Journal of the Oriental Institute ISSN No. 0030-5324
M. S. University of Baroda Impact Factor 7.1 UGC Care Group I

Impact of natural pigments (Betanin, β-Carotene) with reference to colour enhancement in ornamental fishes

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Abstract

The present study investigates the impact of natural pigments—Betanin from *Beta vulgaris* (beetroot) and β-carotene from *Citrus sinensis* (orange peel)—on colour enhancement in ornamental fishes. Colour intensity is a primary factor influencing the aesthetic and commercial value of ornamental species, making pigmentation a crucial area of focus in ornamental aquaculture. Since fish cannot synthesize carotenoids de novo, dietary supplementation becomes essential for achieving optimal skin coloration. In this experiment, multiple ornamental fish species such as Koi, Molly, Gourami, Black Moor Goldfish, Crucian Carp, and Shubunkin were fed diets enriched with beetroot powder and orange peel powder over a 15-week period. Weekly visual assessments were conducted to evaluate pigmentation changes. The findings revealed that beetroot powder significantly enhanced pigmentation in most species, particularly Koi fish, producing brighter and more intense hues compared to the control. Orange peel powder showed limited effectiveness, with noticeable improvement only in Crucian Carp and Shubunkin. These results align with earlier studies indicating that carotenoid-rich natural pigments improve colour intensity, antioxidant activity, immunity, and overall fish health. The study concludes that beetroot powder is a more potent natural pigment source than orange peel powder for colour enhancement. The use of natural carotenoids offers an eco-friendly alternative to synthetic colourants, reducing environmental impact while enhancing commercial value. Incorporating 10 grams of beetroot or orange peel powder per day demonstrated the most effective results, supporting its potential application in ornamental fish feed formulations within the aquaculture industry.

Keywords: Natural Pigments, Betanin, β-Carotene, Ornamental Fish, Colour Enhancement, Carotenoids

1. Introduction

Ornamental fish keeping is a popular and stress-relieving hobby with world-wide interest. Attention to aquarium fish production has steadily increased in the aquaculture industry. Attractive and eye-catching bright colour and physical appearance of ornamental fishes are vital criteria in determining consumer enthusiasm and market demand all over the world. It is therefore of crucial interest for fish farmers to achieve acceptable pigmentation of fish flesh integument by formulating fish feeds using different carotenoids either from natural or synthetic sources. Skin coloration is one of the most important factors which decide the aesthetic value, therefore the market value of ornamental fishes. Pigmentation in the skin is responsible for colouration in the fish. In natural environment, the fishes meet their carotenoid requirements by ingesting aquatic plants or through

their food chains. The enhancement of coloration can be done by administrating pigment enriched feed; it will definitely improve the quality and cost of the fish. Attractive colouration determines the commercial value of ornamental fish. Carotenoids are among the most common natural pigments responsible for many of the hues found in nature as well as a variety of functions. Carotenoids are a class of 800 natural fat-soluble pigments found principally in plants, algae, fungi, animals, photosynthetic bacteria and some non-photosynthetic bacteria. Only plants, bacteria, fungi and algae can synthesize carotenoids; animals cannot biosynthesize them thus, they must be obtained through the diet (Gouveia and Rema, 2005). In the animal kingdom, carotenoids are the most widely occurring pigments after melanin. They play a critical role in the photosynthetic process and they carry out a protective function against damage by light and oxygen. Carotenoids additionally play different vital functions as pro-vitamin A, antioxidants, immunoregulators and which they are mobilized from muscle to ovaries which recommend a function in reproduction. It has also observed that fishes with a high level of carotenoids are more resistant to bacterial and fungal diseases. The majority of carotenoids are derived from a 40-carbon polyene chain, which could be considered as the backbone of the molecule.

Fish coloration can be determined by three factors:

- Genetics: Whether the fish has inherited the necessary genetic material to show certain colours.
- Nervous system and glandular factors: Coloration depends on a fish's mood and general health. All things being equal, a sick fish is probably less colourful than a healthy one. Males also may develop strong colours to attract females, and how the colour of subordinate males lessens in the presence of dominant males.
- Dietary factors: Nutrients and chemical compounds that the fish eats which directly or indirectly influence colour.

