Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43A, No.1,
January-June 2024: P.152-164

Print version ISSN 0970 0765
Online version ISSN 2320 3188
DOI: https://doi.org/10.52710/bpas-zoo.34

Original Research Article
Content Available online at: https://www.bpasjournals.com/

Effect of Dietary Supplementation of Annatto (*Bixa orellana*) Seed Meal on Growth, Survival, Whole-Body Composition and Total Carotenoid Content of Rosy Barb, *Puntius conchonius*

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Received 05.01.2024
Revised 25.04.2024
Accepted 15.05.2024

ABSTRACT:
A 65-day feeding trial was performed on rosy barb, *Puntius conchonius* to evaluate the effects of diets supplemented with 0%(control), 0.5%(B1), 1%(B2), 1.5%(B3), 2%(B4) and 2.5%(B5) annatto seed meal and with 0.01% lucantin pink(positive control) on growth, survival, proximate body composition and total carotenoid content in fish skin and muscle tissue. For the study, 21 aquaria were used, each with 30 juveniles of uniform weight (1.51±0.01gm). At the end of feeding experiment, significantly highest final body weight (2.66±0.01gm) weight gain(1.04±0.01gm), final length (5.16±0.01cm), specific growth rate (SGR) (0.88±0.01%) and condition factor (CF)(1.871±0.003) was found in the fishes those were fed with experimental diet B3, followed by B2 set, B1 set and the lowest values were noted in B5 set. Feed conversion ratio (FCR) of the control fishes was 0.108±0.001 whereas the best FCR was observed in fishes of B3 set (0.079±0.001) and the poorest in B5 diet set (0.193±0.001). Total carotenoid content in fish skin and muscle was highest in B3 set (9.63±0.13), followed by B2 set (7.03±0.10), B1 set (6.21±0.14) and the lowest value was found in B5 set (2.11±0.28). No significant effect of dietary treatments was noticed in whole-body composition of experimental fishes. As survival was 100% in B1, B2 and B3 sets, so annatto seed meal could be supplemented safely up to 1.5% in the diet of rosy barb to ensure enhanced skin pigmentation, growth, feed utilization and survival. Thus, annatto seed meal could be successfully added as an alternative, cost-effective, easily accessible natural carotenoid source in rosy barb diet to enhance skin coloration.

Keywords:
Annatto Seed, Carotenoid Content, Growth, Rosy Barb, Survival.

How to cite this article: Das M. and Haque R. (2024). Effect of Dietary Supplementation of Annatto (*Bixa orellana*) Seed Meal on Growth, Survival, Whole-Body Composition and Total Carotenoid Content of Rosy Barb, *Puntius conchonius. Bulletin of Pure and Applied Sciences-Zoology*, 43A (1), 152-164.

INTRODUCTION

Fishes, reared in confined spaces for their aesthetic values are known as ornamental fishes. They are colorful, attractive and peaceful. In urban areas and cities ornamental fishes are kept as decorative objects that bring peace of mind and keep us away from day-to-day stress. Globally, ornamental fishes are popular pets, with over 4500 freshwater species and 1450 marine species traded worldwide. According to Saxby, Adams, Snellgrove, Wilson, and Sloman (2010), trade in ornamental fishes is an important source of income for many countries. Estimates of economic values of the industry range between U.S. $800 million and $30 billion annually and between 350 million and 1.5 billion live fishes are thought to be traded worldwide (Ploeg, 2007).

Pigmentation is one of the major factors that decides the market value of ornamental fishes. Attractive coloration helps to determine the commercial value of these fishes in the world market (Tiewsoh, Singh, Nath, Surnar, and Priyadarshini, 2019). Pigments found in fish muscle and skin are responsible for coloration in ornamental fishes (Miller-Morgan 2010; Ahilan, Jegan, Felix, and Raveneswaran, 2008). Experiments conducted by Saxby et al. (2010) showed that carotenoids are the main source of pigmentation of fish skin and produce a wide spectrum of vibrant colors. Tetraterpenoids, the carotenoids can be yellow, orange, and red organic pigments produced by plants, algae, bacteria, and by several fungi as well. There are over 1100 known carotenoids which can be further categorized into two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons and contain no oxygen) (Miller-Morgan, 2010), both are the derivatives of tetraterpenes which are produced from eight isoprene molecules and contains forty carbon atoms being built from four terpene units each containing ten carbon atoms. Structurally, carotenoids take the form of a polyene hydrocarbon chain which sometimes terminates by rings, and may or may not have additional oxygen atom attached and belong to the category of tetraterpenoids. Xanthophylls are carotenoids with molecules containing oxygen, such as lutein and zeaxanthin. The non-oxygenated (oxygen-free) carotenoids such as alpha-carotene, beta-carotene, and lycopene, are known as carotenes. Carotenes typically contain only carbon and hydrogen. Hence, they are the hydrocarbons under the subclass of unsaturated hydrocarbons.

