

**EFFECT OF β -CAROTENE-ENRICHED DIET ENHANCED SKIN
COLORATION, GROWTH PERFORMANCE,INTESTINAL
HISTOMORPHOLOGY,and IMMUNE RESPONSES OF ORNAMENTAL
FISH**

PROJECT WORK

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Science degree in Biotechnology

By

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Pavithra. M(41770020) who carried out Project Work entitled "EFFECT OF β -CAROTENE-ENRICHED DIET ENHANCED SKIN COLORATION, GROWTH PERFORMANCE, INTESTINAL HISTOMORPHOLOGY, and IMMUNE RESPONSES OF ORNAMENTAL FISH" under my supervision from October 2023 to April 2024.


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DECLARATION

I, Pavithra. M(41770020), hereby declare that the Project Report entitled "EFFECT OF β -CAROTENE-ENRICHED DIET ENHANCED SKIN COLORATION, GROWTH PERFORMANCE, INTESTINAL HISTOMORPHOLOGY, AND IMMUNE RESPONSES OF ORNAMENTAL FISH" done by me under the guidance of Ms. A. Kavipraba (Internal) and Dr. M.V. Rajeswari (External) at the Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Biotechnology.

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ABSTRACT

Ornamental aquaculture is a growing industry with an estimated value between 6 and 15 billion dollars. Ornamental fish is characterized by an attractive color that originates from high levels of carotenoids in the fish tissue, and this color is responsible for customer acceptability and competitive price. Proper feeding of ornamental fish, like the other animals, is essential for the development of natural pigmentation, as well as healthy fish because of their inability to perfectly biosynthesize the carotenoids. Therefore, under intensive rearing conditions, fish do have access to a limited diet, which should be supplemented with carotenoids. Food supplements are used to increase growth efficiency and health, along with food attractiveness. This study aimed to assess the impact of beta carotene (spirulina, astaxanthin and carrot peel)-enriched diet on molly fish (*Poecilia sphenops*) growth, survival and skin pigmentation. Molly fish (N = 100, 3.5 ± 0.2 g) were tested in four experimental treatments, three replicates each: (Control) fish fed an un-enriched experimental diet; (Exp-I) fish fed an experimental diet enriched with spirulina; (Exp-II) fish fed an experimental diet enriched with astaxanthin; and (Exp-III) fish fed an experimental diet enriched with carrot peel for 30 days. The results revealed that the growth performance and feed utilization parameters of *P. sphenops* were significantly increased by using β -carotene enriched fish diet. Furthermore, *P. sphenops* skin carotenoid levels are increased by utilizing β -carotene enriched fish diet. Lightness (L^*) values showed a slight increase in skin colour when supplemented with carrot peel rich diet. Redness (a^*) values of the fish fed with astaxanthin enriched diet had higher a^* values followed by astaxanthin. Finally, dietary supplement of spirulina, astaxanthin and carrot peel has shown the highest efficiency for improving skin pigmentation in *Poecilia sphenops*.

CHAPTER 1

1. INTRODUCTION

1.1 AQUACULTURE SYSTEM

Aquaculture, or the rearing of aquatic animals and plants for food, is complex and covers a wide range of variables. Aquaculture systems may vary in their environment, that is, fresh-, brackish- and sea-water; coastal, riverine and land-based; tropical to temperate regions; in type, that is, farmed species may include seaweeds, molluscs, crustaceans and finfish species; in scale, that is, extensive, semi-intensive and intensive farming; relatively low numbers of high-value finfish to large numbers of low-value invertebrates; in input, that is, natural and artificial diets; and wild harvested stocks to cultured progeny from eggs to adults. Variations in the applicability of technologies to control the aquaculture environment depend on national and commercial economies and infrastructures, as well as the species under culture. Consequently, aquaculture varies from large, international high technology farming of high-value species, to labour-intensive, low-technology subsistence farming in earthen ponds. To meet the growing demands for aquatic food production, aquaculture has expanded rapidly since the 1980s to become the world's fastest-growing food production sector, particularly in Asian countries that supply 89% of the global aquaculture production (FAO 2019).

1.2 AQUACULTURE PRODUCTION

Aquaculture (the farming of aquatic animals and plants) continues to dominate aquatic food production in Asia and globally, over 91% of global aquaculture production currently being produced within the Asian region (102.9 million tonnes in 2017), and

total global aquaculture production now exceeding global capture fisheries production by over 18.32 million tonnes. Moreover, in contrast to most terrestrial agricultural food production systems, over 95% of global aquaculture production is currently realized within developing countries, with production increasing within these countries at an average annual rate of 6.13% per year (FAO 2019).

With total aquaculture production valued at over US\$250 billion, the aquaculture sector represents a very diverse group of different aquatic plant and animal species (328 species reported in 2017). As with capture fisheries, fish still represent the largest major species group of aquaculture production by weight (53.4 million tonnes or 47.7% total aquaculture), followed by aquatic plants (31.8 million tonnes or 28.4% total), molluscs (17.4 million tonnes or 15.4% total), crustaceans (8.4 million tonnes or 7.5% total), amphibians and reptiles (471,784 tonnes) and miscellaneous invertebrate animals (422,124 tonnes; FAO 2019).

