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Effect of dietary natural carotenoid sources on colour enhancement of guppy, *Poecilia reticulata* (Wilhelm Peters, 1859)

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Abstract
A 55-day feeding trial was carried out to investigate the effects of natural dietary carotenoids on the survival and colour enhancement of guppy fish (*Poecilia reticulata*). Four aquaria, each measuring 40 cm x 30 cm x 30 cm and stocked with 15 guppy fish, were used in the study. Four isonitrogenous @50%, (Ingredients: Fishmeal, wheat flour, mustard oil cake), were formulated with various natural carotenoid supplements: carrot powder, beetroot powder, turmeric powder and control (Devoid of carotenoid supplement). Additionally, 1% vitamin (Agrimin) was added to each diet. The study found that the highest survival rates were observed in T_2 and T_3 groups, where fish were fed with diets supplemented with beetroot and turmeric, respectively. The T_1 group, which received the carrot diet, had a slightly lower survival rate, while the control group (T_0) had the lowest survival rate. Colour analysis, performed using spectrophotometry to measure carotenoid content, revealed that the highest carotenoid content (1296±131.45 $\mu g.g^{-1}$) was in T_3 (Turmeric diet), followed by T_2 (beetroot diet) with 1056±118.65 $\mu g.g^{-1}$, and T_1 (Carrot diet) with 976±381.15 $\mu g.g^{-1}$. The control group had the lowest carotenoid content (57.6±19.91 $\mu g.g^{-1}$). The t-test for two samples assuming equal variance indicated that adding dietary carotenoid sources such as carrot, beetroot, and turmeric significantly increased the total carotenoid content in *Poecilia reticulata*, thereby enhancing their coloration.

Keywords: Ornamental fishes, colour enhancement, carotenoid, guppy fish

Introduction
Ornamental fish farming is a growing area within aquaculture that offers substantial income and job opportunities worldwide. This sector plays a pivotal role in boosting economic growth, particularly in underdeveloped tropical areas. The market value of ornamental fish is primarily based on their color, with more brightly colored fish commanding higher prices. In the wild, fish achieve their coloration naturally. However, when kept in high-density, captive environments without added dietary carotenoids, their colors can diminish, leading to a decrease in their commercial worth.

In the ornamental fish industry, the visual appeal of fish, particularly their coloration, significantly impacts market value and consumer preference. Enhanced coloration can lead to higher market prices and increased profitability for breeders and sellers. Carotenoids are also known to have antioxidant properties that can improve the overall health and immunity of fish. Using natural carotenoids as dietary supplements supports environmentally friendly and sustainable aquaculture practices, reducing the reliance on synthetic pigments and their potential negative environmental impacts. This study paves the way for future research into the prolonged impacts of natural carotenoid supplementation on fish health and reproduction, as well as the potential benefits for other ornamental fish species. The study demonstrates that dietary natural carotenoids are a valuable tool for enhancing the coloration and overall well-being of guppy fish, benefiting both the ornamental fish industry and promoting sustainable aquaculture practices. This study could be beneficial for enhancing natural coloration in commercial production, not only in *Poecilia reticulata* but also in various other freshwater ornamental fish species with similar feeding behaviors.

Methodology

Experimental site

This research was carried out at the Meghalaya State Fisheries Research & Training Institute, located in Mawpun, Ribhoi District, Meghalaya-793103, during the period from August to September 2023.

Source of ingredients and diets preparation

Fishmeal, wheat flour, mustard oil cake, carrot, beetroot, and agrimin (Vitamin) were purchased from the market. The carrot, beetroot, and turmeric were cut into pieces and left to dry in the sun for three days. Once dried, they were ground into a fine powder with a grinder and sieved to ensure uniform particle size. The other ingredients were also ground and sieved. Four experimental diets were created: three with different carotenoid sources (Carrot, beetroot, turmeric) and one without any carotenoids. Using the Pearson square method, the protein content was set at 50%. For every 100 g of the diet, 5 g of powdered carotenoid sources and 1 g of agrimin (vitamin) were added. The ingredients were thoroughly mixed by hand, water was added, and the mixture was blended into a dough-like consistency. The diets were pelletized using a 1.5 mm pellet press (kitchen press) and then sun-dried for four days to ensure complete drying and prevent fungal growth. The dried pellets were stored in airtight, well-labelled containers.