In the natural environment, carotenoid requirements are met by fishes by ingesting aquatic plants or through the food chain. Ploeg (2007), Ahilan et al. (2008), Hata (1972), Simpson, Katayama and Chichester (1981) and Meyers (1994) experimentally proved that fish are unable to synthesize carotenoids *de novo.*

Annatto plant (*Bixa orellana*) is a tropical shrub, found in central and southern America (Brazil, Mexico and Guatemala) and East Asia (Philippines) (Karimi, 2011; Giridhar et al., 2014). It produces fruits that bear nearly 50 orange-reddish seeds. Annatto seeds contain natural, non-toxic, and non-carcinogenic pigment. Of the seed weight, about 4.5% to 5.5% is the total carotenoids (Giridhar et al., 2014) including bixin which is oil soluble, and norbixin which is alkaline water soluble, comprising up to 80% and 20% of total carotenoid content, respectively (Safari and Atash, 2014; Yolmeh, Habibi-Najafi, and Farhoosh, 2014). Annatto seeds are known as poor man's saffron, used for coloring human foods as well as animal feeds (Franco et al., 2002) and also used in cosmetics, pharmaceuticals, and textiles. These seeds are used in medicine for the treatment of high lipid blood levels (Ferreira et al., 2013). Few earlier studies include annatto seed meal in fish feed for the enhancement of fish skin coloration. Safari and Atash (2014) revealed that the dietary inclusion of annatto seed meal enhanced the fillet pigmentation of rainbow trout. In a similar feeding trial, Dananjaya et al. (2019) showed that annatto seed meal could be used as an effective natural carotenoid source for enhancing pigmentation and color in goldfish.

One of the popular aquarium fish, the rosy barb (*Puntius conchonius),* is an indigenous colorful ornamental fish. It is a freshwater cyprinid fish found in Southern Asia from Afghanistan to Bangladesh having great market demand. It

grows up to 6 inches (14cm) in length. Their color becomes bolder during their mating periods. It is very popular for its attractive pinkish color. Effects of different carotenoids on the coloration, growth, and survival of rosy barb have been studied by several researchers like Pailan, Sinha, and Kumar (2012), Jagadeesh et al., (2015), and others.

The present study aimed to investigate whether annatto seed meal could be used as a low-cost natural carotenoid source to enhance the skin coloration of experimental fish, rosy barb (*Puntius conchonius).*

MATERIAL AND METHODS

Rosy barb juveniles were purchased from the Galiff Street ornamental fish market, West Bengal for the commencement of the feeding experiment. Then, they were acclimatized to laboratory conditions for two weeks in a glass aquarium (36"×12"×12") with a control diet containing 32% crude protein. For the 65-day feeding experiment at the laboratory of Vidyasagar College, Salt Lake, Kolkata, a total number of 630 juveniles of uniform weight of 1.51±0.01gm, were chosen and distributed in 21 glass aquariums (18"×12"×12") having seven dietary treatment sets with three replications, each aquarium with 30 juveniles. During the feeding trial, diets were given at the rate of 5% of their body weight to the experimental fishes twice a day at 9 am and 6 pm, at the amount of satiation level, and daily food consumption of each set was recorded. All the waste like uneaten food particles and fecal matter, were siphoned daily. In each aquarium, one-third level of water was exchanged twice a week. To ensure better survival of fishes, each experimental tank was provided with continuous aeration by mini air blower pumps throughout the feeding trial.

Experimental diet

Annatto seeds were purchased from the nearby local market, dried in a hot air oven at 40˚C for three days, and then ground by electric grinder into powder form to prepare the annatto seed meal. All the feeding ingredients, used for the preparation of the experimental diets, were also ground into powder form and kept in airtight plastic zipper bags at room temperature until used for diet preparation.

The feeding experiment was carried out with one control diet, one positive control diet, and five treatment diets with five different proportions of ground annatto seed meal. These seven different fishmeal-based iso-proteic experimental diets were formulated with 32% of crude protein to meet the nutritional requirement of rosy barbs. Practical diets were prepared by inclusion of ground annatto seed meal at the level of 0%(control), 0.5%(B1), 1%(B2), 1.5%(B3), 2%(B4) and 2.5%(B5) by replacing the amounts of rice bran. Similarly, a positive control diet was prepared with the same proportion of feed ingredients, but instead of an annatto seed meal, lucantin pink was added at a level of 0.01%.

