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Papaya Epicarp Powder Meal as Natural Carotenoid Source for Pigmentation and Growth in Indigenous Ornamental Fish, Rosy Barb (*Pethia conchonius*)

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Abstract: Experiment was conducted to observe the efficacy of experimental feeds supplemented with graded levels of papaya epicarp powder (PEP) meal as a natural source of carotenoid on growth and colouration in the muscle and skin of indigenous ornamental fish, rosy barb (*Pethia conchonius*). PEP was incorporated in the control feed (T0) at four different levels (1.5, 3, 4.5, and 6%) to prepare the experimental feeds. During the experimental period of 120 days, water quality parameters remained in optimum range for fish culture. Fish growth in terms of total body length gain was significantly higher in treatment with PEP @ 3% and @ 6%, whereas net weight gain and SGR was significantly higher in T2 (PEP @ 3%), FCR also significantly reduced in T2, whereas PER and condition factor did not show any significant differences. Fish muscle and skin colour in terms of carotenoid content and digital analysis showed significant improvement in diet with PEP @ 6%. Histological changes observed in muscle tissue in terms of increased muscular striations and number of nuclei were also coincides with improvement in growth in PEP @ 3%. The fish growth in terms of weight gain significantly improved in T2, but colouration was significantly enhanced in PEP @ 6%. Based upon these results and considering the value of ornamental fish in terms of colouration, papaya epicarp powder meal can be incorporated @ 6% in diet of ornamental fish, rosy barb.

Keywords: Ornamental fish, Papaya epicarp meal, Rosy barb, Carotenoid, Pigmentation

Ornamental fish keeping is 2^{nd} most popular hobby after photography worldwide due to their high aesthetic value. It is a well-known fact that ornamental fish have highly attractive skin colouration and physical appearance, which is responsible for their high commercial value (Khairnar and Kaur 2021). The ornamental fish industry faces a major challenge when replicating fish colours in captivity. To achieve acceptable pigmentation of ornamental fish integuments, carotenoids (natural or synthetic) need to be added in the regular feed. Further, the colour of the integument is determined by three significant pigments: melanins, pterins, and carotenoids; although fish synthesize pterins and melanins, they cannot synthesize carotenoid pigments (Grether et al., 2001). Therefore, fish rely on dietary carotenoids in natural or synthetic forms to achieve their optimal pigmentation. In addition to pigmentation, the carotenoids also play significant role in growth, reproduction and disease resistance due to antioxidant and anti-inflammatory properties (Nakano and Wiegertjes 2020). Number of natural carotenoid pigments are present in the fruits, flowers, seeds, roots, and leaves of higher plants and several studies have shown an increased interest of researchers in exploiting carotenoid pigments of plant origin (Sathyaruban et al., 2021). Among various plant pigment sources, papaya is one such source, which hold great potential as natural source of carotenoids. India accounts for around 43% of papaya output, producing more than 13.9 million tonnes per year (Sharma et al., 2022). After peeling, papaya fruit is typically served as a salad. The peel accounts for 12.5% of papaya waste and has a high concentration of protein, fat, fibres, phenolic compounds, vitamin C, potassium and other nutrients (Pathak et al., 2019). The orange and red colours of papayas are due to carotenoids such as beta-carotene and beta-cryptoxanthin (Jha et al., 2010). Considering nutritional value of papaya and its pigments status, the study was designed to evaluate the efficacy of papaya epicarp meal as a potential dietary carotenoid supplement for colour enhancement in rosy barb, *Pethia conchonius*, one of the indigenous colourful ornamental fish having great market demand.

MATERIAL AND METHODS

Experimental Details

Preparation of the experimental fiber reinforced plastic (FRP) pools: Experiment was conducted in triplicate in FRP pools (1000 litres capacity) for 120 days at Instructional cum Research Farm of College of Fisheries. Prior to the fish stocking, FRP pools were thoroughly cleaned. One-inch-thick layer of soil was spread at the bottom of each tank followed by liming to disinfect the tanks and maintain the water pH in the optimum range (7.5-8.5) for fish culture. All the tanks were manured (pre-stocking) with cow dung @20, 000 kg ha^{-1} yr^{-1}. The tube well water was used for filling and maintaining the water level in the pools during the experimental period.

