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Use of Natural Carotenoid Sources for an Indian Major Carp, *Labeo rohita* (Hamilton, 1822)

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ABSTRACT

A 90-day feeding trial was conducted to determine how natural carotenoid sources influence the growth performance, carcass composition, carotenoid deposition, flesh color, and palatability of an Indian major carp, *Labeo rohita*. Four diets were formulated: a carotenoid-free control feed (CF) and three carotenoid-supplemented feeds containing 5% tomato (TEF), 5% carrot (CEF), and a combination of 2.5% tomato plus 2.5% carrot (TCEF). Fish were randomly distributed into four treatments with triplicate groups and fed to apparent satiation twice daily. Standard procedures and formulas were used to measure growth parameters, including mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), and survival, along with proximate compositions, carotenoid content, coloration and palatability attributes (flavor, taste, and texture) of fish flesh. Fish fed the CEF diet exhibited significantly ($p<0.05$) higher MWG, PWG, and SGR, and a lower FCR compared with other treatment groups. No significant differences ($p>0.05$) were found in proximate composition among the treatments. Carotenoid accumulation was greatest in CEF, followed by TCEF and TEF. Color measurements showed that fish receiving carotenoid-enriched diets, particularly CEF and TCEF, had significantly elevated lightness (L*), redness (a*), yellowness (b*), and chroma (C*) values ($p<0.05$). Palatability evaluation further confirmed a stronger preference for the fish fed with CEF, while the control fish scored the lowest. Overall, the results indicate that dietary inclusion of 5% carrot is highly effective in enhancing growth, pigmentation, and palatability in *L. rohita*.

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Introduction

In Bangladesh, fish production is increasing through feed-based aquaculture practices. As the country's fish production increases, the industrial production of fish feed correspondingly rises. Different varieties of fish feed are currently available on the market from various producers [1]. Disappointingly, most manufacturers fail to provide quality feed due to a lack of quality feed ingredients, the use of contaminated ingredients, and other factors [2]. The company uses lower-quality alternative raw materials because of fluctuating feed component availability and variable costs [3].

Since animal-origin protein and lipid sources are commonly used in the feed industry, aquaculture production will likely face sustainability challenges. This has led to an increased focus on locally available plant-based ingredients to create sustainable aquafeed. Moreover, the increasing demand for fish feed necessitates that possible natural sources be considered as potential feed ingredients. So, the available natural sources of carotenoids can be used as feed ingredients that improve growth and color, quality, ultimately increasing the market demand and value of the fish. Bangladesh produces a substantial amount of carotenoid-pigmented vegetables such as tomatoes, carrots. There is a drop in the price of crops when they are produced in large amounts in the peak production season. In this situation, adding these vegetables to fish feed as a possible source of feed ingredients can be advantageous for the aquaculture industry. Numerous studies have assessed various possible sources of carotenoids, including tomatoes, carrots, beetroot, marigold flowers, and spirulina [4-6].

Carotenoids are common pigments found in aquatic animals and play a significant role not only in coloration but also in growth, reproduction, and maintenance [7]. Researchers have shown interest in using these carotenoids as supplements in aquaculture to boost the growth and flesh quality in fish [6,8]. Moreover, demand and customer choice for fish and fish products are influenced by various factors, including the product's flavor, aroma, and color [9]. Consumer purchasing decisions can be swayed by color and smell, which can have physiological and psychological effects on the mind, ultimately affecting market demand for fish [10]. The growth, flesh color, and quality of carp fish can be enhanced through dietary natural carotenoids, which may promote market demand and price for the fish. However, several studies have been conducted worldwide on the impact of carotenoid-enriched feed on the enhancement of growth and coloration in various fish species, including *Puntius tetrazona, Cyprinus carpio, Dicentrarchus labrax, and Xiphophorus helleri* [4, 11-14]. Research on the effects of natural carotenoid-enriched feeds on the growth and flesh quality of cultured species in Bangladesh is scarce. Hence, a feeding trial was conducted to elucidate the effects of feeds enriched with natural carotenoid pigment sources (carrot and tomato) on the growth, flesh composition, flesh carotenoids and coloration, and palatability of *L. rohita* as a candidate species in aquaculture.

