CHAPTER-1

INTRODUCTION

Ornamental fishes are now rapidly gaining importance because of their aesthetic value as well as their immense commercial importance world over. The commercial value of any ornamental fishes is determined by the attractive coloration and pigmentation in the skin (Joseph et al., 2011). Carotenoids are the key element of the pigmentation of the skin of fishes. In aquatic environment, fishes fulfill their carotenoids demand by ingesting different types of aquatic plants or through their food chains. Coloration in fishes has a great importance in camouflaging and breeding season as well (Wagde et al., 2018). Ornamental fish culture is one of the most important and most promising fields of aquaculture. Various factors involved in ornamental fish culture, and among these quality and quantity of food, water hardness and density are the most important (James., 2008).

Fish depends on aquatic environment to meets their carotenoids requirement in the natural environment. Fishes are unable to produce de novo (inside the body) the synthesis of pigments such as other animals. The ingestion of carotenoids have vital role in resulting to the pigmentation of fish. Fish diets contain different sources of carotenoid pigments (Swian et al., 2014).

Carotenoids are a group of naturally occurring lipid soluble organic pigments that are responsible for the orange, red, and yellow color in the skin, flesh, shell and an exoskeleton of an aquatic animal. Both male and female fishes were evaluated separately for their carotenoid concentration in a muscle tissue after the end of feeding trial (Jagadeesh et al., 2015). To enhance the pigmentation of fish and crustaceans, various synthetic pigments like a carotene canthaxanthin and the astaxanthin has been used as dietary supplements (Shahidi et al., 1998).

In ornamental fish, apart from the body shape and lineages, the coloration is one of the major traits which determine the fish quality and the pricing in the commercial trade. Pigments are responsible for the coloration and the quality of the most fishes. Principally, the skin coloration of fishes is genetically determined and generally fishes have the capacity to absorb, metabolize from the food they eat and deposit pigments in their skin/flesh. Hence, the color expression of fishes is closely related to nature of food it eats and its pigment content (Monica et al., 2019).

Ornamental fish is important which provides high income and employment opportunity to the people across the globe. It has potential to contribute to the economic development in underdeveloped countries, especially in tropics (Yanar et al., 2008). Like other fishes the price of the ornamental fish is highly determined by the pigmentation. Without supplementary feed, fishes gain color from wild source and culture in captive condition with high density leads to decrease coloration which decrease the commercial value of the fish (Harpaz and Podowic., 2007). Fish use carotenoids, as one of the major groups of natural pigments for their pigmentation (Swian et al., 2014).

Now a day's ornamental fish culture is important in aquaculture. Fish is one of the most important ornamental fish in aquaculture production. Sword tail fish is a species live bearing that is come from the family Poeciliidae. Most of the popular ornamental fish being produced in Singapore, Malaysia, Indonesia, Thailand, India and China. Many reasons are involved in the ornamental fish production. Some of these are quality and quantity of feed, stocking density and water hardness. Higest produces of artificial feed but less production of live feed cause lacking of suitable feed in an ornamental fish production stage and that is the most important problems in the ornamental fish development industry (Anjur and Sandakan., 2017).

The ornamental fish sector has high profitable that is contributed to the income the foreign currency upon the economic income of underdeveloped countries, especially in the tropical region. Sword tail fish has its advantage compared to other ornamental fishes. Most of the countries as the price value of sword tail fish will be greatly determined by the color of ornamental fish since it is one of the important factors for the great demand (Paripatananont et al., 1999).

Carotenoids are not easily synthesized in the ornamental fish body. The carotenoid pigmentation of fishes is attributed to the pigment present in the different formulated diets. Loss of their skin coloration when kept in the captive condition, henceforth the loss of pigments can gain by the addition of carotenoids to the artificial diet. The diet for aquarium fish should be nutritionally enriched, palatable and resistant to crumbling, water stable and buoyant, but it should also improve the body pigmentation in the fish in the captive condition (Das and Biswas., 2020).

Now a day’s carotenoid pigments can be produced commercially and are commonly used for the pigmentation of fish including salmonids or other families. In the industry of aquaculture, feed additives like carrots, red peppers, marigold flowers, rose petals, china roses, chestnut flowers, spirulina, crustacean waste, yeast, synthetic astaxanthin, vitamin C and vitamin E have long been used to obtain the color development and desired quality of fish (Ellis., 1979; Ezhil et al., 2008). Red pepper and marigold flower can be used to improve color in rainbow trout. Marigold flower and beetroot are easily found sources of pigmentation within the lower stretches of the Himalayan region. So, this experiment we elucidate the effects of marigold flower and beetroot, which are inexpensive, abundant, and rich in carotenoids, on growth performance, carcass composition, and total carotenoids in snow trout fingerlings (Jha et al., 2012).

Pigmentation is the main factors, which determine the worth of the decorative fish within the world market aquaculture sector (Saxena, 1994; Torrissen., 1989). According to Hulshof., (2013) many color and scent influence on people’s mood and mind in naturally. On three things for the color: a light source, an object and an observer. The fishes in their natural environment get coloration from the wild source from the adequate live fed but culture of ornamental fish under high density in captive condition without applying of dietary carotenoid leads to fade coloration, which decreases the commercial value of the fish (Kumar et al., 2017). The cichlid family, with a complete of 1330 species, is the second largest family within the Perciformes order. Species in the cichlid family have a variety of colors that can easily culture in a captive condition (Yilmaz and Ergun., 2011).

To get the good performance of the skin coloration of ornamental fish, generally their diet it is supplemented with synthetic carotenoids such as astaxanthin and cantaxanthin. Natural carotenoids are less costly and environmentally friendly but synthetic carotenoid affects the fish body and the environment. Hence, there is a great demand for an inclusion of natural carotenoids in the aqua feed to ensure the bright coloration in fish. The utilization of low-cost pigment ingredients should be supplied in the ornamental fish diet (Kurnia et al., 2019).

The experimental fish is largely elongated, laterally compressed having a pointed head. On the other poeciliids, sword tail fish, is both males and females may reach 140 mm and 160 mm or less respectively Tamaru et al. (2001). Pigmentation is more variable due to the availability and variability of natural habitats, and the availability for sword tail fish to hybridize. Brood females may have a dark abdominal mark or brood spot, and the abdomen may appear quite distended close to birth Tamaru et al. (2001); Allen et al., (2002).

Objectives of the research work:

- To improve coloration of the swordtail fish
- To increase market demand and aesthetic value of swordtail fish

CHAPTER-2

REVIEW OF LITERATURE

Before conducting any experiment, it is essential to know the information about the previous work related. This chapter is designed to review the past studies conducted by different researchers to the related field. The following information was briefly reviewed in the favor of the present study that was done around the world and relevant to the study.

Anjur and Sandakan. (2017) stated that the ornamental fish production has been profitable industry in the aquaculture. Sword tail fish is very important ornamental fish in aquaculture production. Sword tail fish is a live bearing species. It was the most popular ornamental fish being produced in Singapore, Malaysia, Indonesia, Thailand, India and China.

DoF, (2001) found that the Siamese Gourami (*Trichogaster pectoralis*) was first introduced into Bangladesh brought from Singapore in 1952. Then the Goldfish (*Carassius auratus*) was imported into Bangladesh brought from Pakistan in 1953. At the first time it was used as recreational purposes in aquaria and cement tanks.

Mostafizur et al. (2009) stated that the professional culture of aquarium fish was started in 1980. At the first-time aquarium was set in the restaurant for aesthetic enjoyment to attract people. Then the fishes with an aquarium were practiced in shopping centers. Generally rich people keep the aquarium in their house or an office for their aesthetic enjoyment. The day by day the ornamental fish culture practice was increasing. For increasing the demand, the aquarium fish culture was oriented in the mid-1980, at Kataban in Dhaka.

