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ROLE OF PALASH FLOWER (*Butea monosperma*) POWDER IN IMPROVING THE COLOUR STABILITY OF ORNAMENTAL KOI CARP (*Cyprinus carpio* L, 1758)

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ABSTRACT

The colour of ornamental fish influence their market value. Koi carp (*Cyprinus carpio*) are globally renowned for their vibrant colours and patterns. Palash flower (*Butea monosperma*), commonly known as the flame of forest, is a natural carotenoid source. It is seasonally (April-May) available in Midnapore, Bankura, and Purulia districts of West Bengal (India). At flowering time, a large number of flowers are scattered on ground and usually get wasted. The present study evaluated the effect of dietary incorporation of palash flower powder as a natural carotenoid source for Koi carp skin and muscle pigmentation. The experiment was conducted in indoor aquarium tanks for 60 days. The fish were divided into 6 groups and fed twice a day @ 4% of their body weight with palash flower powder-supplemented diet with different graded levels T_2 (2.5%), T_3 (5%), T_4 (7.5%), and T_5 (10%) along with a control feed [no supplementation (T_1)] and commercial feed (T_6). The experimental feed was prepared by following Pearson's square method. Pigment concentration was determined spectrophotometrically. At the end of experiment, the highest total carotenoids concentration found in fish skin and muscle was 25.09 ± 0.20 and 15.78 ± 1.6 µg g^{-1}, respectively, in T4 tank ($p < 0.05$) and the specific growth rate and weight gain were high in T_4 treatment with 100% fish survival. The result revealed that T_4 (7.5%) feed had best positive effect on pigmentation as compared to the control and commercial feed treatments. The treatment was also cost-effective.

Keywords: Cost-effective feed, Koi carp, palash flower, pigmentation

INTRODUCTION

In fish, of the most extensively researched phenotypic characteristics is pigmentation. Commercially, ornamental fish species with a good colour appearance are significant since they not only fetch the attention of buyers but also provide information about the fish's health and other physiological condition. If the colour intensity of ornamental is satisfactory, the fish will have the highest possible selling price. Producers of ornamental fish constantly look for ways to improve the colour of their skins. Also, they look for the natural pigments to replace synthetic pigments, because synthetic pigments are expensive (Ramamoorthy *et al.,* 2010). The fish, like other animals, are incapable of producing pigments by *de novo* synthesis. Accordingly, the ingested carotenoids give fish their colour (Goodwin, 1984). Feeds containing carotenoids play a role in the formation of fish body colours. Koi carps, one of the major ornamental fish species, are distinguished by their great variety of colours and patterns (Gomelsky *et al.,* 2003). As the carotenoids are absorbed and deposited in the body of Koi carp, their skin colour intensity improves which enhances the fish value.

The major problem affecting the ornamental fishes is fading of their appealing colours during rearing under captive conditions, which is not preferred by hobbyists. Hence, in order to have constant and bright colour under indoor conditions, the colour needs to be enhanced through dietary synthetic or natural carotenoids. In order to improve the colouration of ornamental fish, a range of plant sources, including leaves, fruit skins, and flowers, are frequently used as natural carotenoid sources. For example, flowers like marigold, china rose, rose petals, butterfly pea flower, etc. might be important in improving the intensity of fish chromatophores, and consequently induc colour change because it is cheap, easily available, natural and rich in pigments.

Butea monosperma (Lam.) Taub., 1894, is commonly known as 'the flame of the forest' and locally as "Palash", and belongs to the family 'Fabaceae'. The bright orange-red flowers of *B. monosperma* are usually 2.5 cm long which contain carotenoid pigments. The natural colour extracted from the petals of palash flower is used as natural dye as an alternative to synthetic dyes (Sinha *et al.*, 2012). The supplementation of palash flower to fish diet may enhance the colour in ornamental fishes. It is assumed that the supplementation of palash flour in fish feed may probably intensify the colour in ornamental Koi fish. Perusal of literature has revealed that no study has so far been conducted on assessing the use of palash flowers (as natural carotenoid pigments) on the colour of Koi fish. Therefore, this study was aimed to assess the effect of pigment source on skin and muscle colour in Koi fish.

MATERIALS AND METHODS

An experiment was conducted in August-September, 2024 (for 60 days) at the Department of Fishery Sciences, Vidyasagar University, Midnapore, West Bengal (India) to assess colour retention by Koi carp in captive conditions. The 180 Koi carp fingerlings of 4-5 month's age were procured from an ornamental fish farm in Dasnagar, Howrah (India) and acclimatized for seven days prior to the experimentation.

