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Original Article

The effect of ripe papaya, *Carica papaya*, as natural carotenoids meal on body pigmentation and growth performance in banded gourami, *Trichogaster fasciata*

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Abstract: The present investigation elucidates the synergistic effects of improvised ecological parameters and ripe papaya (*Carica papaya*) meal on skin pigmentation, growth performance and survival of Banded gourami, *Trichogaster fasciata*, under confined environment. A feeding trial of 60 days was done with initial length groups from 6.6 to 9.7 cm using five isonitrogenous experimental diets formulated by supplementing graded levels of carotenoids at 1 to 5% and a control without carotenoids. Two ecological parameters temperature and light intensity were elevated using artificial modulators. At the onset of the feeding trial, the total carotenoid concentration in fish muscle in both male (2.94±0.07 $\mu g.g^{-1}$) and female (2.54±0.05 $\mu g.g^{-1}$), respectively, which significantly increased, highest being in male (6.86±0.12 $\mu g.g^{-1}$) and female (5.96±0.07 $\mu g.g^{-1}$) fishes fed with 4% papaya meal. Positive correlation, (0.98) in male and (0.97) female was observed between elevated levels of dietary carotenoids and body pigmentation which revealed that incorporation of dietary carotenoids resulted in a significant increase in total carotenoid concentration in chromatophores. Congenial effects were observed on body indices was revealed by positive correlation of weight (0.79) to elevated levels of carotenoid and 100% survival rate of the fishes. The feeding regimes showed ripe papaya meal as cheap natural colour enhancer source can be safely supplemented at 4% levels in the diets to increase the skin pigmentation and optimum conditioning in optimized captive environment having temperature range of 26-28ºC and light intensity 344.0-346 Lux, without any xenobiotic effect on Banded gourami.

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Introduction

Globally ornamental fish trade is estimated to be more than US$ 15 billion with trading of more than 2 billion live ornamental fishes, is emerging as a much lucrative industry (Katia, 2001; Das and Biswas, 2016). Vibrant body pigmentation is one of the major quality attributes for market acceptability of the ornamental fish (Saxena, 1994; Das and Biswas, 2016). Colouration of fish skin is mainly attributed to the presence of chromatophores that contain pigments, including melanins, pteridines, purines and carotenoids (Chatzifotis et al., 2005). Like other vertebrates, fish do not possess the ability of *de novo* synthesis of carotenoids (Goodwin, 1951). The carotenoid pigmentation of fish is attributed to the pigment present in the diet (Hata and Hata, 1973). Hence, a direct relationship between dietary carotenoids and pigmentation in fish exists (Halten et al., 1995). Carotenoids are a group of naturally occurring lipid soluble organic pigments that are responsible for the red, orange and yellow colour in the skin, flesh, shell and exoskeleton of aquatic animal (Pailan et al., 2012). Ornamental fishes, when kept under captivity for long duration have been found to lose their natural skin coloration; henceforth the loss of pigments can be overcome by the addition of carotenoids to the artificial diet. Hence, the diet for aquarium fish should be nutritionally balanced, palatable, and resistant to crumbling, water stable, and buoyant but it should also enhance body pigmentation in the fish in captivity (Das and Biswas, 2016). From commercial point of view, maintenance of the natural skin pigmentation is of great importance, as it is directly associated with acceptance or rejection by the

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consumers (Shahidi et al., 1998) and its market demand. The prominence of the carotenoid based colouration is reflected as an authentic indicator influencing the foraging ability for carotenoid rich foods (Endler, 1980). Aside from colouring muscle and skin, there is evidence of the beneficial effects of dietary carotenoid supplementation, such as growth enhancement through increased metabolism of fish (Tacon, 1981) and better nutrient utilization (Amar et al., 2001).

Ecological factors such as water temperature is a significant abiotic driver of aquatic ecosystems which influence attributes of an organism including feeding, metabolic and growth rates, reproductive performance, behaviour and ultimate survival (Caissie, 2006; Dallas, 2008; Webb et al., 2008). Moreover, daily endogenous rhythms in fish as well as growth, metabolic rates, locomotory activities, body pigmentation, sexual maturation and reproduction is profoundly influenced by photoperiod since it acts as a synchronizer (Duston and Saunders, 1990; Gross et al., 1995; Silva-Garcia, 1996; Boeuf and Le Bail, 1999; Trippel and Neil, 2002; Biswas and Takeuchi, 2002; Biswas et al., 2002; Biswas et al., 2005).

