



EFFECT OF CANTHAXANTHIN ON GROWTH AND PIGMENTATION OF *CYPRINUS CARPIO*

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Abstract

Fish is a major source of protein with high nutritional value for individuals. *Cyprinus carpio* (Gulfam) is the third most widely cultivated, contributing 9% of the total aquaculture production. The problem of pale coloring of common carp as well as less growth with the passage of time was solved with the help of dietary carotenoids "Canthaxanthin". In the present experiment, canthaxanthin was given 2% of the average body weight of the fishes. Fingerlings were taken from Punjab Fish Hatchery Faisalabad, while experiment was held in Fisheries Farms, Department of Zoology, University of Agriculture, Faisalabad. Two glass aquaria having the capacity of 60 liters water, each having 15 fingerlings of *Cyprinus carpio* were used for experiment up to the period of 45 days. Glass aquaria was marked as a T0 and T1. T0 received basic feed while T1 received carotenoids "canthaxanthin". Physico-chemical parameters were also measured. Weekly specific growth rate (SGR) in terms of body weight (g) and body length (cm) was measured. Survival rate and FCR were also measured. At the end of trial duration, red pigmentation was measured. All the data were subjected to statistical analysis using paired t-test. The results of this study illustrated that average (DO) for T0 was 3.95 ± 0.16 mg/l (Dissolved Oxygen) and for T1 was 3.98 ± 0.11 mg/l, average temperature was 26.36 ± 0.41 °C and 27.63 ± 0.47 °C and average pH was 7.36 ± 0.12 and 7.51 ± 0.12 for T0 and T1 respectively. Average gain in body weight for T0 was 0.99 ± 0.09 g specific growth rate (SGR) and for T1 was 1.67 ± 0.22 g (SGR). Average gain in body length for T0 was 0.79 ± 0.06 cm (SGR) and for T1 was 1.23 ± 0.14 cm (SGR). Average feed conversion ratio results for T0 and T1 were 20.70 ± 2.84 (FCR) and 11.00 ± 1.58 (FCR) respectively. Survival rate for T0 was 73.3% and for T1 was 86.6%. The average of red pigmentation was 1.03 ± 0.06 for T0 and 2.40 ± 0.30 for T1. Results showed that there are non-significant relationship among dissolved oxygen, temperature and pH. While, there are highly significant relationship among SGR, FCR, survival rate and pigmentation. So, these results prove that carotenoids have beneficial effects on fish growth and pigmentation.

Keywords: Carotenoids, Canthaxanthin, Growth Performance, Pigmentation, *Cyprinus carpio*

CHAPTER 1 INTRODUCTION

Aquaculture will account for 20% of total global fish output. Carotenoids are capable for the pigmentation of muscles in fish food as well as the color of an ornamental fish's skin. Pigmentation is a significant qualitative trait of aquatic animals in terms of consumer acceptance. Aquaculture is the cultivation of diverse freshwater and marine finfish, shellfish, and mollusk species (Garca-Chavarra and Lara-Flores, 2013).

Carotenoids are to blame for the yellow, orange, and red hues of fish and crabs. Salmonids are characterized by their ability to store carotenoids absorbed in their muscles. Muscle is popular nowadays. The coloring produced by these lipophilic chemicals has become an industry standard (Kop and Durmaz, 2008).

Cyprinus carpio is one of the most famous and valuable decorative fish in the world, as well as in Pakistan. This fish were brought from China, and it is now a part of our culture in many parts of the country. The color of the fish is very important since different spectacular colors have a significant impact on the price of fish. As a result, the vivid colors of ornamental fish, such as *Cyprinus carpio*, are very important in the market. The term "carotenoids" is used to describe the pigments used to color *Cyprinus carpio*. Carotenoids accumulate in the fish's body and cause coloration. When fish are reared in an aquarium, their color becomes pale owing to a lack of carotenoids. Carotenoids could not be produced in fish by the "De Novo" synthesis method. As a result, carotenoids should be included in their diet (Kurnia *et al.*, 2008).