Ahilan et al. (2008) revealed that ornamental fishes are rapidly gaining importance because of their aesthetic value and also due to their immense commercial value in the export trade world over. Fishes with a high coloration may a high attractive value and a high market price.

Ali and Salim. (2004) reported that the color enhancing diets contain additional natural pigments to enhance the color of ornamental fish. The fish does not possess the ability to synthesize carotenoids. The carotenoid pigmentation of fish depends on the supplementary feed.

Wagde et al. (2018) found that ornamental fish trade with a turnover of US $6 Billion and annual growth rate of 8 percent offers a lot of scope for development in India. There are many studies have proved that fish can be pigmented by including processing wastes and plant sources in their diet. Carotenoid is the class of 800 natural fat-soluble pigments found mainly in plants, algae, photosynthetic bacteria, and some non-photosynthetic bacteria, and they play critical role in the photosynthetic process. They also occur in yeast and moulds, and they carry out a protective function against damage by light and oxygen.

Ezhil et al. (2008) conducted that color is one of the major factors, which determines the price of aquarium fish in the world market. Fish are colored in nature show faded coloration under intensive culture conditions. Fish, like other animals do not synthesize carotenoid and depend on dietary carotenoid content for the coloration.

Harpaz and Padowicz. (2007) investigated that culture of ornamental fishes under high density in captive condition without supplementation of the dietary carotenoids lead to faded coloration and decreased commercial value of fish.

Tamaru et al. (2001) studied that the green sword tail fish is one of the popular ornamental fish that belong to the top 4 of a popular aquarium freshwater fish in the world. It has a green sword-like tail shape and a red body color that make the good-looking uniqueness. Fishes body color will determine the market value and the level of consumer demand.

Jagadeesh et al. (2015) stated that the increasing cost of synthetic pigments, and the public concerns on the use of synthetic additives, many alternative natural carotenoid sources have also been studied.

Das and Biswas. (2016) found that many reports have revealed that skin color change over time depend on the level of carotenoid in the diet, and it’s also differed among species. Fish must obtain an optimum level of carotenoids in their diet to increase the color of skin, and flesh in captive condition. Animals cannot biosynthesize carotenoids, so diet is their sole source as only plants, fungi, bacteria, and algae have the capacity for its synthesis.

Monica et al. (2019) found that the best convenient method to enhance and intensify the coloration in ornamental fishes is through their diet and the pigment supplementation through feeds. The red carotene pigments viz., astaxanthin and canthaxanthin, and the yellow xanthophyll pigments viz., lutein and zeaxanthin are the two major carotenoids most commonly fed to fishes to enhance their coloration.

Swian et al. (2014) mentioned that an ornamental fish farming is an emerging sector of aquaculture which provides high income and the employment opportunity to the people across the globe. It has potential to contribute in developing countries, especially tropics. Like other fishes the price of the ornamental fish is highly determined by the pigmentation. Fishes with good color pattern character higher price and demand in the market. The fishes in their natural environment get pigment from the wild source. Culture of ornamental fish under high density in captive condition without supplementation of dietary carotenoid leads to fade coloration which decreases the commercial value of the fish.

Lili et al. (2020) stated that the ornamental fish sector has a high potential to contribute upon the economic income of underdeveloped countries, especially in the tropical region. Certain species belonging to freshwater ornamental fish that is much loved because of its beautiful shape, color and pattern was named sword tail fish. Sword tail fish has advantage compared to other ornamental fishes. The value of sword tail fish will be greatly determined by the color of ornamental fish. Marigold petals in the plant source contain high carotenoid content which is maximum in dry weight. Marigold flower mixed feed significant as it contains the product of natural carotenoids which are cheap and easy to obtain and use. Marigolds is available found in Indonesia and other countries. The marigold contains various type of carotenoid, primarily leutin is a principal component.

Das and Biswas. (2020) conducted ornamental fishes, when kept under captivity for the long duration has been found to lose their natural skin pigmentation; henceforth the loss of pigments can have been overcoming by the addition of carotenoids to the artificial feed. Hence, the supply of a feed for aquarium fish should be nutritionally balanced, palatable, and resistant to crumbling, water stable and buoyant, but it should also enhance the body pigmentation in the fish in captivity.

Jha et al. (2012) studied that therefore, there is a direct relationship between dietary carotenoids, and the pigmentation in fish. Fishes in the nature obtain the food of the quality required for proper growth, the pigmentation and the nutrient profile. But in captive conditions, a lack of nutrients and pigment-bearing substances can result in faded coloration, retarded growth, and a degraded nutrient profile of the fish.

Kumar et al. (2017) studied that aquarium keeping as a hobby in India is nearly 70 years old, and it began with the British who ruled Indian until 1947. the ornamental fish culture is steadily gaining in importance as a main component in Indian, fisheries' scenario, alongside the culture of food fishes. India is one of the countries having a vast potential of ornamental fishes. The fishes in their natural environment get coloration from the wild source however, the culture of ornamental fish under the high density in the captive condition without the supplementation of dietary carotenoid leads to fade coloration, which decreases the commercial value of the fish. More than 125 countries involve in the global trade of ornamental fishes.

Yilmaz and Ergun. (2011) found that the ornamental fish aquaculture is a growing industrial sector. Some important factor that is related to color are fins, scale, skin, etc. that are related to market price and attractive value and skin is more important factor. The cichlid family, with a total of 1330 species, is not the first it is second largest family in the Perciformes order.

Yustiati et al. (2020) found that the aesthetics in ornamental fish also can increase the commercial value of exports. Color is one of the decisive components in the assessment of ornamental fish. Feeds affect the growth and health of fish, which can also affect the color of fish. The red color in red spinach is a betacyanin pigment that can be used as a natural coloring as well as being an antioxidant.

CHAPTER-3

MATERIALS AND METHODS

The research was carried out to observe the effects of natural carotenoids mixed feed on the body coloration of sword tail fish. In this experiment sword tail fish was reared and maintained in the re-circulatory system for four (4) months by providing different dietary levels of natural carotenoid mixed feed meal to observe the body coloration and growth performance of the sword tail fish. The experiment was conducted in aquarium like rectangular plastic tank with recirculation facilities in the Wet Laboratory of the Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University.

3.1 Description of experimental design

The experimental system consists of 12 rectangular aquaria like plastic tank each of containing 20 liters of water. There were another two rectangular aquaria like plastic tank for conditioning and stocking of fish. All the aquaria were placed on cork sheet and foam for easier handling, and it facilitated better observation and accessibility. Underground water from deep tube well was used in the aquaria during experimental period. An adequate level of oxygen in each aquarium was maintained through artificial aeration using aerators.

3.2 Aeration

An electric aerator motor was used to provide aeration among the tank. The motor was connected to the electric line, and continuous electricity was provided.

3.3 Cleaning and siphoning

Experimental aquariums were cleaned manually by siphoning the water along with the fecal matter every alternate day and the same was replaced by 50% of fresh chlorine free water.

3.4 Collection of experimental fish

The sword tail fish were collected from local market of Chattogram. The collected fish were acclimatized in conditioning tank for 10 minutes. Then gently fish were released in conditioning tank for 3 days before stock in the aquaria. During conditioning sufficient oxygen supply was maintained through artificial aeration.

3.5 Experimental design

For the study of the effects of the natural carotenoid on the body coloration of sword tail fish, 12 aquaria were divided into four groups containing 3 aquariums in each group. These four groups corresponded to four experimental treatments (T_0, T_1, T_2 and T_3) with the three replications of each group. Each aquarium stocked with 8 fish. Feed with four different levels of carotenoid percentage- 0% carotenoid as the control treatment, 15% China-rose, 15% marigold and 15% carrot were administered for studying the growth coloration of fish in T_0, T_1, T_2 and T_3 respectively.