Procurement, acclimatization and stocking of the experimental fish: Experimental fish, rosy barb, *Pethia conchonius,* was procured from ornamental fish culture and breeding facility of the College of Fisheries. Fish were kept on a control feed and acclimated under indoor conditions for a month in order to decolorize them. After proper acclimatization, twenty fish were stocked (average initial weight - 0.9-1 g and length - 2-4 cm) in each pool.

Preparation of experimental feeds: Five experimental feeds were tested including one control (T0). In the four experimental feeds, dried papaya epicarp meal powder (PEP) was added @ 1.5% (T1), 3% (T2) 4.5% (T3) and 6% (T4), respectively in control feed composed of mustard meal (23.75%), soybean meal (23.75%), rice bran (47.75%), tapioca flour (3.0%), vitamin –mineral mixture (1.5%) and salt (0.5%). For preparation of experimental feeds, feed ingredients were dried and finely grounded before mixing. Carotenoid source i.e. papaya epicarp was procured from local market and fruit chat shops. Papaya epicarp was separated from other fruit and vegetable wastes and dried in sunlight for 3-4 days followed by oven drying at temperature of 60±5^{0}C. Dried papaya epicarp then finely grounded to form the meal and mixed with other ingredients to make crumbled feed. Following AOAC (2000) guidelines, the proximate analysis (crude protein, crude fat, crude fibre, moisture, and ash) on dry matter (DM) basis of feed ingredients/ experimental feeds was done (Table 1). The values of crude protein, crude fat (ether extract), crude fibre, crude ash and moisture were subtracted from 100 to determine the nitrogen free extract.

Fish feeding: For whole experimental duration (120 days), fish were fed twice a day at 9:30 and 16:30 hr. with control and experimental feeds @ 3% of their body weight (BW). At each sampling (monthly interval), the amount of feed was adjusted based on the increase in fish weight.

Water quality parameters: Water samples were taken every two weeks in the morning hours to analyse physico-chemical characteristics viz. water temperature, pH, dissolved oxygen (D.O.), total Alkalinity (TA), total hardness (TH), ammoniacal - nitrogen (NH3-N), nitrite and nitrate as per standard methods (APHA 2005).

Fish survival and growth: By comparing the number of live fish collected at the end of the experiment with the total number of fish stocked, the survival rate of each treatment was ascertained. Fish were sampled every month to measure body weight and total body length. The total length gain (TLG), net weight gain (NWG), percent total length gain (% TLG), percent net weight gain (% NWG), specific growth rate (SGR), condition factor (K), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated.

TLG = Average final total body length (cm) – Average initial total body length (cm)

%TLG = Final total body length (cm)-initial body length (cm) /initial total body length (cm) x100

NWG = Average final body wt. (g) – Average initial body wt. (g)

%NWG = Final body weight (g) – initial body weight (g) /initial body weight (g) x 100

SGR (% increase in weight /day) = [(*ln* final body wt. – *ln* initial body wt.)/ Culture days] x 100

ln – Natural logarithm

$$\text{Condition Factor (K)} = \frac{\text{Body weight}}{(\text{Body length})^3} \times 100$$

Table 1. Proximate composition (% DM basis) and gross energy (Kcal g^{-1}) of different feed ingredients and experimental feeds