Table 1: Composition of the experimental diet per 100 g, featuring varying amounts of different carotenoid sources

Ingredients	Meal 1 (Control)	Meal 2 (Carrot)	Meal 3 (Beetroot)	Meal 4 (Turmeric)
Fish meal	41.1 gm	41.1 gm	41.1 gm	41.1 gm
Mustard oilcake	41.1 gm	41.1 gm	41.1 gm	41.1 gm
Wheat flour	17.7 gm	17.7 gm	17.7 gm	17.7 gm
Carrot	0 gm	5 gm	0 gm	0 gm
Beetroot	0 gm	0 gm	5 gm	0 gm
Turmeric	0 gm	0 gm	0 gm	5 gm
Agrimin (vitamin)	1 gm	1 gm	1 gm	1 gm

Experiment Procedure

Four aquariums, each with dimensions of 40 cm x 30 cm x 30 cm, were set up for the experiment, and each housed 15 guppy fish (*Poecilia reticulata*). Tap water was used and allowed to settle beforehand to eliminate chlorination problems. An air-stone was installed in each aquarium for aeration, and the tanks were disinfected with 20 ppm potassium permanganate before adding the fish. The guppies were fed with experimental diets that included different carotenoid sources: carrot powder, beetroot powder, turmeric powder, and a control diet without carotenoids. Each aquarium was clearly labeled to indicate its specific treatment. The fish were provided with feed twice daily, once in the morning and once in the evening, at a quantity equating to 3% of their body weight throughout the experiment. Any remaining feed and waste were removed from each aquarium.

Water quality parameters

A 50% water change was conducted daily in all aquariums throughout the experiment. The water's physical and chemical parameters-such as temperature, pH, dissolved oxygen, total alkalinity, and total hardness-were measured weekly over the 55-day culture period.

Determination of survival and mortality rate

After 55 days of administering the control and experimental diets to the fish, the number of surviving fish in each tank was recorded. The survival and mortality rates were subsequently calculated using the following formulas:

$$\text{Survival rate (\%)} = \frac{\text{Number of fishes survived at the end of the experiment}}{\text{Number of fishes stocked}} \times 100$$

$$\text{Mortality rate (\%)} = \frac{\text{Number of fishes died}}{\text{Number of fishes stocked}} \times 100$$

Carotenoid Analysis

Carotenoid content was assessed following Olson's (1979) [24] technique. A 0.5 g portion of fish flesh and skin was placed in a 10ml screw-capped vial with 1.25 g of anhydrous sodium sulfate. The sample was lightly crushed with a glass rod, and 3ml of chloroform was added. The vial was then refrigerated at 0 °C overnight, resulting in a 1-2cm layer of chloroform. A 0.3ml sample of this chloroform layer was taken into a clean test tube, and 3ml of absolute ethanol was added. A blank was made with 3ml of absolute ethanol. Optical density was recorded at 380nm, 450nm, 475nm, and 500nm using a spectrophotometer, and the wavelength showing the highest absorption was used for calculations. The carotenoid content was calculated in micrograms per gram of tissue.

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

Where, 10= Dilution factors 0.25= Extinction coefficient

Statistical Analysis

The results from this study (Table 4) were analyzed statistically using Microsoft Excel. A t-test (two-sample assuming equal variances) was performed to identify significant differences between the control and each of the carrot, beetroot, and turmeric diets, as well as among the carrot, beetroot, and turmeric diets. Comparisons were conducted at a 5% probability level.

ResultS and Discussion

Water parameters

Water parameters for each treatment were recorded every 7 days throughout the 55-day experimental period, as detailed in Table 2.