Table-1: Layout of the experiment showing the distribution of 'sword tail' fishes in tank and the applied treatments

Replications	Treatment (carotenoid dose)	T_0 0% (control feed)	T_1 15% (china rose feed)	T_2 15% (marigold feed)	T_3 15% (carrot feed)	Total fish
	R_1	8	8	8	8	32
	R_2	8	8	8	8	32
	R_3	8	8	8	8	32
	Total	24	24	24	24	96

3.6 Stocking of fish

The fishes were then randomly released into different treatment groups. Eight (8) fishes were stocked in each aquarium. There were four treatments each containing three replications. Before stocking, weight of every individual fish was taken.

3.7 Feed formulation and preparation

3.7.1 Selection and collection of natural carotenoid sources

For experimental feed preparation purposes, marigold flower, china rose flower and carrot were used as a natural carotenoid source. "Tiger Brand EON Nursery Powder Feed-1" used as normal feed (without natural carotenoid) in the whole experimentation.

3.7.2 Collection of raw ingredients

Raw ingredients for preparing feed were collected from local market located at Kotouali and Chattogram Metropolitan.

3.7.3 Preparation of mixed feed

The feed which was applied in the aquaria as treatment for 'sword tail' was prepared in 'Wet Lab' of CVASU by adding appropriate of carotenoids.

3.7.4 Feed proximate analysis

Table-02: Proximate composition of raw feed and raw carotenoid Content

Sl. No.	Ingredients Name	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)	Carotenoid Value (mg/g)
1.	Commercial feed	30	6	16	12	0.007
2.	China Rose Flower	15.7	6.8	6.2	71.02	0.029
3.	Carrot	22	8.7	5.6	75.54	0.096
4.	Marigold Flower	12.3	9.3	7.21	68.05	0.733

Proximate composition of Carotenoid content mixed feed:

Table-03: Proximate composition of Carotenoid content mixed feed

Content	T_0 (Commercial feed)	T_1 (China Rose Mixed Feed)	T_2 (Marigold Mixed Feed)	T_3 (Carrot Mixed Feed)
Protein %	30	27.82	27.34	28.8
Lipid %	06	5.47	5.11	5.58
Ash %	16	14.53	14.681	14.44
Moisture %	12	11.38	11.47	12.24

3.8 Feeding of fish

Three percent of body weight feed of the experimental diet was weighed out for each aquaria each week. Handling methods were followed during feeding fish in aquariums. Each time a small amount of feed was dropped in to the aquarium.

Feeding rate, and feeding frequency was adjusted by their bodyweight. Dry powdered feed was fed with fish in twice daily.

3.9 Sampling

The sampling of the experimental fish was done at regular interval of 15 days. The sampling acted as an important tool for checking the growth performance of fish, and also adjusting the carotenoids content of the body coloration. The growth of fish in each sampling was taken by weight machine and carotenoids content determination by spectrophotometer.

3.10 Measurement of carotenoid

The carotenoid content of fish skin was extracted according to the method of Torrissen and Naevdal (1984). Three fish were randomly sampled from each treatment per sampling period and used for carotenoid analyses. The samples of 1 gram muscle were collected from both sides between the abdominals and dorsal regions of the fish. These samples were transferred into 10-ml pre-weighed glass tubes. After the samples were ground in acetone containing 1.5gm of anhydrous sodium sulfate with a homogenizer, the extractions were made up to 10 ml with acetone. The samples were stored for three days at 4°C refrigerators, and then extracted three or four times until no more colors could be obtained. The solution was centrifuged at 5000 rpm for 5 minutes, and then absorption was measured in a spectrophotometer. A similar method was adapted for total carotenoid analysis of Marigold, China rose and Carrot. Carotenoid content of fish body was measured by the following formula:

$$\text{Carotenoid Value} = \frac{\text{Abs x 10000 x V}}{1900}$$

Where,

Abs= Pigment Absorption rate which measured by spectrophotometer

V=10

3.11 Growth performance

Growth performance was determined by using following formula based on sampling data.

- Length gain (cm) = Mean final length- Mean initial length
- Weight gain (g) = Mean final weight- Mean initial weight

3.12 Water quality parameters

The water quality parameters as water temperature, dissolved oxygen (DO), and pH were monitored weekly throughout the experimental period.

3.13 Statistical Analysis

All the data entered into MS excel (Microsoft Office Excel-2013, USA). Data management and data analysis done by SPSS (Version 26)

