dry feed fed/Wet weight gain

Analysis of the proximate composition of fish flesh

At the end of the feeding trial, the flesh of three fish from each cage was collected and homogenized in a blender. Then the samples were analyzed according to the methods of the Association of Official Analytical Chemists (2005). The micro-Kjeldahl technique (VELP Scientifica, Italy) was used to determine crude protein as N×6.25. The lipid content was determined through ether extraction by the Soxhlet apparatus (Wincom, Hunan, China). Moisture and ash were estimated by drying at 105°C for 24 hours (POL-EKO, Germany) and incineration at 550°C for 12 hours in a muffle furnace (Nabertherm GmbH, Germany), respectively. The crude fiber content was determined by solvent extraction, followed by acid and alkali digestion, and finally ashed in the muffle furnace at 600°C for 2 hours. Carbohydrate content was calculated by difference as 100 – (% moisture + % crude protein + % crude lipid + % ash + % fiber).

Analysis of carotenoid content of fish flesh

Samples of fish flesh were weighed and mashed in a mortar and pestle with 90% methanol. The resulting mixture was then centrifuged for 12 minutes at 12,000 rpm. After that, the supernatant was transferred to a centrifuge tube, and its optical density was determined at wavelengths of 662, 653, and 470 nm using a spectrophotometer (Analytik Jena, Germany). The total concentration of carotenoids was measured following the method of Lichtenthaler and Wellburn (1983).

$$Ca = 15.65\ A_{666} - 7.340\ A_{653}$$

$$Cb = 27.05\ A_{653} - 11.21\ A_{666}$$

$$\text{Total carotene } (\mu g/g) = \frac{1000A470 - 2.86Ca - 129.2Cb}{245}$$

Where, A_{666} = OD at 666 nm, A_{653}= OD at 653nm and A_{470}= OD at 470 nm

Color measurement

At the start of the feeding trial and subsequently at two-week intervals, three fish were randomly selected from each cage and anesthetized using a clove oil solution. The skin coloration was evaluated using a tristimulus colorimeter (Model NH310, Shenzhen ThreeNH Technology Co., Ltd., China). Color parameters were expressed as L*, a*, and b* values, where L* denotes lightness (ranging from 0 for black to 100 for white), +a* indicates redness, –a* greenness, +b* yellowness, and –b* blueness, following the guidelines of the International Commission on Illumination (CIE, 1977). The hue angle (H°) and chroma (C*) values were calculated from a* and b* to describe the tone and intensity of color, respectively. The H° represents the angular position of color within the visible spectrum, where 0°, 30°, and 60° correspond to red, orange, and yellow hues, respectively. The C* indicates the degree of color saturation or vividness. These parameters were derived using the following equations (Hunt, 1977):

$$H^\circ = \tan^{-1}(\frac{b^*}{a^*})$$
$$C^* = \sqrt{a^{*2} + b^{*2}}$$

Palatability test of cooked fish flesh

Initially, fish samples collected from each replicate of the four treatments were scaled, gutted, and filleted into loins. After being washed, 500g of fish loins from each treatment were cooked following a traditional recipe with spices. To avoid cooking bias, the fish loins were labeled and cooked at the same time. Then, the cooked loins were served to a randomly selected panel of consumers for sensory assessment based on flavor, taste, and texture, using the structured scaling system (Table 2) described by Huss (1996).

Table 2. Organoleptic/Sensory scoring scale for palatability test

Palatability indicators			**Score**
Flavor	**Taste**	**Texture**	
Species-specific	Meaty flavor	Firm/elastic	10
Fresh fish	Sweet	Firm/springy	8
Slightly fishy or sour	Slightly fishy	Less firm	6
Sour and stale	Slightly sour/off flavor	Softer	4
Strong ammonia	Slightly rotten	Very soft	2
Rotten smell	Spoiled	Slippery	0

Economic feasibility of the experimental feeds

The economic feasibility of the experimental feeds was determined by feed cost/kg feed and feed cost/kg fish production following the methods of Zamal *et al.* (2009).

- Feed cost/kg feed = summation of the ingredient's price and manufacturing cost per kg
- Feed cost/kg fish production = Feed cost/kg feed × Amount of feed required per kg of fish production (FCR)

Data analysis

The statistical analysis of the data was performed using one-way analysis of variance (ANOVA) and Duncan's multiple-range test, employing SPSS, USA. A significance level of $p < 0.05$ was used to determine significant differences among the values.