1.3 AQUACULTURE PRODUCTION—FISH

Total reported fish production was 53.4 million tonnes in 2017, with fish production growing at an average annual rate of 5.7% per year since 2000, with over 208 different fish species reported in 2017, valued at US\$ 139.7 billion. Moreover, despite that over 70% of our planet is covered with marine or brackish water, over 83.6% of fish production were freshwater fish species, compared with only 13.4% for capture fisheries. As mentioned previously, freshwater fish species currently dominate aquaculture fish production, with freshwater carps and cyprinids constituting over 53.1% of total fish production, followed by miscellaneous freshwater fish species (19.5%), tilapia and other cichlids (11.0%), followed by the diadromous salmonids (6.5%), and miscellaneous coastal fishes (2.8%) (FAO 2019).

1.4 ORNAMENTAL AQUACULTURE

"Ornamental Aquaculture, a fast developing 'Aquatic Rainbow Sector' in India demands true innovations and implementation of advanced technologies to promote itself to the next level to compete in the international market (Felix, 2014). Ornamental

fish are characterized by a wide diversity of colours and colour patterns and success in the ornamental fish trade is very much dependent on the vibrant colour of the fish. Color is one of the major factors which determines the price of aquarium fish in the world market (Saxena and Health 1994). Fish are colored in nature often show faded coloration under intensive culture conditions. Fish like other animals do not synthesize carotenoids and depend on dietary carotenoid content for the coloration. Hence, a direct relationship between carotenoids and pigmentation exists in them (Halten et al., 1997). If enhancement of coloration can be done by administering pigment-enriched feed, it will improve the quality and cost of the fish because it contains the colour enhancing pigments like carotenoids, xanthophylls etc. (Ramamoorthy et al., 2010). Ornamental fishes are nowadays rapidly gaining importance because of their aesthetic value and also due to their immense commercial value in the export trade world over. Feed additives facilitate feed ingestion and consumer acceptance of the product. Carotenoids are the primary source of pigmentation on the skin of fishes. In the natural environment, the fishes meet their carotenoid requirements by ingesting aquatic plants or through their food chains. But, fishes cannot synthesize carotenoid de novo. Carotenoids are responsible for many of the red, orange and yellow hues of plant legumes, fruits and flowers. The colour-enhancing diets should contain additional natural pigments to enhance the colours of ornamental fishes (Ahilan et al., 2010).

1.4.1 MOLLY FISH (*POECILIA SPHENOPS*)

Poecilia sphenops (Valenciennes, 1846) is a freshwater fish species commonly named molly fish. They are natural inhabitants of freshwater streams and the coastal brackish water of Mexico and Colombia and mostly occur in swarms below the floating vegetation as they feed principally on algae and other herbal resources, and have been marked in many countries around the world through the aquarium fish trade (Ramírez-García et al., 2018). Because of their higher birth size, growth rate, reproduction, and brood number, mollies are categorized as one of the most widely consumed fish. Moreover, this fish is a viviparous species (Alda et al., 2013). Molly is a tropical hardy and highly adaptable fish species that comes in many different colors and varieties such as orange, green, black, sailfin, ballon, etc. *Poecilia sphenops* comes under Molly

group. 'Poecilia' means "many coloured" and 'Sphenops' means "wedge appearance" (Nithya Jeniffer et al., 2012).

CLASSIFICATION

Phylum	:	Chordata
Class	:	Actinopterygii
Order	:	Cyprinodontiformes
Family	:	Poeciliidae
Genus	:	<i>Poecilia</i>
Species	:	<i>sphenops</i>

1.5 FISH PIGMENTS

Fish 'skin' has chromatophores, a type of cell that contains color pigments. These pigments utilize carotenoids to bring forth shades of yellow (Xanthophylls), red and orange (Carotenoids), and brown and black (Melanin). Genetics dictate where these colors are, while diet impacts the actual pigment. Xanthophylls and carotenoids are the most important classes of pigments for fish and crustaceans. Yellow and Red shades are the two colors most effectively influenced by color enhancing foods, which utilize chromatophores. However, protein and foods such as seaweed are effective with the chromatophores to produce brilliant blues, purples and greens in fish. Combine that with the possibilities opened by layering and you have a virtual rainbow of fish shades (McKinnon, 2013).

1.6 CAROTENOIDS AND THEIR TYPES

Carotenoids are tetraterpenoid organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some bacteria, and some types of fungus. Carotenoids are a diverse family of organic compounds that act as immunostimulant and antioxidant (Emeka et al., 2014). Carotenoids also provide the basis for a wide range of animal pigmentation. Carotenoids are absorbed in animal diets, sometimes transformed into other carotenoids, and incorporated into various tissues. Dietary carotenoids play a

significant part in the regulation of skin and muscle color in fish. Carotenoids are compounds produced by plants, algae, and certain fungi (Armstrong and Hearst, 1996). Generally, they're produced to help collect light, most often for photosynthesis, the chemical process by which plants generate their own food. Canthaxanthin and Astaxanthin add colour to the flesh and eggs. Carotenoids can be subdivided into: a. Carotenes (oxygen-free carotenoids) such as lycopenes (in tomatoes) and α , β and γ carotenes (in carrots). Carotenes have a yellowish color but do not color the fish. b. Xanthophylls or oxy-carotenoids (oxygen-containing carotenoids, xanthophylls means yellow leaves). These compounds have in contrast to carotenes one or more oxygen atoms. These compounds can color the fish, either the fillet or the skin and can also color egg yolks (Kidd and Parris, 2011).

1.7 CAROTENOIDS IN FISHES

Fishes contain various kinds of carotenoids, most dominant of them with their colors are tunaxanthin (yellow), lutein (greenish-yellow), beta-carotene (orange), alpha, betadoradexanthins (yellow), zeaxanthin (yellow-orange), canthaxanthin (orange-red), asthaxanthin (red), eichinenone (red) and taraxanthin (yellow). Among these, dominant carotenoid is astaxanthin, which is common in red fishes (Gupta et al., 2007). Carotenoids range from red, to orange to yellow. Most commercial color formulas contain astaxanthin, a red carotenoid that can bring out red color.

