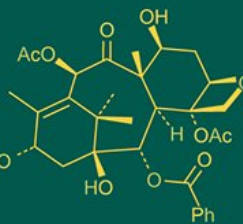
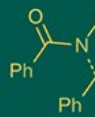
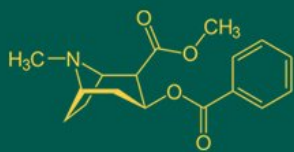


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Effects of dietary supplementation of carrot and beetroot on pigmentation, growth and survival of orange molly, *Poecilia sphenops*

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Abstract

The present study was conducted to assess the effects of dietary supplementation of carrot and beetroot on pigmentation, growth and survival of Orange molly, *Poecilia sphenops* for 60 days at Wet laboratory, Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval. The experiment was carried out with six experimental diet and control group. The control diet was prepared without any supplementation. Treatment C1, B1, C2, B2, CB1 and CB2 consisted of 5% carrot, 5% beetroot, 10% carrot, 10% beetroot, 5% mixture of carrot and beetroot and 10% mixture of carrot and beetroot respectively. The experimental design used was completely randomized with three replications for each treatment group. The weight, weight gain, FCR, SGR, carotenoid content and survival of the experimental fish (*Poecilia sphenops*) was recorded every 15 days during the experimental period. The results of the present study showed that the highest carotenoid content was found in treatment B2 ($5.55 \pm 0.024 \mu\text{g/g}$) followed by treatments C2 ($5.42 \pm 0.006 \mu\text{g/g}$), CB2 ($5.32 \pm 0.011 \mu\text{g/g}$), CB1 ($5.02 \pm 0.011 \mu\text{g/g}$), B1 ($4.89 \pm 0.037 \mu\text{g/g}$) and C1 ($4.78 \pm 0.011 \mu\text{g/g}$). The highest weight was also observed in treatment B2 compared to all treatments and control group ($p < 0.01$). The better feed conversion ratio was found in treatment B2 which contained 10% beetroot. The highest survival rate of the fish was found in treatment B2 and C1 compared to all other treatments and control group. Based on the results of present study, it can be concluded that dietary supplementation of carrot and beetroot is effective for enhancing growth and colouration of the orange molly (*Poecilia sphenops*).

Keywords: Carrot, beetroot, pigmentation, orange molly, carotenoid content

1. Introduction

Aquaculture or ornamental fish culture, has become increasingly popular worldwide because of the aesthetic and recreational importance of ornamental fish. These fishes are often referred to as “living gems” because of their bright colours, unique body shapes, and attractive swimming behaviour. They are commonly maintained in aquariums and garden ponds, enhancing the beauty of indoor and outdoor environments. In recent years, consumer interest in ornamental fishkeeping has increased rapidly due to the growing preference for visually attractive home décor and the relaxing effect associated with aquariums (Kumar *et al.*, 2015) [20]. The ornamental fish industry also provides considerable economic opportunities. Ornamental fish are highly valuable compared to food fish and therefore contribute significantly to income generation and livelihood development. According to Pandey *et al.* (2017) [28], ornamental aquarium fish can be sold at prices much higher than food fish, while marine ornamental fishes often have greater market value than freshwater species. This increasing commercial demand has created opportunities for employment, economic diversification, and sustainable income generation in several countries, including India (MPEDA, 2013) [24].

India possesses rich ornamental fish diversity because of its extensive freshwater and marine ecosystems. Marine ornamental fishes are widely distributed in the lagoons and coral reef ecosystems of Lakshadweep and Minicoy Islands, the Andaman and Nicobar Islands, the Gulf of Kutch, Kerala coast, Gulf of Mannar, and Palk Bay. These coastal ecosystems are rich in biodiversity and serve as important centres for ornamental fish collection and trade (Pandey *et al.*, 2017) [28]. Globally, ornamental fish trade has expanded rapidly over the past few decades and continues to grow. The total industry value, including exports, domestic sales, labour, and associated products, is estimated at nearly 15 billion

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USD (FAO, 2022) ^[6]. The global ornamental fish market was valued at 5.88 billion USD in 2022 and is expected to continue growing steadily. Although India has rich fish biodiversity, its contribution to the global ornamental fish trade remains limited, accounting for only about 0.4%, with an estimated market share of 500 crore ₹ (PMMSY, 2020).

Among freshwater ornamental fish, Orange molly (*Poecilia sphenops*) is one of the most popular and widely cultured species. It belongs to the family *Poeciliidae* and is well known for its bright orange colour, attractive appearance, and adaptability. The species is distributed widely across North America, Europe, Asia, and India. Orange mollies are primarily freshwater fish but can also tolerate brackish water conditions. They are commonly found in coastal waters, estuaries, lagoons, and brackish swamps. Their ability to tolerate changes in salinity and water quality makes them a hardy and suitable ornamental fish for aquarium culture (Froese *et al.*, 2019) ^[7].

Colour plays an important role in determining the quality and market value of ornamental fish. Fish with bright and attractive pigmentation are preferred in the aquarium trade and generally receive higher prices in the market. However, fish are unable to synthesize most pigments naturally and depend on dietary sources such as plants, algae, and microorganisms for colour development. Maintaining the natural body colour of ornamental fish under captive conditions is one of the major challenges faced by ornamental fish producers. Poor pigmentation reduces market value and affects commercial acceptance. Among various colouring agents, carotenoids are considered the most effective for improving pigmentation in ornamental fish (Gupta *et al.*, 2007) ^[9].