For the preparation of diets, all the dried dietary ingredients were mixed thoroughly and dough was made by adding required amount of double-distilled water, and then pellets were formed by the pelletizer with a 1.5 mm die. Then moist noodles were dried under shade for 72 hours and then crushed into desirable particle sizes and kept in airtight plastic zipper bags at room temperature until used. The dietary ingredients proportion and proximate composition of all seven experimental diets are listed in Table 2.

Growth efficiency

At the end of the feeding trial, fishes from each experimental set were collected, and their final weights and lengths were noted to estimate their growth performance parameters such as weight gain and specific growth rate (SGR%) and feed utilization parameters like food conversion ratio (FCR).

Proximate Composition Analysis

At the termination of the feeding experiment, fishes from each experimental set were collected to estimate whole-body composition. The proximate composition such as crude protein, crude lipid, ash, and moisture contents of the seven experimental diets and whole-body of the fishes from the seven experimental sets, were analyzed by following the methods of AOAC (2006).

Estimation of carotenoid

Carotenoid contents of the seven experimental diets were estimated by following the method of Cyanotech (2002). At the end of the feeding experiment, colored region of skin and muscle tissue of fishes from the seven experimental sets were collected to estimate the total carotenoid content by Tiewsoh et al. (2019) method.

Total carotenoid concentration = [(Absorption at maximum wavelength/0.25) × sample weight in gm] × 10

Where, 10 = Dilution factor; 0.25 = Extinction coefficient

Survival

Survival percentage of experimental fishes from the seven different sets, was calculated by subtracting the number of fishes collected at the end of the feeding trial from the fishes, stocked at the start of the feeding trial.

Water Quality Analysis

Water quality parameters such as water temperature, dissolved oxygen, pH, free carbon dioxide, total hardness and total dissolved solids (TDS) of experimental aquariums were analysed and recorded twice a month, throughout the feeding trial period using the standard methods of APHA (2012).

Statistical Analysis

The experimental data were presented as mean ± standard error (SE) of the three replications. One-way analysis of variance (ANOVA), followed by Duncan's multiple range tests (DMRT) for multiple comparisons at the significance level of 0.05 was used to compare the differences among the seven dietary treatments.

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RESULTS AND DISCUSSION

One of the most popular hobbies throughout the world is ornamental fishkeeping. The vibrant coloration of the ornamental fishes is one of the aspects that gives them tremendous popularity. The present study was carried out to determine the effect of annatto seed meal on the color enhancement of ornamental fish like rosy barb, in order to produce aquarium fishes with proper vibrant coloration at a low-cost fish feed that will ultimately help the fish traders to gain a huge profit.

Water quality

The average physicochemical parameters of water like water temperature, pH, dissolved oxygen, free carbon dioxide, TDS, and total hardness (Table 1) did not vary significantly among the different experimental aquarium sets and was maintained within an acceptable range during the entire experimental period (Rinna, Jansi and Vasudhevan, 2013) except the water hardness and TDS, their value ranges were found to be quite high. In this respect, Chapman (2000) stated that ornamental fishes can withstand a wide range of water quality in their habitat and are highly adaptable to cultural conditions

Table 1: Water quality parameters of experimental aquarium sets during the experiment

Parameter	Minimum	Maximum
Temperature (^{o}C)	26	32
Dissolved Oxygen (ppm)	8.1	8.4
pH	7.2	7.4
Free Carbon dioxide (ppm)	1.46	2.18
Hardness (ppm)	590	660
TDS (ppm)	910	1070

Experimental diet

Dietary ingredients proportions and nutrient composition of all the seven experimental diets were displayed in Table 2. Fishmeal was incorporated at the level of 35 gm per 100 gm in all the experimental diets and 0%, 0.5%, 1%, 1.5%, 2% and 2.5% of powdered annatto seed meal was added in the diet by replacing the amount of rice bran and named the diets as control, B1, B2, B3, B4 and B5 respectively. A positive control diet was prepared with the same proportion of all the dietary ingredients as the control diets, but lucantin was added at a level of 0.01%. Relatively, a consistent percentage of crude protein content among the experimental diets (32% to 32.30%) was found.