1.7.1 REDS

Food containing astaxanthin really brings out the reds in white cloud mountain minnows, *Fundulopanchax scheeli*, *Gymnogeophagus rhabdotus*, and in the fins of clown loaches. Another source is ground, dried shrimp or krill.

1.7.2 ORANGE

Paprika, is made from a ground, dried pepper, *Capsicum annum*. Paprika is rich in a number of carotenoids, including beta carotene, capsanthin and capsorubin. Ground marigold petals also a good source of orange carotenoids.

1.7.3 YELLOW

Zeaxanthin and lutein are perhaps the most well-known yellow carotenoids. They're present in the yellow vegetables like corn (maize) and probably in the yellow variety of snap beans and the yellow form of bell peppers. They're also found in egg yolk.

1.7.4 BLUE

The non-carotenoid pigment, phycocyanin, brings out the blue color in fish. Phycocyanin is produced by blue green algae, which are contained in spirulina preparations (Berman et al., 2016). Normally these are obtained through carotenoid-containing organisms in the aquatic food chain, but commercial feed ingredients such as yellow corn, corn gluten meal, and alfalfa are used as sources of carotenoids such as zeaxanthin and lutein (Lovell, 1992). Other carotenoid-rich ingredients used are marigold meal (lutein), red pepper (*Capsicum* sp.) extract (capsanthin) and krill or crustacean meals (astaxanthin) (Boonyaratpalin et al., 1989). Canthaxanthin has been shown to be an effective pigmenter for the tropical fish *Trichogaster leeri* (Fey and Meyers, 1980), and especially to enhance reproductive processes.

1.8 NUTRITION OF ORNAMENTAL FISH

Over the last decade, a number of studies have pursued to boost the profitability of ornamental fish industry by improving their production efficiency and enhancing their skin pigmentation through the incorporation of various non-conventional, alternative, carotenoid loaded, cheap, easily available animal protein sources by manipulating ornamental fish nutrition (Besen et al., 2019). Dietary protein of fish feed, is an important nutrient of fish nutrition. Proper growth and colouration of ornamental fishes rely on the quality and appropriate quantity of dietary protein (Erdogan et al., 2012). Aqua-feed cost mostly depends on the cost of primary protein source used in feed (Aragão et al., 2022). In aquaculture, fish feed is most expensive input²⁴ due to high cost of fishmeal which is the most accepted primary protein source of fish feed due to its nutritional profile and its quality protein and amino acid composition (Tacon and Metian 2015). The increasing cost of fishmeal and its scarcity due to its upscaling

demand, resulted to the search for non-conventional alternative cost-effective animal protein sources with a good amount of natural carotenoids that could replace fish meal completely or partially in the fish feed.

1.9 CARROT PEEL

Carrot (*Daucus carota*) peel waste as cheap carotenoid source. Carrot contains high quantities of alpha-carotene and beta-carotene and is a good source of vitamin K and vitamin B6. Raw carrots are 88% water, 9% carbohydrates, 0.9% protein, 2.8% dietary fiber, 1% ash and 0.2% fat. The carrot gets its characteristic bright orange to red color from beta carotene, and lesser amount of alpha-carotene, gamma-carotene and lutein (Afzal et al., 2000).

1.10 SPIRULINA

Spirulina is used as a prominent feed additive for fish (Al-Deriny et al., 2020). Spirulina is rich with bioactive substances such as chlorophyll, carotenoids, and phycobiliproteins which are colored substances that might be used as food pigments (Li et al., 2022). Despite the fact that spirulina-derived pigments are less stable than their synthetic ones, they may give extra health advantages upon ingestion, since carotenoids contain provitamin A and their consumption improves immune response of fish (Rodriguez-Concepcion et al., 2018). Apart from being high in protein, microalgae also contain a lot of vitamins, fat (particularly polyunsaturated fatty acids, or PUFAs), which are good for aquatic animals' growth, reproduction, and immunity, a lot of polysaccharides, which can boost an animal's immunity, and a lot of pigments, like lutein, astaxanthin, and β -carotene, which can enhance an animal's color (Kumar et al., 2023).

1.11 ASTAXANTHIN

Astaxanthin is a carotenoid that can be found in freshwater and the largest concentration can be found in green algae (*Haematococcus pluvialis*), which is 10.000-

40.000 mg/kg. Comparative studies between astaxanthin and other types of carotene have shown that astaxanthin has antioxidant activity 10 times stronger than the carotene group in the form of beta-carotene, cantaxanthin, lutein, zeaxanthin (Gouveia et al., 2003). The keto-carotenoid astaxanthin (3,3'-dihydroxy- β,β -carotene-4,4'-dione) is the main carotenoid responsible for the reddish or pinkish color of most wild fish (Carvalho and Caramujo, 2017). The continued growth of the aquaculture industry has led to a massive demand for astaxanthin (Lim et al., 2018).

1.12 APPLICATION OF COMPUTER ASSISTED IMAGE ANALYSIS

The application of computer assisted image analysis in ornamental fish breeding has been very limited so far. Taniguchi et al. reported on the application of an image analyzer (Shimadzu, Tokyo, Japan) for measuring the area of red and black spots in ploidy-manipulated fancy carp. The different colors recognized with normal visual activity are the result of a mixture of light of different wavelengths. It has been determined that certain wavelengths of red (R), green (G) and blue (B), when combined with each other in various proportions (intensities) will produce a wider range of colors than any other combinations of the three colors. These colors are thus referred as the primary colors of light.⁶ The 'true color' digital images consist of 24 bits (3 bytes): 1 byte red, 1 byte green, 1 byte blue (RGB) in each pixel (dot). The color of a certain pixel is determined by the so-called gray scale values (256 shades, 8 bites) of R, G and B (Hancz et al., 2003).