The skin shade of plate fish is one of the main quality and worth viewpoints in the aquarium exchange and specialist acknowledgment, with shades of orange, yellow and red coming about because of carotene testimony. Carotenoids, (for example, beta-carotene, astaxanthin, lutein, lycopene, canthaxanthin and zeaxanthin) can't be biosynthesized anew by most fish, including disk, hence they should be given in their eating regimen (Ninwichian *et al.*, 2020).

One of the most difficulties in the decorative fish industry is repeating the normal shade of the fish breed in a restricted setting. The emphasis is on adding carotenoids to the diets of ornamental fish (Meilisza *et al.*, 20210).

Previously, natural chemicals produced from red yeast, sea bacteria, and green algae were shown to be equally effective as synthetic carotenoids in increasing skin pigmentation in a variety of ornamental fish species, especially gold fish *Carassius auratus* (Karadal *et al.*, 2017).

Absolute carotenoids which were present in the skin, scales, and serum were examined. If such natural carotenoid supplemented diets were efficient in enhancing the skin pigmentation of fancy carp, the costs and problems associated with utilizing synthetic carotenoids may be lower without a loss in market value, which is dependent on pigmentation (Savun and Ince, 2017).

Carotenoids are pigments found in microorganisms and plants; beta-carotene is one carotenoid structure that is a good source of vitamin A. It is also regarded as an essential natural aquaculture color producing. Carotenoids also be treated as natural immune stimulants to improve the antioxidant capacity and immunological condition of fish (Komoto *et al.*, 2018).

Color is one of the most important factors influencing the value of ornamental fish on the global market. The fish color is principally determined by chromophores, which include pigments like melanin, carotenoids like zeaxanthin, and purines. Goodwin demonstrated that fish do not have the capacity to synthesize catalyzed by their own pigment found in their feed (Ho *et al.*, 2014).

Carotene-rich fish have also been discovered to be more resistant to fungal and bacterial infections. Two major carotenoids, astaxanthin and canthaxanthin, have key roles in aquaculture. Another pigment thought to be a precursor to retinal in Salmon sp. is canthaxanthin (Garca-Chavarra and Lara-Flores, 2013).

Specifically, Astaxanthin, is generally dispersed in fishes and advances undeveloped and larval turn of events, cell security from photodynamic harm, development and development, the arrangement of in-chain epoxides that capacity as oxygen holds under anoxic conditions, O₂ quenchers or free

extremist searching, skin tinge during sexual development, and sign substances in generation (Tizkar *et al.*, 2015).

A number of carotenoids, both natural and synthetic, have been introduced into fish diets for color improvement, including astaxanthin, cantaxanthin, beta-carotene, lutein, and xanthophyll (Boonyapakdee *et al.*, 2015).

The chemical formula of canthaxanthin is $C_{40}H_{52}O_2$. It was first isolated in edible mushrooms *Cantharellus cinnabarinus*. The skin pigmentation of extravagant carp is the main quality boundary deciding the market worth and shopper adequacy. A successive issue found in extravagant carp culture is that, the fish will generally lose their shading as though blurring when kept up with in bondage, and this reductions their fairly estimated worth. Canthaxanthin can be used to enhance growth and pigmentation in *Cyprinus carpio* in present research (Venil *et al.*, 2021).

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Protocols

Fingerlings of (*Cyprinus carpio*) were collected from the Punjab Fish Hatchery near Satyana Road, Faisalabad. About 30 samples were collected of different sizes and weights. The average initial weight was measured. A good quality aquarium (2×3 feet) having a water capacity of 60L was selected in the lab which did not have any leakage. Firstly, the aquarium was cleaned with warm water to remove any trash present in it. The aquarium consisted of two equal parts; one was a control group and the other was an experimental group, and each group was contained 15 fish. Every day, about one-third of the water was changed with fresh water.