PHOTO GALLERY

Plate 1: Experimental setup

Plate 2: Experimental fish

Plate 3: China rose sample

Plate 4: **Marigold flower sample**

Plate 5: **Carrot sample**

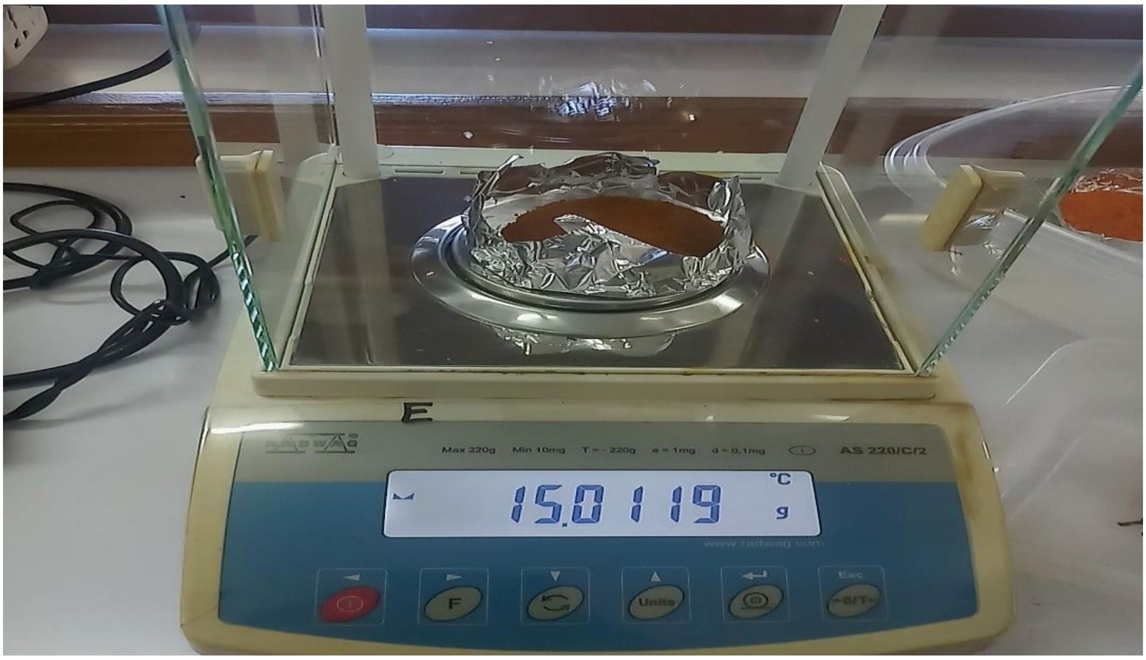

Plate 6: Weight of carotenoids sample

Plate 7: Prepared carotenoids mixed feed

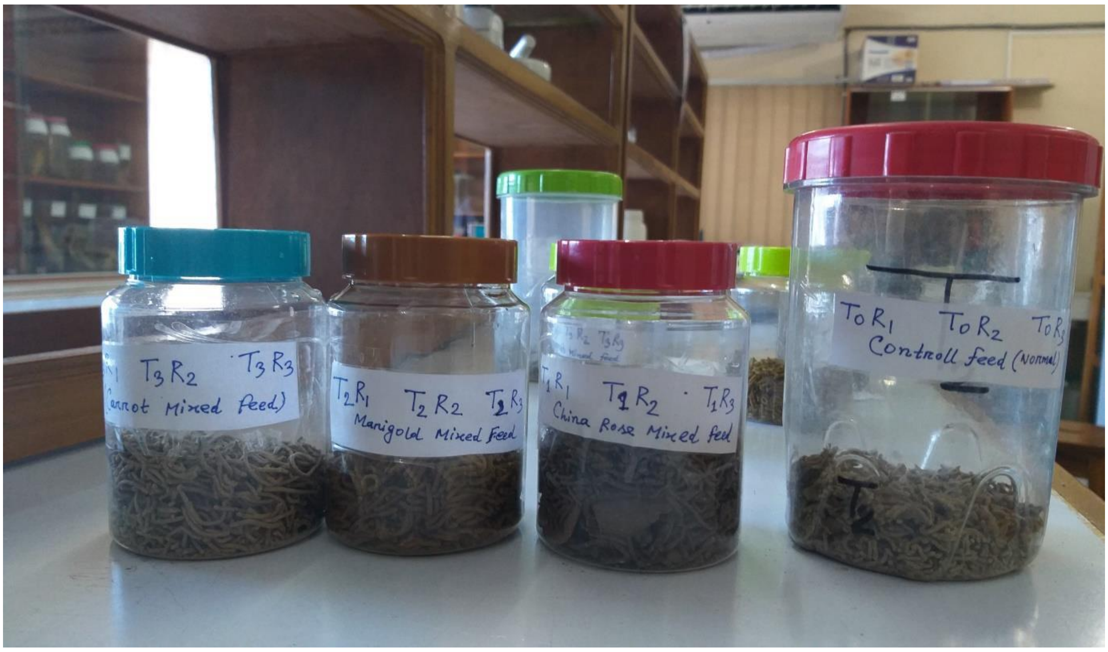

Plate 8: Prepared carotenoids mixed feed

Plate 09: Feeding of fish

Plate 10: Siphoning

Plate 11: Weighing of fish

Plate 12: Sample preparation for analysis.

Plate 13: Proximate composition analysis of prepared feed

Plate 14: Proximate composition analysis of prepared feed

Plate 15: Proximate composition analysis of prepared feed

Plate 16: Titration of experimental feed

Plate 17: Sacrifice of fish

Plate 18: Take 1 g fish sample in test tube

Plate 19: **Keep sample in refrigerator**

Plate 20: **Measurement of carotenoids absorption of experimental species**

CHAPTER-4

RESULTS

4.1 Total carotenoid gain in fish tissue

After each fifteen days of feeding, the carotenoid content in skin, and muscle of fishes were measured by the spectrophotometer's optical density calculation. The carotenoid absorbance reading of sword tail fish during the experimental period is presented in Figure-1. Average initial carotenoid contents absorption of fish in four treatments were 0.083±0.0621 mg/10 kg, 0.085±0.0457 mg/10 kg, 0.085±0.0214 mg/10 kg, and 0.084±0.0105 mg/10 kg in treatments T_0, T_1, T_2 and T_3 respectively. At the end of the 120 days experimental period, average final carotenoid absorption of the fishes of four treatments were 0.1742±0.0851 mg/10 kg, 0.2053±0.0955 mg/10 kg, 0.3524±0.2054 mg/10 kg and 0.2341±0.1128 mg/10 kg in treatments T_0, T_1, T_2 and T_3 respectively. In case of carotenoids gain higher result was found in T_2 (marigold) (0.3524±0.2054 mg/10 kg) followed by T_3, T_1 and T_0 and the lower carotenoid gain result found in T_0 (control) (Figure-2). Statistical analysis by ANOVA showed there was no significant difference ($P>0.05$) among the carotenoid gain of T_0, T_1, T_2 and T_3 at the end of 120 days experimental period.

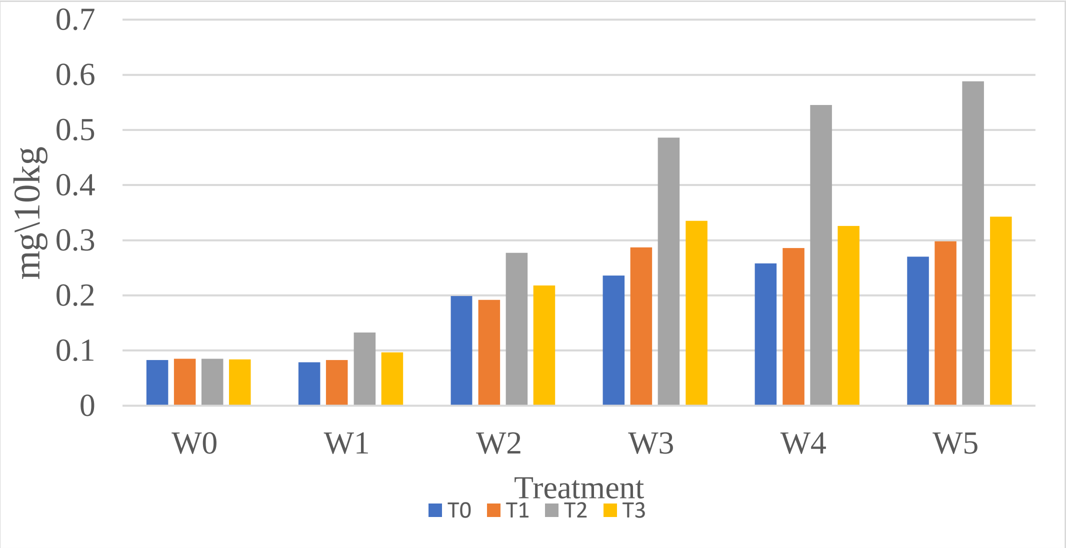

Figure- 01: Carotenoids gain of Sword tail Fish

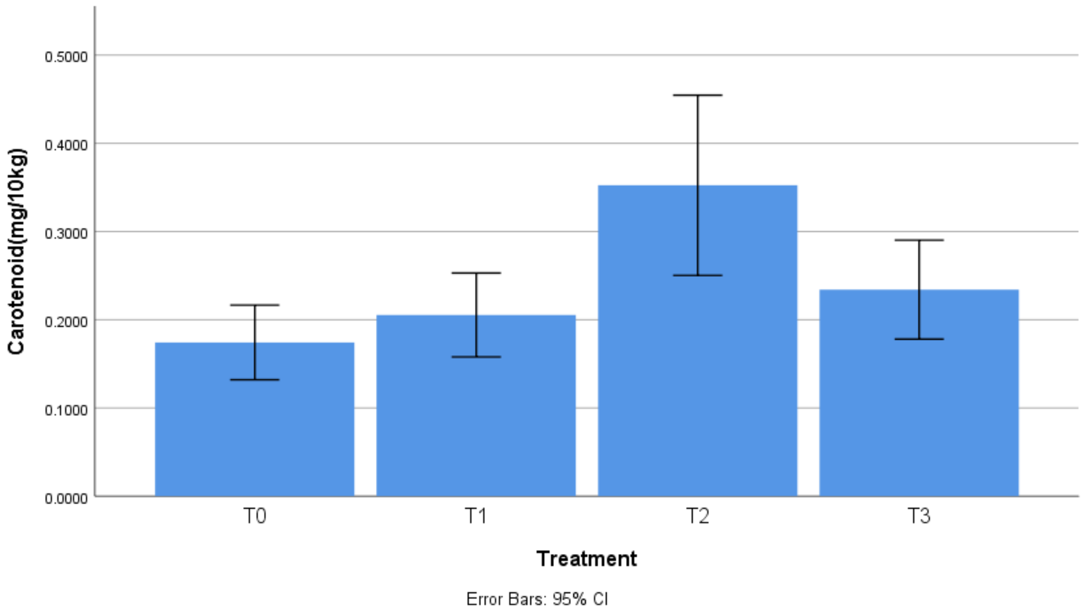

Figure-: 2 Effects of carotenoid on sword tail fish in terms of Carotenoids gain at the end of the experiment.