Table 2: Ingredients, their proportion and proximate composition of experimental diets

Diet ingredients (gm/100gm)	Control	Positive Control	B1	B2	B3	B4	B5
Fishmeal	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Wheat	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Rice bran	20.00	20.00	19.50	19.00	18.50	18.00	17.50
GOC	6.74	6.74	6.74	6.74	6.74	6.74	6.74
Soybean	20.76	20.76	20.76	20.76	20.76	20.76	20.76
Vit-Min Premix*	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Lucantin pink	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Annatto seed meal	0.00	0.00	0.50	1.00	1.50	2.00	2.50
Total	100	100	100	100	100	100	100
Crude protein	32±0.58ns	32.17±0.57ns	32.28±0.832ns	32.09±0.55ns	32.27±0.81ns	32.30±0.85ns	32.22±0.86ns
Crude lipid	3.33±0.15ns	3.39±0.20ns	3.44±0.23ns	3.27±0.24ns	3.26±0.27ns	3.32±0.27ns	3.32±0.48ns
Ash	2.84±0.21ns	2.85±0.20ns	2.82±0.51ns	2.82±0.47ns	2.82±0.36ns	2.84±0.36ns	2.83±0.34ns
Crude fibre	9.33±0.97ns	9.07±1.02ns	9.03±1.05ns	8.97±0.97ns	7.95±0.74ns	8.79±0.75ns	8.66±0.79ns

*GOC- groundnut oil cake

*Vit-Mineral Premix (mg/kg diet): retinol-18,000 IU, Choleclaciferol-2000 IU, thiamine-15, menadione sodium bisulphate-10, riboflavin-25, pyridoxine-5, α-tocopherol-35, nicotinic acid-200, Ca-pantothenate-50, biotin-1.5, folic acid-10, cyanocobalamin-0.03, ascorbyl monophosphate-50, inositol-400, copper sulphate-20.2, dibasic calcium phosphate-5.9, sodium fluoride-2.21, potassium iodide-0.78, zinc oxide-37.5, ironsulphate-200, magnesium oxide-840, manganese oxide-26, cobalt sulphate-1.85, sodium selenite-0.65, potassium chloride-1.17, sodium chloride-0.45.

Values of proximate composition are presented as mean ± SE. Values with ns letters are not significantly different (P>0.05) in one way ANOVA

Growth performance

Annatto seed meal added diet-fed fishes showed acceptable results for growth performances when this meal was added to the diets at a certain level. Carotenoids play a crucial role in the intermediary metabolism of fish that enhances the utilization of feed and finally results in improved growth (Amar et al., 2001). Prior studies showed the positive role of carotenoids in the metabolism of fishes (Tacon, 1981) and also in better nutrient utilization and improved growth (Amar, Kiron, Satoh and Watanabe, 2001). Growth is one of the important criteria that indicates the physiology and health of fishes (Huntingford et al., 2006).

The effect of different dietary treatments on the growth performance and feed utilization of experimental rosy barbs are shown in Table 3. The initial length and weight of the juveniles of all seven dietary sets did not vary significantly (P>0.05; DMRT). After the 65-day feeding trial,

significant variations were found in the growth efficiency parameters like final body weight, weight gain, specific growth rate in percent (SGR%) and also in feed utilization parameters such as total food consumption and food conversion ratio (FCR).

Significantly highest final body weight ($P>0.05$; DMRT) was noted in the fishes fed with the B3 diet, followed by the B2 set and then B1 set and a significantly lower value was recorded from the B5 set. The final body weight of the fish fed with the positive control diet was significantly ($P<0.05$; DMRT) higher than the control set. Whereas, fishes fed with B1, B2, and B3 diets, were recorded with higher final body weight and B4 and B5 sets with lower values than both the control and positive control sets. A similar trend of results was observed in the case of the final length of the experimental fishes, where maximum length was attained by the fishes fed with the B3 diet and minimum by the B5 sets ($P>0.05$; DMRT).

In the treatment of B4 and B5 where the incorporation of annatto seed meal at a level above 1.5%, the final body length, final weight and gain in weight of rosy barbs were reduced. This finding follows the study of Bordner et al. (1986), where diets, mixed with astaxanthin concentrate were found to enhance the growth of *Homarus americanus* up to a certain dose. In a similar feeding trial with zebrafish was revealed that the growth performances of the experimental fishes were improved with the increased level of carrot meal inclusion up to a certain level, beyond that level with a high percentage of carrot meal supplemented diet, a reduced growth of experimental fishes was recorded (Das, 2023). Correlation between growth performance and carotenoid content in the supplemented diets was observed in characins and koi carps (Pan, Chien and Wang, 2010; Maiti et al., 2017). An increase in weight was found to be positively related to the increased length, this observation was similar to the studies conducted by Pailan et al. (2012).