Carotenoids are natural pigments obtained from both synthetic and natural sources. These pigments are responsible for yellow, orange, and red coloration in fish skin. Fish possess pigment cells called chromatophores in their skin. Carotenoids supplied through feed are absorbed in the intestine, transported through the bloodstream, and deposited in tissues, which enhances skin pigmentation and body colour (Swain *et al.*, 2020) ^[36]. Natural carotenoids are mainly obtained from plant and animal sources. Green vegetables and brightly coloured fruits are rich in carotenoids such as beta-carotene, lycopene, lutein, and zeaxanthin. Among these, carrot and beetroot are important natural pigment sources and are commonly available.

Carrot (*Daucus carota* subsp. *sativus*) is an important root vegetable and a rich source of beta-carotene, vitamins, and

antioxidants. It is widely consumed and valued because of its nutritional importance. Carrots contain carotenoids, phenolic compounds, ascorbic acid, and tocopherols, which are beneficial in pigmentation and health improvement (Hashimoto and Nagayama, 2004) ^[11]. Beta-carotene is especially important as a natural pigment source and has several beneficial properties including antioxidant and immune-enhancing effects. Beetroot (*Beta vulgaris* L.) is another important root vegetable widely recognized for its nutritional and pigment content. It contains carotenoids, flavonoids, vitamins, minerals, and betalain pigments such as betacyanins and betaxanthins, which provide strong antioxidant activity and contribute to natural colour enhancement. Beetroot is also considered a valuable natural feed additive because of its pigmentation properties and nutritional value (Panghal *et al.*, 2017) ^[29].

Considering the growing importance of ornamental fish culture and the commercial need for improved pigmentation, natural plant-based carotenoid supplementation has become an important area of research. Carrot and beetroot are economical and easily available sources of natural pigments that may improve body colour along with growth and survival of ornamental fishes. Therefore, the present study was undertaken to evaluate the effect of dietary supplementation of carrot and beetroot on pigmentation, growth, and survival of Orange molly (*Poecilia sphenops*).

Materials and Methods

The experiment entitled “Effects of dietary supplementation of carrot and beetroot on pigmentation, growth and survival of Orange molly, *Poecilia sphenops*” was conducted in the Wet laboratory, Department of *Aquaculture*, College of Fisheries Science, Kamdhenu University, Veraval for 60 days from 22nd March to 20th May 2024. Experimental animal (*Poecilia sphenops*) was procured and transported in polythene bags by road from the local aquarium shop near Veraval to the experimental site. After the proper acclimatization of the fish for about 7 days, healthy fish were added to into the plastic aquariums for the experiment. A total of 15 fish were stocked in each of the tank. The experiment was carried out with six treatments and a control group in triplicates. Aeration systems were installed in each tank to maintain optimal oxygen levels. Continuous aeration was provided through aerators, with each tank equipped with an aeration pipe, air stones and pressure regulators to precisely control the airflow and ensure consistent oxygen concentrations throughout the experiment.

Table 1: Proximate composition of feed ingredients (%)

Ingredients	Crude protein (%)	Crude-fat (%)	Ash (%)	Moisture (%)
Fish meal	58.00	10.39	21.68	7.63
Groundnut oil cake	40.45	7.50	4.51	11.72
Wheat flour	10.67	0.74	1.15	5.95
Tapioca flour	4.00	10.23	0.25	7.63
Carrot	1.30	1.97	0.98	4.27
Beetroot	2.20	0.28	1.40	5.12

Seven experimental diets containing 35 % protein were formulated. Treatment diet CB0 was a control diet that did not contain either carrot or beetroot supplementation. The other six diets were containing different levels of carrots and beetroot supplementation. Carrots and beetroot were added

at the rate of 5% and 10% in each of the four treatments. In the other two treatments mixture of carrots and beetroot was added at the rate of 5 % and 10 %.

The treatments were designated as follows:

CB0	= Diet supplemented without carrots and beetroot
C1	= Diet supplemented with 5% carrot
B1	= Diet supplemented with 5 % beetroot
C2	= Diet supplemented with 10 % carrot
B2	= Diet supplemented with 10 % beetroot
CB1	= Diet supplemented with a mixture of 5 % carrot and beetroot
CB2	= Diet supplemented with a mixture of 10 % carrot and beetroot

Table 2: Composition of Experimental Diets

Ingredients (gm)	CB0 Control	C1 (5%)	B1 (5%)	C2 (10%)	B2 (10%)	CB1 (5%)	CB2 (10%)
Carrot	0	5	0	10	0	2.5	5
Beetroot	0	0	5	0	10	2.5	5
Fish meal	37	38	38	39.5	38	38	39
GNOG	27	27	27	26	28	27	26.5
WF	21	15	15	11	10.5	15	11.5
TP	9	9	9	7.5	7.5	9	7
Plant oil	2	2	2	2	2	2	2
Fish oil	2	2	2	2	2	2	2
Vit mix	2	2	2	2	2	2	2
Total	100	100	100	100	100	100	100

2.1 Experimental Procedure

2.1.1 Effects of carrot and beetroot on the growth, survival and FCR (feed conversion ratio) of orange molly, *poecilia spheps*.

The effect of the carrot and beetroot on the growth and survival of Orange Molly was measured at the interval of 15 days by collecting fish from individual tanks and the average weight of the fish was recorded by using electronic balance. The survival of the fish was observed daily during the research work. FCR was measured after 60 days of the experiment by the data obtained from weight gain during the experimental period. The fish were fed with different experimental diets @ 2% of body weight twice daily.