In comparison, the pigmentation effectiveness of synthetic carotenoids (which are always a single carotenoid) can be obviously determined by research. However, due to high costs of synthetic pigments, attempts were created to assess the potential of natural compounds. Fish farmers need color analysis that is easy, fast and precise. Although there are several techniques for evaluating color, reflectance spectroscopy measurements are a direct and increasingly common method, especially as measured by parameters based on tristimulus values, L^* , a^* and b^* of lightness, redness and yellowness.

The present work was undertaken to effect of β -carotene-enriched diet obtained from Carrot peel, Spirulina and Astaxanthin as a dietary carotenoid source for colouring the skin of ornamental species and using as negative control to a diet with no colouring added. The study was performed with freshwater ornamental molly fish (*Poecilia sphenops*).

CHAPTER 2

2. LITERATURE SURVEY

Sagarika Swain et al., (2020) reported the use of carotenoid supplementation for enhancement of pigmentation in ornamental fish environmental degradation, residue and other pollution related problems recent aquaculture practices give more importance to organic farming as the synthetic supply of feed additive is restricted in the animal diet. The research attempts must be more focused with special reference to natural carotenoids and incorporation in aqua feed and productivity has to reach the feed formulation stage.

Ebeneezar et al. (2020) evaluated that dietary oleoresin on the enhancement of skin coloration and growth in the marine ornamental clown fish, *Amphiprion ocellaris* (Cuvier, 1830) study confirm that paprika oleoresin and the combination of the pigments produced a significant effect on red and yellow hues and also on the growth performance of the fish. Further studies need to be focused to elucidate the biochemical and molecular mechanisms of pigmentation in fish and the underlying mode of the action of different pigments. Optimization of dietary inclusion of pigments in fish and the underlying mode of action of different pigments.

Azab et al. (2016) revealed that effect of some food additives on colour enhancement of koi fish, *Cyprinus carpio* (Linnaeus, 1758) to study best enhancement of red color in koi fish, *C. carpio* was recorded in the fish fed on the diet containing 5% of the mixture of all experimental additives (carrot roots + grape leafs + turnip leafs and roots). The enhancement of yellow color was recorded in fish fed on the diet containing 5% "carrot

roots” for more than 30 days. The enhancement of total color of koi fish, *C. carpio* was recorded in fish fed on the diet containing 5% “carrot roots, or carrot roots with turnip leafs and roots.

Jiang et al., 2019 reported that enhancing the colouration of the marine ornamental fish *Pseudochromis fridmani* using natural and synthetic sources of astaxanthin. There was a significant effect of astaxanthin source, dietary concentration of astaxanthin, and time of supplementation, on the colouration of the marine ornamental fish *P. fridmani*. There were four main outcomes of this study: (1) the dietary supplementation of astaxanthin (both natural and synthetic), for treatments above 25 ppm, changed the colour of un-supplemented fish away from a light, dull, violet colour towards a dark, intense, magenta/raspberry colour; (2) the colour of fish.

Ramya et al. (2016) reported that among the natural colour enhancers, the spirulina fed fishes recorded the maximum mean weight gain of 1.11g at 3 % level and in the artificial colour enhancers, a higher mean weight gain was recorded in the juvenile fishes fed with beta carotene at 0.02 % level. The maximum specific growth rate of 1.613 was recorded in the spirulina fed fishes at 3% concentration. The maximum specific growth rate of beta-carotene fed fishes was recorded at 0.02 % concentration.

Derya Güroy et al. (2022) reported that color intensity and growth performance of common clownfish (*Amphiprion ocellaris*) enhanced by dietary Spirulina (*Arthrospira platensis*) The growth performance of clownfish fed with different dietary Spirulina levels was increased. At the end of the 12 weeks, the final mean weight (FMW), specific growth rate (SGR), and protein efficiency ratio (PER) of Spirulina groups (SP15 and SP30) were significantly higher than the control (C) group ($P < 0.05$). The FMW of the SP30 group (2.05 g) was higher than the SP15 group (1.95 g), even if there are no statistical differences between these groups. Feed conversion ratio (FCR) of the C was the highest among the experimental groups ($P < 0.05$). There were no statistical differences between the feed intake (FI) and the survival rates (SR) of the experimental groups ($P > 0.05$).

Rekha et al. (2024) reported Marine yeast *Rhodotorula paludigena* VA 242 a pigment enhancing feed Additive for the ornamental fish Koi Carp. The improved growth rate in Koi Carps fed with carotenogenic marine yeast *R. paludigena* VA242 provides scope for further studies in this Field with respect to the growth and survival of fishes. Furthermore, the effect of carotenogenic marine yeast on the Feed Conversion Ratio (FCR), disease prevention and other biological activities may also be a subject of research.