Specific growth rate helps to measure the percentage increase in fish weight per day. Prior researches revealed that dietary carotenoids showed positive effects on SGR percent in red tilapia (Boonyaratpalin and Unprasert, 1989). In this regard, Tacon (1981) depicted from his experiment that carotenoids play positive roles in metabolism of fish.

In the present study, the obtained result of SGR was found to follow a similar trend as noted in the result of weight gain. Juveniles, fed with the B3 practical diets containing 1.5% annatto seed meal, reached a significantly higher ($P<0.05$; DMRT) SGR, followed by the B2 set and then in the B1 set, and a lower SGR was noted in the fishes fed with the diet containing 2.5% annatto seed meal (B5). Values of SGR were significantly ($P<0.05$; DMRT) higher in the fishes fed with B1, B2, and B3 diets when compared with the control set.

Surprisingly, it was found that increasing the concentration of annatto seed meal beyond 1.5% in the experimental diets resulted in decreased SGR values. This observation was in accordance with the findings of the earlier studies, conducted on goldfish fed with bixin and zebrafish fed with carrot meal (Dananjaya et al., 2017; Das, 2023). Thus, for optimum growth performances, determination of the appropriate inclusion level of natural carotenoid sources in fish feed is essential.

In the case of total food consumption, the maximum amount of food was taken by the fishes of the B3 set, followed by the B2 set and then the B1 set, while a minimum amount was consumed by the fishes of set B5. Fishes of the sets B1, B2 and B3, consumed a higher amount of diet when compared with the control set.

Feed conversion ratio is the ratio that depicts the efficiency of an animal to convert the feed into desired output i.e body mass gained by that animal. It is considered as an index of feed utilization. The FCR values are useful in the estimation of the amount of feed required for the optimum growth of fishes during the growing phase and thereby help the aquaculture industry to make wise choices in selecting the fish feed to maximize their profitability.

In the current feeding experiment, the FCR values obtained from the different diet-fed sets, ranged from 0.079 to 0.193 (Table 3). All the

prepared experimental diets were nutritionally balanced for the proper growth and maintenance of the experimental fishes. However, improved FCR was noticed in the B3 with the best value, followed by the B2 set and then in the B1 set, whereas the poorest FCR was noticed in the B5 set among all the seven dietary treatments. Hence, the amounts of carotenoid in the experimental diets could have a role in feed utilization by the selected fish. Feed utilization of the rosy barbs was maximum when they were fed with a 1.5% annatto seed meal added diet (B3 diet). Amar et al. (2001) reported that carotenoids by playing a positive role in the intermediary metabolism of fish increase their feed utilization and finally result in improved growth.

Table 3: Growth and food utilization parameters of rosy barb, *P. conchonius* fed with seven experimental diets.

Growth parameters	Diets						
	Control	Positive Control	B1	B2	B3	B4	B5
Initial weight (gm)	1.51±0.01a	1.51±0.01a	1.51±0.01a	1.51±0.01a	1.50±0.01a	1.51±0.01a	1.51±0.01a
Initial length (cm)	4.13±0.01a	4.11±0.01a	4.12±0.01a	4.11±0.01a	4.11±0.01a	4.13±0.01	4.11±0.01a
Final weight (gm)	2.09±0.01c	2.20±0.01d	2.34±0.01e	2.55±0.01f	2.66±0.01g	1.95±0.02b	1.80±0.02a
Final length (cm)	4.86±0.01c	4.95±0.01d	5.05±0.01e	5.16±0.01f	5.22±0.01g	4.71±0.01b	4.62±0.01a
Weight gain (gm)	0.57±0.01c	0.68±0.01d	0.82±0.01e	1.04±0.01f	1.15±0.01g	0.44±0.02b	0.29±0.02a
SGR (%)	0.49±0.01c	0.57±0.01d	0.67±0.01e	0.81±0.01f	0.88±0.01g	0.39±0.01b	0.27±0.01a
Condition factor (CF)	1.814±0.003a	1.815±0.004a	1.826±0.011a	1.855±0.007b	1.871±0.003c	1.859±0.007b	1.811±0.002a
Food consumption (gm)	0.062±0.001c	0.067±0.001d	0.073±0.001e	0.084±0.001f	0.091±0.001g	0.060±0.001b	0.056±0.001a
FCR	0.108±0.001e	0.098±0.001d	0.089±0.001c	0.081±0.001b	0.079±0.001a	0.138±0.001f	0.193±0.001g

Values are mean ± SE. Values with different letters are significantly different ($P<0.05$) using DMRT after one way ANOVA.

