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Pritam Paul
Research Scholar, Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Surya Kanta Sau
Assistant Professor, Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Anindya Nayak
Subject Matter Specialist (Fisheries), North 24 Parganas Krishi Vigyan Kendra, Ashok Nagar, West Bengal, India

TJ Abraham
Department of Aquatic Animal Health, Faculty of Fishery Sciences, West Bengal University of Animal & Fishery Sciences, Kolkata, West Bengal, India

Corresponding Author:
Pritam Paul
Research Scholar, Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Influence of papaya pulp powder on fin pigmentation of an ornamental fish banded cichlid, *Heros severus*

Pritam Paul, Surya Kanta Sau, Anindya Nayak and TJ Abraham

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Abstract
The monetary worth of ornamental fish in the market mainly depended on the presence of carotenoids which activate the coloration of fish. Nevertheless, there is limited exploration of various natural carotenoid sources as potential feed supplements for ornamental fish. The present feeding trial was conducted to know the influence of papaya pulp powder meal on the fin pigmentation of banded cichlid, *Heros severus*. Five experimental diets were prepared, each containing varying amounts of papaya pulp powder **{**0 g/100 g (C-0), 1 g/100 g (T_1), 2 g/100 g (T_2), 3 g/100 g (T_3), 4 g/100 g (T_4), 5 g/100 g (T_5)**}**. Ten fish per tank, each weighing 4.18±0.3 g and measuring 4.12±0.2 cm were randomly stocked to 12 glass aquaria (L 60 cm x W 30 cm x H 30 cm). The experiment was conducted in duplicate for 60 days. The feed was given at 2% of body weight with equal split doze at 09:00 and 18:00 hours. To maintain a healthy state, any uneaten food and waste were syphoned out on daily basis and water was replaced with fresh water at a rate of 10% to 15% every three days. The carotenoid level variation was observed at 380, 450, 475 and 500 nm wavelength. After the experiment, it was found that there was increase in the total carotenoid concentration (TCC) in the fin of banded cichlid in all five treatments than that of control ones significantly (p<0.01). The results suggest that the supplementation of 3 g/100 g papaya pulp powder in the diet can be effective in enhancing fin pigmentation of banded cichlid.

Keywords: Ornamental fish, *Heros severus*, papaya pulp powder, banded cichlid and carotenoids

Introduction
The banded cichlid, *Heros severus* locally known as “The poor man's discus” an indigenous coloured fish belonging to the family Cichlidae and is a calm and popular ornamental fish to the hobbyists. Due to its consistent behaviour and colour, it commands a fair price in the market, making it of tremendous economic significance. Colour is regarded as a primary determinant influencing the price of the ornamental fish [1]. The colour of the fin of ornamental fish is thought to be caused by chromatophores, which are composed of three primary pigments i.e. purines, carotenoids, and melanins [2]. Carotenoids are the dominant pigment in fish. Carotenoids are natural lipid-soluble yellow to red colour compounds, mostly found in plants, algae, and photosynthetic bacteria [3]. The carotenoids constitute the major pigment in the carrot root, *Daucus carota* [4]. Since fish cannot synthesise carotenoid pigments, the amount and duration of dietary carotenoids in natural or synthetic forms, as well as the type of carotenoids given, determines the intensity of colouration of the integument [5]. Therefore, carotenoids must either be obtained directly from the feed or transformed into feed carotenoid precursors via metabolic processes [6]. In fish, carotenoids are primarily employed for colouration as well as camouflage, signalling, healthy growth, metabolism, and breeding [7]. In addition to their potent colouring effects, carotenoids guard against oxidative stress by acting as antioxidants and radical scavengers. [8]. In their natural habitat, fish satisfy their need for carotenoids by consuming aquatic plants or by way of their food chains. There are more than 600 different types of carotenoids in nature, but only a limited selection finds application in pharmaceuticals, animal feed, food colouring, or chemical polishing [9]. In captivity, deficiency of nutrients and pigment-bearing chemicals may cause the fish's nutrient profile to deteriorate as well as stunted growth and faded colouration [10]. The freshwater colour fish has encountered the issue of fish with faded pigmentation, particularly when the fish are remained in captivity for long time [11]. Now-a-days, numerous scientists are dedicated to enhancing the coloration of the fins or skin of