Results

Growth and feed utilization of fish

The recorded values of growth and feed efficiency parameters, including MWG, PWG, SGR, FCR, and SR, for fish under the four different treatments are shown in Table 3. Fish in the TCEF group showed significantly ($p<0.05$) higher MWG, PWG, and SGR, followed by the fish of CEF and TEF, with the lowest values found in CF (Table 3). The TCEF group also exhibited the most efficient FCR, while CF had the least efficient value. No mortality of fish was observed in any treatment group during the study period.

Table 3. The mean values of growth parameters under four treatments

Parameters	**Treatments**			
	CF	**TEF**	**CEF**	**TCEF**
MIW (g)	240.65±6.86^a	237.65±5.94^a	245.64±7.11^a	241.55±6.75^a
MFW (g)	465.15±15.57^d	514.80±16.09^c	563.84±18.74^b	599.45±17.24^a
MWG (g)	224.50±12.17^d	276.84±17.41^c	318.20±16.67^b	357.91±12.91^a
PWG (%)	93.29±2.83^d	119.29±3.27^c	136.07±4.97^b	152.33±3.57^a
SGR (%)	0.73±0.07^d	0.86±0.05^c	0.93±0.05^b	1.01±0.06^a
FCR	2.12±0.07^a	1.81±0.08^b	1.75±0.07^b	1.62±0.05^c

**Values are expressed as mean±SD. CF was assigned to the control fish fed with feed without pigments, while TEF, CEF, and TCEF were assigned to fish groups fed with 5% tomato, 5% carrot, and a mixture of 2.5% tomato + 2.5% carrot-enriched feeds, respectively. Data with varying superscripts in the same row differ significantly (p<0.05).*

Proximate composition of fish flesh

The results of proximate parameters, including crude protein, lipid, carbohydrate, moisture, and ash content in fish flesh, are presented in Table 4. Although crude protein and lipid levels did not differ significantly among treatments, they were relatively higher in fish fed with TCEF. The carbohydrate, moisture, ash, and fiber contents showed no significant variation (*p*>0.05) among the treatments.

Table 4. Flesh composition (% wet basis) of fish under four treatments

Parameters	Treatments			
	CF	**TEF**	**CEF**	**TCEF**
Protein	16.74±0.53[a]	17.20±0.51[a]	17.29±0.22[a]	17.37±0.62[a]
Lipid	2.45±0.37[a]	2.56±0.35[a]	2.59±0.21[a]	2.78±0.27[a]
Carbohydrate	4.08±0.34[a]	3.95±0.27[a]	4.13±0.22[a]	3.88±0.19[a]
Moisture	73.59±2.59[a]	73.21±2.71[a]	71.87±3.36[a]	72.56±2.88[a]
Ash	2.11±0.37[a]	2.02±0.45[a]	2.07±0.27[a]	2.16±0.29[a]
Fiber	1.02 ± 0.09[a]	1.05 ± 0.11[a]	1.25 ± 0.17[a]	1.13 ± 0.10[a]

**Values are expressed as mean±SD. CF was assigned to the control fish fed with feed without pigments, while TEF, CEF, and TCEF were assigned to fish groups fed with 5% tomato, 5% carrot, and a mixture of 2.5% tomato + 2.5% carrot-enriched feed, respectively. Data with the same superscripts in the same row do not differ significantly (p>0.05).*

Carotenoid content

The results demonstrated that incorporating natural carotenoid sources into the feed significantly enhanced carotenoid deposition in the fish flesh compared to fish given feed without carotenoid sources (Figure 1). At the start of the experiment, no significant (*p*>0.05) differences in carotenoid content were observed among the dietary treatment groups. Among the treatments, the CEF and TCEF exhibited significantly (*p*<0.05) greater carotenoid accumulation in the fish flesh compared to the other test feeds.