Researches indicates that ornamental fish require between 50 and 400 mg/L of synthetic or natural carotenoids (e.g., red pepper and marigold extracts) in their diet to develop coloration identical to those of fish consuming live foods (Boonyaratpalin and Lovell, 1977; Fey and Meyers, 1980; Lovell, 1992). In spite of significance of body pigmentation in depth studies on colouration enrichment in native ornamental fish are lacking (Das and Biswas, 2016). Plant sources have been extensively harnessed for inducing pigmentation in ornamental fish. For example, *Arthrospira platensis* (Spirulina) have been used as a source of carotenoid pigments for rainbow trout and fancy carp (Choubert, 1979; Boonyaratpalin and Phromkunthong, 1986; Alagappan et al., 2004). Gouveia et al. (2003) worked with microalgal biomass supplementation have shown that *Chlorella vulgaris* is as efficient as synthetic pigments in the pigmentation of *Cyprinus carpio* and *Carassius auratus*. Alagappan et al. (2004) studied the utilization of algae *Spirulina* sp. as a source of carotenoid pigment for *Trichogaster trichopterus*. Marigold petal meal was used for the tiger barb (*Puntius tetrazona*), red swordtail (*Xiphophorus helleri*) and gold fish (*C. auratus*) to enhance body pigmentation (Boonyarapatin and Lovell, 1977; Ezhil et al., 2008; Alma et al., 2013). In fruits, such as papaya, with the progression of ripening, the content of carotenoids increases (Wall, 2006). The main carotenoids that have been identified in papaya are Lycopene, β-cryptoxanthin, and β-carotene (Marelli de Souza et al., 2008). The banded or giant gourami, *Trichogaster fasciata* (Bloch and Schneider 1801) has high nutritional and ornamental value in India and global market. The present study was carried out to evaluate the efficacy of supplementation of graded levels of ripe papaya (*Carica papaya*) meal on the skin pigmentation and body indices of the Banded gourami, one of the beautiful and hardy native gourami species for tropical aquarium culture.

Materials and Methods

Experimental feeding design: The experimental feeding trial was conducted in the Department of Life Sciences, Dibrugarh University from December 2018 to January 2019. Banded gourami of size group with initial length groups from 6.6 to 9.7 cm was collected from a wetland of Dibrugarh district. Following collection, the fishes were transferred to aquaria of 50 litre with continuous aeration and acclimatized for 8 days in laboratory conditions. The experiment was conducted for 60 days and carried out in 15 aquaria of 50 litre. These 15 aquaria were grouped into five sets and each set consists of 3 replicates. The fishes were stocked at the rate of 15 per aquarium in which 10 were males and 5 were females in each trough. The length and weight of fishes were recorded weekly. The fishes were fed twice daily viz. morning and evening at the rate of 2% of body weight per day. The feed was adjusted after every 10 days according to the body weight of the fish. Aquaria were cleaned properly by siphoning off feed particles and metabolic wastes daily from the bottom. Water exchange was carried out weekly for the sufficient supply of oxygen to the

Table 1. Rearing conditions for experimental *Trichogaster fasciata* during acclimatization.

Days	Diet	Feeding frequency/amount	Water exchange rate (per day)	Density
0-3	None	NA	Twice	15
4-6	Rice bran	Ad libitum	Twice	15
7-10	Rice bran	2% of body weight	Once	15

fish (Table 1).

Diet formulation: For the feeding trail, dry pelleted feed was formulated following Pearson Square method and trial and error method of Hardy (1980). The control feed was prepared using the basic ingredients like fish meal 25%, soya bean meal 22%, groundnut oil cake 15%, rice bran 20% and wheat flour 12%. The selected ingredients were grounded, thoroughly mixed and dough was prepared by adding required amount of luke warm water. This dough was steamed in water bath at 70°C for 40 min. After this, the dough was cool for a while and added other ingredients like Soya oil 2%, starch powder 3%, vitamins and minerals premixes 1% were added and mixed thoroughly. Ripe papaya was dried in Lypholizer until complete moisture was removed, finely powdered and subsequently added in respective concentration of 1, 2, 3, 4 and 5 g/100 g of basal diet by replacing same quantity of rice bran for the preparation of treatment diets (C1 to C5). The pelleted feed was air dried and kept in airtight containers until further use.