ornamental fish, recognizing its pivotal role in influencing the market value of these fish. Most of the time, artificial pigments are employed to intensify colour, even though they are costly and can harm the fish health. This prompt the scientist to utilise relatively inexpensive and widely accessible natural additives to intensify the fish colour. However, detailed studies on color enrichment through the different dietary sources in ornamental fishes are still not available. So, the current research aimed to investigate how varying concentrations of papaya pulp powder affect the fin pigmentation of the ornamental fish *Heros severus* in laboratory settings.

Materials and Methods

Experiment site

The present trial took place at the Krishi Vigyan Kendra, Ashok Nagar, North-24-Parganas, affiliated with the West Bengal University of Animal and Fishery Sciences, West Bengal.

Collection and acclimatization of fishes

Adult males of Banded Cichlid (*Heros severus*) of same size group (weight 4.18±0.3 g & length 4.12±0.2 cm) were bought from the same batch of Bengal Aqua Fish Farm, Ashoknagar, North 24 Parganas, West Bengal. The fish were undergone a bath treatment with a 3 ppm $KMnO_4$ solution for 3 to 5 minutes at the experimental site as prophylactic measures to disinfect them. They were then carefully transferred to acclimatization tanks filled with chlorine-free tap water so they could get used to the natural environment. Fish were acclimated in the lab condition for three weeks before the experiment. At 09:00 and 18:00 hours, the fish were given prepared pellet feed in two equal portions at 2% of their body weight (without papaya powder) for three weeks.

Preparation of the experimental tanks

Healthy fish were stocked to the experimental tanks after the acclimatization period. Five treatment sets, each with a duplicate, and a control set of twelve glass aquaria of the same dimensions (L 60 x W 30 x H 30 cm) were employed. The experiment was done for 60 days. Ten fish were maintained in each experimental tank throughout the experiment. To provide aquariums natural aquatic vegetation, a small amount of floating aquatic weed Hydrilla (*Hydrilla verticillata*), was utilised.

Formulation of diet

Papaya pulp powder was blended during the preparation of pelleted feed in varying amounts: 1 g per 100 g (T_1), 2 g per 100 g (T_2), 3 g per 100 g (T_3), 4 g per 100 g (T_4), and 5 g per 100 g (T_5) and one group served as the control (C-0) without papaya pulp powder. The feed ingredients utilized in formulating the experimental diet are listed in Table 1. Fish fed at a rate of 2% of body weight, twice a day, at 9 am in morning and 6 pm in evening. In order to keep the environment healthy, food scraps and waste were syphoned out every day, and 10% to 15% of the water was replenished with fresh water in every three days.

Table 1: Percentage of components in diet formulations.

Ingredients	Amount (%)
Fish meal	25
Soyabean meal	22
Rice bran	20
Groundnut oil cake	15
Wheat flour	12
Starch	3
Soya powder oil	2
Vitamin & mineral mix	1

Analysis of total carotenoid content

Olson's method was used to analyze the total carotenoid content (TCC) in the experiment fish's fin. [12]. A UV-VIS Spectrophotometer (UV 3200, manufactured by LABINDIA Analytical) was used to measure the optical density of the fin samples at 380, 450, 475, and 500 nm. Using the following formula, the carotenoid content (µg/g) was determined-

$$\text{Total Carotenoid Content} = \frac{\text{optical density}}{0{:}25 \text{ x sample weight (g)}} \text{ X } 10$$

where,
The dilution factor is 10 and the extinction coefficient is 0.25.

Statistical analysis

The carotenoid contents in the fin of banded cichlid (*Heros severus*), were statistically analysed through multivariate ANOVA model. Microsoft Excel 2010 software was utilised to exhibit the data graphically, and SPSS for Windows (standard version 21.0) software packages were utilised for statistical analysis of the data. The statistical findings were interpreted at % level of significance.