Ingredients/Experimental feed		Crude protein	Ether extract	Crude fibre	Ash	Moisture	NFE	Gross energy
Ingredients	Rice bran	19.49	1.46	14.27	9.67	4.43	50.68	3.32
	Mustard meal	45.54	2.04	11.31	7.00	1.34	32.77	4.11
	Soybean meal	43.27	2.05	5.10	5.67	3.27	40.64	4.30
	Papaya Epicarp Meal (PEP)	17.68	2.03	8.74	8.34	3.67	59.54	3.63
Experimental Feeds	T0	32.34	1.66	10.68	7.63	3.20	44.49	3.81
	T1	32.77	1.69	10.81	7.76	3.26	43.71	3.80
	T2	33.20	1.72	10.94	7.88	3.31	42.95	3.80
	T3	33.63	1.75	11.07	8.01	3.37	42.17	3.79
	T4	34.06	1.78	11.20	8.13	3.42	41.41	3.79

FCR Weight gain (g) = Feed given (g) / Weight gain (g)

PER = Weight gain (g) / Protein intake (g)

Colouration in Fish

Total carotenoid content: Carotenoid analysis in fish muscle and skin colour analysis was analysed by following method Olson (1979) at initiation and termination of the experiment.

Digital analysis of fish skin: Sony full HD camera (DSC-HX 300) was used to take digital pictures of the experimental fish once a month. At least three fish were taken from each replicate to record their skin colour at three different locations: i) directly above the opercular region, ii) at the base of the dorsal fin, and iii) at the caudal peduncle. All camera settings were maintained during the photo shoot. The digital pictures were analysed in CIE and RGB colour scales

CIE colouration scale: Using digital photos, the CIE (Commission International de l'Eclairage) Lab is used to measure the colour of the fish skin. The "Lab colour mode" colour space in Adobe Photoshop is based on CIE Lab with L*, a *, and b* are its three parameters. L* stands for luminosity, which is the lightness that ranges from 0 for black to 100 for white. The balance between red and green is represented by the a* channel, while the balance between yellow and blue is described by the b* channel.

RGB colouration scale: Using RGB (Red, Green, and Blue) values, photographs were examined in Adobe Photoshop to assess the colour intensity of fish skin from various treatments (Hancz et al., 2003, Tlusty 2005).

Histological studies: Three muscle samples per tissue per treatment were taken and were processed as per method of Kong et al., (2008). Every tissue was sliced into 4-6 µm serial pieces using a rotary microtome (Leica RM2125RT, Germany). After being placed on frosted slides (Abdo's Labtech Pty., Ltd., India), the sections were dried for 15 to 20 hours at 35 °C. Hematoxylin and eosin was used to stain tissue sections, which were then examined using a compound microscope (Shanthanagouda et al., 2014). The NIKON 80i microscope was used to view the tissues' histomorphology at a 40× objective, and a digital camera mounted on the microscope was used to take images.

Statistical analysis: The SPSS statistical package (v16.0 for Windows, SPSS Inc., Richmond, CA, USA) was used to do the statistical analysis of the data with Duncan's multiple range test.

RESULTS AND DISCUSSION

Water quality: During the experimental period, temperature, pH, dissolved oxygen, total hardness, total alkalinity, ammonia-nitrogen, nitrite, and nitrate varied from 27.67 to 31.68°C, 6.43 to 7.69, 4.93 to 6.40 mg l^{-1}, 385.33 to 248.67 $CaCO_3$ mg l^{-1}, 254 to 404 $CaCO_3$ mg l^{-1}, 0.005 to 0.049 mg l^{-1}, 0.007 to 0.174 mg l^{-1}, and 0.003 to 0.042 mg l^{-1}, respectively, in the various treatments (T0-T4). Variations were observed for all of the water quality parameters during the culture period, but they remained within the optimum range for ornamental fish growth. Supplementation of feed with PEP for rosy barb had no negative effect on water quality.