Flower collection and flower powder preparation

Fresh palash flower [*Butea monosperma* (Lam.) Taub.] was collected from Vidyasagar University campus, Midnapore (India). The flower petals were separated from sepals and fresh scarlet red petals were washed, shade-dried and then dried in a dryer at 37-40°C. The dried flowers were finely powdered and kept in an airtight zip lock pouch till use.

Feed preparation and experimental design

Sixty fingerlings of Koi carp were randomly distributed in six treatment groups with three replications per treatment as per an completely randomized design. Ten fish with an average size group of 8-10 cm were distributed in each replicate tank. Experimental diet was formulated following the Pearson's Square method (Wagner and Stanton, 2012) to obtain the desired protein percentage of 35. Fish meal, soybean meal, rice bran, wheat flour, tapioca powder, vegetables oil, vitamin and mineral mixture were used as raw additive materials in the preparation of diets for fish feeding. No palash flower powder was added to the control feed, while the test feeds were prepared by supplementing the varied amounts of palash flower powder [2.5% (T_2), 5.0% (T_3), 7.5% (T_4), and 10% (T_5) level], to the basal feed without changing the chemical property by compensating the amount of rice bran and wheat flour in other feed groups. Commercial feed (T_6) namely "Optimum - Highly Nutritious Food for All Aquarium Fish", bought from local market, was included in the experiment as positive control for comparison. The commercial feed had 28% crude protein, 3% crude fat, 4% crude fiber and 10% moisture. The proximate composition of experimental diets was analysed by standard methods (AOAC, 2007).

Feeding of experimental diet

The Koi carps were fed twice a day @ 5% of their body weight. The daily allowance was divided into two equal portions which was fed at 10 am and 5 pm**.**

Assessment of growth and survivability

Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate were calculated by using the following equations:

$$\text{Specific growth rate (\%)} = \frac{\ln W2 - \ln W1}{T2 - T1} \times 100$$

Here, W_2 is the final live body weight (g) at time T_2 (final) day; W_1 is the initial live body weight (g) at time T_1 (starting) day; and T_2 - T_1 is the duration of the total experiment (in day).

$$\text{Weight gain (WG\%)} = \frac{\text{Mean of final weight (g)} - \text{Mean of initial weight(g)}}{\text{Mean of initial weight (g)}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed given to fish (g)}}{\text{Weight gain of fish (g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Numbers of fish at the end of experiment}}{\text{Numbers of fish at the start of experiment}} \times 100$$

Pigment extraction from fish tissue

The total carotenoids present in different tissues were estimated as per Olson (1979). At the start of the feeding trial, the skin and muscle of fishes were analysed to get initial carotenoid content. Then the total carotenoid content in the skin and muscle of fish were analysed at every 15 days-interval of feeding trial.

For carotenoid extraction, 1 g body tissue was taken in 10 mL screw capped clear glass vial and 2.5 g anhydrous sodium sulphate added to it. The sample was gently mashed with a glass rod against the side of vial. Then 5 mL chloroform was added and the solution left as such overnight at 0°C. Chloroform formed a clear 1-2 cm layer. Its optical density was measured at 380, 450, 475 and 500 nm in a UV spectrophotometer (Shimadzu UV-1800, Japan) by taking 0.3 mL aliquots which was diluted to 3 mL with absolute ethanol before estimation. Blank was prepared with absolute ethanol.

$$\text{Carotenoid content} = \frac{\text{Absorption at max wavelength} \times \text{Dilution Factor (10)}}{0.25 \times \text{Sample weight in g}}$$

where 0.25 is the extinction coefficient

Statistical analysis

A completely randomized design was used to conduct the experiment. The analysis of variance (ANOVA) and test of significance was determined by using SPSS 27.0 software. The level of significance used was $p < 0.05$. Post hoc Duncan multiple range test was performed for comparison of the groups.

RESULTS AND DISCUSSION

The proximate composition of palash flower supplemented feeds is presented in Table 1. The analysis revealed that the chemical composition of experimental diets *viz*., carbohydrates, protein, fats, ash and moisture contents remained almost uniform.