Table 2: Physical and chemical water parameters over the course experiment. (Mean ± SD)

Parameters	Control (T_0)	Carrot (T_1)	Beet (T_2)	Turmeric (T_3)
Temperature(oC)	24.67±0.68	24.67±0.68	24.67±0.68	24.67±0.68
pH	7±0	7.5±0.54	7.5±0.54	7.8±0.4
Dissolved oxygen (mg.L-1)	6.17±0.42	5.8±0.34	5.6±0.39	5.3±0.58
Total alkalinity (mg.L-1)	63±1.15	63.67±0.51	63.8±0.95	65.5±1.2
Total hardness (mg.L-1)	47.9±1.5	48.6±1.2	49.6±1.43	51.7±1.3

Environmental factors such as temperature, pH, and dissolved oxygen play a crucial role in determining fish skin pigmentation. Additionally, handling stress and methods of sacrifice can negatively affect fish coloration. In the current 55-day study, the temperature varied from 24- 26 °C, which is within the ideal range of 24-29 °C for guppy fish. The water pH was between 7 and 8, considered optimal for freshwater ornamental species. Dissolved oxygen levels ranged from 5-7 mg/L, which falls within the ideal range of 5-10 mg/L for these fish. Total alkalinity was measured between 62-67 mg/L, also within the preferred range of 60-250 mg/L for freshwater ornamental fish. Total hardness was recorded at 46-54 mg/L, which is suitable given the desirable range of 45-200 mg/L.

Survival percentage

Table 3 presents the percentages for survival and mortality rates, and Chart 1 depicts these percentages graphically.

Table 3: Survival and Mortality rate after 55 days of feeding with the experimental diets.

Tank	Survival rate	Mortality rate
Tank 0 (Control)	66.67%	33.33%
Tank 1 (Carrot)	80%	20%
Tank 2 (Beetroot)	86.6%	13.34%
Tank 3 (Turmeric)	86.6%	13.34%

In this study, Tank 2 and Tank 3, where fish were fed diets containing beetroot and turmeric respectively, exhibited the highest survival rate of 86.6%. This was followed by Tank 1, where fish were fed a diet with carrot. Tank 0, which had a control diet, recorded the lowest survival rate of 66.67%. These results align with the findings of Keleştemur *et al.*, (2016), who reported similar effects of β-carotene on growth and skin pigmentation in rainbow trout (*Oncorhynchus mykiss*) [14]. The results are also consistent with Swian *et al.*, (2014), who found that adding 180 ppm of marigold oleoresin to the diet improved growth, survival, and coloration in *C. carpio* [35].

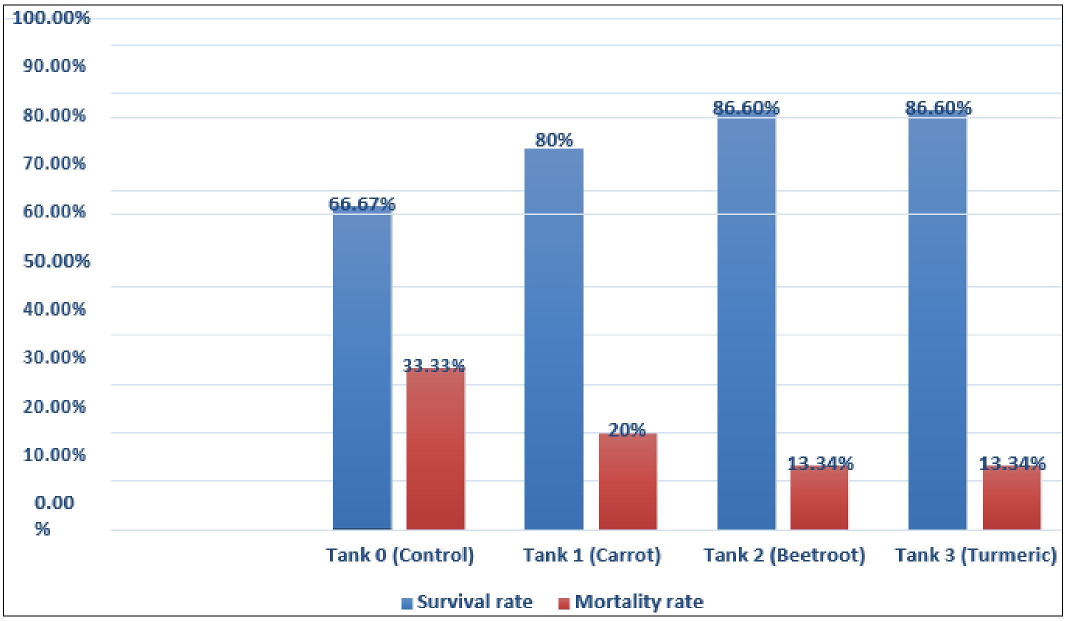

Chart 1: Showing survival and mortality rate in all the treatments

Carotenoid content: The carotenoid levels in guppy fish were assessed using Olson's (1979) [24] method after 55 days of feeding them diets with various carotenoid sources. Table 4 indicates that *Poecilia reticulata*, which were given different carotenoid sources, exhibited varying carotenoid concentrations in their skin.