4.2 Effects of marigold on weight gain

The growth pattern of sword tail fish can be determined with different dietary levels of natural carotenoid content in terms of weight gain during the experimental period is presented in Figure-4. The average initial weights of fish in four treatments were 3.413±0.0742 gm, 3.213 ±0.0875 gm, 3.31±0.0324 gm, 2.313±0.0291 gm in T_0, T_1, T_2 and T_3 respectively. At the end of the 120 days experimental period, average final weight of the fishes of four treatments were 4.455±0.821 gm, 3.504±0.219 gm, 3.764±0.727 gm and 2.776±0.418 gm in treatments T_0, T_1, T_2 and T_3 respectively. In case of weight gain higher result was found in T_0 (4.455± .821 gm) followed by T_3, T_2 and T_1. Statistical analysis by ANOVA showed that there was no significant difference ($P > 0.05$) among the weights gain of T_0, T_1, T_2 and T_3 at the end of experimental period.

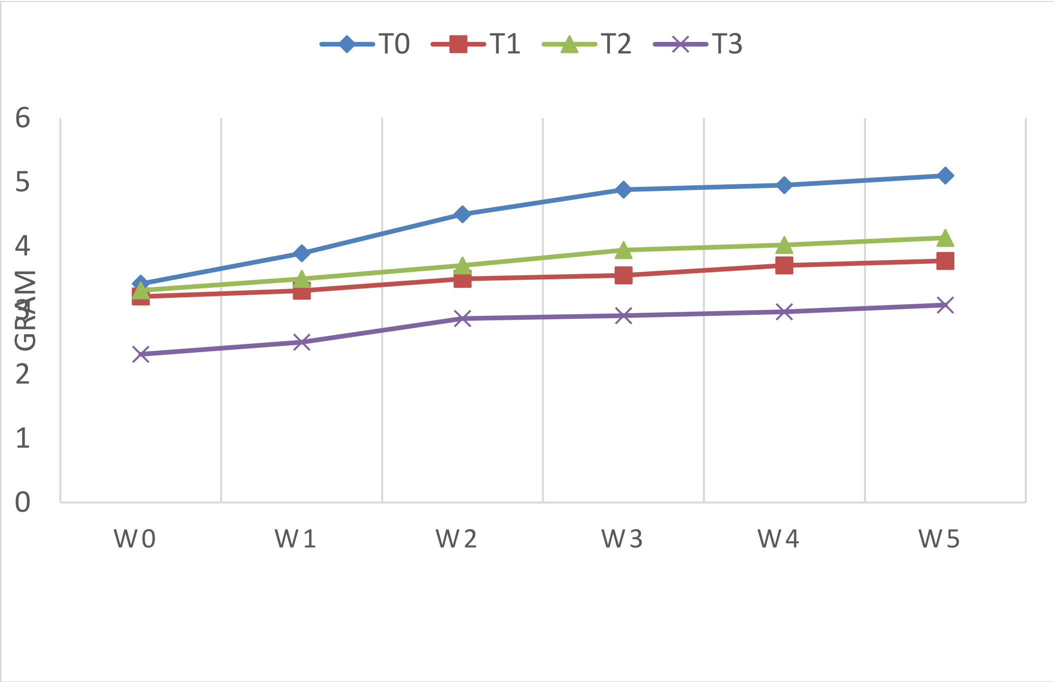

Figure- 3: Weight gain of Sword tail Fish

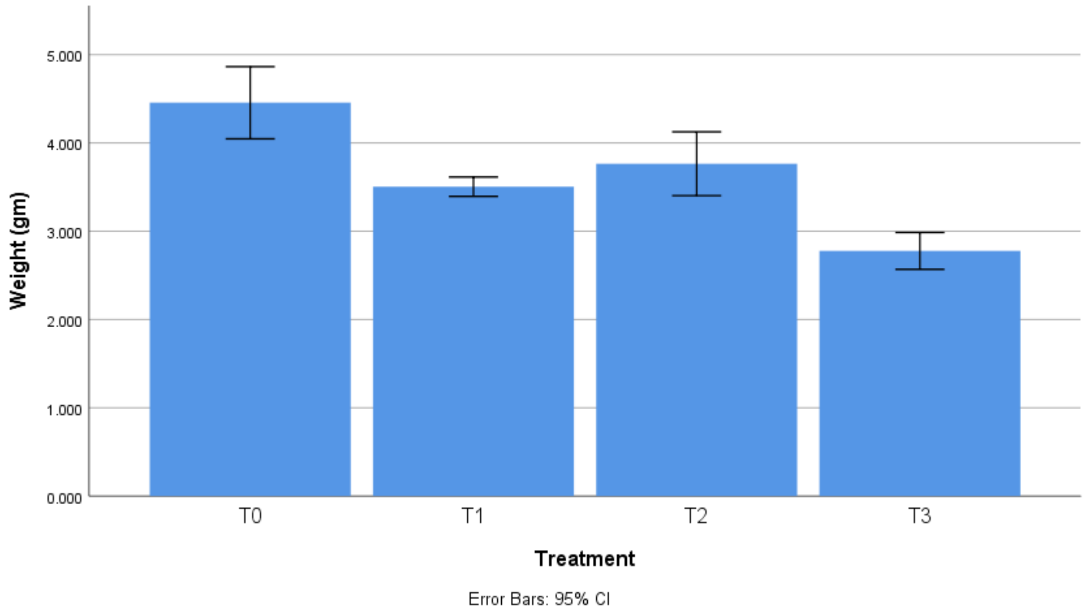

Figure -04: Effects of carotenoid on the growth (weight gain) of sword tail under different treatments.

4.3 Effects of marigold on length

The average initial lengths of fish in four treatments were 3.9±0.0828 cm, 3.8 ±0.0349 cm, 3.76±0.0271, 3.76±0.0211 cm in T_0, T_1, T_2 and T_3 respectively. At the end of experimental period, average final weight of the fishes of four treatments were 3.594±0.250 cm, 3.505±0.233 cm, 3.583±0.297 cm and 3.583± 0.311 cm in treatments T_0, T_1, T_2 and T_3 respectively. In case of length gain higher result was found in T_0 (3.594±0.250 cm) followed by T_3, T_2 and T_1. Statistical analysis by ANOVA showed there was no significant difference (P>0.05) among the lengths gain of T_0, T_1, T_2 and T_3 at the end of 120 days experimental period Figure-6.