2.1.2 Effects of carrot and beetroot on the pigmentation of orange molly, *poecilia spheps*

The total carotenoid concentration of the fish was estimated at 15 days interval during the experimental period by the standard method given by Olson *et al.* (1979) [26]. The skin and muscle tissue of the fish were used to estimate the carotenoid concentration. After the feeding trial, skin and muscle samples were collected from the dorsolateral region of the fish to determine total carotenoid content. One gram of tissue was transferred to a 10 ml screw-capped vial and homogenized with a glass rod. Then, 2.5 g of anhydrous sodium sulfate and 5 ml of chloroform were added, and the vial was tightly sealed. The samples were kept overnight in the dark to allow extraction. Once a clear chloroform layer formed above the residue, 0.3 ml of the extract was taken and diluted with 3 ml of absolute ethanol in a cuvette. The absorbance was measured using a spectrophotometer at wavelengths of 375, 400, 450, and 500 nm. A blank sample was prepared following the same method without adding

fish tissue for comparison.

By using the following formula, the total carotenoid content was measured:

$$\text{Total carotenoid content} = \frac{\text{Absorption at maximum wavelength} \times 10}{0.25 \times \text{sample weight}}$$

Where, 10 = Dilution factor

0.25 = Extinction coefficient

3. Results and Discussion

The aim of the study was to assess the effects of dietary supplementation of carrot and beetroot on various aspects of the orange molly, *Poecilia spheps* such as growth performance, survival rate and pigmentation. Observations such as weight, weight gain, feed conversion ratio (FCR), specific growth rate (SGR), survival rate and carotenoid content were recorded throughout the experiment. The results of the study are presented in tables and illustrated graphically. The experiment consisted of six treatments and a control in triplicates to ensure reliability and statistical significance of the results.

3.1 Effects of dietary supplementation of carrot and beetroot on growth of orange molly, *poecilia spheps*.

3.1.1 Weight (g): During the 60-day experiment, 15 *Poecilia spheps* were randomly sampled from each treatment group every two weeks to monitor changes in body weight. The weight of the fish was recorded at each sampling, and the average weight was calculated for each treatment group. The total weight of the fish in each treatment group throughout the experiment is summarized in Table 3.1. and graphically represented in Figure 3.1.

Table 3: Weight (g) of orange molly (*Poecilia spheps*) fed with different experimental diets (Mean ±SE, n=3)

DAYS	TREATMENT							Mean
	CB0	C1	B1	C2	B2	CB1	CB2	
0	0.68 ^a ±0.003	0.68 ^a ±0.003	0.68 ^a ±0.003	0.68 ^a ±0.003	0.68 ^a ±0.003	0.68 ^a ±0.003	0.68 ^a ±0.003	0.68 ±0.001
15	0.86 ^f ±0.012	0.88 ^{ef} ±0.012	0.91 ^e ±0.009	1.08 ^b ±0.012	1.15 ^a ±0.012	0.96 ^d ±0.015	1.01 ^c ±0.015	0.98 ±0.040
30	0.95 ^f ±0.023	0.98 ^f ±0.020	1.06 ^e ±0.019	1.23 ^b ±0.009	1.32 ^a ±0.012	1.11 ^d ±0.012	1.18 ^c ±0.009	1.12 ±0.050
45	1.03 ^g ±0.018	1.12 ^f ±0.009	1.16 ^e ±0.012	1.33 ^b ±0.012	1.45 ^a ±0.015	1.22 ^d ±0.023	1.27 ^c ±0.003	1.23 ±0.050
60	1.22 ^g ±0.012	1.25 ^f ±0.012	1.31 ^e ±0.015	1.53 ^b ±0.015	1.60 ^a ±0.012	1.37 ^d ±0.015	1.43 ^c ±0.019	1.38 ±0.050
Mean	0.95	0.98	1.03	1.17	1.24	1.07	1.11	-
TRETMENT		SEM (±)		CD @ 1%			CD @ 5%	CV (%)
D(Days)		0.000		0.019			0.014	2.101
T(Treatment)		0.000		0.022			0.017	
T X D		0.000		0.049			0.037	