Mousumi Das (2023) reported that effect of dietary protein replacement of fishmeal with mealworm meal on growth, feed utilisation, survival and colour enhancement of Goldfish, *Carassius auratus* present experiment elucidate that the experimental goldfishes were able to efficiently utilize mealworm meal protein as well as its carotenoid. Dietary substitution of fishmeal with mealworm meal at 50% level improve growth and survival of the fish, and promoted efficient carotenoid concentration in skin and tissue of goldfish. Moreover, mealworm meal could be incorporated in the diet of goldfish up to 75% of fishmeal replacement without any negative influence on growth, survival and skin pigmentation of the fishes. Therefore, the outcome of this study is of special importance in ornamental fish culture. Hence, follow-up research works Should be conducted aiming at optimizing the potential percentage of the mealworm meal replacing the fishmeal in the fish feed.

Manikandan et al. (2020) review on the application and effect of carotenoids with respect to canthaxanthin in the culture of fishes and crustaceans role of individual additives are becoming more and more debatable as many researchers tend to specify the unique use of that particular additive in relation to improved and enhanced growth of aquatic animals. Though the carotenoid astaxanthin has an important role in the aquafeed industry across the world, canthaxanthin to a lesser extent also plays a major role in the supplementation as both of them are widely recognised as safe and more efficacious pigments. Apart from the use for pigmentation of aquatic animals, they also find application in dairy foods, meats, cosmetics, beverages, cereal products and

pharmaceuticals. The development of new strains of microbes yielding the carotenoid pigments are an important breakthrough in the development of carotenoid research.

CHAPTER 3

3. AIM AND SCOPE

AIM

To study the effect of β -Carotene-enriched diet (Carrot peel, Spirulina and Astaxanthin) enhanced skin coloration, growth performance, and survival of Ornamental molly fish.

OBJECTIVE:

- Preparation of β -Carotene-enriched (Carrot peel, Astaxanthin, and spirulina) and un-enriched experimental diet
- Experimental design – Freshwater ornamental molly fish were divided into three different group and control group
- To study the growth performance and survival analysis of all experimental groups.
- To examine the intensity of colour in all experimental group by spectrophotometry analysis.

CHAPTER 4

4. MATERIALS AND METHODS

4.1 PREPARATION OF UN-ENRICHED AND ENRICHED EXPERIMENTAL DIET

The experimental feeds were prepared with basic ingredients such as wheat flour, rice bran, tapioca flour, soybean, fish meal and groundnut oilcake (Table 1 and Fig. 1). They were split into three equal portions. These ingredients were mixed until very soft biscuit-textured dough was obtained. The material was then steam sterilized (autoclaved at 120°C for 20 min) and pelletized by hand to get pellets of 3 mm size. The pellets were dehydrated in a forced-air oven at 55–60 °C for 48 h when the natural moisture content <15%. After drying, the two natural carotenoid sources such as Spirulina, Astaxanthin and Carrot peel were mixed with 2.5% vegetable oil onto the experimental diets (T4, and T5) respectively. After that, dough was heated in a drying oven at 35 °C for 30 min and broken by crumbling and then stored at -20°C to avoid oxidation of the carotenoids.



Fig 1. Preparation of experimental feed

Table 1: Shows Formulation of Experimental Diets (g/100g)

Control	Exp-I (T1)	Exp-II (T2)	Exp-III (T3)
Soybean (15.22)	Soybean (15.22)	Soybean (15.22)	Soybean (15.22)
Fishmeal (15.22)	Fishmeal (15.22)	Fishmeal (15.22)	Fishmeal (15.22)
Groundnut oilcake(11.28)	Groundnut oilcake(11.28)	Groundnut oilcake(11.28)	Groundnut oilcake(11.28)
Wheat flour (13.04)	Wheat flour (13.04)	Wheat flour (13.04)	Wheat flour (13.04)
Tapioca flour (13.04)	Tapioca flour (13.04)	Tapioca flour (13.04)	Tapioca flour (13.04)
Rice bran (12.2)	Rice bran (12.2)	Rice bran (12.2)	Rice bran (12.2)
Vitamins (5. 0)	Vitamins (5. 0)	Vitamins (5. 0)	Vitamins (5. 0)
Nil	Spirulina (15%)	Astaxanthin (15%)	Carrot peel (15%)

4.2 MOLLY FISH AND REARING SYSTEMS

The molly fish ($N = 100$, 3.5 ± 0.2 g) were obtained from a private commercial hatchery and transported in polyethylene plastic bags (1 water : 2 pure oxygen) to the glass aquarium laboratory. Tap water was used in this experiment and aerated for 72 h to ensure that there is no residual chlorine content. The water temperature was 28.45°C , pH was 7.20, dissolved oxygen was $> 6 \text{ mg L}^{-1}$, and unionized ammonia was less than 0.001 mg L^{-1} . The water was filtered through a $0.45 \mu\text{m}$ membrane filter prior to use. The koi carp fish were acclimated for two weeks before the start of the trial. The proximate composition of the commercial diet used during the adaptation period contained 90% dry matter (DM), 44% crude protein, 9% ether extract, 7% ash, 36.62% Nitrogen-free extract (NFE), 3.38% crude fiber and $4520 \text{ kcal kg}^{-1}$ gross energy. After the acclimatization period, the fish ($n = 20$) were distributed into five FRP tanks representing four experimental treatments and one control (Fig 2). Each tank was supported with an aeration system from a radial compressor with ne-porous stones placed at the bottom of each aquarium. The experimental units were placed in a wet laboratory with artificial lighting and a controlled photoperiod (12 light:12 dark) and the experiment lasted for 60 days.