Table 4: Carotenoid content (μg.g^{-1}) of *Poecilia reticulata* fed with different diet containing different carotenoid sources

Sl. No.	T_0 (Control)	T_1 (Carrot)	T_2 (Beetroot)	T_3 (Turmeric)
1	40	320	1040	1280
2	32	1040	1040	1200
3	72	1280	880	1200
4	72	1040	1120	1520
5	72	1200	1200	1280
Mean	57.6	976	1056	1296
SD	19.9198393	381.156136	118.659175	131.453413

In this study, the control and experimental diets, which included carotenoids from carrot, beetroot, and turmeric, provided valuable insights. After 55 days of feeding guppy fish with these diets, the highest carotenoid content of 1296±131.45 μg/g was observed in T_3, where turmeric was used (Fig. 4). This was followed by T_2 with 1056±118.65 μg/g from beetroot supplementation (Fig. 3) and T_1 with 976±381.15 μg/g from carrot (Fig. 2). The control group, with no carotenoid supplementation, had the lowest carotenoid content of 57.6±19.91 µg/g (Fig. 2), indicating significant results. These findings suggest that the tested diets not only affect the survival rates but also improve the coloration of the guppy fish. This is consistent with Xu *et al.*, (2006), who found that adding Astaxanthin to goldfish diets enhanced red pigmentation [39]. Similarly, Maiti *et al*. (2017) reported that natural carotenoids like beetroot, carrot peel, and tomato peel improved growth and coloration in koi carp at a 1% inclusion rate [18]. Ezhil *et al.*, (2008) and Swain *et al.*, (2014) found that Marigold petal meal, at 180 ppm, boosted growth and color in tiger barbs and red swordtails [7, 35]. Additionally, Mirzaee *et al.*, (2012) observed that a diet containing a mixture of tomato and carrot at 50 mg/kg enhanced color and carotenoid levels in guppy fish [19].