Materials and Methods

Experimental Site

The study was carried out in 12 cages that were set in a research pond located on the north side of the Department of Fisheries, University of Rajshahi, Bangladesh, for 90 days (June to September 2023). Each cage (measuring 2.73 m3) was constructed with an iron frame that was covered by a monofilament nylon net with a 5 mm mesh size. To facilitate feeding and fish handling, a small opening was maintained on the upper side of the cage.

Collection and Preparation of Natural Carotenoids

Natural pigment sources such as carrots and tomatoes were purchased from a local market in Rajshahi. After being collected, they were cleaned with water, cut into tiny pieces, and dried in the sun. Following drying, they were crushed and transformed into powder for feed preparation, and they were stored in a polythene bag.

Feed Preparation

Four experimental feeds were prepared, such as control feed (CF), tomato-enriched feed (TEF), carrot-enriched feed (CEF), and both tomato and carrot-enriched feed (TCEF). To prepare the feed, each ingredient was weighed (Table 1), and the required amount of water was mixed with it to form the dough. Using a pelletizer, the dough was formed into pellets, dried, and stored in sealed polythene bags at room temperature. The proximate compositions of the feed samples were analyzed and the data showed no significant difference among the feeds (Table 1) [15].

Table 1: Dietary Inclusion of each Ingredient and Proximate Compositions of the Feeds

Ingredients/Parameters (%)	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
Mineral Premix	0.5	0.5	0.5	0.5
Protein	29.35±0.48[a]	29.62±0.16[a]	29.67±0.08[a]	29.60±0.54[a]
Lipid	6.33±0.11[a]	6.52±0.34[a]	6.56±0.21[a]	6.34±0.10[a]
Carbohydrate	37.71±0.06[a]	37.29±0.56[a]	37.97±0.70[a]	37.68±0.34[a]
Moisture	12.52±0.29[a]	12.64±0.03[a]	12.39±0.12[a]	12.22±0.35[a]
Ash	7.7±0.22[a]	7.78±0.12[a]	7.72±0.07[a]	7.55±0.06[a]

*Values are presented as mean ± SD. CF is the pigment-free control diet; TEF, CEF, and TCEF contain 5% tomato, 5% carrot, and a mixture of 2.5% tomato + 2.5% carrot, respectively. Different superscripts in a row indicate significant differences ($p < 0.05$).

Experimental Design

The study was carried out by using four test feeds designated as four distinct treatments, viz., CF, TEF, CEF, and TCEF. Where CF was designated as the control fish (the fish fed with feed without a pigment source), TEF was designated as the fish group fed with 5% tomato enriched feed, CEF was designated as the fish group fed with 5% carrot enriched feed, and TCEF was designated as the fish group fed with a mixture of 2.5% tomato and 2.5% carrot enriched feed. Each treatment was replicated three times. A completely randomized design was used to arrange these four treatments across the settled cages.

Test Fish Releasing and Feeding

One hundred and thirty juvenile L. rohita were collected from a nearby aquaculture farm. The collected fish were acclimatized to the experimental conditions for one week. After acclimatization, 10 fish (starting at an average weight of about 100 g each) were released into twelve cages. Over ninety days, the test feeds were administered to fish twice daily at a rate of 5% (2.5% + 2.5%) of their body weight. The ration rate was adjusted by weighing the fish twice a month.

Monitoring of Water Quality Parameters

Throughout the study period, the water quality indicators, including temperature, pH, dissolved oxygen (DO), total alkalinity (TA), and ammonia-nitrogen (NH_3-N), were measured periodically using standard methods. The estimated values of these parameters showed no discernible variations and stayed within the permissible ranges for fish culture.