Growth and survivability

The growth and survivability of Koi carp in various experimental tanks revealed higher specific growth rate and weight gain in T_4 tank (Table 2). The feed conversion ratio (FRC) was high in T_1 tank and low

Table 1: Proximate analysis (%) of experimental diets supplemented with palash flower extracts

Chemical components (%)	T_1	T_2	T_3	T_4	T_5
Carbohydrates	35.85	35.30	34.70	34.08	33.65
Proteins	34.40	34.34	34.35	34.39	34.40
Fats	10.45	10.47	10.50	10.46	10.49
Ash	8.60	8.63	8.62	8.61	8.62
Moisture	8.20	8.20	8.30	8.10	8.30

Palash flower powder added @ 0, 2.5, 5.0, 7.5 and 10.0% in the experimental diets T_1, T_2, T_3, T_4 and T_5, respectively, in feed groups. Commercial feed 'Optimum' (T_6) served as positive control.

in T_4 tank. The survival rate was 100% in carp fed with palash flower incorporated experimental diet.

The effect of palash flower (*Butea monosperma*) powder on skin and muscle pigmentation of ornamental Koi fish was monitored during the experimental period. The carotenoid concentration in fishes fed with palash flower supplemented diets is given in Table 3. The data were computed using the highest reading

Table 2: Growth and survivability of Koi carp (*Cyprinus carpio*) in different feed tanks

Parameters	T_1	T_2	T_3	T_4	T_5	T_6
Specific growth rate (%)	0.21 ± 0.00^{a}	0.21 ± 0.00^{a}	0.24 ± 0.00^{bc}	0.26 ± 0.00^{c}	0.24 ± 0.00^{b}	0.24 ± 0.00^{b}
Weight gain (%)	34.38 ± 1.05^{a}	35.48 ± 0.78^{a}	41.24 ± 0.73^{bc}	44.53 ± 1.60^{c}	40.15 ± 0.86^{b}	40.84 ± 1.37^{b}
Feed conversion ratio	6.99 ± 0.20^{c}	6.76 ± 0.15^{c}	5.82 ± 0.10^{ab}	5.40 ± 0.19^{a}	5.98 ± 0.12^{b}	5.89 ± 0.20^{ab}
Survival rate (%)	70 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	80 ± 0.00

Values are means ± SEM of 3 replications; The values in the same row sharing different superscript differ significantly ($P<0.05$) from each other.

Palash flower powder added @ 0, 2.5, 5.0, 7.5 and 10.0% in the experimental diets T_1, T_2, T_3, T_4 and T_5, respectively, in feed groups. Commercial feed 'Optimum' (T_6) served as positive control.

at 450 nm. At the end of experiment, maximum colouration in fish muscle and fish skin was recorded in T_4 tank with values of 15.78 ± 1.6 and 25.09 ± 0.20 µg g^{-1}, respectively. The least carotenoids concentration was found in T_1 tank (12.53 ± 0.35 and 3.29 ± 0.44 µg g^{-1} in fish skin and muscle, respectively) ($p < 0.05$).

The plant materials having pigments are of immense interest as these influence the nutritional quality and serve as excellent source of carotenoids (Ghiasvand *et al.*, 2006). Adding carotenoid

Table 3: Carotenoid content (µg carotenoids g^{-1} tissue), measured at 450 nm, in skin and muscle of Koi carp (*C. carpio* L.) in different treatment tanks

Fish organ	Tank with different feeds	Carotenoid content (µg carotenoids g^{-1} tissue)				
		Initial ± SEM	15 days ± SEM	30 days ± SEM	45 days ± SEM	60 days ± SEM
Muscle	T_1	10.67 ± 0.54	7.10 ± 0.29^{a}	5.05 ± 0.12^{a}	4.18 ± 0.23^{a}	3.29 ± 0.44^{a}
	T_2	10.67 ± 0.54	9.87 ± 0.40^{c}	8.74 ± 0.07^{c}	9.42 ± 0.36^{bc}	12.29 ± 0.90^{c}
	T_3	10.67 ± 0.54	8.89 ± 0.32^{b}	11.87 ± 0.17^{e}	12.29 ± 0.90^{d}	13.57 ± 0.70^{cd}
	T_4	10.67 ± 0.54	9.07 ± 0.15^{bc}	10.46 ± 0.18^{d}	12.28 ± 0.80^{d}	15.78 ± 1.60^{d}
	T_5	10.67 ± 0.54	9.00 ± 0.14^{b}	10.77 ± 0.14^{d}	11.67 ± 1.60^{cd}	12.8 ± 0.51^{c}
	T_6	10.67 ± 0.54	8.33 ± 0.13^{b}	8.10 ± 0.25^{b}	7.69 ± 0.04^{b}	7.11 ± 0.11^{b}
Skin	T_1	18.80 ± 0.23	17.09 ± 0.40^{a}	15.11 ± 0.29^{a}	13.73 ± 0.35^{a}	12.53 ± 0.35^{a}
	T_2	18.80 ± 0.23	18.13 ± 0.35^{abc}	19.33 ± 0.26^{c}	20.80 ± 0.23^{c}	22.40 ± 0.23^{c}
	T_3	18.80 ± 0.23	18.66 ± 0.58^{bc}	20.66 ± 0.26^{d}	21.20 ± 0.23^{c}	23.33 ± 0.13^{d}
	T_4	18.80 ± 0.23	19.15 ± 0.43^{c}	21.21 ± 0.12^{d}	23.39 ± 0.32^{d}	25.09 ± 0.20^{e}
	T_5	18.80 ± 0.23	18.40 ± 0.23^{abc}	19.73 ± 0.35^{c}	20.40 ± 0.23^{c}	22.53 ± 0.13^{c}
	T_6	18.80 ± 0.23	17.73 ± 0.35^{ab}	17.46 ± 0.26^{b}	16.66 ± 0.35^{b}	14.53 ± 0.35^{b}