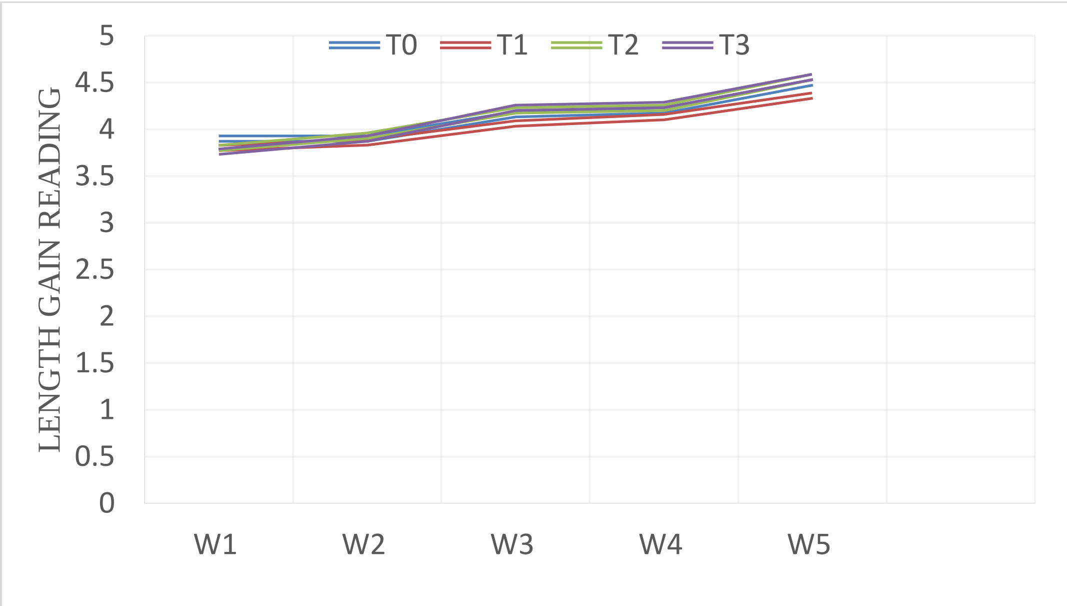

Figure-5: length gain of sword tail fish under different treatments.

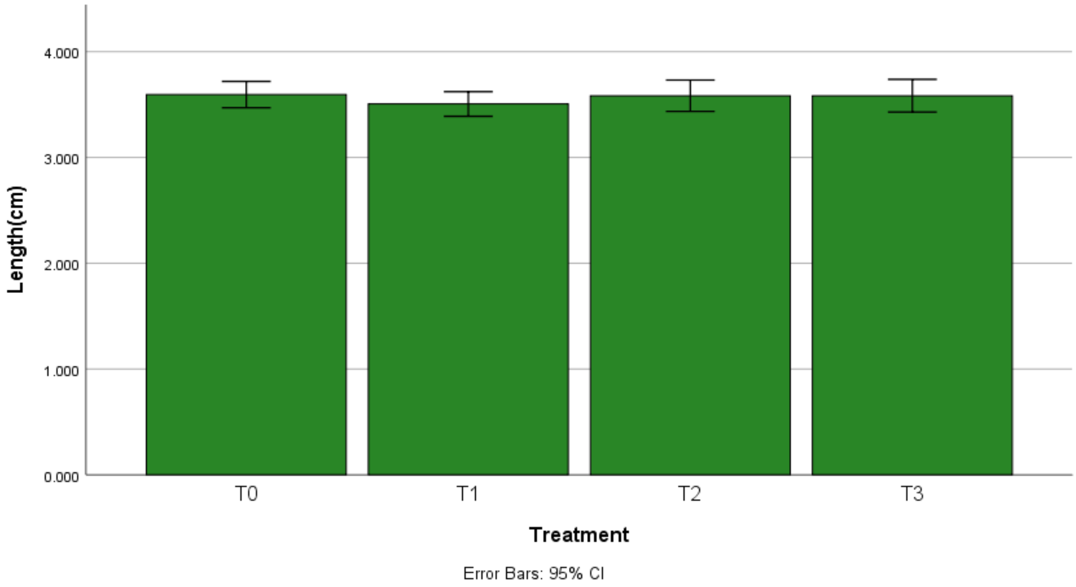

Figure-6: Final length gain data of sword tail fish under different treatments.

Table -4: Growth performance, length and Carotenoid gain of experimental fish under different treatments:

Treatment	Weight	Length	Carotenoid
T_0	4.4555 ± 0.821876[c] (4.04679-4.86421)	3.59444 ± 0.250816 [b] (3.46972 -3.71917)	0.174278 ± 0.0851116[b] (0.131953 - 0.216603)
T_1	3.50422 ± 0.219680[a] (3.39498-3.61347)	3.50556 ± 0.233823[b] (3.38928 - 3.62183)	0.205389 ± 0.0955874[b] (0.157854 - 0.252923)
T_2	3.76450 ± 0.727063[a] (3.40294-4.12606)	3.58333 ± 0.297539[b] (3.43537 - 3.73130)	0.352444 ± 0.2054190[a] (0.250292 - 0.454597)
T_3	2.77672 ± 0.418587[b] (2.56856-2.98488)	3.58333 ± 0.311070[b] (3.42864 - 3.73802)	0.234111 ± 0.1128981[b] (0.177968 - 0.290254)
Level of significance	0.001	.232	0.001

4.4 Effects on survival rate

Survival rate of experimental fish of different treatment are given in the figure-7. Experimental fish showed good performance in T_2 in which survival rate is 100% and the lowest performance was showed in T_1.

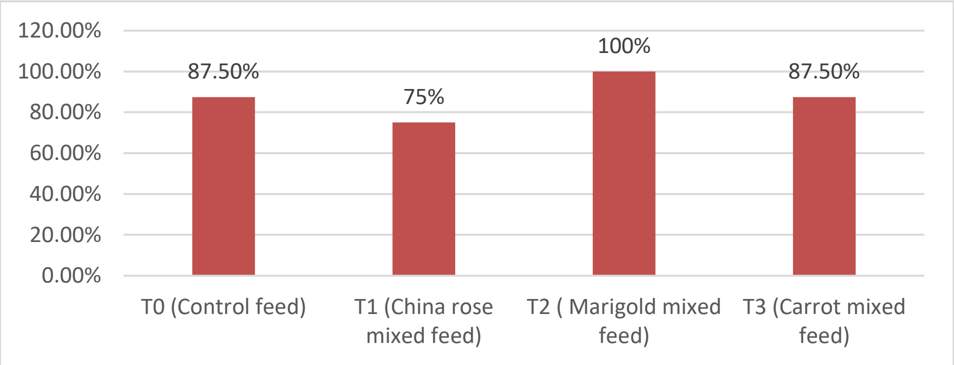

Figure-7: Survival rate data of sword tail fish under different treatments.

4.5 Water quality parameters

The water quality parameters as water temperature, dissolved oxygen (DO) and pH were monitored at 15 days interval throughout the experimental period. Average water quality parameters (P^H, DO and temperature) during the research work are shown in table 5.

Table-5: Water quality parameters

Treatments	p^H	DO (dissolved oxygen) (mg/l)	Temperature (℃)
T_0	7.5-8.5	5-7	24-27.8
T_1	7.2-8.0	6-7.2	24-27
T_2	7.5-8.2	5.5-7.5	25-28
T_3	7.0-8.0	5-7.5	23-28

CHAPTER-5
DISSCUSSION

5.1 Total carotenoid gain in fish tissue

Skin coloration is an important attribute for ornamental fishes which determines the demand and value in ornamental fish industry. Some important factor that is related to color are fins, scale, skin, etc. that are also related to market price and attractive value (Paripatananont et al., 1999). Compared to traditional fish feed ingredients such as fish meal, meat by-product meal, soybeans and cereals, reliable sources of carotenoids are comparatively costly. Therefore, the current research, which uses natural carotenoids in fish feed, is effective in improving coloration in fish without hindering growth, and survival rates. There was also no adverse effect on the quality of the water. The primary source of pigmentation in ornamental tropical fish are carotenoids, which are responsible for different colors, such as yellow, red, and other similar colors. But sources of carotenoids such as zeaxanthin and lutein are used for commercial feed ingredients such as yellow corn, corn gluten meal and alfalfa. Marigold meal (lutein), red pepper (*Capsicum sp)* extract (capsanthin) and krill or crustacean meals are other carotenoid-rich components used (astaxanthin) (Boonyarapatin et al., 1989). Several studies have shown improved coloration and significant positive effects of dietary pigments in fish, especially the use of natural pigments from plant sources to improve skin coloration in ornamental fish (Sinha and Asimi., 2007).