The feeding trial was conducted for 45 days (6 weeks). After every week, the growth, length, weight, and color of fish in both groups, i.e., control and experimental, was measured and noted. Damaged fishes were removed from the aquarium so that these fishes did not damage the other healthy fish.

3.2 Experimental Feed:

Experimental feed was consisted of basic feed with carotenoids, especially canthaxanthin, and was given 2% of the total average body weight of fish. Basic feed was given to control group. **Canthaxanthin** is a keto-carotenoid pigment widely distributed in nature. Carotenoids belong to a larger class of phytochemicals known as terpenoids. The chemical formula of canthaxanthin is $C_{40}H_{52}O_2$. It was first isolated with edible mushrooms.

Canthaxanthin is approved for use as a food coloring agent in different countries, including the United States and the EU; however, it is not approved for use in Australia and New Zealand. It is generally authorized for feed applications in at least the following countries: US, Canada and EU. In the EU, canthaxanthin is allowed by law to be added to trout feed, salmon feed and poultry feed. Canthaxanthin is a potent lipid-soluble antioxidant. The biological functions of canthaxanthin are related, at least in part, to its ability to function as an antioxidant (free radical scavenging/vitamin E sparing) in animal tissues.

Experimental feed was measured by this formula.

Feed measuring: $2/100 \times (\text{Average weight of Fishes})$

Table 3.1: Proximate experimental diet composition with Canthaxanthin in control and experimental groups

Ingredients %	Basic Feed %	Experimental Feed %
Fish Meal	30.0	30.0
Wheat Brain	20.0	20.0
Sunflower	2.0	2.0
Rice Brain	14.0	14.0
Canthaxanthin	0.0	10.0
Maize	4.0	4.0

Rice Polish	10.0	10.0
Wheat	20.0	10.0
Total	100	100

3.3 Physico-Chemical Parameters:

Physico-chemical parameters of water i.e. Dissolved oxygen (DO), Temperature and pH were monitored for maintaining water quality. Water was removed in separate beakers for checking and measuring these parameters from each aquarium.

3.4 Growth Parameters

Fish weight (g) and length (cm) were recorded on weekly basis. Growth parameters such as Length gain (cm), Weight gain (g), Percent Weight gain (%), Specific growth rate (SGR), Feed Conversion ratio (FCR) and Percent Survival rate (%) were measured at the end of trial.

3.4.1 Length gain

At the end of experimental trial, increase in total length of fish was calculated by using following formula:

$$\text{Length gain} = \text{Final length} - \text{Initial Length}$$

3.4.2 Weight gain

At the end of experimental trial, increase in total weight of fish was calculated by using following formula:

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

3.4.3 Percent Weight gain (%)

Percent weight gain of fish was calculated by this formula:

$$\text{Percent Weight gain (\%)} = \frac{\text{Final fish weight} - \text{Initial fish weight}}{\text{Initial Fish weight}} \times 100$$

3.4.4 Specific Growth rate (SGR)

SGR of fish was calculated by this formula:

$$\text{SGR} = \frac{\text{Final weight} - \text{Initial weight}}{\text{No. of days}} \times 100$$

3.4.5 Percent Survival rate (%)

Percent survival rate of fish was calculated by this formula:

$$\text{Survival rate (\%)} = \frac{\text{Final number of fish}}{\text{Total number of fish}} \times 100$$

3.5 Feed Conversion ratio (FCR)

The FCR is a factor that is usually used in all kinds of farming techniques, in addition to field of research.

$$\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Weight gain in (g)}}$$

3.6 Pigmentation

Cyprinus carpio is one of the most famous and valuable decorative fish in the world, as well as in Pakistan. The color of the fish is very important since different spectacular colors have a significant impact on the price of fish. As a result, the vivid colors of ornamental fish, as well as edible fish like *Cyprinus carpio*, are very important in the market. The term "carotenoids" is used to describe the pigments used to create color in *Cyprinus carpio*. Carotenoids accumulate in the fish's body and cause coloration.