Results and Discussion

Carotenoid level observed in fin at different wavelengths

Carotenoid concentration at 380 nm wavelength

The fluctuation in the fin's total carotenoid concentration (µg/g) measured at 380 nm is displayed in (Figure-1). The fin's initial carotenoid value was 0.25 µg/g. Fish showed maximum carotenoid content in T_5 (6.89 µg/g) in their fins, with T_4, T_3, T_2 and T_1 following in descending order. The effect of all the papaya pulp powder included in the diet on fin colouration on the fifteenth day of sampling was essentially non-existent. However, the impact of every diet that included papaya pulp powder increased noticeably on the 30th day onwards. On the other hand, there was a very slight rise in the control group's carotenoid concentration.

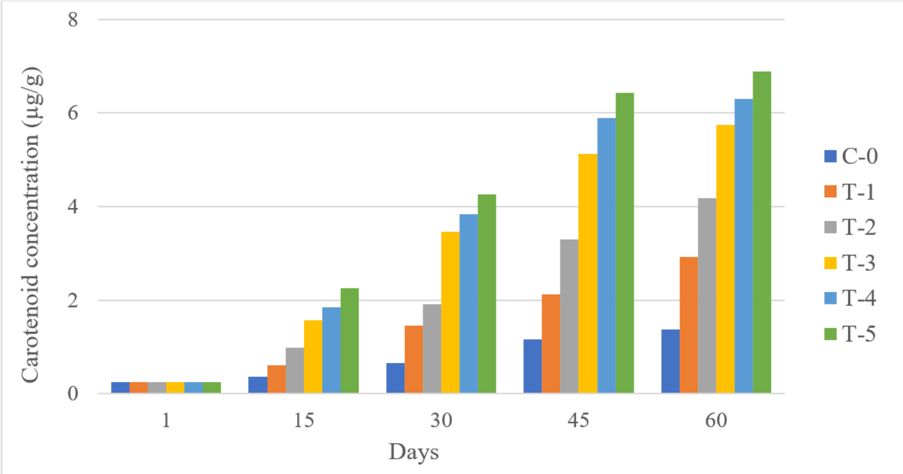

Fig 1: Carotenoid concentration (µg/g) in fin at wave length of 380 nm for different treatments.

Carotenoid concentration at 450 nm wavelength

At 450 nm, the total variation in the carotenoid content of fin is presented in Figure-2. It was evident that all five treatments enhanced fin's total carotenoid concentration over control. At the end of the trial, the carotenoid level in the fin increased to 21.52 µg/g, from an initial concentration of 4.88 µg/g. The highest accumulation of carotenoids was noted in T_3, with T_4, T_5, T_2, and T_1 followed by; whereas, the control group fish showed a negligible increase in the carotenoid level (Figure-2).

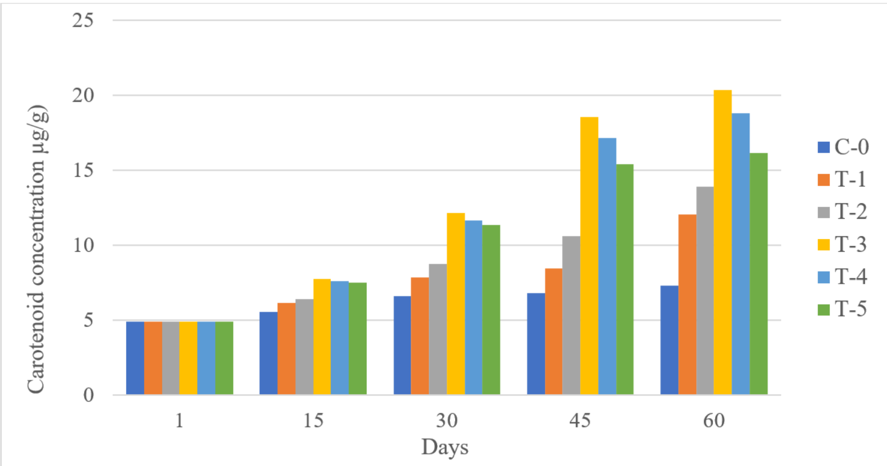

Fig 2: Carotenoid concentration (µg/g) in fin at wave length of 450 nm for different treatments.