In this study, we selected three natural carotenoid sources to enhance pigmentation these marigold flower, china rose flower and carrot. To evaluate of natural carotenoid in sword tail fish, in terms of skin color intensity. The present study concluded that at the end of the 120-day experimental duration, the average final fish carotenoid absorption was 0.1742±0.0851 mg/10kg, 0.2053±0.0955mg/10kg, 0.3524±0.2054 mg/10kg and 0.2341± 0.1128 mg/10kg for the T_0, T_1, T_2 and T_3 procedures respectively. Higher results were observed for carotenoid gain in T_2 (marigold) (0.3524±0.2054 mg/10kg) followed by T_3, T_1 and T_0 and lower carotenoid gain in T_0 (control) respectively. Swian et al. (2014) found that that marigold oleoresin (180 mg/kg of feed) as a carotenoid source was effective on growth and skin pigmentation of Koi carp as it led to nearly maximum carotenoids accumulation in the body of goldfish. Liang *et al*., (2012) found that there was a substantially higher deposition of carotenoids in the fish body is fed a diet supplemented with carotenoids (with 180 mg/kg Marigold oleoresin) diet. The 250 mg of astain is observed to be the highest supplementation per kg of diet was seen deposition of Carotenoids in Koi Carp. The effectiveness of a carotenoid source for pigment deposition is species specific (Ha *et al*., 1993) and also present study demonstrated that marigold meal led to nearly maximum carotenoid accumulation in the skin of goldfish, it should be considered as a valuable source of carotenoids, such as zeaxanthin, lutein or astaxanthin (Matsuno et al., 1981).

5.2 Effects of marigold on growth, length and survival rate

The development of manufactured feed could be considered as one of the contributing factors to the tremendous growth of this hobby's widespread popularity over the past 50 years. The increased acceptability of reliance upon manufactured feed for ornamental fish has focused the attention on the nutritional requirements. In the present study the diet was prepared containing 30, 27.82, 27.34 and 28.8% of protein. In the present study the proximate composition shows china rose flower, marigold and carrot's protein value was 15.7%, 12.3% and 22%. In the present study survival rate of fish is not markedly different within the treatment but growth performance with respect to final mean weight was significantly improved in fish fed with marigold than the other natural carotenoid mixed feed fed. The fish fed with china rose supplemented diets showed lowest growth rate. These results are also in agreement with the finding of Atlantic salmon fry (*Salmo salar*) (Christiansen *et al.*, 1995), rainbow trout (*Oncorhyncus mykiss*) (De la Mora *et al.*, 2006) and goldfish (*Carassius auratus*) (Sinha and Asimi., 2007). Further, observation was made by Ahilan *et al.*, (2008) in gold fish fed with coriander incorporation feed at 3 percent level showed better biological performance like weight gain and specific growth rate when compared to other coriander in corporate diet. According to Tveranger., (1986) and Sommer *et al.*, (1992), the addition of carotenoids rich microalgae and krill meal enhanced the growth of trout. In the present study also showed that high protein content in supplemented feed shows high growth at the end of study. For four treatments, the average initial weights were 3.413±0.0742 gm, 3.213±0.0875 gm, 3.31±0.0324 gm, 2.313±0.0291 gm for T_0, T_1, T_2 and T_3, respectively. After complection days of experimental period average final weight of the fishes of four treatments were 4.455±0.821 gm, 3.504±0.219 gm, 3.764± 0.727 gm and 2.776±0.418 gm in treatments T_0, T_1, T_2 and T_3 respectively. Ezhil. J *et al*., (2008) reported fed with marigold petal 15 gm/100 gm were increased the growth rate of sword tail reared for 60 days. Sinha et al. (2007) studied the growth rate of fishes in the group fed with the China rose petal feed was the highest in terms of weight, with an increased value of carotenoid in skin (4.01µg/g). Several factors such as accumulation of nitrogenous compounds feed availability and quality stocking density etc. that also affects the mortality and survival rate (Randazzo *et al.,* 2017). In the present study the parameters of water were found within the ideal range and hence didn't supposed to have any major influence on the results. Highest survival rate was found in T_2 (100% survived) and lowest survival rate was found in T_0 and T_3 (87.50% survived). Inclusion of carotenoid mixed supplementary feed also had no influence on water quality in this study. Moreover, natural carotenoid content which was insoluble in water do not produce problems due to leaching to the surrounding water medium. According to Harpaz and Padowicz., (2007) found that added of paprika in the preparation of diet had no adverse effects on the quality of water and survival rate in dwarf cichlids. In case of length gain higher result was found in T_0 (3.5944 ± 0.2508 cm) However, Arulvasu*et al.,* (2013) reported a negative effect on survival when *Rosa rubiginosa* was used as the pigment.

CHAPTER-6
CONCLUSION

From the experiment it could be concluded that dietary supplementation of marigold flower mixed feed at the rate of 15 g/100 g of diet can be used for enhancing the coloration of sword tail fish. Natural carotenoids could be effective pigment enhancer in ornamental fish’s coloration as their swimming behavior and survival rate also in very well. Since synthetic carotenoids are pricey but natural carotenoid sources such as marigold can be incorporated into the diet to enhance better coloration in sword tail fish. This will help farmers and other stakeholders realize greater profits in the culture of this species. Considering the present study findings, it is proved that marigold had a positive impact on the coloration of sword tail fish. In addition, the findings also support that the use of marigold flower meal in the diet as a replacement of synthetic meal as a natural carotenoid source for the coloration of sword tail fish.

CHAPTER-7

RECOMMENDATION AND FUTURE PERSPECTIVES

According to this research work, the following recommendations may be done:

- Marigold, china rose and carrot may add new dimension in fisheries and approved by governments and included in national policy level by maintaining consumer safety.
- Natural's carotenoids may hold burning issues for feed industries to create noble products, such as feed supplements and will represent an interesting resource for the feed industry sector as synthetic color replacement
- Natural's carotenoids may be used in improving the total coloration and growth.
- Farmer will be enabling to produce low-cost feed using natural carotenoid as it is locally available and very cheap.
- As it is a pilot study, further studies may be conducted on similar field to make a concrete remark

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APPENDICES

Appendix 1: Carotenoid absorption data of the fishes at different sampling

	W-0	W-1	W-2	W-3	W-4	W-5
Treatment	Carotenoid(mg/kg)	Carotenoid(mg/kg)	Carotenoid(mg/kg)	Carotenoid(mg/kg)	Carotenoid(mg/kg)	Carotenoid(mg/kg)
$T0R_1$	0.082	0.08	0.098	0.234	0.258	0.285
$T0R_2$	0.084	0.076	0.141	0.229	0.263	0.265
$T0R_3$	0.083	0.081	0.118	0.245	0.254	0.261
$T1R_1$	0.085	0.072	0.197	0.272	0.282	0.292
$T1R_2$	0.084	0.089	0.187	0.301	0.286	0.294
$T1R_3$	0.086	0.088	0.194	0.288	0.291	0.309
$T2R_1$	0.084	0.119	0.287	0.415	0.525	0.588
$T2R_2$	0.085	0.138	0.276	0.525	0.532	0.586
$T2R_3$	0.086	0.142	0.269	0.518	0.579	0.59
$T3R_1$	0.084	0.096	0.223	0.337	0.322	0.348
$T3R_2$	0.083	0.097	0.215	0.342	0.333	0.355
$T3R_3$	0.086	0.099	0.217	0.326	0.324	0.327