4.3 EXPERIMENTAL TREATMENTS

The experiment was conducted at Centre for Aquaculture breeding lab, Sathyabama Institute of Science and Technology (SIST), Chennai, India. The experimental fish were randomly allocated into three treatments as follows: treatment one (Control): molly fish fed a commercial diet (5% of body mass, adjusted according to fish till reach 5% at the end of 4th week then continue with 5% till the end of 30 days experimental period); treatment one (T1): fish fed experimental diet mixed with spirulina; treatment two (T2): fish fed experimental diet supplemented with astaxanthin; and treatment three (T3): fish fed carrot enriched experimental diet; The fish received three equal meals (9 a.m., 12 p.m., and 3 p.m.) per day containing commercial diet for control group only. For T1, T2, and T3, Fish received one meal (at 9 a.m.) containing an experimental diet of the same amount introduced to fish. Unconsumed feed was

collected daily, and feces were removed and 25% of the water was refreshed every 2 days.

4.4 GROWTH PERFORMANCE AND CONDITION FACTOR OF MOLLY FISH.

For each experimental treatment, 10 fish were randomly selected bi-weekly to estimate the periodical gain in weight and length. At the end of 60 days experimental period, koi carp fish were anesthetized by clove oil (Himedia, Mumbai, India, 50 $\mu\text{L L}^{-1}$), collected, counted, and weighed. The growth performance and feed utilization parameters were determined as follows:

- o Weight gain (g fish^{-1}), $\text{WG} = \text{Wt} - \text{W0}$;
- o Average daily gain ADG $\text{g fish}^{-1} \text{ day}^{-1} = \text{Wt} - \text{W0 days}^{-1}$;
- o Specific growth rate ($\% \text{ day}^{-1}$);
- o $\text{SGR} = 100 \times \ln \text{Wt} - \ln \text{W0} / \text{experimental period}$, where: W0: initial fish weight (g), Wt: final fish weight (g), $\ln$: natural logarithm;
- o Survival rate (%) = $100 \times (\text{final number of fish} / \text{initial number of fish})$.
- o Length gain (cm fish^{-1}) = $\text{Lt} - \text{L0}$; condition factor (K) = $100 \times \text{W1} / \text{Lt}^3$ where, Lt: final fish length (cm), L0: initial fish length (cm).

4.5 SKIN COLOUR MEASUREMENT

Skin colour measurement was performed on both sides of the dorsal skin by reflectance spectroscopy based on tristimulus values, L^* , a^* and b^* , of lightness, redness and yellowness, respectively, using a 5100 H (YRL II) spectrophotometer equipped with a 5 mm measuring area. The illuminant used was D65 and calibration was done with a white standard and specular component of light was excluded. The colour parameters measured were L^* , lightness, which ranges from 0 for black and 100 for white; a^* for red/green chromaticity; and b^* for yellow and blue chromaticity, in accordance with the recommendations of the International Commission on Illumination, CIE (1976). Measurements were recorded at the beginning and the ending of the experiment.

4.6 STATISTICAL ANALYSIS

Statistical analyses were carried out on various parameters that were measured on the plants to evaluate the effect of each treatment. Data was presented as mean value $\pm$ standard deviation (error bars). Analysis of variance (ANOVA) was used and Tukey's honest significant difference ($P < 0.05$) multiple range test was conducted to determine differences between treatments.

CHAPTER 5

5. RESULTS AND DISCUSSION

5.1 Growth and survival

The mean SGR and the mean rate of survival and the FCR of the fish across the treatments were tabulated in table: 2. the results showed that the survival rate, specific growth rate and feed conversion ratio were not significantly affected by dietary treatments. Fish in all experimental groups, gained significant weight and length during the experimental period of 30 days. The fishes grew normally and showed high activity. Specific growth rate of *P. sphenops* fed on experimental diet was significantly higher than that of the control diet.

The diets used in this study contained various supplement spirulina, astaxanthin and carrot peel as coloration pigment. After 30 days of feeding the

fishes those diets, the results obtained showed that the dietary carotenoid can cause changes in the skin colouration. These results are in agreement with the previous reports, in which it was indicated that dietary carotenoids can induce skin coloration of fishes (Pan and Chien, 2009; Yasir and Qin, 2010). The impact of dietary carotenoids on skin color change was more easily detectable by direct analysis which indicates that carotenoid-supplemented diets trigger the skin pigmentation in *P. sphenops*

Adult fish can grow on vegetable diets, however juvenile fish require a greater protein content in their feeds for better growth (Mohanta, K. & Subramanian 2002). Furthermore, improved growth performance may be attributed to feeding fish live natural food rather than commercial diets which are dependent on the palatability, quality, and adequate quantity of feed, size, type, and physical appearance of the feed, as well as other factors such as attractiveness, feed acceptability, and assimilation of nutrients

Table 2 Growth performance, Survival rate and feed utilization of *P. sphenops* fed with experimental diets for 30 days

Diets	CD	Exp-I	Exp-II	Exp-III
Initial body weight (g)	3.3 ± 0.44	3.5 ± 0.75	3.3 ± 0.83	3.4 ± 0.77
Final Body weight (g)	3.45 ± 0.06*	3.7 ± 0.09*	3.6 ± 0.06*	3.9 ± 0.041*
Weight gain	4.54 ± 0.12	5.71 ± 1.3	9.09 ± 2.1	14.70 ± 1.23
SGR	0.5 ± 0.15	0.66 ± 0.75	1 ± 0.9	0.34 ± 1.45

FCR	0.69 ± 0.01 ^a	0.55 ± 0.02 ^a	0.34 ± 0.01 ^a	0.21 ± 0.02 ^a
Survival (%)	80	90	85	90

*Non-significant difference (P>0.05), ^a Significant difference (P<0.05)