Betanin and beta-carotene are important carotenoids that can be used as feed additives to enhance the coloration of ornamental fish. While betanin, derived from beetroot, contributes to red and pink hues, beta-carotene, found in various sources like algae and vegetables, is responsible for yellow and orange pigments. These pigments are crucial for fish's natural coloration and can significantly impact their market value.

Koi Fish:

Koi fish, also known as Nishikigoi or brocaded carp, are ornamental varieties of the Amur carp (*Cyprinus rubrofuscus*). They are highly valued for their vibrant colors and are often kept in outdoor ponds or water gardens for decorative purposes. Koi can come in a wide range of colors, including red, orange, yellow, white, black, blue, and brown, as well as metallic shades like gold and silver-white. Koi are adaptable omnivores and can tolerate a range of temperatures, but they thrive in cool-water environments. They eat insects, crustaceans, algae, and vegetation. They should be given standard koi food with at least 30% protein.

Benefits of Betanin Pigment:

Betanin, a red pigment found in beetroot, offers several benefits to fish, primarily acting as a natural antioxidant and color enhancer. It can improve growth, reproduction, and overall health, while also adding vibrant colors to their appearance.

- Antioxidant Properties: Betanin acts as a scavenger of free radicals, protecting fish from oxidative stress and potential cellular damage.
- Growth and Reproduction: Studies have shown that adequate betanin intake can promote healthy development in juvenile fish and improve reproductive success in breeding setups, leading to higher hatch rates and healthier offspring.
- Color Enhancement: Betanin can contribute to vibrant colors in fish, making them visually appealing.
- Anti-inflammatory Effects: Betanin may help reduce inflammation in fish, contributing to overall health and well-being.
- Other Potential Benefits: Research suggests betanin may also have anti-lipidemic, antimicrobial, and antimalarial effects, potentially beneficial for fish health.

Benefits of Beta Carotene:

Beta carotene, a carotenoid pigment, offers several benefits for fish, including enhancing their coloration, supporting their immune system, and potentially improving their growth and reproductive success. It acts as a precursor to vitamin A and functions as an antioxidant, helping to neutralize free radicals and protect against cellular damage.

- Enhanced Coloration: Beta carotene accumulates in the skin and scales of fish, intensifying red, orange, and yellow pigments, making them more vibrant.
- Immune Support: As an antioxidant, beta carotene helps neutralize free radicals, which can damage cells and contribute to disease. This support can strengthen the fish's immune system.
- Growth and Reproduction: Adequate beta-carotene intake may be crucial for optimal growth and reproductive success in some fish species.
- Stress Resistance: Beta carotene can help fish cope with various stressors, including infectious diseases, heavy metal toxicity, and temperature changes.
- Antioxidant Properties: Beta carotene's antioxidant activity helps protect fish from the harmful effects of free radicals, which can lead to cellular damage and disease.

2. Review of Literature

Research carried out by Hong K. No & Trond Storehakken. The effects of frozen storage (3 and 6 months at -20 and -80°C) on stability of carotenoids and colour [CIE, (1976) L*a*b* and H(o)J in vacuum-packed fillets of rainbow trout were studied in a paired sample experiment. The trout were fed diets containing astaxanthin or canthaxanthin at different water salinities and temperatures. Carotenoids in the flesh were stable (up to 5% loss) for 6 months at-20 or -80°C, regardless of carotenoid sources and rearing conditions. Frozen storage resulted in increased L (lightness), a* (redness) and b* (yellowness), and decreased H(o)ab (hue) values. Colour characteristics from different parts of the fillet differed significantly.

Amiri et al.,(2019) reported the effects of using B. vulgaris pulp in the diet of carp improved the fillet composition. The results showed that adding B. vulgaris leaf powder to the fish diet had positive effects on their feed conversion index. Because of the high nutritional value and low price of B. vulgaris leaves, they are used to feed animals. The different compounds present in B. vulgaris had positive effects on fish growth.