Carotenoid concentration at 475 nm wavelength

The carotenoid level variation in the fin of *Heros severus* was also measured at a wavelength of 475 nm (Figure-3). The estimated results showed that total carotenoid level in fin was increased for all the five concentrations of papaya pulp powder added to the diet. In fin, the carotenoid content increased from 0.88 µg/g to 8.12 µg/g throughout the experimental period. The 45th and 60th day of the sampling period in T_3 showed the highest carotenoid pigmentation, which was subsequently followed by T_4, T_5, T_2, T_1, and C-0 (Figure-3).

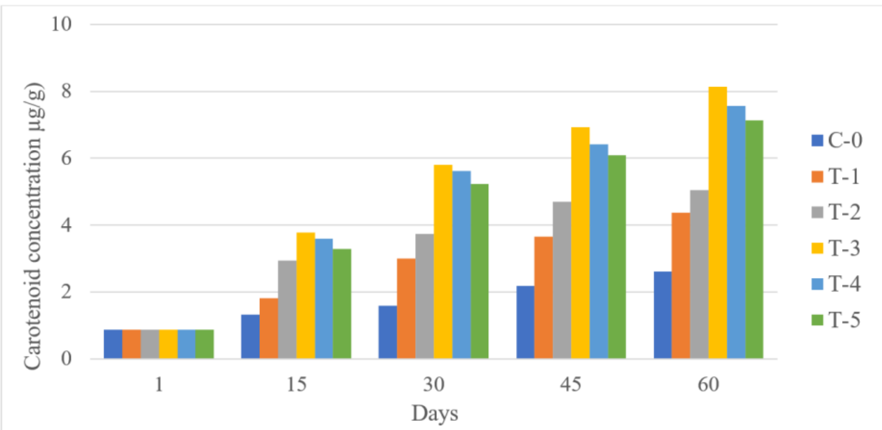

Fig 3: Carotenoid concentration (µg/g) in fin at wave length of 475 nm for different treatments.

Carotenoid concentration at 500 nm wavelength

The carotenoid level variation in the fin was also measured at 500 nm (Figure-4). At the conclusion of the trial, the carotenoid level in the fin increased to 3.94 µg/g from the initial level of 0.17 µg/g. A highest amount of carotenoid was found in T_3 with T_4, T_5, T_2, T_1 and C-0 following in descending order (Figure-4).

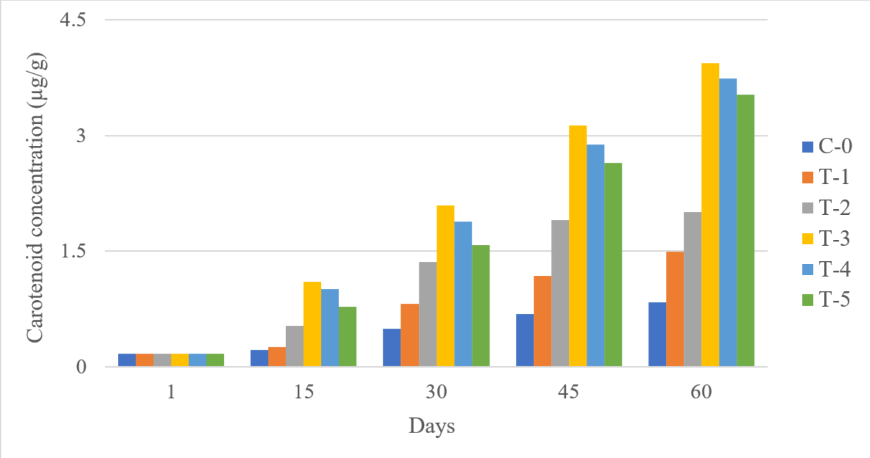

Fig 4: Carotenoid concentration (µg/g) in fin at wavelength of 500 nm for different treatments.

The experiment was done to find out how the inclusion of papaya pulp powder in the diet affected the fin pigmentation of *Heros severus*, a whitish-yellow variety of male banded cichlid. Two-way ANOVA statistical calculation was used to determine how varied diet dosages of papaya pulp powder affected the colour of the fins. The "p" value is 0.01, which was at a significant level ($p<0.01$) according to the two-way ANOVA result. The outcome demonstrated that all the diets that included papaya pulp powder affected the banded cichlid's fin colour significantly.