5.2 Pigment Analysis and Experimental Fish

The diets used in this study comprised different β -carotene enriched feeds as coloration pigment. After 30 days of feeding the fish those diets, the results revealed that the dietary carotenoids can alter the skin color of the *P. sphenops* fish. Lightness (L^*), Redness (a^*) and Yellowness (b^*) values of the dorsal skins of *P. sphenops* were displayed in Fig 2a-c. At the end of the feeding trial, the maximum L^* , a^* and b^* value were found in the treatment group fed with β -carotene enriched diet, especially Astaxanthin (T3) when compared to others (Fig 2). The diet of β -carotene rich live feed showed its effectiveness in increased skin red color deposition at end of the 30th day. These results were in agreement with the previous study, in which it was indicated that dietary-supplemented carotenoids can enhance the skin coloration of fish (Yasir and Qin 2010). The effect of dietary carotenoids on fish skin color change was more easily visible by direct analysis, indicating that carotenoid-supplemented diets induce skin coloration in *P. sphenops*.

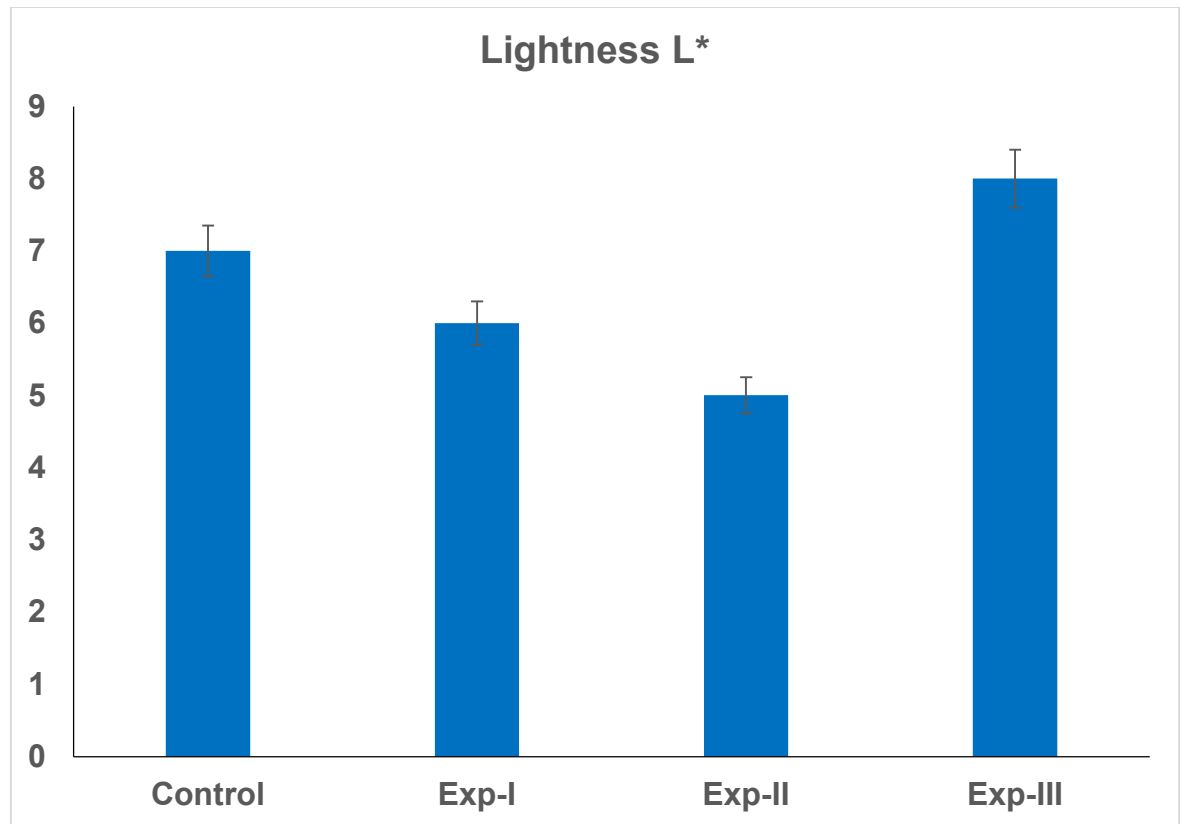


Fig: 2a Lightness (L*) values of the dorsal skin of *P. sphenops*

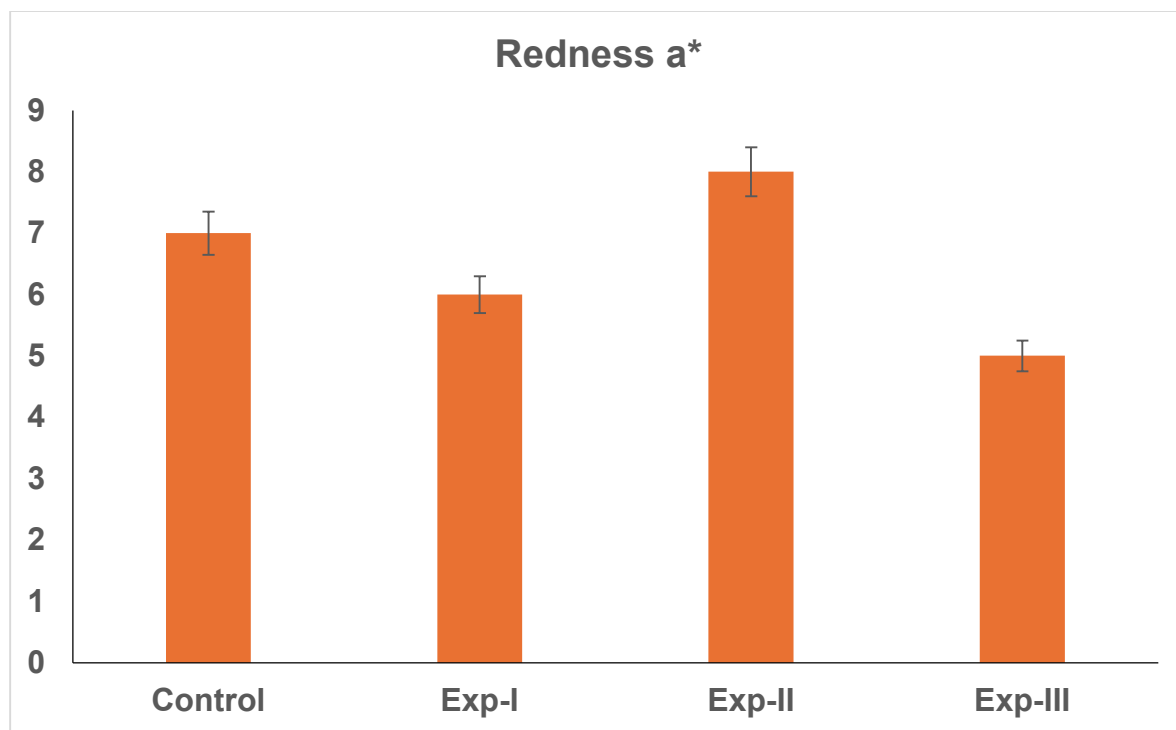