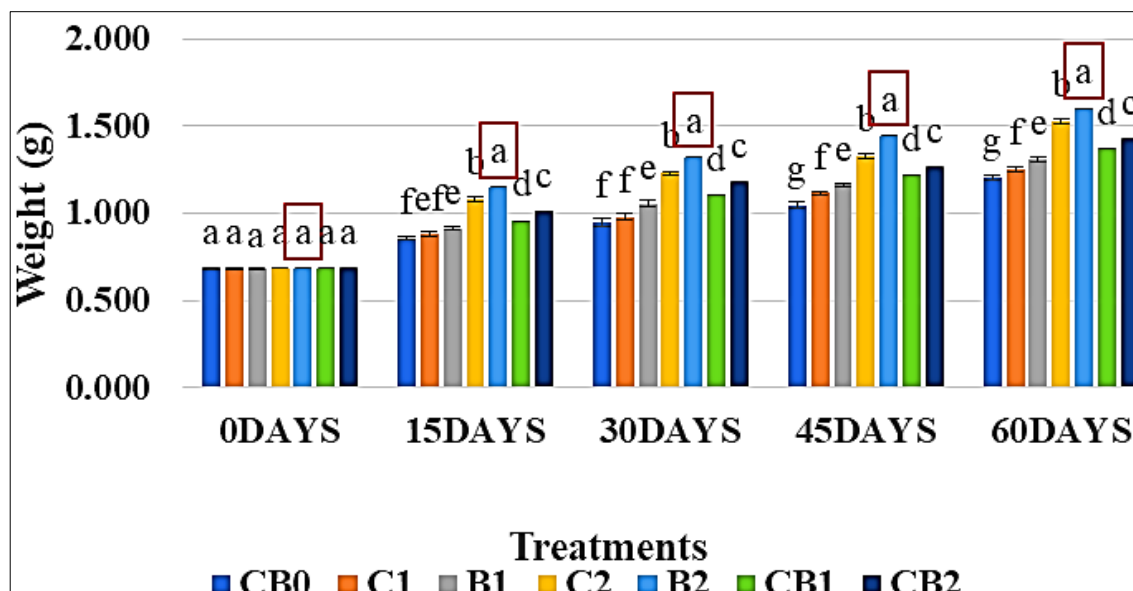


Fig 1: Weight (g) of orange molly (*Poecilia sphenops*) fed with different experimental diets

The initial average weight of *Poecilia sphenops* was observed 0.68 ± 0.001 g in all the treatment group. Significantly highest weight was observed in treatment B2 (1.60 ± 0.012 g) among all the treatments at the end of the experiment. Treatment B2 showed the significantly higher weight (1.60 ± 0.012 g) followed by treatments C2 (1.53 ± 0.015 g), CB2 (1.43 ± 0.019 g), CB1 (1.37 ± 0.015 g), B1 (1.31 ± 0.015 g), C1 (1.25 ± 0.012 g) and control (1.22 ± 0.012 g) after 60 days of the experiment. All the treatments showed significantly higher weight than the control group. The results indicated that fish fed a diet containing 10% beetroot (B2) exhibited the significantly highest growth rates compared to the control group.

Monica *et al.* (2019) [23] reported that dietary inclusion of 0.4% beetroot peel significantly increased weight gain in swordtail fish compared to the control, which supports the findings of the present study. Similar results were reported by Hosen *et al.* (2022) [12] and Amiri *et al.* (2023) [1], who

observed improved growth performance in fish fed beetroot-supplemented diets. Zutshi and Madiyappa (2020) [42] and Neverian *et al.* (2014) [25] also reported enhanced growth with beetroot or carotenoid-rich plant ingredients, confirming their positive role in fish growth. In the present study, cooked beetroot may have contributed to better growth by reducing *anti-nutritional* compounds such as tannins and oxalates. However, Gil *et al.* (2017) [30] and Castro-Castellón *et al.* (2023) [4] reported contrasting findings, where higher beetroot inclusion had no positive effect or reduced growth, likely due to differences in inclusion level, preparation method, and experimental species.

3.1.2 Weight gain (g)

According to the data obtained from the weight, the weight gain was calculated in all the treatment and the average weight gain of the experimental animal is presented in table 3.2 and graphically presented in Fig. 3.2.

Table 4: Weight gain (g) of orange molly (*Poecilia sphenops*) fed with different experimental diets (Mean $\pm$ SE, n=3)

Days	TREATMENT							Mean
	CB0	C1	B1	C2	B2	CB1	CB2	
15	0.17 ^f ±0.009	0.20 ^{ef} ±0.014	0.23 ^e ±0.010	0.39 ^b ±0.009	0.47 ^a ±0.009	0.27 ^d ±0.017	0.33 ^c ±0.015	0.29 ±0.041
30	0.27 ^f ±0.023	0.29 ^f ±0.023	0.37 ^e ±0.017	0.55 ^b ±0.009	0.64 ^a ±0.010	0.42 ^d ±0.013	0.49 ^c ±0.012	0.43 ±0.051
45	0.36 ^g ±0.018	0.43 ^f ±0.012	0.48 ^e ±0.012	0.64 ^b ±0.012	0.76 ^a ±0.012	0.53 ^d ±0.020	0.58 ^c ±0.007	0.54 ±0.051
60	0.52 ^f ±0.018	0.57 ^f ±0.015	0.63 ^e ±0.012	0.84 ^b ±0.015	0.92 ^a ±0.010	0.68 ^d ±0.018	0.74 ^c ±0.018	0.70 ±0.054
Mean	0.40	0.44	0.48	0.62	0.69	0.52	0.57	-
TRETMENT		SEM (±)		CD @ 1%		CD @ 5%		CV (%)
D(Days)		0.000		0.019		0.014		4.344
T(Treatment)		0.000		0.022		0.017		
T X D		0.000		0.050		0.038		

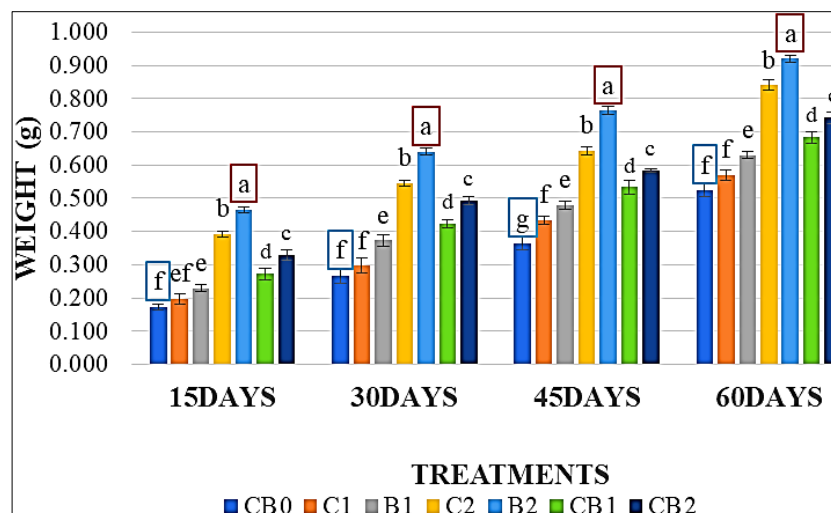


Fig 2: Weight gain (g) of orange molly (*Poecilia sphenops*) fed with different experimental diets