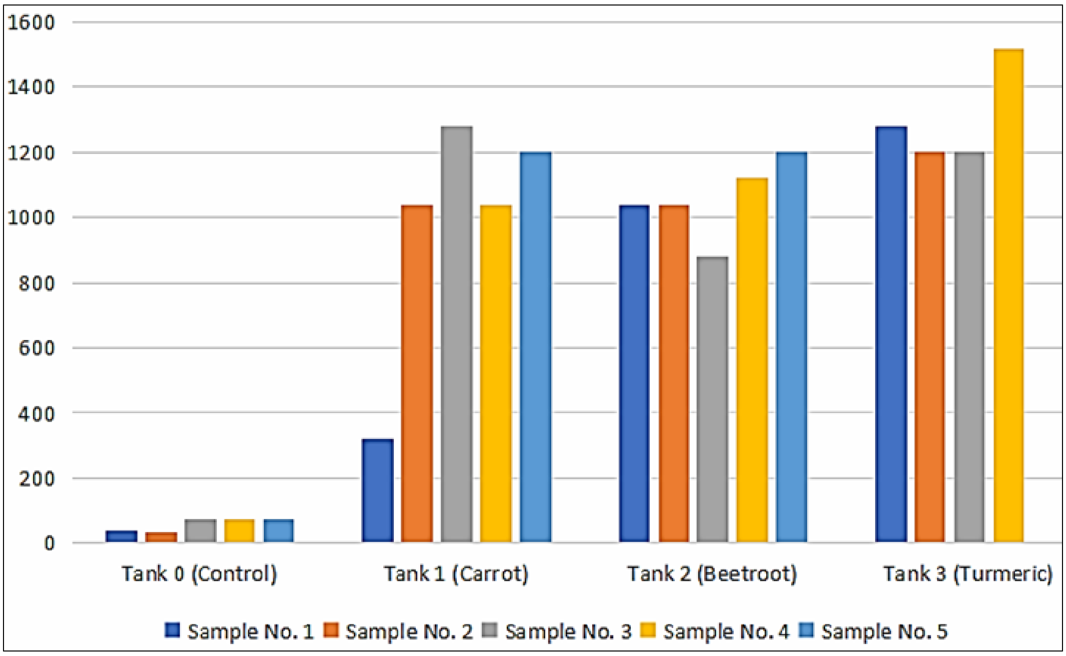

Chart 2: Carotenoid content variation in all the treatments

Fig 1: Guppy fish from Tank 0 (Control)

Fig 2: Guppy fish from Tank 1 (Carrot)

Fig 3: Guppy fish from Tank 2 (Beetroot)

Fig 4: Guppy fish from Tank 3 (Turmeric)

Statistical Analysis (t-test: Two-sample with equal variance assumption)

Table 5: T-test (Two samples assuming equal variances) - Control & Carrot

	Tank 0 (Control)	Tank 1 (Carrot)
Mean	57.6	976
Variance	396.8	145280
Observations	5	5
Pooled Variance	72838.4	
Hypothesized Mean Difference	0	
Df	8	
t Stat	-5.380488168	
P(T<=t) one-tail	0.000330626	
t Critical one-tail	1.859548033	
P(T<=t) two-tail	0.000661251	
t Critical two-tail	2.306004133	

Inference: From the given (Table 5), the calculated absolute t Stat value > t Critical value (i.e., 5.380488168> 2.306004133), therefore we reject the null hypothesis.
The difference is significant. Hence, we can say that there is a significant difference between two fish diets (i.e., Control and Carrot) with respect to the total carotenoid content.
In other words, feeding the fish with a carrot diet will enhance the carotenoid content of the guppy fish compared to the control diet.

Table 6: T-test (Two samples equal variances) - Control & Beetroot

	Tank 0 (Control)	Tank 2 (Beetroot)
Mean	57.6	1056
Variance	396.8	14080
Observations	5	5
Pooled Variance	7238.4	
Hypothesized Mean Difference	0	
Df	8	
t Stat	-18.55467224	
P(T<=t) one-tail	3.66978E-08	
t Critical one-tail	1.859548033	
P(T<=t) two-tail	7.33956E-08	
t Critical two-tail	2.306004133	

Inference: From the given (Table 6), the calculated absolute t Stat value > t Critical value (i.e., 18.55467224 > 2.306004133), therefore we reject the null hypothesis.
The difference is significant. Hence, we can say that there is a significant difference between two fish diets (i.e., Control and Beetroot) with respect to the total carotenoid content.
In other words, feeding the fish with beetroot diet will enhance the carotenoid content of the guppy fish compared to control diet.

Table 7: T-test (Two samples assuming equal variances) - Control & Turmeric

	Tank 0 (Control)	Tank 3 (Turmeric)
Mean	57.6	1296
Variance	396.8	17280
Observations	5	5
Pooled Variance	8838.4	
Hypothesized Mean Difference	0	
Df	8	
t Stat	-20.82783466	
P(T<=t) one-tail	1.48068E-08	
t Critical one-tail	1.859548033	
P(T<=t) two-tail	2.96135E-08	

Inference: From the given (Table 7), the calculated absolute t Stat value > t Critical value (i.e., 20.82783466 > 2.306004133), therefore we reject the null hypothesis.
In other words, feeding the fish with turmeric diet will enhance the carotenoid content of the guppy fish compared to the control diet.

Table 8: T-test (Two samples assuming equal variances) - Carrot & Beetroot

	Tank 1 (Carrot)	Tank 2 (Beetroot)
Mean	976	1056
Variance	145280	14080
Observations	5	5
Pooled Variance	79680	
Hypothesized Mean Difference	0	
Df	8	
t Stat	-0.448110715	
P(T<=t) one-tail	0.33298019	
t Critical one-tail	1.859548033	
P(T<=t) two-tail	0.665960381	
t Critical two-tail	2.306004133	

Inference: From the given (Table 8), the calculated absolute t Stat value > t Critical value (i.e., 20.82783466 > 2.306004133), therefore we reject the null hypothesis.
In other words, feeding the fish with turmeric diet will enhance the carotenoid content of the guppy fish compared to the control diet.