Pigmentation was checked by color flow-chart at the end of trial.

L* = Lightness, a* = Redness, b* = Yellowness

3.7 Statistical analysis

All data were placed to paired t-test analysis. Some differences were regarded as highly significant at $p < 0.01$ and some were significantly different at $p < 0.05$ among treatment groups.

CHAPTER 4

RESULTS

4.2 Calculation of Growth Parameters

4.2.1 Growth in term of Weight Gain (g)

Table 4.10: Profile of growth performance of total (6) weeks in term of weight gain (g)

Weeks	Control Group	Experimental Group	P-Value
1 st	0.58	0.68	0.00
2 nd	0.84	1.38	
3 rd	1.08	1.72	
4 th	0.97	1.54	
5 th	1.29	2.1	
6 th	1.23	2.61	
Mean	0.99±0.09	1.67±0.23	

Table 4.10.1: T-test on comparison of specific growth rate in term of weight gain of *Cyprinus carpio* for T0 and T1

Treatments	Mean	St.E	N	Mean Difference	Df	t-table value	P-Value
T0	0.99	0.09	7	0.67	6	4.66	0.00
T1	1.67	0.23					

Non-significant ($P > 0.05$) Significant ($P < 0.05$) Highly significant ($P < 0.01$)
 T0= Control Group T1 = Experimental Group

Weekly calculations on average of specific growth rate in term of weight gain of total 6 weeks were noted and applied paired t-test. Above results show that T0 have 0.99 ± 0.24 (SGR) and T1 have 1.67 ± 0.59 (SGR). Above results show highly-significant results between T0 and T1.

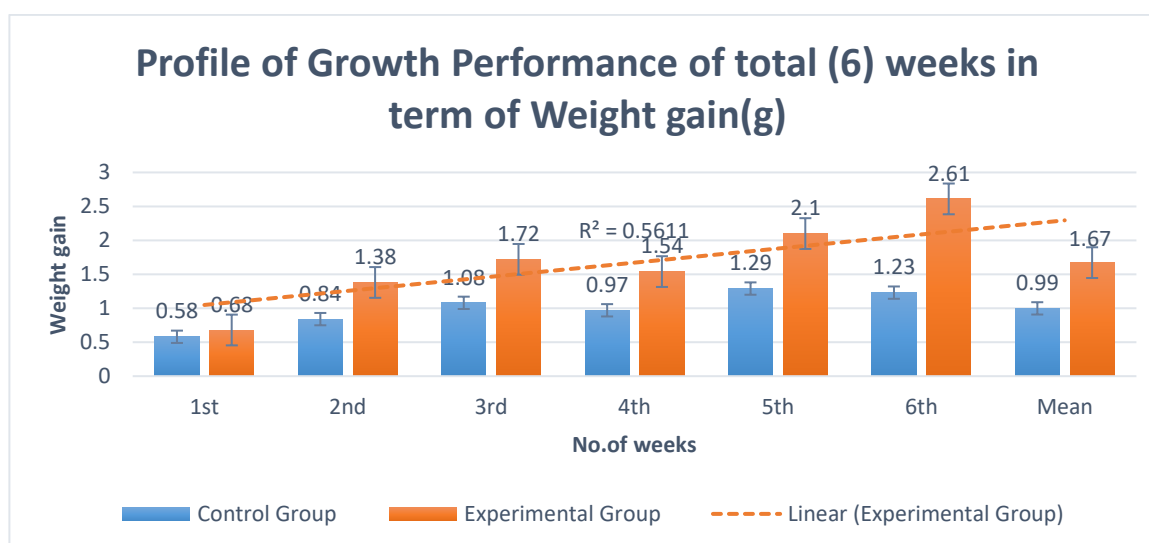


Fig. 4.10: Graph of weight gain of *Cyprinus carpio* for T0 and T1 (Total 6 weeks)