Nath Jha et al.,(2013) reported that The presence of B. vulgaris root (10%) in. the fish diet not only improved their growth but also enhanced the total carotenoid content and body composition significantly. Therefore, cheap and easily available natural carotenoid sources like beetroot can be incorporated into B. bendelisis diet at 10% level to obtain better pigmentation and market value .

Asadi Sharif et al. (2014) reported that the red beet juice powder up to 6% represented good effect on growth performances of Oscar fish. It was found that the most appropriate dose of red beet in the diet for best growth performances of rainbow trout was 4%. These results were agreeing with the results of our study that showing the increase in the percentage of the fish fillet protein had directly proportional to the increase in the concentration of the B. vulgaris additive.

Iwuozor & Afiomah, et al., (2020) reported that the Beta vulgaris is popular for its abundance of a nitrogenous pigment which is water soluble called betalains. Betalains are used in the processing of food products as an additive due to its low toxicity, high water solubility, and natural colorant characteristics. Two major categories of betalains are found to exist in the plant. The first is betacyanin, which is a red pigment. The pigment's major effects are the antioxidant property where it enriches the low-density lipoprotein which increase the body resistance to oxidation, stimulates the immune system, gives kidney and liver protection, increases cognitive functioning, and guards one's blood vessels against damage and inflammation. Beta vulgaris is undoubtedly a very important and underutilized plant worldwide. Its approximate properties have shown that its leaves are a good source of food and animal feed. Most of its medicinal value is due to the phytochemical properties of the plant.

3. Objectives

- To improve the fish coloration through a natural pigment source: *Beta vulgaris* and *Citrus sinensis*.
- To use Pigment enriched feed to improve the quality and cost of ornamental fish.

4. Materials And Methods

Experimental Design:

A 15 weeks experiment (December 2024 to March 2025) was organized to examine the effect of applying orange peel powder and beet root powder to ornamental fishes(Sailfin Molly , Crucian Carp, Black Moor Gold Fish, Gourami, Koi, Shubunkin, Japanese Crucian Carp). The study was conducted in a fish pot (14×25×24 cm). Fish were acclimatized under optimal conditions for 2 weeks before starting the trial. Fish were fed twice a day at 09:00 am, and 08:00 pm. Water was changed twice a week.

Materials Required:

1. Five glass bowl. (Size : 14cm × 25cm × 24cm)

2. Fish catching band net.

3. Temperature meter.

Species collection:

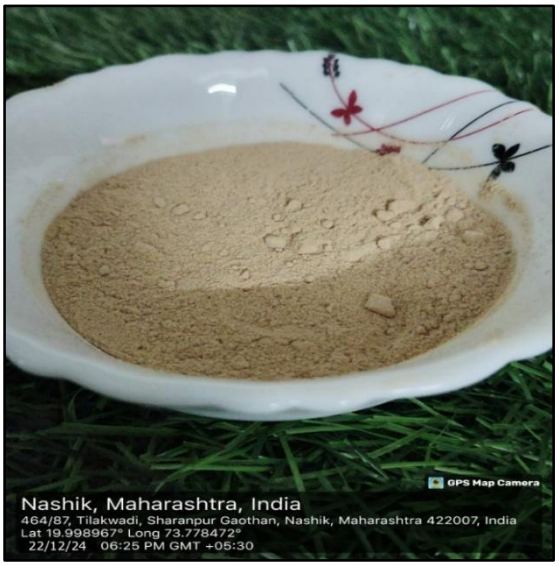
Ornamentl fishes were collected from Bharat Aquarium, Near Nehru garden, Shalimar, Nashik. Cost of the each pair of fish is Rs.80/-

Food source:

- Common fish feed
- **Beet Root:** Beetroot were obtained from the market. Beetroot was then cut to small pieces and sundry for three days, then powder with the help of grinder, the powder form was further sieved through fine mesh size sieve to ensure small and equal particle sized. The sieve material was then thoroughly mix together, by hand. Water was added to the premix ingredients and a dough

like pest and these diets were sundry for hour, days to ensure complete drying and no fungal formation and then store in air tight well-developed container.