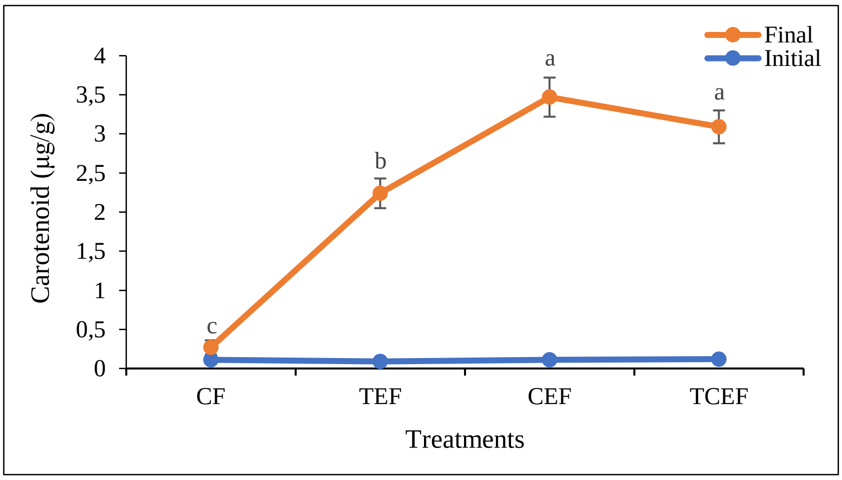

Figure 1. Carotenoid content in fish flesh under four treatments

**CF was assigned to the control fish fed feed without pigments, while TEF, CEF, and TCEF were assigned to fish groups fed with 5% tomato, 5% carrot, and a mixture of 2.5% tomato + 2.5% carrot-enriched feed, respectively.*

Effects on coloration

Dietary carotenoids significantly influenced the flesh color of fish (Table 5). At the end of the feeding trial, significantly higher L* values were observed in fish fed carotenoid-enriched diets (CEF and TCEF) compared to the control (*p*<0.05), indicating lighter flesh coloration in these groups. The a* and b* increased markedly in carotenoid-supplemented groups, with the highest values observed in CEF (a*: 7.53±0.63; b*: 9.74±0.72). Moreover, C* was significantly higher in all carotenoid-supplemented groups than in the control (CF), with the highest value in CEF, whereas H° exhibited a significant reduction in the carotenoid-fed groups, with the lowest in TEF.

Table 5. Effects of carotenoid-enriched feeds on the color parameters of fish flesh

Parameter	Treatments			
	CF	TEF	CEF	TCEF
L^*	31.42±0.67[c]	37.56±1.27[b]	41.08±0.91[a]	40.35±0.76[a]
a^*	2.45±0.35[c]	6.74±0.58[b]	7.53±0.63[a]	7.26±0.55[a]
b^*	6.07±0.73[c]	7.92±0.70[b]	9.74±0.72[a]	9.12±0.68[a]
H°	68.8 ± 3.8[a]	49.4 ± 3.5[b]	52.0 ± 3.1[b]	51.8 ± 3.0[b]
C^*	6.55 ± 0.69[c]	10.40 ± 0.65[b]	12.32 ± 0.69[a]	11.66 ± 0.63[a]

**Values are expressed as mean±SD. CF was assigned to the control fish fed feed without pigments, while TEF, CEF, and TCEF were assigned to fish groups fed with 5% tomato, 5% carrot, and a mixture of 2.5% tomato + 2.5% carrot-enriched feed, respectively. Data with varying superscripts in the same row differ significantly (p<0.05).*

Palatability of cooked flesh

After sensory evaluation, the consumer panel provided scores for the palatability attributes of the cooked fish flesh (Table 6). Though the flesh of fish fed with CEF showed a higher flavor score, no significant variation in the flavor of cooked fish was found among the treatments. Significantly higher taste and texture scores were recorded in fish from CEF, whereas the lowest scores for both attributes were observed in CF.

Table 6. Organoleptic score of cooked fish flesh of four treatments

Organoleptic criteria	Treatments			
	CF	TEF	CEF	TCEF
Flavor	7.91±0.85[a]	8.35±0.74[a]	8.79±0.81[a]	8.45±0.67[a]
Taste	7.26±0.63[c]	8.03±0.47[b]	8.93±0.53[a]	8.04±0.48[b]
Texture	7.48±0.85[b]	7.84±0.63[b]	8.67±0.74[a]	8.17±0.83[b]
Total Score	22.65±1.23[c]	24.22±1.06[b]	26.39±1.13[a]	24.66±1.31[b]
Rank	4th	3rd	1st	2nd

**Values are expressed as mean±SD. CF was assigned to the control fish fed feed without pigments, while TEF, CEF, and TCEF were assigned to fish groups fed with 5% tomato, 5% carrot, and a mixture of 2.5% tomato + 2.5% carrot-enriched feed, respectively. Data with varying superscripts in the same row differ significantly (p<0.05).*

Cost-benefit analysis of the experimental feeds

The result showed that the TCEF feed was the most cost-efficient among other experimental feeds and was 13.51±2.62% cheaper than CF regarding fish production. The other two feeds (TEF and CEF) also exhibited significant cost efficiency and were 5.98±1.82 and 5.73±1.65%, respectively (Table 7).