Prior studies revealed that better FCR was observed in rosy barb and zebrafish when were fed a diet containing natural carotenoid sources rose petal and carrot meal respectively (Pailan et al., 2012; Das, 2023). A similar finding was obtained by Christiansen and Torrissen (1996) in the case of Atlantic salmon juveniles when fed with astaxanthin-supplemented feed.

The condition factor is used to compare the well-being or condition of fish. It gives information about the physiology and health of the fish population (Blackwell Brown, and Willis, 2000). High condition factor indicates healthy fish and healthy habitat. Early investigations stated that the condition factor is one of the significant parameters for calculating growth level and provides an overview of the health of fish (Richter, 2007).

In the current study, the effect of annatto seed meal on the condition factor of rosy barb was presented in Table 3. The highest value was recorded in the fishes fed with the B3 diet and the lowest in the B5 set. Thus, fishes fed with a diet having 1.5% annatto seed, were in the healthiest condition among the fishes of the other experimental sets. While increasing the level of annatto seed meal inclusion in the diets, may adversely affect the physiological conditions of the rosy barbs.

Carotenoid concentration of experimental meal and of rosy barb skin and muscle tissue

Skin pigmentation is one the important criteria for deciding the commercial value of ornamental fishes. Duration of supplementation and concentration of dietary pigment affect the skin coloration of fishes (Torrisen, 1985). Studies conducted by Gouveia, Rema, Pereira and Empis (2003) stated that coloration helps to determine the market value of ornamental fish. Past investigations on natural carotenoid supplementation in the basal diet of fishes revealed that increasing the concentration of green algae (*Haematococcuss pluviallis*) significantly increased carotenoid levels in the skin of *Oncorhynchus mykiss* (Sommer D'souza and Morrissy, 1992). Similar results were shown by Choubert, Milicua JC and Gomez (1994) and Boonyaratpalin et al. (1989) where diets containing higher levels of spirulina and marigold petal resulted in increased pigmentation and higher coloration in the skin of rainbow trout and tiger barb (*Puntius tetrazona)* respectively.

In the present study, it was observed that the amount of carotenoid in the seven different experimental diets as well as in the muscle and skin of fishes fed with these experimental diets showed significant variations (P<0.05; DMRT) among them as displayed in figure 1 and figure 2 respectively. With the increasing level of carotenoids by the increasing amount of annatto seed meal in the experimental diets, the carotenoid concentration in muscle and skin tissue of rosy barb was progressively increased from the control to B3 set and was the highest in B3 set (9.63±0.13) but no further increase in carotenoid concentration was observed beyond this level, which is in accordance with the results obtained by Pailan et al. (2012) and Dananjaya et al. (2017). The probable cause is that there is a saturation and limitation level of carotenoids uptake and transportation to tissue and this amount of carotenoids in the diet is responsible for the maximum pigmentation in fish skin and muscle, (Dananjaya et al., 2017). In this study, carotenoid concentration in the diet of positive control (19.84±0.10) containing lucantin pink was found to be close to the B3 diet (19±0.51) and this level of carotenoid in the diet of rosy barb might be its saturation level which was found accountable for maximum fish skin coloration. Results of the feeding trial revealed that annatto seed meal might be a potent skin color enhancer to obtain desired coloration in rosy barb without any adverse effect on their growth or survival if its incorporation level to the basal diet is 1.5%.

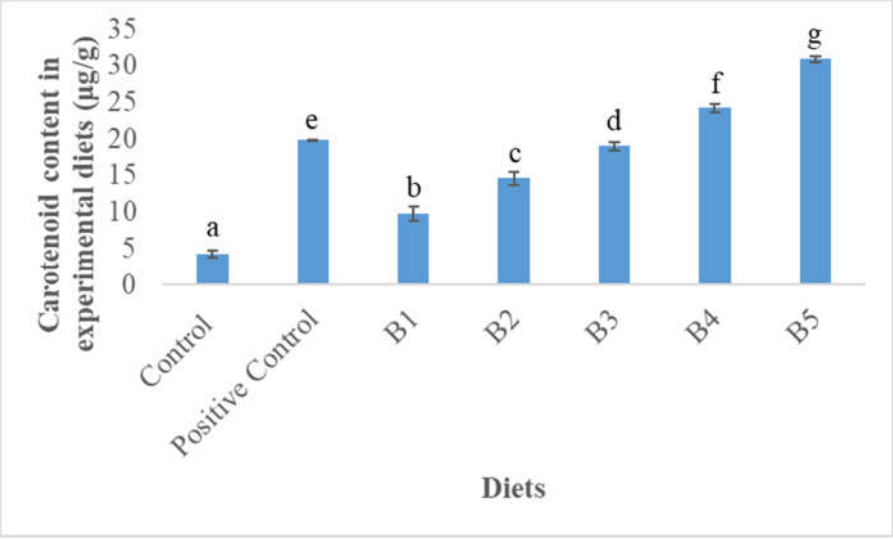

Figure 1: Carotenoid content (µg/g) in seven experimental diets.
Values are mean ± SE. Bars with different letters are significantly different (P<0.05) using DMRT after one way ANOVA.