Table 4.17.2 Growth performance of both parameters of all 6 weeks

Weeks	Weight gain		Length gain	
	T0	T1	T0	T1
1 st	0.58±0.07	0.68±0.07	0.57±0.04	0.74±0.04
2 nd	0.84±0.07	1.38±0.10	0.70±0.04	1.01±0.05
3 rd	1.08±0.10	1.72±0.13	0.62±0.63	0.96±0.08
4 th	0.97±0.10	1.54±0.12	0.94±0.10	1.67±0.12
5 th	1.29±0.17	2.1±0.22	0.92±0.12	1.28±0.13
6 th	1.23±0.19	2.61±0.26	1.00±0.16	1.76±0.18
Mean	0.99±0.09	1.67±0.23	0.79±0.06	1.23±0.14

4.3 Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) or feed conversion rate is a ratio or rate measuring of the efficiency with which the bodies of livestock convert animal feed into the desired output. The less value of FCR showed better results in fish growth. So, in my results, less values of FCR were found in experimental group than control group which showed the better growth in experimental group.

Table 4.18 Weekly observation of FCR

Weeks	Control Group	Experimental Group	P-Value
1 st	11.5	7.8	0.00
2 nd	31.3	19	
3 rd	27	12	
4 th	11.5	12.2	
5 th	18.7	8.9	
6 th	24.2	6.1	
Mean	20.7±2.84	11±1.58	

Table 4.18.1: T-test on comparison of specific growth rate in term of FCR of *Cyprinus carpio* for T0 and T1

Treatments	Mean	St.E	N	Mean Difference	df	t-table value	P-Value
T0	20.70	2.84	7	-9.70	6	-3.98	0.00
T1	11.00	1.58					

Non-significant ($P>0.05$) Significant ($P<0.05$) Highly significant ($P<0.01$)

T0 = Control Group

T1 = Experimental Group

Weekly calculations on average of (FCR) of total 6 weeks were noted and applied paired t-test. Above results show that T0 have 20.70±7.50 (FCR) and T1 have 11.00±4.19 (FCR). Above results show highly-significant results between T0 and T1.