Table 7. Cost-benefit analysis of the experimental feeds

Economics	Treatments			
	CF	TEF	CEF	TCEF
Feed cost/kg (Tk.)	61.50	63.50	62.60	65.75
Feed cost/kg fish production (Tk.)	125.10±2.16[c]	119.07±2.63[b]	118.81±2.72[b]	110.89±2.65[a]
Cost reduction (%) compared to control	-	5.73±1.65[b]	5.98±1.82[b]	13.51±2.62[a]

**Values are expressed as mean±SD. CF was assigned to the control fish fed feed without pigments, while TEF, CEF, and TCEF were assigned to fish groups fed with 5% tomato, 5% carrot, and a mixture of 2.5% tomato + 2.5% carrot-enriched feed, respectively. Data with varying superscripts in the same row differ significantly (p<0.05).*

Discussion

Growth and feed utilization

Several studies have demonstrated that the addition of carotenoids to fish diets significantly improves their development and survival when compared to diets prepared without these substances (Azab *et al*., 2016; Maiti *et al*., 2017; Lim *et al*., 2018; Maoka, 2020). The results of the present study also showed that dietary pigment inclusion notably improved the growth performance and feeding efficiency of *H. molitrix* when compared to those that were not fed pigment-enriched feed. The current findings are roughly in line with those of Jain *et al*. (2019), who showed that *Cyprinus carpio* treated with a carrot-enriched diet had better weight gain, SGR, and FCR. Kowalska *et al*. (2022) observed that providing *C. carpio* with a diet enriched with carrots enhanced the weight gain, SGR, and FCR in fish, which aligns with the findings of the current study. Weerakkody and Cumaranatunga (2016) reported comparable results that carotenoids have growth-promoting properties, and carrot-enriched diets exhibit higher weight gain, SGR, and improved FCR in *Catla catla* compared to diets enriched with tomato or beetroot. In another study, Goda *et al*. (2018) found notable improvements in weight gain, daily growth index, and growth efficiency in European sea bass that were fed carotenoid-enriched feed. More or less similar results were also recorded by Das (2023), who also found that a 5% carrot-enriched diet resulted in better growth and survival outcomes in fish compared to other diets. According to Wagde *et al*. (2018), diets including carrots had a higher net weight increase than the control in swordtail fish (*Xiphophorus helleri*), but diets with spinach were even more efficient. Likewise, adding carrot flour to the diet of lemon cichlids (*Labidochromis caeruleus*) significantly boosted their length and absolute weight growth (Octaviani *et al*., 2022), which also roughly coincides with the present study outcomes. However, our results showed that a combination of tomato and carrot enrichment improved the growth performance of *H. molitrix*, which may be due to the variation in fish species and their metabolic activities.

Proximate composition of fish flesh

There were available studies on the effects of natural pigment-enriched feed on growth performance and carotenoid deposition in fish, but only a few were available on the flesh proximate composition of fish. In the study, the proximate analysis of fish flesh showed no significant differences in crude protein, lipid, moisture, and ash content among the treatments. However, fish fed with carrot and tomato-carrot mix-enriched feed exhibited a relatively higher protein and lipid content, as well as lower moisture content. The results of the study are in agreement with the findings of Das (2023), who reported that zebrafish fed with a carrot-enriched diet showed no significant variations in proximate composition among the different treatment groups. Hosen *et al*. (2024) also reported similar outcomes that supplementing fish diets with natural pigments such as carrot, tomato does not significantly alter the proximate composition of *B. gonionotus*. More or less comparable results are also registered by Pailan *et al*. (2012) and Jorjani *et al*. (2019), who noted that natural carotenoid sources such as *Hibiscus rosasiensis* and rose petals had no significant impacts on the flesh composition of *Puntius conchonius* and *Trichogaster trichopterus*, respectively. However, Jha *et al*. (2012) observed that Snow Trout fed with diets incorporating marigold flower and beetroot meals showed comparatively higher flesh protein and lipid content, which differs from the results of the present study. The findings of this study are different from those of Kelestemur and Çoban (2016), who found that a diet supplemented with beta-carotene improved the crude protein content of rainbow trout flesh. The differences observed in the findings of the researchers on the proximate composition of fish flesh that were fed with natural pigment source-enriched diets might be due to the variation in fish species or the difference in pigment sources used in their study.