Analysis of proximate composition of feed, carotenoid concentration, feed conversion ratio (FCR), protein energy ratio (PER) and survival: Proximate compositions of the experimental diets were analyzed for moisture, crude protein, ether extract and total ash based on AOAC (2005). Quantitative estimation of crude fat was done following the protocol developed by Das and Biswas (2019). Estimation of carotenoids in fish feed was carried out following Britton (1995) with improvisations. Feed conversion ratio (FCR) was calculated by relating the feed consumption to gain in wet weight of fish following Vasudhevan et al. (2013), protein energy ratio (PER) was calculated by relating wet weight gain to protein fed and the survival rate was estimated following Francis (1995).

Fish skin color determination: Total carotenoid concentration (TCC) in the muscle tissue from dorsal and abdominal skin of both male and female fishes was analyzed at an interval of 15 days and after the completion of experiment following Olson (1979). All procedures were performed in low light and temperature as carotenoids are very sensitive to light, temperature and oxygen.

Improvisation and monitoring of water quality parameters: Water parameters viz. water temperature was elevated to 26-28 using water heater and light intensity using candescent fluorescent light (CFL). Parameters such as pH, dissolved oxygen, total hardness, and alkalinity from all the aquaria’s were analyzed at weekly intervals as per standard methods of APHA (2005) and light intensity was measured using software Lux Meter of Microsoft Lumia.

Results

Water quality parameters: Physico-chemical parameters of water in the experimental tanks were estimated weekly during the experimental period are presented in Table 2. The water quality parameters were improvised to maintain within the normal range tolerable for tropical fishes viz., temperature 24 to 30ºC, dissolved oxygen > 5 mg and pH 7 to 8.5 (Santhosh and Singh, 2007) throughout the experimental period.

Proximate composition analysis, growth and survival: Analyzing the proximate composition of the experimental diets was almost similar (Table 3), which can be traced to the inclusion of feed ingredients, which were only differentiated by the level of carotenoids. The protein content of the diets varied from 30.39 to 31.47%. The survival and growth rates of the fishes at end of the experimental period are shown in Table 4. There were significant effects of papaya meal supplementation on growth parameters

Table 2. Ecological parameters recorded in optimized experimental tanks.

Parameter	Minimum	Maximum	Improvised
Temperature (°C)	16±1.5	18±1	26±2
pH	7.2±0.3	7.4±0.1	
Dissolved oxygen (ppm)	5.3±0.9	6.1±0.2	
Light intensity (Lux)	50.6	54.0	344.0±2.4

*Temperature and light intensity was elevated using Seibo water heater and using CFL bulb in the aquaria.

Table 3. Feed composition and proximate analyses of diets.

Ingredients (%)	Control	1 % CF	2% CF	3% CF	4% CF	5% CF
Fish meal	15	15	15	15	15	15
Soybean meal	20	22	22	22	22	22
Groundnut cake	23	23	23	23	23	23
Rice bran	20	19	18	17	16	15
Wheat flour	14	14	14	14	14	14
Starch powder	3	3	3	3	3	3
Soybean oil	2	2	2	2	2	2
Vitamin mineral premix	2	2	2	2	2	2
Carotenoids (dried papaya)	-	1	2	3	4	5
Vitamin C	1	1	1	1	1	1

Chemical composition (% dry wt.)

Diet	Moisture	Crude fat	Crude protein	Crude Fibre	Carotenoids (mg/gm)	NFE	Total Ash
Control	7.56±0.02	8.3±0.01	30.67±0.02	6.28±0.02	0.15±0.01	35.77	11.42±0.02
CF1	7.54±0.03	8.1±0.03	30.63±0.01	6.26±0.01	1.27±0.03	36.02	11.45±0.01
CF2	7.63±0.01	8.3±0.01	30.60±0.03	6.24±0.02	2.13±0.02	35.80	11.43±0.02
CF3	7.59±0.02	8.1±0.02	30.59±0.02	6.26±0.01	2.72±0.01	35.80	11.41±0.01
CF4	7.56±0.02	8.3±0.01	30.67±0.02	6.28±0.01	3.21±0.01	35.77	11.42±0.02
CF5	7.58±0.01	8.2±0.01	30.61±0.03	6.26±0.02	3.46±0.03	35.91	11.44±0.01