*W= Week, TR= Treatment × Replication

Appendix 2: Weight data of the fishes at different sampling

	W-0	W-1	W-2	W-3	W-4	W-5
Treatment	weight(gm)	weight(gm)	weight(gm)	weight(gm)	weight(gm)	weight(gm)
$T0R_1$	4.060	4.270	4.820	4.950	5.000	5.200
$T0R_2$	1.970	3.070	3.890	4.810	4.950	5.100
$T0R_3$	4.210	4.330	4.789	4.880	4.900	5.000
$T1R_1$	3.380	3.386	3.490	3.520	3.730	3.760
$T1R_2$	3.270	3.276	3.560	3.610	3.650	3.770
$T1R_3$	2.990	3.250	3.420	3.510	3.720	3.784

$T2R_1$	2.430	2.489	2.990	3.457	3.574	3.668
$T2R_2$	4.500	4.520	4.560	4.596	4.606	4.712
$T2R_3$	3.000	3.460	3.551	3.757	3.880	4.011
$T3R_1$	2.150	2.372	2.745	2.770	2.896	2.957
$T3R_2$	2.000	2.242	2.634	2.686	2.698	2.711
$T3R_3$	2.790	2.886	3.231	3.295	3.341	3.577

*W= Week, TR= Treatment × Replication

Appendix 3: Length data of the fishes at different sampling

	W-0	W-1	W-2	W-3	W-4	W-5
Treatment	Length(cm)	Length (cm)	Length (cm)	Length(cm)	Length(cm)	Length(cm)
$T0R_1$	3.8	3.8	3.8	4	4	4.4
$T0R_2$	4	4	4	4.3	4.3	4.5
$T0R_3$	3.9	3.9	3.9	4.2	4.3	4.6
$T1R_1$	3.9	3.9	3.9	4.1	4.2	4.3
$T1R_2$	3.9	3.9	3.9	4.1	4.2	4.4
$T1R_3$	3.6	3.6	3.8	4	4	4.4
$T2R_1$	3.8	3.8	4	4.2	4.3	4.6
$T2R_2$	3.8	3.9	3.9	4.3	4.3	4.6
$T2R_3$	3.7	3.7	3.9	4.1	4.1	4.5
$T3R_1$	3.8	3.8	3.9	4.2	4.3	4.5
$T3R_2$	3.7	3.7	3.8	4.2	4.2	4.5
$T3R_3$	3.8	3.8	4	4.3	4.3	4.7

*W= Week, TR= Treatment × Replication

Appendix 4: Carotenoid gain data of the fishes at different weeks

	W-1	W-2	W-3	W-4	W-5
Treatment	Carotenoid(mg/kg)	Carotenoid(mg/kg)	Carotenoid(mg/kg)	Carotenoid(mg/kg)	Carotenoid(mg/kg)
$T0R_1$	-0.002000	0.018000	0.136000	0.024000	0.027000
$T0R_2$	-0.008000	0.065000	0.088000	0.034000	0.002000
$T0R_3$	-0.002000	0.037000	0.127000	0.009000	0.007000
$T1R_1$	-0.013000	0.125000	0.075000	0.010000	0.010000
$T1R_2$	0.005000	0.098000	0.114000	-0.015000	0.008000
$T1R_3$	0.002000	0.106000	0.094000	0.003000	0.018000
$T2R_1$	0.035000	0.168000	0.128000	0.110000	0.063000
$T2R_2$	0.053000	0.138000	0.249000	0.007000	0.054000
$T2R_3$	0.056000	0.127000	0.249000	0.061000	0.011000
$T3R_1$	0.012000	0.127000	0.114000	-0.015000	0.026000
$T3R_2$	0.014000	0.118000	0.127000	-0.009000	0.022000
$T3R_3$	0.013000	0.118000	0.109000	-0.002000	0.003000

*W= Week, TR= Treatment × Replication

Appendix 5: Weight gain data of the fishes at different weeks

	W-1	W-2	W-3	W-4	W-5
Treatment	Weight gain	Weight gain	Weight gain	Weight gain	Weight gain
$T0R_1$	0.21	0.55	0.130	0.05	0.2
$T0R_2$	1.1	0.82	0.920	0.14	0.15
$T0R_3$	0.12	0.459	0.091	0.02	0.1
$T1R_1$	0.006	0.104	0.030	0.21	0.03
$T1R_2$	0.006	0.284	0.050	0.04	0.12
$T1R_3$	0.26	0.17	0.090	0.21	0.064
$T2R_1$	0.059	0.501	0.467	0.117	0.094
$T2R_2$	0.02	0.04	0.036	0.01	0.106
$T2R_3$	0.46	0.091	0.206	0.123	0.131
$T3R_1$	0.222	0.373	0.025	0.126	0.061
$T3R_2$	0.242	0.392	0.052	0.012	0.013
$T3R_3$	0.096	0.345	0.064	0.046	0.236

*W= Week, TR= Treatment × Replication

Appendix 6: Length gain data of the fishes at different weeks

	W-1	W -2	W-3	W-4	W-5
Treatment	Length gain	Length gain	Length gain	Length gain	Length gain
$T0R_1$	0	0	0.2	0	0.4
$T0R_2$	0	0	0.3	0	0.2
$T0R_3$	0	0	0.3	0.1	0.3
$T1R_1$	0	0	0.2	0.1	0.1
$T1R_2$	0	0	0.2	0.1	0.2
$T1R_3$	0	0.2	0.2	0	0.4
$T2R_1$	0	0.2	0.2	0.1	0.3
$T2R_2$	0.1	0	0.4	0	0.3
$T2R_3$	0	0.2	0.2	0	0.4
$T3R_1$	0	0.1	0.3	0.1	0.2
$T3R_2$	0	0.1	0.4	0	0.3
$T3R_3$	0	0.2	0.3	0	0.4

*W= Week, TR= Treatment × Replication

Appendix 7: Average carotenoid absorption data of fishes per treatment per week

Treatment	W0 (mg/10kg)	W1 ((mg/10kg)	W2 (mg/10kg)	W3 (mg/10kg)	W4 (mg/10kg)	W5 (mg/10kg)
T0	0.083	0.079	0.199	0.236	0.258	0.270000
T1	0.085	0.083	0.192	0.287	0.286	0.298000
T2	0.085	0.133	0.277	0.486	0.545	0.588000
T3	0.084	0.097	0.218	0.335	0.326	0.343000

*W= Week, TR= Treatment × Replication

Appendix 8: Average weight data of fishes per treatment per week

Treatment	W0 (gm)	W1 ((gm)	W2 (gm)	W3 (gm)	W4 (gm)	W5 (gm)
T0	3.413	3.89	4.5	4.88	4.95	5.100000
T1	3.213	3.304	3.49	3.547	3.7	3.771000
T2	3.31	3.49	3.7	3.937	4.02	4.130000
T3	2.313	2.5	2.87	2.917	2.978	3.082000

*W= Week, TR= Treatment × Replication

Appendix 9: Average length (cm) data of fishes per treatment per week

Treatment	W0(cm)	W1 (cm)	W2 (cm)	W3 (cm)	W4 (cm)	W5 (cm)
T0	3.9	3.9	3.9	4.16	4.2	4.500000
T1	3.8	3.8	3.86	4.06	4.13	4.360000
T2	3.76	3.8	3.93	4.2	4.23	4.560000
T3	3.76	3.76	3.9	4.23	4.26	4.560000

*W= Week, TR= Treatment × Replication

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