Carotenoid content

Natural carotenoid-based dietary additives have been consistently shown to improve carotenoid accumulation and intensify the coloration of fish flesh and skin in different species. The findings demonstrated that fish fed a diet enriched with 5% carrot had the highest carotenoid accumulation in their flesh, followed by fish fed a diet that contained 2.5% tomato and 2.5% carrot. The present results are consistent with those of Das (2023), who reported that feeding zebrafish with 5% carrot powder-incorporated diet increased carotenoid accumulation and improved coloration. The findings coincide with those of Jain *et al*. (2020), who stated that diet enriched with 5% carrots provides a better level of carotenoid accumulation in the flesh of *C. carpio*. Maiti *et al*. (2017) similarly observed that *C. carpio* fed a carrot-enriched diet had increased carotenoid levels compared to those receiving the control diet. The present outcomes also align with those of Weerakkody and Cumaranatunga (2016) and Tiewsoh *et al*. (2019), who found a greater amount of carotenoid deposition in the flesh of *C.*

catla and Carassius auratus when provided with carrot-enriched diets.

Effects on coloration

Dietary carotenoids significantly enhanced the flesh coloration of fish in this study. Fish fed carotenoid-enriched diets (CEF and TCEF) exhibited higher L* values than the control, indicating lighter flesh, while a* (redness), b* (yellowness), and C* were markedly increased, with the highest values in CEF. These results suggest effective assimilation of carotenoids into muscle tissue, consistent with previous findings in *C*. catla and *C. carpio*, *Badis badis* species (Weerakkody and Cumaranatunga, 2016; Maiti *et al*., 2017; Jain *et al*., 2019; Biswas *et al*., 2023). The pronounced effect of CEF may be attributed to the high β-carotene content in carrot, a potent pigmenting agent, while the mixed carotenoid diet (TCEF) produced slightly lower pigmentation, possibly due to variations in bioavailability or pigment interactions (Wagde *et al*., 2018). Overall, Dietary carotenoids from tomato and carrot are effective, natural enhancers of fish flesh coloration, increasing lightness, redness, yellowness, and color saturation while shifting hue toward more desirable tones.

Palatability of cooked flesh

In the current study, the organoleptic assessment revealed that the texture, taste, and odor of the experimental fish were influenced by the inclusion of natural carotenoids in their diet. According to the panelists' evaluation, fish fed with a 5% carrot-enriched diet were valued as the most palatable. This enhanced palatability may be attributed to the lipid content in the flesh of the carrot-fed fish. Lipids, which are present in foods either as emulsions or free fats dispersed in a solid matrix, are known to influence both texture and flavor by forming volatile oxidation products and contributing to the taste of short-chain free fatty acids (Shahidi and Weenen, 2006). These findings are consistent with those of Hosen *et al*. (2024), who reported improved palatability scores in silver barbs fed a carrot-supplemented diet. Nonetheless, limited research exists on the effects of natural pigments on fish palatability, indicating a need for further investigation.

Economics of the experimental feeds

The present study demonstrates that, in terms of feed cost/kg fish production, the addition of tomato and carrot (110.89±2.65 Tk.) in the diet of *H. molitrix* is the most economical approach for its production using locally available feed ingredients, followed by CEF (118.81±1.72 Tk.) and TEF (119.07±2.63 Tk.). Initially, TCEF was more costly than the control feed in terms of cost per kg feed, but it was significantly more cost-efficient due to lower FCR values. As the prices of feed and feed ingredients have been steadily increasing, the cost of feeding cannot be directly compared to previous scientific reports. However, while comparing the economics of the present study with earlier works of Jewel *et al.* (2018) and Zamal *et al.* (2009), it becomes clear that the feeding cost in fish farming has alarmingly increased over the last decade in Bangladesh. In this context, using available pigment sources in feed formulation can assist farmers and feed manufacturers in achieving their objectives more efficiently.

Conclusions

In comparison to the other treatments, the inclusion of combined carotenoid sources (2.5% tomato and 2.5% carrot) in the diet is more economical and effectively enhances the growth, flesh quality, color performance, and palatability of *H. molitrix* without causing any negative effects. However, additional studies are necessary to determine the optimal inclusion levels and evaluate the long-term effects of natural carotenoid supplementation in aquaculture feeds.

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Ethical approval

The author declares that this study complies with research and publication ethics.

Conflicts of interest

There is no conflict of interest in publishing their study.

Data availability statement

The authors declare that data are available from the authors upon reasonable request.

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Author contribution

Author 1: Conceptualization, Supervision, Validation, Funding acquisition, Resources, Review, Editing.

Author 2: Writing original draft, Investigation, Methodology, Data curation, Visualization, Formal analysis.

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