- **Orang Peel:** Rinse the orange peels well under running water to remove dirt or pesticides. Peel the oranges, trying to avoid too much of the white pith (the bitter part). The thinner the orange layer, the better. Spread the peels on a clean tray or cloth in a single layer. Place under direct sunlight for 3–5 days, turning occasionally. Once the peels are completely crisp and dry, grind them in a blender, spice grinder, or food processor until you get a fine powder. Store in an airtight jar in a cool, dry place.



Method:

Each aquarium was stocked with ornamental fish, using tap water as the water source. Prior to stocking, the aquaria were disinfected using potassium permanganate. Initially, all fishes were placed together in a control setup. Later, each aquarium was stocked with different types of ornamental fish. The experimental groups were fed a diet supplemented with beetroot and orange peel powder. Each aquarium was clearly labeled as either experimental or control using glass bowls.

Feeding was carried out twice daily at 9:00 a.m. in the morning and 8:00 p.m. in the evening throughout the experiment. Small balls of the dietary powder were added to the experimental tanks, while the control tanks were given regular fish feed. Water in all aquaria was replaced three times a week during the entire project. The experiment lasted for a total of 15 weeks.

Pigmentation and colour enhancement will be check visually every week in whole project duration.

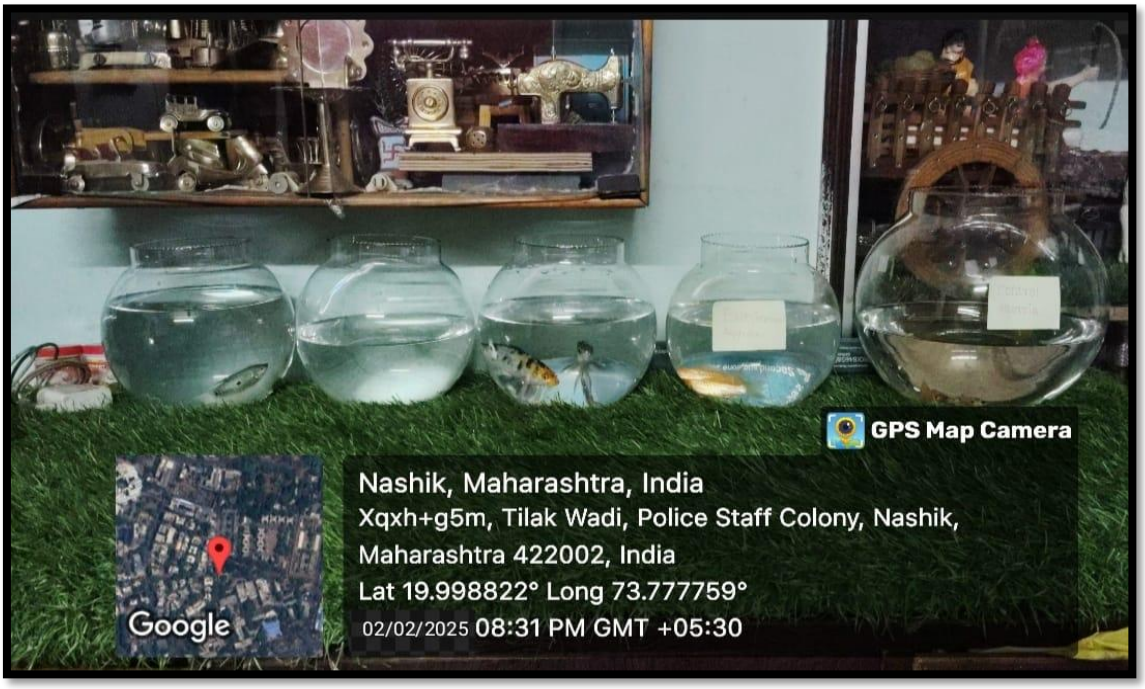
A. First day of Aquaria:

B. After 20 days:

C. After 40 days:

D. After 60 days:

E. After 80 days:



F. After 100 days:

5. Result

Control:

1st day 40th day 100th day

Impact of Beet Root Powder on Koi Fish Pigmentation:



1st day 20th day 40th day
60th day 80th day 100th day





- **Control:**

1st day

40th day

100th day

- **Impact of Orange Peel Powder on Koi Fish Pigmentation**

1st day

20th day

40th day

60th day

80th day

100th day

This result indicates that beetroot powder enhances the coloration of Koi fish more effectively than orange peel powder, which shows minimal or no improvement in pigmentation.

Table No. 1: Impact of Beet Root Powder and Orange Peel Powder on Ornamental Fish Species.

Sr. No.	Fish	Beet Root Powder	Orange Peel Powder
1	Koi Fish	More Effective	Less Effective

Present research indicates that beetroot powder enhances pigmentation in Koi fish. Therefore, beetroot powder is considered more effective than orange peel powder for improving color in ornamental fish.

6. Discussion

Similar, to our results, increasing supplementation of carotenoids could improve the skin colour of gourami fish after 60 days of feeding trial was also reported by Andram et al (2018) Enhancement of colour intensity in skin coloration of koi indicates that carotenoid or colour pigment that originated from RDFM supplemented in the diet could improve colour intensity of koi fish. Some authors had proved that increasing pigment supplementation in the diet could increase the colour brightness of some fish species (e.g. Duncan & Lovell 1993; Storebakken et al 1987: Chatzifotis et al 2005, Dharmaraj & Dhevendaran 2011. Ho et al 2014).

The diets used in this study contained different dosage of red dragon meal. After 45 days of rearing, the skin colour of the fish fed with test diet had differed from the initial colour of the fish. Studies reported differentiation of skin colour on omamental goldfish fed with an astaxanthis-supplemented diet against fish feed control diet after 7 days (Paripatanarunt et al 1999). In a case of orange-red skin colour oranda goldfish fed commercial diets that contained astaxanthin, luten, or zeaxanthin a wok about 28 days to observe differences from contest (Wallat et.al., 2005).

The fish fed with diet contained RDFM resulted higher of total score in black skin colour brightness than orange skin colour brightness. The results indicated that RDFM supplemented in the diet tend to increase black pigment comparing with orange pigment RDFM meal contains anthocyanın. Similar to our study, Baron et. al., (2008) reported that supplementation of anthocyanin-based (Overseal Carantho powder) pigments in the diet did not affect orange skin colour of dwarf gourami. On the other hand, this experiment found that RDFM supplemented in the diet could improve black skin colour of koi fish. This founding is similar with the work of Peres-Escalame et al (2012), which revealed that anthocyanin supplemented in the diet could increase melanophors cell as black pigment.

7. Conclusion

Present research indicates that beetroot powder enhances pigmentation in Molly fish, Koi fish, Gourami, and Black Moor Goldfish, whereas orange peel powder only affects Crucian carp and Shubunkin fish. Therefore, beetroot powder is considered more effective than orange peel powder for improving color in ornamental fish.

The use of pigment-enriched feed can improve the quality and cost of ornamental fish. In view of the deteriorating effects on the environment due to use of synthetic pigments, the researchers are emphasizing the need for natural pigment colouring agents which will act as an alternative to synthetic chemicals. As the aqua feed industry seeks a natural, environment friendly source of

pigment to improve coloration and to enhance commercial acceptability, there is a great potential for use of natural plant-based carotenoids for pigmentation in aquaculture. It paves the way too many aqua feed industries to promote their products as natural with a distinct shift away from synthetic ingredients and colorants.

The present research concluded that supplementation of beta vulgaris, as a natural pigment source in the diet. In this the fish show colour enhancement.

The colour enhancement resulted much brighter in uses of beetroot and orange peel powder than normal fish feed. Therefore, the optimum dosage beetroot and orange peel powder in the diet to increase the skin colour brightness of koi fish was 10 gram per day.

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