CF- Carotenoids feed with numbers referring to percentage of carotenoids incorporated per 100 gm of basal ingredients, FCR- Feed conversion ratio,
Nitrogen free extract = 100 - (% moisture + % crude protein + % crude lipid + % crude fibre + % ash)

of banded gourami. Fishes fed with CF4 (4%) papaya supplemented diet showed considerable increase in values in net weight gain (1.9±0.08 g) in comparison to the other treatments and control group. Feed conversion ratio (FCR) in control group was 1.74±0.05 and the corresponding values in different experimental groups ranged from 1.75±0.06 to 1.79±0.07, highest being in CF4 and lowest being in CF1 (P<0.05) (Table 4). Feed conversion ratio and feed efficiency ratio are considered as indices for feed utilization. The results showed that increased level of supplementation of carotenoids in diets increased FCR and a significant affect was observed on the survival rate of the Banded gourami with treatment diets and control diet ranged from 90 to 100% (Table 3). The results may be attributed to lower densities and/or the hardiness of species, since banded gouramies are very hardy species and survive under extreme conditions.

Effects of the experimental diets on total carotenoid concentration (TCC) of banded gourami skin: At the beginning of the experiment, the total carotenoids concentration in the muscle and skin of banded gourami was 2.37 µg/g wet weights. Enhancement of body pigmentation was witnessed with the brightening from 3rd week of feeding trial. Total carotenoids concentration in the muscle and skin of banded gourami after 60 days of experimental feeding trial showed that the total carotenoids concentration increased with the supplementation of papaya meal in the diet, highest being in 4% levels (6.82±0.10 µg/g

Table 4. Mean survival, weight, length, and FCR of *Trichogaster fasciata* after 60 days of feeding trial.

Diet	Increase in weight (gm)	Increase in length (cm)	Feed intake (g)	FCR	Survival %	Carotenoids% (µg/gm wet weight)	
						Male	Female
Control	1.6 ±0.07	1.2±0.01	97.2	1.74±0.05	90	3.07±0.08	2.64±0.09
CF 1	1.4±0.04	1.2±0.01	92.4	1.75±0.06	100	3.46±0.04	3.21±0.06
CF 2	1.7±0.07	1.4±0.01	95.4	1.77±0.03	100	4.34±0.10	4.04±0.03
CF 3	1.7±0.05	1.3±0.01	99.6	1.78±0.04	100	5.74±0.07	4.88±0.06
CF 4	1.9±0.08	1.6±0.01	96.3	1.79±0.07	100	6.82±0.10	5.92±0.09
CF 5	1.7±0.05	1.5±0.01	95.2	1.78±0.04	100	6.73±0.04	5.81±0.07

The means with different superscript in each row indicate a significant difference ($P<0.05$).

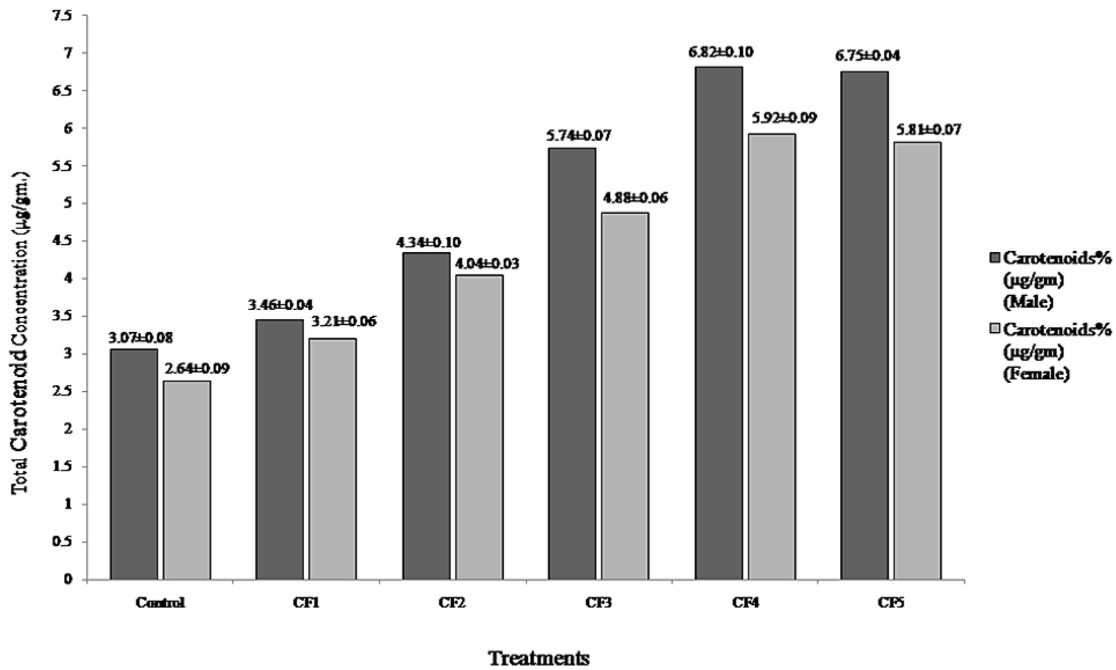

Figure 1. Total carotenoid concentration (µg/gm) of male and female *Trichogaster fasciata* fed with diets containing graded levels of papaya meal (Means with different superscript indicate significant difference ($P<0.05$)).