The values are means ± SEM (standard error of mean) of three replicates. The values in the same column sharing different superscript differ significantly ($P<0.05$) from each other.

Palash flower powder added @ 0, 2.5, 5.0, 7.5 and 10.0% in the experimental diets T_1, T_2, T_3, T_4 and T_5, respectively, in feed groups. Commercial feed 'Optimum' (T_6) served as positive control.

content to the diets like carrot and marigold meal improves the colour quality of ornamental fish. Research findings have revealed that Koi fish treated with 5% *Spirulina* meal had different colour quality than those untreated ones (Andriani *et al.,* 2016). The greater colour intensity revealed the fish's ability to absorb the carotenoid present in feed made from spirulina meal. The ability of ornamental fishes to adapt well to culture conditions increases their capacity to live in a variety of habitats (Chapman, 2000). For successful ornamental fish-rearing, a steady supply of reasonably priced nutritionally balanced feed is required. A significant impact on banded gourami survival has been reported by Das and Biswas (2020) when the amount of papaya meal was varied in treatment diets. The enhancement of body pigmentation begins in the 3rd week of feeding trial, this is the time required for the dietary carotenoids to break down, convert, and rise in pigment cells (chromatophores). The body pigmentation stabilizes as the feeding trial progresses due to the availability of dietary carotenoids to maintain carotenoids in chromatophores. The aforementioned outcome is consistent with Sitorus *et al.* (2015). Kalinowski *et al.* (2005) observed improvement in growth and skin colour of *Pagrus pagrus* when various carotenoid sources were fed as per their dietary levels. Ezhil *et al*. (2008) observed improvement in pigmentation when *X. helleri* was fed a prepared meal containing *Calendula officinalis* and concluded that this lutein can be utilized as a naturally occurring pigmenting source. As per Alma *et al.* (2013) the best amount of carotenoids to boost goldfish skin pigmentation is 200 mg marigold meal. Further, they did not observe any more carotenoid accumulation above this amount. Comparable works on *Etroplus maculatus* fed with a meal rich in marigold oleoresin revealed greater accumulation of total carotenoid (Jagadeesh *et al.,* 2014). The quantity and kind of carotenoid in pigment source or ingredients affect the rate of colour development in fish (Boonyaratpalin and Unprasert, 1989). The use of marigold petal powder and rose petal powder though increased the colour of *Puntius sophore* fish, yet the body growth and storage of flower meal were adversely affected due to rapid decomposition (Jagadeesh *et al.,* 2015; Jain and Kaur, 2016). When Boonyaratpalin and Lovell (1977) gave marigold petal meal to tiger barbs, they discovered that the barbs were more colourful than the fish fed a control diet. Another study found that using petals of Asian pigeonwings meal retain the body colour of Indian rosy barb positively (Mukherjee and Maity., 2024).

In the present study, the carotenoid content of palash flower powder was used as the natural pigment source in fish diets; and good pigmentation with carotenoids accumulation in skin was observed in 7.5% (T_4) feed tank with high specific growth rate and weight gain values, and low FCR value with 100% survivability. The cost effectiveness of the formulated feed is good as compared to the commercial feed. Commercial feed 'Optimum' has market price of ₹ 550 kg^{-1}; whereas the formulated feed costed just ₹ 360 kg^{-1}. Hence, one can easily say that the formulated feed is cost-effective as well as a natural source of carotenoids. Based on these findings, it may be concluded that ornamental fish should always have a suitable pigment-enhancing substance in their diet over an extended time period in order to ensure proper colour.

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