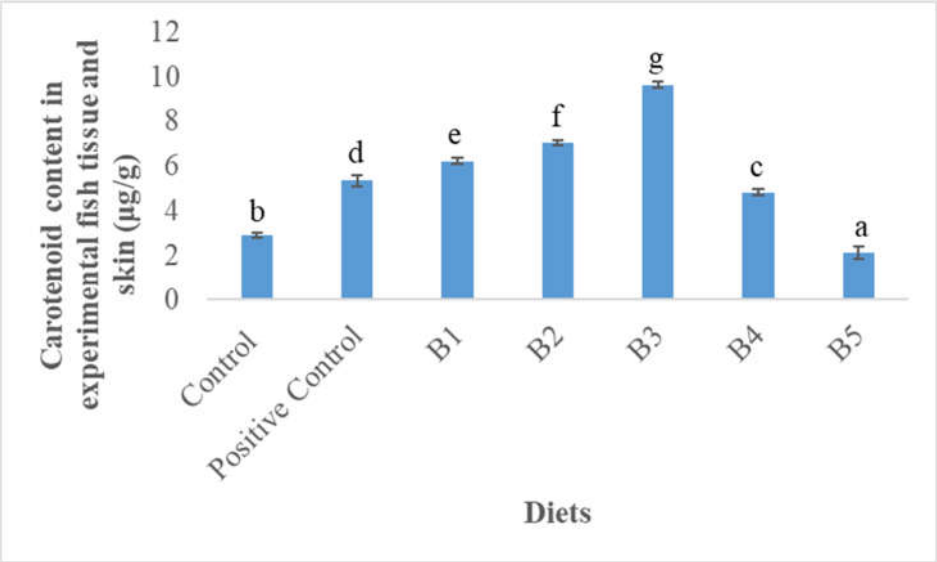

Figure 2: Carotenoid content (µg/g) in seven experimental diets fed fish tissue and skin.
Values are mean ± SE. Bars with different letters are significantly different (P<0.05) using DMRT after one way ANOVA.

Natural carotenoids had a growth-promoting role in fishes. Through carotenoid bioconversion, carotenoids produce essential nutrients, which act as growth promoter (Maiti et al., 2017). Jha, Sarma and Qureshi (2012) also stated that the natural carotenoid sources like spirulina and marigold when supplemented in fish diets, resulted in improved growth and feed utilization of *Barilius bendelisis*. A similar finding was observed by Ezhil, Jeyanthi, and Narayanan (2008) and Jagadeesh et al. (2015), where marigold incorporated diet enhanced the growth and nutrient utilization of red sword tail and rosy barb respectively. Earlier researchers like Pailan et al. (2012), Maiti et al. (2017), Jain, Kaur, and Hollyappa (2019), Bano, Kashyap and Serajuddin. (2020), Tiewsoh et al. (2019) and Pan et al. (2010) reported that there exists a correlation between carotenoid inclusion and growth improvement in rosy barb, koi carp, gourami, goldfish and characins respectively. The present feeding experiment also supports these earlier observations and revealed that incorporation of annatto seed meal up to 1.5% in basal diet, significantly improved the growth performance and feed utilization in rosy barb as well as enhanced their skin coloration.

Whole-body composition

The effect of dietary supplementation of annatto seed meal on the whole-body proximate composition in terms of crude protein, crude lipid, moisture, and ash content of rosy barb from the dietary sets were presented in Table 4. Whole-body composition of the fishes was relatively consistent across all the dietary treatments with no significant differences (P>0.05). This observation supports the finding of Pailan et al. (2012) when rosy barb fed with rose petal-supplemented diets

Table 4: Proximate composition (%) of the whole-body of rosy barb, *P. conchonius* fed with seven experimental diets.