At 380 nm wavelength, the TCC in the fin was increased with the increasing concentration of papaya pulp powder. The fish showed maximum carotenoid content in T_5 (6.89 µg/g) in their fins, with T_4, T_3, T_2 and T_1 followed by. The results of a similar experiment done on goldfish by feeding them *Ulva reticulata* supplemented supported the results of the current study (Rama *et al.*, 2014) [13]. Khieokhajonkhet *et al.* (2023) who conducted a comparable experiment in goldfish by feeding them turmeric powder incorporated diet [14]. Schiedt *et al.* (1985) claimed that reductive metabolic routes were established from muscle to the skin and fins in order to obtain pigmentation [15]. This could be the reason that T_5 resulted in the highest carotenoid deposits from muscle to fin, compared to other concentrations. Das and Biswas (2020) observed a very similar outcome in banded gourami, *Trichogaster fasciata* administered with papaya powder as like the result of 450 nm wavelength [16]. Our findings also in line with the observation of Vasudhevan *et al.* (2013) who also observed that goldfish's carotenoid accumulation in their fins rose in tandem by the addition of azolla in their diet [17]. The largest accumulation of carotenoid was evident on the 45th and 60th day of sampling in T_3, as indicated by the 475 nm wavelength analysis. This could be because the fin has a disproportionately large amount of carotenoids that were absorbed from papaya and transferred there instead of anywhere else. Song *et al.* (2017) found exact similar findings relevant to our study in discus fish by feeding them synthetic astaxanthin incorporated diet [18]. However, Kim *et al.* (2012) discovered that the carotenoid content of Fancy Carp (*Cyprinus carpio*) did not improve with spirulina powder, paprika powder, astaxanthin, and canthaxanthin [19]. In light of this, it may be said that different species exhibited different levels of carotenoids' effects and intake from diet. The changes of carotenoid concentration measured at 500 nm showed that 3 g/100 g of papaya pulp powder incorporated diet resulted in the best. Mukherjee *et al.* (2009) discovered a comparable outcome in banded gourami, *Trichogaster fasciata*, when incorporating a dosage of 45mg/50 gm of turmeric powder into their diet. [20]. The investigation's outcomes concur with the conclusions of Patel *et al.* (2023) who carried out a comparable experiment on three spot gourami, *Trichopodus trichopterus* by feeding them spinach powder incorporated diet [21]. According to Ranjan (2016), the reduced degree of carotenoid utilization from diet is mainly caused by a low absorption rate in the gastrointestinal tract, metabolic conversion into colourless chemicals and accumulation in other organs which may be eliminated in the near future [22]. This might be due to these reasons: T_1 and T_2 showed comparatively lower fin colouration than T_3, T_4 and T_5. Carotenoids must be absorbed from the digestive tract and delivered to a specific tissue in order for them to be deposited, according to Mirzaee *et al.* (2013) [23]. Fish fed a diet containing 3 g/100 g powdered papaya pulp may absorb the most carotenoids from the diet, which travel from the digestive tract to the fin. For this reason, banded cichlids fed 3 g/100 g papaya pulp showed the greatest increase in carotenoid levels in their fins.

Conclusion

The price of aquarium fish in the global market is influenced by their coloration. In ornamental fish, colouration of fin is very much important. Papaya pulp powder, readily accessible in local markets, can be seamlessly integrated as feed ingredients. The current study has successfully achieved its objectives on a broader scale, yielding some noteworthy findings. The findings of the research will be helpful in understanding the effects of papaya pulp powder on the fin pigmentation of *Heros severus*. The results of the study indicated a maximum rise in the total carotenoid concentration in the fin when fish were provided with a diet supplemented with 3 g/100 g of papaya pulp powder in compared to other treatments. So, it can be concluded that, the feed supplemented with 3 g/100 g of papaya pulp powder must be given to get the best pigmentation in the aquarium fish banded cichlid (*Heros severus*).

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