Fig 2b Redness (a^*) values of the dorsal skin of *P. spheonops*

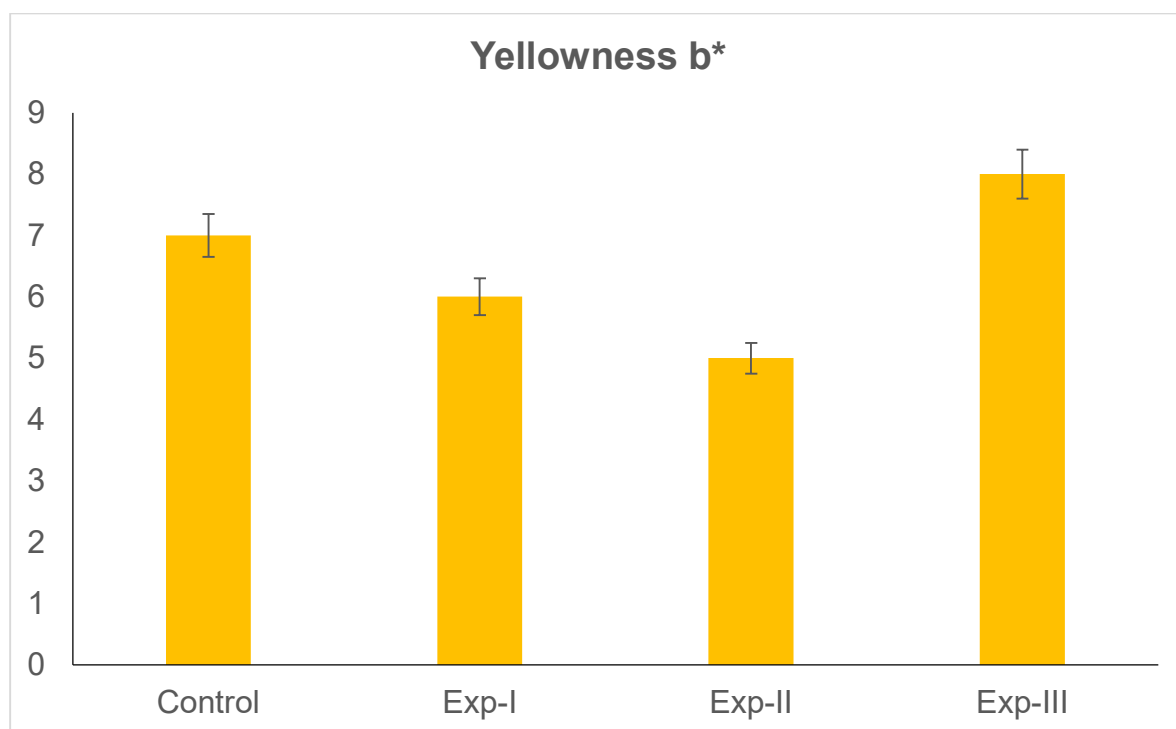


Fig 2c Yellowness (b^*) values of the dorsal skin of *P. spheonops*

CHAPTER 6

6. SUMMARY & CONCLUSION

In this study, a 30 days trial experiment to investigate skin colour enhancement was conducted in *Poecilia sphenops* (molly fish) by feeding different natural dietary of spirulina, astaxanthin and carrot peel and a control diet with no colouring added for comparison. The results revealed that the growth performance and feed utilization parameters of *P. sphenops* were significantly increased by using β -carotene enriched fish diet. Furthermore, *P. sphenops* skin carotenoid levels are increased by increasing the level of astaxanthin in the fish diet. Lightness (L^*) values showed a slight increase in skin colour when supplemented with carrot peel rich diet. Redness (a^*) values of the fish fed with astaxanthin enriched diet had higher a^* values followed by astaxanthin. No significant differences were seen in the Yellowness (b^*) for all the treatments during the experimental trial. Finally, dietary supplement of spirulina, astaxanthin and carrot peel has shown the highest efficiency for improving skin pigmentation in *Poecilia sphenops*.

CHAPTER 7

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