Sampling and Analysis of the Growth and Feed Utilization of the Fish

On the first day of the study, the weight of the fish in each cage was measured and recorded. Subsequently, the sampling was carried out at regular intervals of two weeks. Mean weight gain (MWG), specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR) were used to calculate growth performance and feed utilization using the standard formula.

Chemical Analysis of Fish Flesh

Three fish were sacrificed from each cage at the trial's end. The flesh was taken from different parts of the body and put in the fridge for subsequent analysis. The proximate composition (crude protein, lipid, carbohydrate, moisture, and ash content) of the flesh samples was analyzed using standard methods [15].

Carotenoid Content of Fish Flesh

Samples of the fish's flesh were weighed and ground with 90%

methanol using a mortar and pestle. Sample mixtures were then centrifuged for 12 minutes at 12,000 rpm. The supernatant was then poured into a different centrifuge tube. At wavelengths of 662 nm, 653 nm, and 470 nm, a spectrophotometer (Analytik Jena, Germany) was used to measure the optical density of the supernatant. The total concentration of carotenoids was measured according to Lichtenthaler and Wellburn [16].

Color Measurement

At the start and end of the feeding trial, three fish from each cage were randomly selected and anesthetized with a clove oil solution. Flesh color was measured using a tristimulus colorimeter (Model NH310, Shenzhen ThreeNH Technology Co., Ltd., China). Color readings were recorded as L*, a*, and b* values in accordance with the International Commission on Illumination (CIE, 1977), where L* represents lightness (0 = black, 100 = white), a* ranges from green (–) to red (+), and b* from blue (–) to yellow (+). Hue angle (H°) and chroma (C*) were calculated from a* and b* to describe color tone and saturation. H° denotes the hue position on the visible spectrum (0°, 30°, and 60° indicating red, orange, and yellow, respectively), while C* reflects color intensity. According to Hunt, hue angle and chroma were determined from a* and b* values using the following formulas [17]:

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

Palatability Test of Cooked Fish

At first, the collected fish (from each replication of four treatments) were scaled, gutted, and cut into loins. After washing, 500 g of fish from each treatment was cooked following the conventional procedure with spices. The fish loins from each treatment were marked and cooked together to avoid any cooking bias. After eating the cooked fish, the selected expert panelists blindly evaluated it based on its flavor, taste, and texture, using the structured scaling system (Table 2) according to Huss [18].

Table 2: Organoleptic/Sensory Scoring Scale for Palatability Test

Flavour	Taste	Texture	Score
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

Data Analysis

The statistical analysis of the data was preceded by one-way analysis of variance (ANOVA) and Duncan's multiple-range test using SPSS-21 (computer software developed by SPSS, USA). To determine significant differences in the values, $p < 0.05$ was used.

Results

Growth Performance and Feed Utilization

Significantly higher MWG and SGR were found in the fish of CEF, followed by the fish of TCEF and TEF, while the lowest value was recorded in the fish of CF (Table 3). During the study period, no mortality of the fish was observed among the treatments. Significantly lower (better) FCR values were calculated from the fish of CEF, while the higher FCR value was calculated from the fish of CF.

Table 3: Growth and Feed Utilization Parameters under Four Treatments

Parameters	Treatments (means ± SD)			
	CF	TEF	CEF	TCEF
MIW (g)	100.86±6.86^a	101.42±5.94^a	101.72±7.11^a	100.58±6.75^a
MFW (g)	223.22±5.57^c	239.46±6.09^b	258.96±8.74^a	242.06±6.24^b
MWG (g)	122.37±2.17^c	138.04±1.45^b	157.24±1.64^a	141.48±2.96^b
PWG (%)	121.32±0.80^c	138.06±3.21^b	153.11±2.94^a	142.59±3.17^b
SGR (%)	1.06±0.06	1.16±0.07^b	1.25±0.05^a	1.18±0.06^b
FCR	2.16±0.07^c	1.86±0.08^b	1.68±0.06^a	1.81±0.07^b

*Values are presented as mean ± SD. CF is the pigment-free control diet; TEF, CEF, and TCEF contain 5% tomato, 5% carrot, and a mixture of 2.5% tomato + 2.5% carrot, respectively. Different superscripts in a row indicate significant differences ($p < 0.05$).