The weight gain of orange molly was calculated during the experiment at an interval of every 15 days. The results revealed that treatment B2 had the highest value of weight gain and control treatment showed the lowest weight gain during the experiment. The statistical analysis revealed a significant difference in weight gain among all treatment groups ($p < 0.01$). The results of the present study showed that dietary supplementation with carrot and beetroot significantly improved the growth performance of *Poecilia sphenops*. Fish fed the supplemented diets recorded higher final weight and weight gain than the control group, indicating the positive effect of these natural additives. The improved growth may be due to the presence of antioxidants, vitamins, minerals, and other bioactive compounds in carrot and beetroot, which could enhance metabolism, nutrient utilization, and overall fish health. Similar findings were reported by Jha *et al.* (2012) [16], while

studies by Sathiyaruban *et al.* (2024) [33], Hosen *et al.* (2022) [12], Sung and Abol-Munafi (2020) [35], Verma *et al.* (2015) [37], and Pailan *et al.* (2011) also supported the beneficial role of plant-based carotenoid sources in promoting fish growth. These ingredients may improve gut microbial activity and feeding efficiency, resulting in better nutrient utilization and growth. However, Gil *et al.* (2017) [30] reported reduced growth at higher beetroot inclusion levels, possibly due to *anti-nutritional* compounds such as tannins and oxalates.

3.1.3 Feed conversion ratio (fcr): FCR is the ratio of the feed consumed by the organism to increase the body weight for a given time. It is the amount obtained by dividing the total amount of the feed given by the weight gain in a certain time. Table 3.3 represents the data recorded for FCR. Graphical representation for FCR is showed in figure 3.3.

Table 5: Feed Conversion Ratio (FCR) of orange molly (*Poecilia sphenops*) fed with different experimental diets (Mean $\pm$ SE, n=3)

TRETMENT	MEAN $\pm$ SE
CB0	3.14 ^a $\pm$ 0.089
C1	2.90 ^b $\pm$ 0.096
B1	2.86 ^{bc} $\pm$ 0.110
C2	2.36 ^{ef} $\pm$ 0.040
B2	2.23 ^f $\pm$ 0.037
CB1	2.64 ^{cd} $\pm$ 0.070
CB2	2.52 ^{de} $\pm$ 0.030

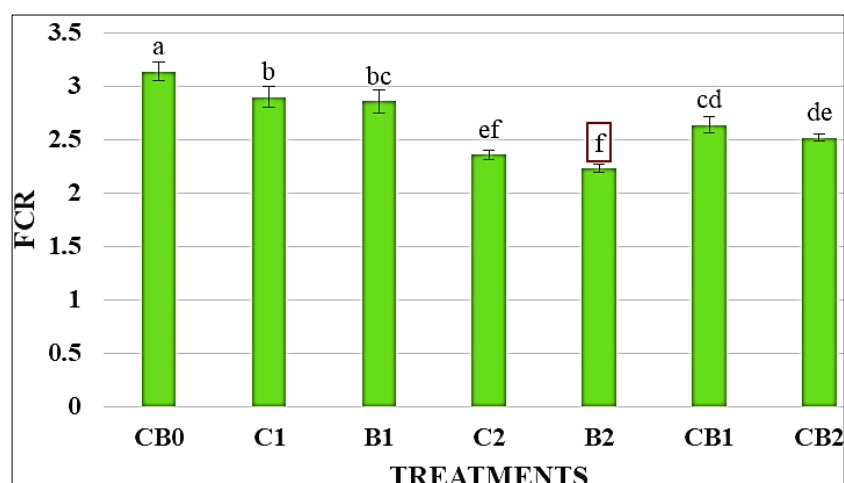


Fig 3: Feed Conversion Ratio (FCR) of orange molly (*Poecilia sphenops*) fed with different experimental diets

Statistical analysis showed that feed conversion ratio (FCR) was significantly influenced by the dietary treatments, with the best FCR recorded in treatment B2 and the poorest in the control group ($p < 0.01$). Treatment B2 showed the lowest FCR (2.23 ± 0.037), followed by C2 (2.36 ± 0.044), CB2 (2.52 ± 0.030), CB1 (2.64 ± 0.073), B1 (2.86 ± 0.110), and C1 (2.90 ± 0.096), while the control group recorded the highest FCR (3.14 ± 0.089). Treatments B2 and C2, C1 and B1, and CB1 and CB2 remained statistically at par, but all treatments performed significantly better than the control. These findings are supported by Jha *et al.* (2012) [16], who reported improved FCR in *Schizothorax richardsonii* fed a 10%

beetroot diet. Similar observations were also reported by Prabhath *et al.* (2019) [31], Sun *et al.* (2012) [34], and Jain and Kaur (2016) [17, 13], supporting the positive effect of beetroot supplementation on feed utilization in fish.