Fish survival and growth: After completion of the experiment, fish survival ranged between 75 to 78.33% in treatments and 70% in control diet. Although maximum fish survival was recorded in T3, however the differences were insignificant, for all the treatments (Table 2). Fish growth in terms of total body length gain (TBLG) was significantly higher in T2 & T4 i.e. Papaya epicarp Powder @ 3 % & 6 %, whereas net weight gain (NWG) & specific growth rate (SGR) were significantly higher in T2 i.e. PEP @ 3.0 %. FCR also

Table 2. Survival and growth parameters in different treatments

Parameters	Treatments				
	T0	T1	T2	T3	T4
Survival (%)	70.00^{a}	76.67^{a}	75.00^{a}	78.33^{a}	76.67^{a}
Final total body length (cm)	5.60^{ab}	5.50^{b}	5.90^{a}	5.67^{ab}	5.73^{ab}
% TBLG	31.09^{b}	39.96^{ab}	42.07^{a}	37.55^{ab}	44.11^{a}
Final body weight (g)	3.87^{cd}	3.70^{d}	5.20^{a}	4.20^{b}	4.00^{bc}
NWG	2.91^{c}	2.77^{c}	4.25^{a}	3.24^{b}	3.05^{ab}
%NWG	301.62^{c}	298.32^{c}	445.86^{a}	338.39^{b}	318.91^{c}
SGR	1.16^{c}	1.15^{c}	1.41^{a}	1.23^{b}	1.19^{ab}
K-Value	2.21^{a}	2.32^{a}	2.58^{a}	2.31^{a}	2.14^{a}
FCR	3.60^{a}	3.01^{ab}	2.91^{b}	2.88^{b}	3.10^{ab}
PER	0.62^{a}	0.56^{a}	0.78^{a}	0.73^{a}	0.76^{a}

TLG = Total length gain, NWG = Net weight gain, FCR = Feed conversion ratio, PER = Protein efficiency ratio, SGR= Specific growth rate, CF = Condition factor
Values with same superscript in row do not differ significantly ($P \leq 0.05$)

showed significant improvement in T2 and T3 i.e. PEP @ 3 and 4.5%. Values for condition factor (K value) and PER showed insignificant differences (Table 2). The growth parameters revealed that feed having PEP @ 3% resulted in growth increment in rosy barb with efficient feed utilization. Das and Biswas (2020) observed that supplementing ripe papaya meal @ 4% can be one of the effective and cost-efficient way to promote growth and survival of banded gourami (*Trichogaster fasciata*).

Pimpimol et al. (2020) also revealed that pineapple juice (PA) and dried papaya peel (PP) @ 5% resulted in improved growth performance of channel catfish in a recirculating aquaculture system. Hamid et al., (2022) reported that papaya leaf extract acted as a growth promoter in seabass (*Lates calcarifer*) and red hybrid tilapia (*Oreochromis mossambicus* X *Oreochromis niloticus*) besides reduced hatching time in seabass. Papaya fruit and its parts including leaves, roots, bark, peel, seeds and flesh are rich source of protein, fat, vitamins and pigments, due to which it must have resulted in improved growth of rosy barb in the present experiment (Rodrigo and Perera 2018).

Muscle and skin colouration: PEP supplementation in rosy barb feed at all levels, i.e. 1.5 - 6% enhanced total carotenoid content of skin and muscle significantly as compared to control feed. After completion of the experiment, significantly higher carotenoid (µg g^{-1} wet weight basis) content (13.21) was in fish fed with feed having PEP @ 6% followed by T3, T2 and T1 (Table 3).

Pigmentation is the one of the most important factors in determining the value of ornamental fish. Digital parameters i.e. red, green and blue colouration on RGB and L*a*b* values on CIE scale also reflected corresponding results (Table 4, 5). The pigmentation in fish body is affected by different factors like concentration of carotenoid, dietary lipid, fish species, environment and feeding period (Lee et al., 2010). In the present study, papaya epicarp meal @ 6% resulted in significantly enhanced carotenoid content, which is also reflected in intense colouration of fish skin. One of the earlier studies too reported that incorporation of ripe papaya meal (RPM) @ 4% resulted in enhanced colouration in banded gourami (*Trichogaster fasciata*) without any adverse effect (Das and Biswas 2020). The major component of the papaya epicarp powder meal used in the study is beta-carotene and beta-cryptoxanthin. The colour improvement in rosy barb might be due to the effective utilization of beta-carotene and beta-cryptoxanthin by the fish and revealed in terms of intense orange-red colour.