Flesh Composition

The flesh composition of fish was analyzed to evaluate the changes in flesh quality due to natural carotenoid pigment sources in the feeds. The analyzed data of flesh crude protein, lipid, carbohydrate, moisture, and ash content are presented in Table 4. The crude protein and lipid contents were not significantly ($p>0.05$) different among the treatments, but they were estimated to be higher in the fish flesh of CEF. A significantly higher carbohydrate content was found in the fish flesh of TCEF, while the lower in CEF. In the case of flesh's moisture and ash contents, no significant differences were found among the treatments.

Table 4: Flesh Composition (% Wet Basis) of Fish under Four Treatments

Parameters	Treatments (means ± SD)			
	CF	TEF	CEF	TCEF
Protein	16.24±0.52^a	16.70±0.11^a	16.99±0.22^a	16.57±0.62^a
Lipid	2.02±0.33^a	2.06±0.35^a	2.48±0.27^a	2.19±0.21^a
Carbohydrate	2.08±0.12^b	2.15±0.07^b	2.03±0.10^b	2.57±0.19^a
Moisture	75.59±2.49^a	74.41±2.51^a	72.87±3.66^a	73.76±2.98^a
Ash	3.17±0.35^a	3.12±0.43^a	3.07±0.26^a	3.16±0.19^a

*Values are presented as mean ± SD. CF is the pigment-free control diet; TEF, CEF, and TCEF contain 5% tomato, 5% carrot, and a mixture of 2.5% tomato + 2.5% carrot, respectively. Different superscripts in a row indicate significant differences ($p < 0.05$).

Flesh Carotenoid Content

The findings revealed that the inclusion of natural carotenoid sources in the feeds markedly increased carotenoid deposition in fish flesh compared to the carotenoid-free feed (Table 5). At the beginning of the trial, carotenoid levels did not differ significantly among the treatment groups (p>0.05). By the end of the study, fish fed the CEF showed significantly higher (p<0.05) carotenoid accumulation than those receiving the other feeds.

Table 5: Carotenoid Content in Fish Muscle under Four Treatments

Parameters	Treatments (means ± SD)			
	CF	TEF	CEF	TCEF
Initial (µg/g)	$0.11{\pm}0.04^{a}$	$0.13{\pm}0.03^{a}$	$0.12{\pm}0.05^{a}$	$0.11{\pm}0.03^{a}$
Final (µg/g)	$0.13{\pm}0.07^{d}$	2.15 ± 0.19^{c}	3.39 ± 0.34^{a}	2.74 ± 0.21^{b}

*Values are presented as mean ± SD. CF is the pigment-free control diet; TEF, CEF, and TCEF contain 5% tomato, 5% carrot, and a mixture of 2.5% tomato + 2.5% carrot, respectively. Different superscripts in a row indicate significant differences (p < 0.05).

Effects on Coloration

Dietary carotenoids had a marked effect on fish flesh coloration (Table 6). Fish receiving the carotenoid-enriched feeds (CEF and TCEF) displayed significantly higher L* values (p<0.05), indicating lighter flesh than the control fish. Both a* and b* increased notably in the supplemented groups, and CEF recorded the greatest values for these parameters (a*: 8.16±0.67; b*: 9.04±0.65). Chroma was also significantly greater in all carotenoid-fed groups compared to CF, with the highest C* in CEF. Conversely, hue angle decreased markedly in the treated groups, with TEF showing the lowest H°.