3.1.4 Specific growth rate (sgr)

The specific growth rate is a coefficient that is used to measure the percentage increase in fish weight per day. It is used to estimate the production of the fish after a certain time. The data calculated for SGR is presented in Table 3.4. Figure 3.4 showed graphical representation of specific growth rate of Orange molly.

Table 6: Specific Growth Rate (%) of orange molly (*Poecilia spheps*) fed with different experimental diets (Mean $\pm$ SE, $n=3$)

TREATMENT	MEAN $\pm$ SE
CB0	$0.94^g \pm 0.028$
C1	$1.01^f \pm 0.023$
B1	$1.08^e \pm 0.012$
C2	$1.33^b \pm 0.019$
B2	$1.42^a \pm 0.009$
CB1	$1.16^d \pm 0.025$
CB2	$1.23^c \pm 0.019$

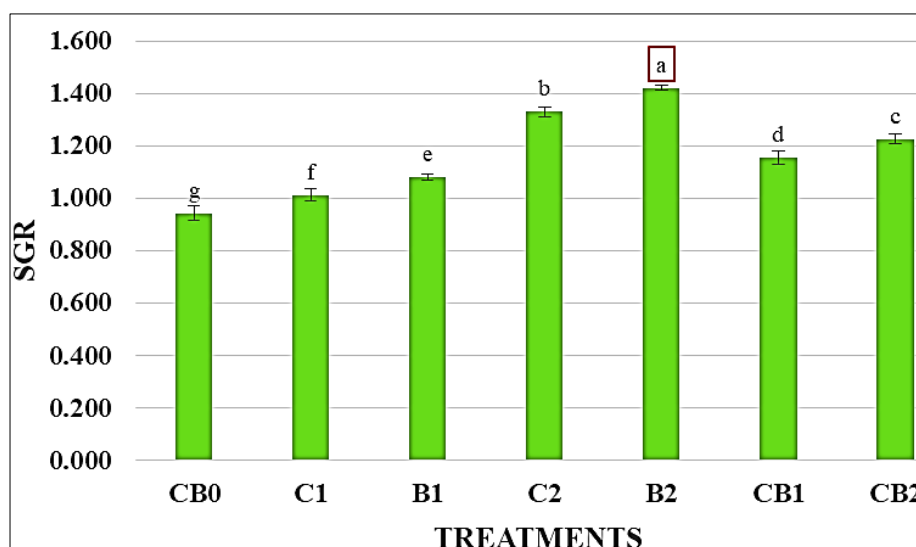


Fig 4: Specific Growth Rate (%) of orange molly (*Poecilia spheps*) fed with different experimental diet

The results of the study showed that the control group without supplementation recorded the lowest specific growth rate (SGR), while all supplemented treatments showed significantly improved growth. Among them, treatment B2 recorded the highest SGR (1.42 ± 0.009), followed by C2 (1.33 ± 0.019), CB2 (1.23 ± 0.019), CB1 (1.16 ± 0.025), B1 (1.08 ± 0.012), and C1 (1.014 ± 0.023), whereas the control group showed the lowest value (0.94 ± 0.028). Statistical analysis indicated that treatment B2 had significantly higher SGR than all other treatments ($p < 0.01$). Similar findings were reported by Jana *et al.* (2023) [15], who observed the highest SGR in *Poecilia spheps* fed with 5% carrot meal. Comparable results were also reported by Das (2024) [5] and Bisht *et al.* (2022) [3], supporting the beneficial effects of natural carotenoid-rich plant ingredients on fish growth. However, Yanar *et al.* (2016) [40] found no significant difference in SGR in *Oncorhynchus mykiss* fed

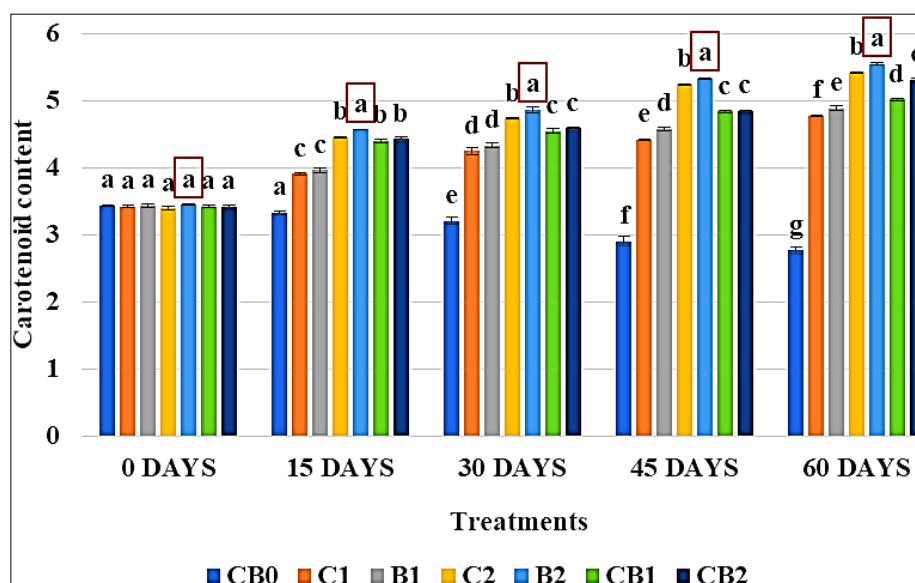
diets supplemented with plant-based carotenoid sources, indicating that the response may vary depending on fish species and dietary composition.