wet weights) in male and (5.92±0.09 µg/g wet weights) in case of female, and beyond that no further significant increase in carotenoids content was found in both the sexes (Fig. 1). Positive correlation is observed between increasing dietary carotenoids level to increasing skin and muscle carotenoids concentration in both male (0.96) and female (0.97) fishes.

Discussions

High adaptability of ornamental fishes to culture conditions enhances their capability of living in environments having wide ranges (Chapman, 2000). Successful ornamental fish culture necessities a continuous supply of nutritionally balanced, cost-effective feed. The results showed that different level of supplementation of papaya meal in the treatment diets has notable effect on survival of banded gourami. Lower survival of Atlantic salmon (*Salmo salar*) fry (Christiansen et al., 1995) and juveniles (Christiansen and Torrissen, 1996) was found when fed with diets without asthaxanthin supplementation in comparison to the groups that were fed diets containing asthaxanthin. In the present work, increased FCR in the fish was observed with increasing level of carotenoids supplementation in diets. Optimum FCR was obtained by Christiansen and Torrissen (1996) in Atlantic salmon juveniles fed with astaxanthin supplemented diet. Similarly, in *Penaeus monodon*, better FCR was observed with prawn fed on the diet containing spirulina (*Arthrospira platensis*).

The onset of enhancement of body pigmentation from the 3rd week of feeding trial is due to time intake for breaking of dietary carotenoids and subsequent transformation and increase of carotenoids in pigment cells (chromatophore). The stabilization of body pigmentation during the progression of feeding trial is due to the consequent supply of dietary carotenoids for maintaining the quantity of carotenoids in chromatophores. The above result coincides to the results of Sitorus et al. (2015). Kalinowski et al. (2005) observed positive effect on growth and skin colour in *Pagrus pagrus* when fed with different carotenoid sources with their dietary levels. Ezhil et al. (2008) observed enhancement of pigmentation in *X. helleri* when fed with formulated feed containing *Calendula officinalis* concluding that this lutein can be used as pigmenting source of natural origin. The carotenoid source and its effectiveness on deposition of pigments in fish body is species specific (Ha et al., 1993). Sexual dichromatism is often common in several fish species. In general males are more brightly coloured than their female counterparts (Gogoi et al., 2013). In the present feeding trial, even female fish fed with carotenoid diets also exhibit the remarkable pigmentation due to increased total carotenoid concentration in their body. Alma et al. (2013) reported that 200 mg of carotenoids from marigold meal is optimum to increase the pigmentation in skin of goldfish and over that level they have not found any additional accumulation of carotenoids. Similar studies on *Etroplus maculatus* fed with diet having marigold oleoresin contained the highest total carotenoid concentration (Jagadeesh et al., 2014). The rate of colour development observed to depend on the amount and nature of carotenoid present on the pigment source/ingredients (Boonyaratpalin and Unprasert, 1989). The present results confirm that percentage of colour development was higher in carotenoids fed fishes and directly attributed to the higher level of carotenoid content in the particular ingredients.

The study enunciates formulated diets incorporated with carotenogenic biomass revealed stabilization and enhancement of body pigmentation during the maximum study period. The most successful period in inducing the skin colouration was after 3 week of feeding papaya meal at 4% concentration and retained further. Therefore, the supplementation of ripe papaya meal at 4% concentration in the feeds of Banded gourami significantly improved its colouration without any adverse effect on growth, feed conversion efficiency and survival in aquarium conditions. Due to the adverse effects of synthetic carotenoids on aquatic environment, many natural plant sources can be harnessed and incorporated in formulated feeds for colour retention or enhancement of ornamental fishes in captive environment. It will aid in creation of avenues for promotion of the ornamental fish industry as well as colour enhancer feed industry and employment generation (Das and Biswas, 2016).

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