Table 9: t-test (Two samples assuming equal variances) - Carrot & Beetroot

	Tank 1 (Carrot)	Tank 2 (Beetroot)
Mean	976	1056
Variance	145280	14080
Observations	5	5
Pooled Variance	79680	
Hypothesized Mean Difference	0	
Df	8	
t Stat	-0.448110715	
P(T<=t) one-tail	0.33298019	
t Critical one-tail	1.859548033	
P(T<=t) two-tail	0.665960381	
t Critical two-tail	2.306004133	

Inference: From the given (Table 9), the calculated absolute t Stat value < t Critical value (i.e., 0.448110715 < 2.306004133), therefore we fail to reject the null hypothesis.
The difference is insignificant. Hence, we can say that there is no significant difference between two fish diets (i.e., Carrot and Beetroot) with respect to the total carotenoid content.

Table 10: T-test (Two samples assuming equal variances) - Carrot & Turmeric

	Tank 1 (Carrot)	Tank 3 (Turmeric)
Mean	976	1296
Variance	145280	17280
Observations	5	5
Pooled Variance	81280	
Hypothesized Mean Difference	0	
Df	8	
t Stat	-1.774713019	
P(T<=t) one-tail	0.056932503	
t Critical one-tail	1.859548033	
P(T<=t) two-tail	0.113865007	
t Critical two-tail	2.306004133	

Inference: From the given (Table10), the calculated absolute t Stat value < t Critical value (i.e., 1.774713019 < 2.306004133), therefore we fail to reject the null hypothesis.
The difference is insignificant. Hence, we can say that there is no significant difference between two fish diets (i.e., Carrot and Turmeric) with respect to the total carotenoid content.
In other words, feeding the fish with carrot diet will not make much difference in the carotenoid content from feeding the guppy fish with turmeric diet.

Table 11: T-test (Two samples assuming equal variances) - Beetroot & Turmeric

	Tank 2 (Beetroot)	Tank 3 (Turmeric)
Mean	1056	1296
Variance	14080	17280
Observations	5	5
Pooled Variance	15680	
Hypothesized Mean Difference	0	
Df	8	
t Stat	-3.030457634	
P(T<=t) one-tail	0.008149034	
t Critical one-tail	1.8595480338	
P(T<=t) two-tail	0.016298067	
t Critical two-tail	2.306004135	

Inference: From the given (Table 11), the calculated absolute t Stat value > t Critical value (i.e., 3.030457634 > 2.306004135), therefore we reject the null hypothesis.
The difference is significant. Hence, we can say that there is significant difference between two fish diets (i.e., Beetroot and Turmeric) with respect to the total carotenoid content.
In other words, feeding the fish with turmeric diet will give higher carotenoid content in guppy fish compared to beetroot diet.
In this study, the carotenoid levels in the skin of *Poecilia reticulata* varied depending on the diet. The t-test for two samples assuming equal variances revealed that the carotenoid content in the skin of fish fed diets supplemented with carrot and beetroot was relatively similar. Whereas the carotenoid content of *Poecilia reticulata* in turmeric was significantly higher than the other diets. Also, from the t-test two sample assuming equal variance, it could be interred that, addition of carrot, beetroot, turmeric dietary carotenoid sources obviously enhanced the total carotenoid content in *Poecilia reticulata* and hence improved its colouration.

Conclusion
The current study indicates that adding dietary carotenoid sources such as carrot, beetroot, and turmeric increases the total carotenoid content in *Poecilia reticulata*. These sources can also be combined with other carotenoid additives to enhance pigmentation in freshwater ornamental fish like *Poecilia reticulata*. This research may benefit the commercial production of natural coloration in *Poecilia reticulata* and other freshwater ornamental fish with similar feeding habits. The study suggests that natural carotenoid sources natural carotenoid sources are an efficient method for improving the coloration of ornamental fish, making it a cost-effective and user- friendly approach.

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