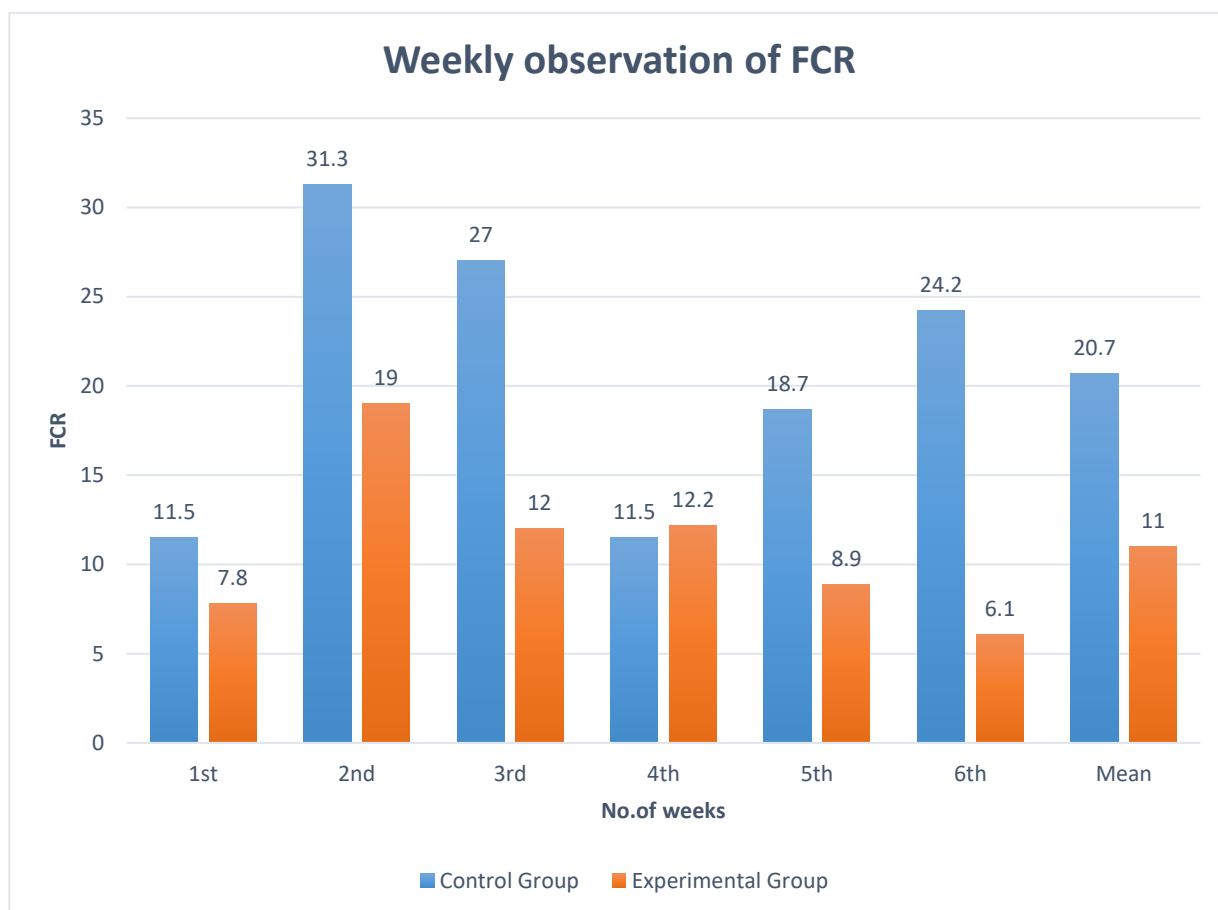


Fig. 4.18: Graph of FCR of *Cyprinus carpio* for T0 and T1 (Total 6 weeks)

4.4 Percent Survival Rate (%)

Table 4.19 Survival rate of T0 and T1

Treatments	Total No. of Fishes	Final No. of Fishes	% Survival Rate
Control Group	15	11	73.3%
Experimental Group	15	13	86.6%