Table 6: Effects of Carotenoid-Enriched Feeds on the Color Parameters of Fish Flesh

Parameters	Treatments			
	CF	TEF	CEF	TCEF
L*	29.34±0.87c	36.32±1.04b	42.18±0.75a	41.325±0.86a
a*	2.33±0.35c	6.83±0.62b	8.16±0.67a	7.76±0.61a
b*	5.68±0.53c	7.12±0.63b	9.04±0.65a	8.42±0.57a
H°	65.34 ± 2.84^{a}	45.37 ± 3.08^{b}	49.40 ± 3.14^{b}	48.18 ± 3.15^{b}
C*	6.46 ± 0.64c	9.44 ± 0.58b	11.23 ± 0.61a	10.12 ± 0.55a

*Values are presented as mean ± SD. CF is the pigment-free control diet; TEF, CEF, and TCEF contain 5% tomato, 5% carrot, and a mixture of 2.5% tomato + 2.5% carrot, respectively. Different superscripts in a row indicate significant differences (p < 0.05).

Palatability of Fish Flesh

After consuming cooked fish flesh, the selected panel of consumers provided their scores on the palatability attributes, which are summarized in Table 7. No significant (p>0.05) differences were observed in the flavor of cooked fish flesh across treatments; however, a relatively higher score was noted for fish from CEF. The fish from CEF and TCEF received significantly higher taste scores, while lower scores were recorded for CF and TEF. Similarly, a significantly higher texture score was noted for fish from CEF and TCEF, with lower scores for CF and TEF. Based on the total scores across the three organoleptic criteria, the flesh of cooked fish from the CEF treatment ranked first compared to the other treatments.

Table 7: Organoleptic Score of Cooked Fish Flesh under Four Treatments

Organoleptic criteria	Treatments (means ± SD)			
	CF	TEF	CEF	TCEF
Flavor	$7.11{\pm}1.45^{a}$	$7.55{\pm}1.94^{a}$	$8.22{\pm}1.20^{a}$	$7.55{\pm}1.33^{a}$
Taste	$6.96{\pm}1.10^{b}$	$7.33{\pm}1.21^{b}$	$8.66{\pm}1.42^{a}$	$8.44{\pm}0.88^{a}$
Texture	$6.88{\pm}1.05^{b}$	$7.77{\pm}0.63^{b}$	$8.88{\pm}1.04^{a}$	$8.37{\pm}1.13^{a}$

*Values are presented as mean ± SD. CF is the pigment-free control diet; TEF, CEF, and TCEF contain 5% tomato, 5% carrot, and a mixture of 2.5% tomato + 2.5% carrot, respectively. Different superscripts in a row indicate significant differences (p < 0.05).

Discussion

Growth Performance and Feed Utilization

Various studies show that there is a notable enhancement in the growth and survival of fish when they are fed a diet containing carotenoids as opposed to a diet lacking carotenoids [11,19,20]. The data from the current research showed that incorporating carotenoids into the diet resulted in a marked enhancement in the growth performance of L. rohita compared to the fish that did not receive the diet with carotenoids. The data also demonstrated that the fish given feed enriched with 5% carrot experienced the highest MWG, PWG, SGR, and lowest FCR, followed by the fish fed a combination of 2.5% carrot and 2.5% tomato enriched feed, and then the fish fed with 5% tomato enriched feed. The outcomes of the present study are comparable to the report of Jain et al., who found superior weight gain, specific growth rate, and positive feed conversion ratio in C. carpio, which was supplemented with 5% carrot-enriched feed [21]. Supportive evidence for the present study was also found in the report of Weerakkody and Cumaranatunga, who stated that the carotenoids have growth-promoting properties, and 100 mg/kg carrot-enriched feed resulted in higher weight gain, specific growth rate, and better feed conversion ratio in C. catla fed compared to tomato and beetroot-enriched feeds [22]. In another study, Kowalska et al. found better weight gain, specific growth, and feed conversion ratio in C. carpio when the fish were supplemented with 20% carrot-enriched feed [23]. Comparable results were also reported by Goda et al., who stated that carotenoid supplementation noticeably enhanced weight gain, daily growth index, and growth coefficient in the case of European sea bass [12]. The findings of the present investigation are also in accordance with the findings of Das, who observed that fish fed with 5% carrot-enriched feed exhibited superior growth and survival rates in comparison to those fed with 3% and 7% carrot powder [6].