3.2 Effects of dietary supplementation of carrot and beetroot on pigmentation of orange molly, *poecilia spheps*:

The present study was conducted to evaluate the effect of dietary supplementation of carrot and beetroot on the pigmentation of *Poecilia spheps* over a 60-day experimental period using a completely randomized design. Carotenoid content was analyzed following the method described by Olson *et al.* (1979) [26]. The carotenoid concentration in fish samples was estimated at 15-day intervals using a spectrophotometer, and the optical density of each sample was recorded at 500 nm. The carotenoid content obtained in $\mu\text{g/g}$ is presented in Table 3.5, while its graphical representation is shown in Figure 3.5.

Table 7: Carotenoid content ($\mu\text{g/g}$) of orange molly (*Poecilia spheonops*) fed with different experimental diets (Mean $\pm$ SE, $n=3$)

Days	Treatment							Mean
	CB0	C1	B1	C2	B2	CB1	CB2	
0	3.44 ^a ±0.014	3.43 ^a ±0.013	3.44 ^a ±0.023	3.40 ^a ±0.023	3.45 ^a ±0.013	3.43 ^a ±0.013	3.43 ^a ±0.013	3.43
15	3.33 ^d ±0.027	3.91 ^c ±0.018	3.97 ^c ±0.029	4.46 ^b ±0.006	4.57 ^a ±0.007	4.40 ^b ±0.023	4.43 ^b ±0.024	4.15
30	3.21 ^e ±0.048	4.25 ^d ±0.048	4.34 ^d ±0.030	4.74 ^b ±0.009	4.87 ^a ±0.044	4.55 ^c ±0.035	4.59 ^c ±0.018	4.36
45	2.91 ^f ±0.071	4.42 ^e ±0.011	4.58 ^d ±0.028	5.24 ^b ±0.011	5.34 ^a ±0.009	4.84 ^c ±0.011	4.86 ^c ±0.009	4.59
60	2.77 ^g ±0.053	4.78 ^f ±0.011	4.89 ^e ±0.037	5.42 ^b ±0.006	5.55 ^a ±0.024	5.02 ^d ±0.011	5.32 ^c ±0.011	4.82
Mean	3.13	4.16	4.24	4.65	4.76	4.46	4.53	-
TRETMENT		SE(m)		CD @ 1%		CD @ 5%		CV%
D(Days)		0.001		0.038		0.029		1.094
T(Treatment)		0.001		0.045		0.034		
T X D		0.001		0.101		0.076		

**Fig 5:** Carotenoid content ($\mu\text{g/g}$) of orange molly (*Poecilia spheonops*) fed with different experimental diets

In the present study, the initial average total carotenoid content was $3.43 \mu\text{g/g}$ in all treatment groups. Carotenoid concentration showed a gradual increase in all supplemented groups throughout the 60-day experimental period, while the control group showed a decline. Statistical analysis revealed significant differences among all treatments ($p < 0.01$). At 15 days, treatment B2 recorded significantly higher carotenoid content than the control and other treatments, while C2, CB1, and CB2 showed comparable results and remained significantly higher than the control. Similar trends were observed after 30 and 45 days, with B2 consistently recording the highest carotenoid concentration. At the end of the experiment, treatment B2 showed the highest total carotenoid content ($5.55 \pm 0.024 \mu\text{g/g}$), followed by C2 ($5.42 \pm 0.006 \mu\text{g/g}$), CB2 ($5.32 \pm 0.011 \mu\text{g/g}$), CB1 ($5.02 \pm 0.011 \mu\text{g/g}$), B1 ($4.89 \pm 0.037 \mu\text{g/g}$), and C1 ($4.78 \pm 0.011 \mu\text{g/g}$), whereas the control group recorded the lowest carotenoid content ($2.77 \pm 0.053 \mu\text{g/g}$). The results indicate that dietary supplementation with carrot and beetroot significantly enhanced carotenoid deposition and pigmentation in *Poecilia spheonops*, with 10% beetroot supplementation (B2) showing the most effective pigmentation response.

The results of the present study showed that dietary supplementation with carrot and beetroot significantly enhanced the pigmentation of *Poecilia spheonops*. Fish fed diets supplemented with these natural carotenoid sources exhibited improved coloration compared to the control group. Similar findings were reported by by Castro-Castellón *et al.* (2023) [4] and Lili *et al.* (2018) [21], where