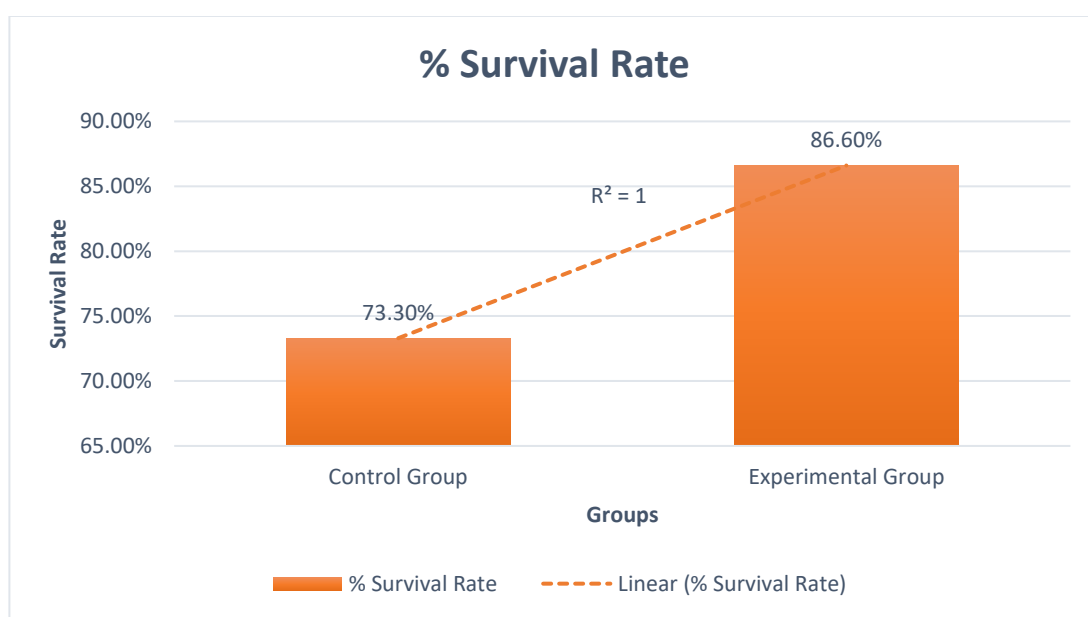


Fig. 4.19: Graph of survival rate of *Cyprinus carpio* for T0 and T1 (Total 6 weeks)

CHAPTER 5

DISCUSSION

Fish is a major source of protein with high nutritional value for individuals. Freshwater fish species play an important role in aquaculture. The proposed study was directed to measure the growth performance and pigmentation of *Cyprinus carpio* fed with carotenoids (Canthaxanthin). The experimental trial was carried out for 45 days at Fisheries Research Farms in Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad.

The proposed study was directed to measure the growth performance and pigmentation of *Cyprinus carpio* using supplemental diets i.e. Carotenoids especially “Canthaxanthin”. Carotenoids are hydrophobic compounds that are not easily solubilized in the aqueous environment of the gastrointestinal tract of fish; therefore, digestion, absorption and transport processes are associated to lipids. The intestinal absorption of carotenoids involves several steps, including disruption of the matrix, dispersion in lipid emulsions and solubilization into mixed bile salt micelles, so, carotenoids are act as a growth promoters. The results showed that maximum growth performance in terms of weight gain and length gain was observed in experimental group in which fish were fed with canthaxanthin for 45 days ($P < 0.05$) and less growth was found in control group in which fish were fed with basic diet for 45 days ($P > 0.05$). No literature could be found regarding the effect of canthaxanthin on growth performance and skin pigmentation in *Cyprinus carpio*. However, the results of present study are in conformity with the findings of (Nurhadi T *et al.*, 2019, Sinha and Asimi, 2007, Ytrestøy *et al.* 2021, Meilisza *et al.*, 2021, Barkallah *et al.*, 2019) all references showed who studied the effect of carotenoids on growth performance of *Nile Tilapia* and revealed that significantly maximum growth performance was attained in the diet that contained carotenoids instead of basic feed.

Considerably, experimental group had a better survival rate than control group, with a small increase in death rate during the trial period. Maximum percent survival rate (86.6%) was observed in experimental group that was fed with carotenoid and less percent survival rate (73.3%) was shown by control group that was fed with basic diet without carotenoids. The order of efficiency for survival rate was observed $T1 > T0$). The results of present study are in conformity with the findings of (Ziegelbecker *et al.*, 2021) who studied the effect of carotenoids on survival rate of Cichlid fish and revealed that significantly maximum survival rate was attained in the diet that contained carotenoids instead of basic feed. These results support the recent results of survival rate $T0 < T1$.

The findings of present research showed significant differences of red pigmentation and relate with the results of (Ninwichian P. *et al.*, 2020). There are different chromatophore cells are present on the skin of fishes. Carotenoids activate those enzyme which enhance the activation of these chromatophore cells. So these enzyme act on cells and enhance pigmentation. The impacts of regular carotenoid sources on the skin pigmentation of *Cyprinus carpio* were significantly measured in present research. Red pigmentation was prominent in experimental group due to presence of carotenoids and there was a less red pigmentation in control group in which carotenoids are missing. Carotenoids were found to be more abundant in red tissues than in white tissues. So, order of efficiency red pigmentation (a^*) was $T1 > T0$.