Histomorphological changes in muscle tissue: The histomorphological observations of control and treated groups were recorded and compared at 40x (Plate I). In control, the muscle tissue of Rosy barb showed normal histological characteristics as each muscle bundle was uniform in shape and possessed peripheral nuclei, with muscle fibres slightly spaced and showed striations as

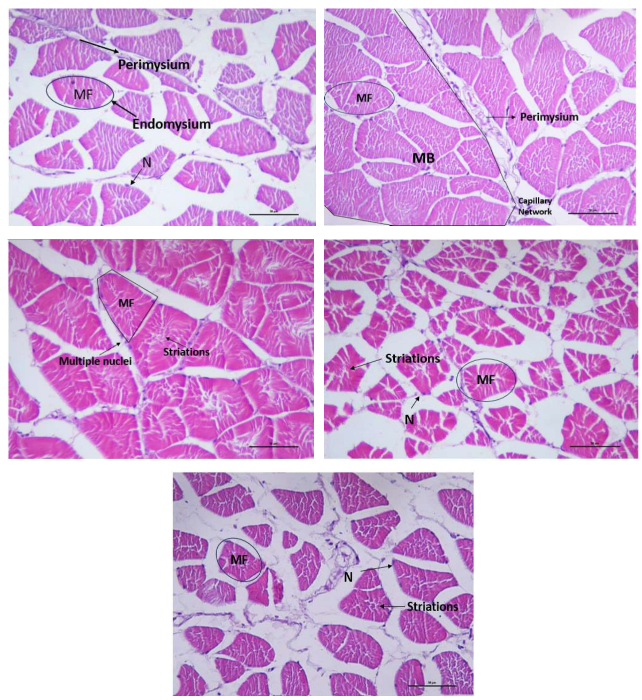

Plate I. Histomorphological changes in muscle tissue of rosy barb at 40x in control and different treatments after completion of the experiment

Table 3. Changes in total carotenoid content (µg g^{-1} wet weight basis) in skin and muscle of rosy barb in different treatments at initiation and termination of experiment

Days	Treatments				
	T0	T1	T2	T3	T4
0	6.79^{a}	6.80^{a}	6.72^{a}	6.58^{a}	6.77^{a}
120	7.385^{c} -----	8.17^{c} (10.70)	8.87^{bc} (20.19)	10.13^{b} (37.26)	13.21^{a} (79.00)

Values with same superscript in row do not differ significantly (P≤ 0.05)
Figures in parenthesis represent % change over control

compared to other treatments. Signs of improvement in muscle tissue quality were observed in T1 and T2 (feed with PEP @1.5 and 3.0%) with enhanced muscle fibres, perimysium and capillary network in muscle fibre along with reduced muscle fibre gapping as compared to control. However, the size of muscle fibre was maximum in T2 (feed with PEP @3%) with increased muscular striation. In this treatment, the number of nuclei in the fish muscle also showed an increase along with less gapping in muscle fibre (Plate I). Similarly, presence of prominent nuclei further supports the notion that PEP promoted cellular activity and growth. The increased muscle fibre size observed in T1 and T2 is a positive indicator of enhanced muscle growth and development. This improvement due to incorporation of PEP

Table 4. Changes in RGB values in skin of rosy barb (*P. conchonius*) in different treatments during the experimental period