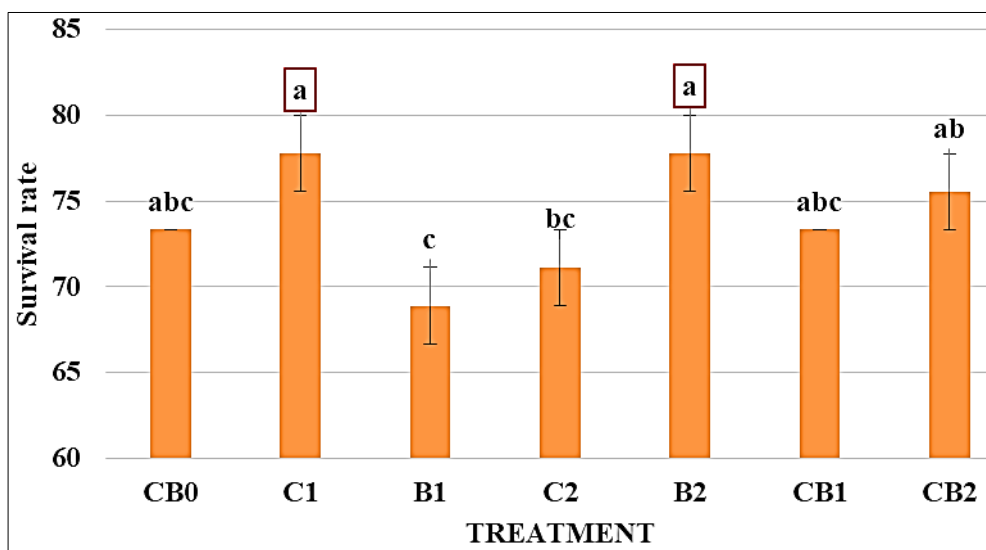
beetroot supplementation resulted in higher carotenoid deposition and better pigmentation in ornamental fish. Gamit *et al.* (2022) [8] and Haetami *et al.* (2020) [10] also observed improved colour enhancement in orange molly and *Carassius auratus* with carrot-based diets. Since fish are unable to synthesize carotenoids on their own, dietary supplementation plays an important role in pigment deposition and colour development. The present findings are further supported by several earlier studies, including Yousefi *et al.* (2023) [41], Bisht *et al.* (2022) [3], Jain *et al.* (2019) [14], Wagde *et al.* (2018) [38], Kumar *et al.* (2017) [19], Maiti *et al.* (2017) [22], Weerakkody and Cumaranatunga (2016) [39], Ramamoorthy *et al.* (2010) [32], and Kop *et al.* (2010) [18], all of which reported positive effects of natural carotenoid-rich feed additives on ornamental fish pigmentation.

3.3 Effects of dietary supplementation of carrot and beetroot on survival rate of *poecilia spheonops*

Throughout the experimental period, the health status and survival of *Poecilia spheonops* were monitored and recorded daily. Fish showing signs of disease were removed from the experimental tanks to prevent the spread of infection to other fish, and mortality was noted regularly during the study period. The percentage survival of the experimental fish during the trial is presented in Table 3.6, while the graphical representation of survival rate at the end of the experiment is shown in Figure 3.6.

Table 8: Survival rate of orange molly (*Poecilia spheonops*) fed with different experimental diets during (Mean $\pm$ SE)

Days	Treatment							Mean
	CB0	C1	B1	C2	B2	CB1	CB2	
15	89.00 ^a ±3.46	93.00 ^a ±0.00	89.00 ^a ±3.46	89.00 ^a ±3.46	91.00 ^a ±3.46	91.00 ^a ±3.46	91.00 ^a ±3.46	90.43
30	87.00 ^{ab} ±0.00	89.00 ^a ±3.46	80.00 ^b ±7.00	80.00 ^b ±0.00	89.00 ^a ±3.46	84.67 ^{ab} ±4.04	84.67 ^{ab} ±4.04	84.90
45	80.00 ^{ab} ±0.00	84.67 ^a ±4.04	75.33 ^{bc} ±4.04	73.00 ^c ±0.00	80.00 ^{ab} ±0.00	75.33 ^{bc} ±4.04	82.33 ^a ±4.04	78.67
60	73.00 ^{abc} ±0.00	77.67 ^a ±4.04	69.00 ^c ±3.46	71.00 ^{bc} ±3.46	77.67 ^a ±4.04	73.00 ^{abc} ±0.00	75.33 ^{ab} ±4.04	73.81
Mean	85.8	88.87	82.67	82.6	87.53	84.8	86.67	-
Tretment		SEM (±)		CD @ 1%		CD @ 5%		CV (%)
D(Days)		5.216		2.458		1.851		
T(Treatment)		5.216		2.909		2.190		
T X D		5.216		-		-		

**Fig 6:** Survival rate of orange molly in each treatment at the end of the experiment

Statistical analysis revealed significant differences in survival rate among the treatments. Treatments B2 and C1 recorded the highest survival rates and were statistically at par with CB1, CB2, and the control group. Treatments B1 and C2 showed no significant difference and were also comparable with CB0 and CB1. The highest survival rate was observed in treatments B2 and C1 (77.67 $\pm$ 4.04 %), while the lowest was recorded in B1 (69.00 $\pm$ 3.46 %). The results indicated that diets supplemented with 5% carrot and 10% beetroot improved the survival of *Poecilia spheonops* during the experimental period. Most treatment groups showed significantly higher survival than the control ($p < 0.01$). Similar findings were reported by Jana *et al.* (2023) [15], who observed 80% survival in orange molly fed carrot peel powder. Comparable results were also reported by Gogoi *et al.* (2017), Kaur *et al.* (2016) [17], and Arulvasu *et al.* (2013) [2], indicating that natural carotenoid-rich feed additives can positively influence fish survival and overall health.

4. Conclusions

The results of the experiment demonstrated that dietary supplementation with carrot and beetroot significantly improved growth performance, pigmentation, and survival of *Poecilia spheonops* compared with the control group. Among all treatments, the diet containing 10% beetroot (B2) recorded the highest weight gain, specific growth rate (SGR), and carotenoid concentration, indicating its strong potential to enhance both growth and body coloration. The diet supplemented with 5% carrot (C2) also showed notable improvement in weight gain, feed conversion ratio (FCR),

and carotenoid content compared with the control. In conclusion, the present study suggests that dietary supplementation with carrot and beetroot positively influences growth, pigmentation, and survival in orange molly. Beetroot, particularly at 10% inclusion, was found to be the most effective treatment and may serve as a promising natural feed additive in aquaculture for improving fish growth performance and colour enhancement.

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