Diets	Crude protein (%)	Crude lipid (%)	Moisture (%)	Ash (%)
Control	16.24±1.45ns	3.52±0.26ns	79.03±3.12ns	3.01±0.33ns
Positive control	16.36±1.34ns	3.51±0.58ns	79.32±2.84ns	2.98±0.31ns
B1	17.81±1.11ns	3.69±0.38ns	79.21±5.23ns	2.64±0.28ns
B2	17.62±1.53ns	3.81±0.65ns	79.08±4.05ns	2.35±0.32ns
B3	17.96±1.42ns	3.73±0.72ns	79.48±5.05ns	2.51±0.25ns
B4	16.01±1.39ns	3.46±0.56ns	79.78±4.60ns	2.72±0.27ns
B5	15.74±1.64ns	3.44±0.35ns	80.12±3.86ns	3.06±0.23ns

Values are mean ± SE. Values with ns letters are not significantly different (P>0.05) one way ANOVA.

Survival percentage

The survival rate of cultured fish is an important factor in fish production. Physicochemical parameters of water also influence the survival of fish. Earlier studies revealed that survival rates might be influenced by the inclusion of colored pigment like carotenoids, which helps to stabilize the quaternary structure of proteins (Cheeseman, Lee, and Zagalsk, 1967; Fox, Smith, and Wolfson, 1976). In this regard, Krinsky (1993) stated that absorption of carotenoids through diet might have a role in enhancing fish survival.

In the current experiment after the trial period, the survival rate recorded in different dietary sets is shown in figure 3. The survival rate was found maximum from the control, B1, B2, and B3 sets (100%) while the lowest survival rate was noted in the B5 set (86.67±1.67%). Hence, the diets containing annatto seed meal up to 1.5% were suitable for rosy barbs as this range of inclusion of annatto seed meal did not provide negative impact on the survival rate in rosy barbs. This finding depicted that carotenoids may play some role in improving fish skin pigmentation which was confirmed by Kumar et al. (2017).

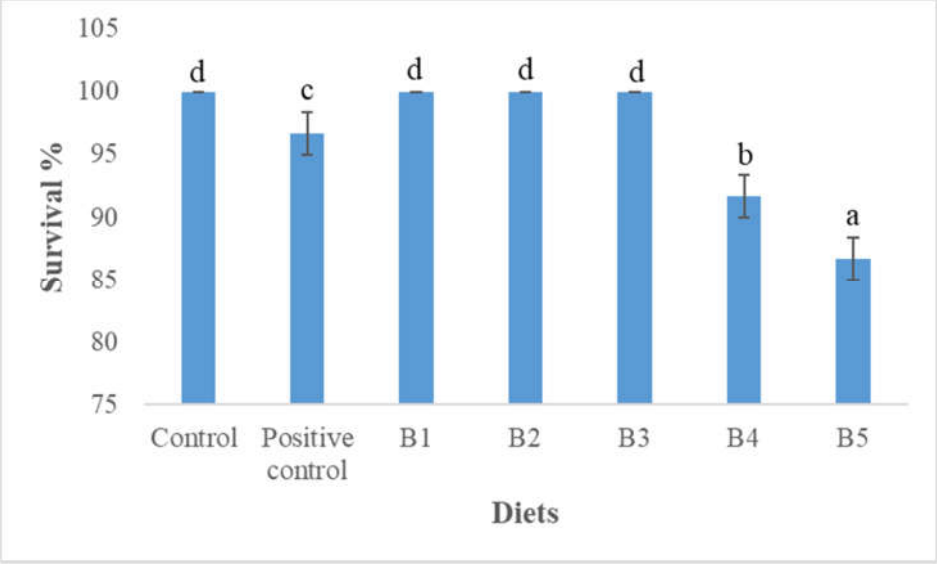

Figure 3: Survival percent of *P. conchonius* fed with seven experimental diets. Values are mean ± SE. Bars with different letters are significantly different (P<0.05) using DMRT after one way ANOVA.

CONCLUSION

The current study showed that the experimental rosy barb could able to efficiently utilize carotenoids from the annatto seed meal supplemented diets having up to 1.5% annatto seed meal and able to deposit the carotenoid in their skin and muscle tissue which will result in an enhanced fish skin coloration.

From the present study, it could be inferred that the annatto seed meal incorporated diets have a positive effect on growth performance, feed utilization, survival rate, and skin coloration of rosy barb, *P. conchonius* when reared in aquariums. Therefore, 1.5% annatto seed (*Bixa orellana*) meal incorporated diet is recommended for rosy barb (*Puntius conchonius*) rearing.

Acknowledgement
The author is thankful to the Head of the Department, Dr. Ankur Bhawal, Vidyasagar College, Kolkata (India) for providing the necessary facilities to carry out the present work.

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