Days	RGB value	Treatments				
		T0	T1	T2	T3	T4
0	R	166.67^{a}	173.33^{a}	170.33^{a}	170.33^{a}	173.33^{a}
	G	148.00^{a}	155.00^{a}	151.33^{a}	151.33^{a}	154.33^{a}
	B	105.67^{a}	114.33^{a}	110.67^{a}	111.67^{a}	112.00^{a}
30	R	170.33^{b}	174.67^{b}	187.00^{ab}	182.33^{ab}	197.00^{a}
	G	156.67^{b}	153.67^{b}	160.00^{ab}	163.00^{ab}	174.00^{a}
	B	131.67^{a}	120.67^{a}	111.33^{a}	124.33^{a}	138.67^{a}
60	R	180.33^{a}	189.33^{a}	198.00^{a}	188.33^{a}	200.67^{a}
	G	156.67^{a}	159.00^{a}	163.33^{a}	156.67^{a}	173.33^{a}
	B	112.00^{a}	112.33^{a}	119.33^{a}	110.67^{a}	123.00^{a}
90	R	186.00^{a}	176.00^{a}	196.00^{a}	191.00^{a}	200.00^{a}
	G	156.00^{a}	158.33^{a}	157.33^{a}	156.00^{a}	167.33^{a}
	B	114.67^{a}	127.00^{a}	118.33^{a}	103.33^{a}	124.33^{a}
120	R	195.67^{c}	199.00^{c}	210.33^{b}	214.67^{b}	225.00^{a}
	G	163.33^{c}	168.67^{bc}	170.33^{bc}	173.67^{ab}	180.67^{a}
	B	116.67^{a}	118.33^{a}	120.67^{a}	120.67^{a}	122.67^{a}

Values with same superscript in a row do not differ significantly (p≤0.05)

Table 5. Changes in Lab values in skin of rosy barb (*P. conchonius*) in different treatments during the experimental period

Days	Lab value	Treatments				
		T0	T1	T2	T3	T4
0	L*	61.96^{a}	64.59^{a}	63.23^{a}	63.40^{a}	64.45^{a}
	a*	0.67^{a}	0.67^{a}	0.89^{a}	0.94^{a}	0.63^{a}
	b*	24.96^{a}	23.75^{a}	23.79^{a}	23.49^{a}	24.89^{a}
30	L*	65.23^{b}	64.48^{b}	67.21^{ab}	67.81^{ab}	72.28^{a}
	a*	1.17^{a}	2.84^{a}	3.25^{a}	1.26^{a}	3.22^{a}
	b*	14.84^{a}	19.95^{a}	29.11^{a}	22.89^{a}	21.25^{a}
60	L*	65.76^{a}	67.34^{a}	69.32^{a}	66.50^{a}	72.13^{a}
	a*	2.20^{a}	5.60^{a}	6.91^{a}	5.62^{a}	3.29^{a}
	b*	26.63^{a}	28.43^{a}	27.73^{a}	28.70^{a}	29.31^{a}
90	L	66.00^{a}	66.00^{a}	67.56^{a}	66.40^{a}	70.51^{a}
	a*	5.28^{a}	1.53^{a}	9.00^{a}	6.42^{a}	6.09^{a}
	b*	25.82^{ab}	18.90^{b}	25.94^{ab}	32.37^{a}	26.68^{ab}
120	L	68.96^{d}	70.70^{cd}	72.26^{bc}	73.44^{b}	76.31^{a}
	a*	5.45^{a}	4.49^{a}	8.20^{a}	8.13^{a}	8.95^{a}
	b*	28.49^{a}	29.87^{a}	31.36^{a}	32.78^{a}	35.60^{a}

Values with same superscript in a row do not differ significantly (p≤0.05) is likely due to the presence of essential nutrients required for muscle growth along with bioactive compounds like carotenoids, which might have promoted muscle growth and improve overall health, which can be positively correlated with reduced oxidative stress leading to enhanced cell cellular activity (Chandran et al., 2024). Additionally, the nutritional composition of floral waste, including vitamins and minerals, supports the synthesis of proteins required for muscle development.

CONCLUSION

Colouration is most important parameters to decide the value of ornamental fish. Hence in view of all the results, PEP can be incorporated @ 3% and above in the feed of Rosy Barb with 6% inclusion giving intense colouration. The present study is a step towards valorization of papaya waste in the form of papaya epicarp meal. However, further studies are warranted to explore the potential of papaya epicarp meal through a holistic approach by considering other aspects including breeding performances, fecundity, gut health etc., so that an unexplored nutrients rich (especially carotenoids) source can be utilized for cost effective feed formulation, which will also be a step towards waste to wealth.

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