The observation made by (El-Gawad *et al.*, 2019) support the findings of current study. They examined the effects of lycopene supplementation at various doses on yellow perch (*Perca flavescens*) growth performance and antioxidant status. Carotenoids are hydrophobic compounds that are not easily solubilized in the aqueous environment of the gastrointestinal tract of fish; therefore, digestion, absorption and transport processes are associated to lipids. The intestinal absorption of carotenoids involves several steps, including disruption of the matrix, dispersion in lipid emulsions and solubilization into mixed bile salt micelles. It was reported that control group and experimental group showed variations in growth performance and antioxidant activity. A visible difference of ($P < 0.05$) was measured in $T0$ and $T1$ groups.

The findings of the current study corroborate with the results of earlier studies by (Liu *et al.*, 2019) who studied the effect of dietary carotenoids on Yellow Catfish (*Pelteobagrus fulvidraco*). It was

reported that FCR in control group was higher than in experimental group. Indication of high FCR in control group showed the lower growth than in experimental group. A visible difference of ($P < 0.05$) was shown in T0 and T1 groups. Similar results were also shown by (Hassaan *et al.*, 2021).

Conclusion: Conclusively, the success of carotenoids supplementation depends on significant concentrations and best management strategies. However, the use of inadequate and less quality carotenoids results in poor performance. Moreover, it is necessary to concentrate the research on the application of carotenoids in the different fish species with commercial importance to increase the sustainability of aquaculture. Canthaxanthin is much better than any other costly feeds for best growth and coloration. Carotenoids are the cheap, best growth and color promoter and easily prepared other than costly feeds. 110 grams of canthaxanthin was available in market only in Rs.750. So, it is recommended that the use of carotenoids for better growth and coloration for different fishes especially *Cyprinus carpio*.

CHAPTER 6 SUMMARY

Carotenoids, additionally called tetraterpenoids, are yellow, orange, and red natural colors that are created by plants, green growth, and a few microorganisms. Carotenoids are substances that have the ability to change the color of a fish's body as well as play a positive role in fish growth. Carotenoids cannot be produced in fish by the "De Novo" synthesis method. So carotenoids are given to fish in the form of supplementary diets.

The proposed study was directed to measure the effects of carotenoids on growth and pigmentation of *Cyprinus carpio*. The experimental trial was carried out for 45 days at Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. Then samples were analyzed at Fish Microbiology and Immunology Lab.

A total of 30 fingerling were taken from Punjab Fish Hatchery, Satyana Road Faisalabad. The average initial weight of fishes was measured. Then these were stocked in two different aquariums. One was control group and second was experimental group. The control group was fed with basic diet whereas, experimental group was fed with experimental feed "Canthaxanthin"

Weekly monitoring of growth, pigmentation and observation of physicochemical parameters were done on daily basis during the trial period and by measuring these parameters, following results were concluded:

- The mean value for dissolved oxygen (DO) measured in control group was 3.95 and mean value in experimental group was 3.98.
- The average value of temperature recorded in control group was 26.36°C while in experimental group, mean value was 27.63°C.
- The mean pH values measured for control group and experimental group were 7.36 and 7.51 respectively.
- At the end of experimental trial of 45 days, maximum average weight gain (1.6716 g) per week was achieved in experimental group and control group showed the less value (0.9983 g) per week.
- Maximum average length gain was observed in experimental group (1.23 cm) per week and control group showed the less value (0.79 cm) per week.
- In short growth performance in terms of specific growth rate (SGR) was maximum in experimental group as compared to control group.
- At the end of experimental trial duration of 45 days, experimental group showed the 11% FCR and control group showed the 20.7% FCR. Indication of less FCR showed the best growth in experimental group because less FCR is best for fish growth.
- Maximum survival rate percent was observed in experimental group (86.6%) and in control group survival rate was (73.3%).
- At the end of experimental trail duration of 45 days, experimental group showed the red pigmentation of (2.40%) while control group showed the less red pigmentation of (1.03%).

In the light of above findings, it is concluded that canthaxanthin proved to be a better growth promoter and color promoter for *Cyprinus carpio* instead of basic diet which have no carotenoids. The order of efficiency was observed $T1 > T0$.

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