Flesh Composition

In the present investigation, the outcome of the fish's proximate analysis revealed no significant variations in the crude protein, lipid, moisture, and ash contents. However, a notably higher protein and lipid content, along with lower moisture and ash content, were observed in the fish fed with 5% carrot-enriched feed. The results of our study are almost akin to the report of Das, who found no significant difference in the proximate composition of zebrafish among the treatments when the fish were fed with carrot meal [6]. Evidence supporting the current findings was also found in the report of Jha et al., who found relatively higher flesh protein and lipid in Snow Trout fed with marigold flower and beetroot meals, whereas Pailan et al. found no significant difference in the proximate composition of P. conchonius fed with rose petal-enriched feed [24, 25]. Conversely, Christiansen and Torrissen recorded higher carcass lipid contents and lower moisture contents in fry and juveniles of Salmo salar when they fed with astaxanthin-containing feed [26]. The variation in findings among researchers regarding flesh compositions of fish fed carotenoid-enriched feed may be due to differences in fish species or variations in carotenoid sources.

Carotenoid Content

Carotenoids are the primary source of pigmentation in fish, typically acquired through the consumption of carotenoid-rich organisms in the aquatic food chain; however, captive culture often requires dietary supplementation. The results of the study revealed that fish fed with 5% carrot-enriched feed deposited the highest amount of carotenoid in the flesh compared to the fish fed with other carotenoid-sources enriched feeds. The result of the study is similar to the report of Das, where the addition of 5% carrot powder to the feed enhanced carotenoid deposition and pigmentation in zebrafish [6]. The results of our study are also analogous to the findings of Jain et al., who found the highest amount of carotenoid deposition in C. carpio, which was fed with 5% carrot-enriched feed [21]. The present results also concur with the study of Ramamoorthy et al., who found that feed containing carrot powder enhances carotene deposition and coloration in Amphipriono cellaris [27].

Effects on Coloration

Dietary supplementation with carotenoids markedly improved fish flesh color. Fish fed the CEF and TCEF diets displayed higher L* values than the control, indicating lighter flesh, and also showed notable increases in a*, b*, and C*, with the CEF group achieving the greatest pigmentation. These results demonstrate efficient carotenoid assimilation and are consistent with previous findings in C. catla, C. carpio, and Badis badis [13,21,22,28]. The pronounced pigmentation effect of CEF may be due to the elevated β-carotene content in carrot, a strong natural pigment, whereas the slightly reduced response in TCEF could be related to differences in carotenoid bioavailability or interactions among the pigments [29]. Overall, tomato and carrot-based carotenoids proved to be effective natural color enhancers, improving flesh lightness, redness, yellowness, and saturation while shifting hue in a favorable direction.

Palatability of Fish Flesh

In the present study, the organoleptic score for the palatability test indicates that the texture, taste, and flavor of the test fish have changed due to the addition of natural carotenoids to the fish feed. Based on the panelists' opinions, the organoleptic score indicates that the fish fed with 5% carrot-enriched feed was the most palatable. This might be due to the outcome of fish lipid content in the flesh of fish that were fed with carrot powder-enriched feed. Lipids exist in foods as emulsions or as free oil/fat dispersed throughout a solid matrix and are known to affect the texture and flavor of foods. Lipids contribute to flavor by producing volatile oxidation products and imparting the taste of short-chain free fatty acids [30]. The present study results align with the report by Hosen et al, who obtained an overall better palatability score from silver barb that was fed with carrot-enriched feed [31]. However, studies on the impact of growth promoters on the palatability of fish are scarce, and additional research is required to draw definitive conclusions.

Conclusion

The study concluded that the addition of 5% carrot powder in fish feed is recommended to enhance the growth, flesh quality, and palatability of L. rohita without any adverse effects. However, more research should be carried out to identify the appropriate dosages and to determine the long-term effects of using natural carotenoid pigment sources in the aquafeed.

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