*Research Journal of Recent Sciences* _____ ISSN 2277-2502
Vol. 4**(ISC-2014),** 37-43 **(2015)** *Res. J. Recent. Sci.*

# Colour enhancement potential of selected local flowers in Sword tail, *Xiphophorus helleri* through dietary incorporation

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**Available online at: www.isca.in, www.isca.me**
Received 1st November 2014, revised 15th January 2015, accepted 28th January 2015

## Abstract

*Ornamental fish keeping has been emerged as an important branch of aquaculture since a few decades with a trade value of more than 8 billion US$ per annum and supports millions of people for their livelihood. Along with other factors like shape, graceful movement, beauty etc., vivid colouration also helps to augment the market demand of the ornamental fishes. In general, the maintenance in captive conditions causes colour-fading in fishes which leads to lesser consumer acceptance and lower price levels. To overcome the problem, an attempt was made in Sword tail, Xiphophorus helleri-a much sought after freshwater aquarium fish-to assess the potential of two pigments derived from local plants viz. Marigold and Ixora (treatments M and I) through dietary incorporation. The growth of the fish assessed simultaneously. The experiment was conducted in the larvae of Sword tail for a period of 90 days. Ninety larvae were randomly exposed to two treatments in triplicates (10 fish per tank). A control was also maintained during the study in which the fishes were fed with a commercial feed. The carotenoid pigment sources were incorporated in the supplementary diets at 5% level of inclusion. Maximum growth was observed in treatment-M followed by treatment-I. Significant difference ($p<0.05$) was observed in the intensity of colouration between the fishes exposed to natural pigments and the control group which could be clearly visible through the naked eye-examination.It was further confirmed by the Spectrophotometer analysis. So, it can be inferred that the dietary incorporation of natural pigments have varying levels of impact on the colouration of Xiphophorushelleri while maintained in captivity. The experiment throws light on the possibility of utilizing other pigment sources for dietary incorporation towards augmenting the colouration in fishes. Developing region and species specific feeds utilizing various types of local ingredients have great potential in the contemporary feed research in the world.*

**Keywords:** Aquarium fish, Xiphophorushelleri, colouring pigments, carotenoids, market demand.

## Introduction

Ornamental fish keeping, an expensive hobby known for more than 1000 years[1] can be made profitable as other hobbies. Countries like Japan, Malaysia, Singapore, Taiwan, USA and India have made the ornamental fish culture and trade as a flourishing business. The Sword tail, *Xiphophorus helleri* -red variety livebearer- is one of the most commercially important ornamental fishes and frequently seen in aquarium trade. They are distributed geographically in the south of Mexico and Guatemala. It is a fact that wild fishes are usually more colourful than the fish reared under artificial conditions hence enjoy top priority in the trade. The colouration of ornamental fish is mainly due to the yellow, orange and red hues found in skin and flesh, resulted from the deposition of carotenoid pigments which are found naturally in the food items. In addition to colour development carotenoids also play an important role in growth, metabolism and reproduction.

Colour is one of the major factors which determine the price of the aquarium fish in the world market[2]. A direct relationship between dietary carotenoid and pigmentation exists in fishes[3]. Unlike other animals, fish lack the ability to synthesis cartenoids and entirely depend on their dietary sources. If enhancement of colouration can be done by administering pigment enriched feed, it will definitely improve the quality and cost of the fish. However, detailed studies on colour enhancement in ornamental fish are scanty.

Synthetic carotenoids are costly and have deteriorating effects on the environment. Hence, there is a great demand for inclusion of natural carotenoids in aqua feed to enfsure bright colouration in fish. The possible use of naturally available carotenoid rich ingredients such as micro algal pigments (*Chlorella vulgaris) Beijer*, (*Haematococcus pluvialis*, *Dunaliella salina) Dun,* Teodor, Yeast extract (*Phafia rhodozyma*, *Xanthophyllomyces dendrorhous*) have received much attention[4]. Plant sources like *Spirulina*have been used as a source of carotenoid pigments for trout and koi[5,6,7]while marigold petal was used for ornamental fishes like sword tail and barb[8].

According to Ahilan *et.al.*[9] in a 60-day trial on gold fish, the growth and body colouration were enhanced by the incorporation of Amaranth leaves or mint leaves to the diet at 1% level. While, Dharmaraj and Dhevendaran[10] reported that

the microbial carotenoid also effectively enhanced the pigmentation and growth in sword tail. In a study by Bagree*t al.* (2011)[11] the incorporation of *Tagetus erectus* at 6% and 8% as feed additives was sufficient to promote growth, however, 10 % of *Hibiscusrosasiensis* was required to promote pigmentation in fishes.

With these in background, the present study was conducted to evaluate the color enhancement potential of indigenous flower pigment sources like Marigold (*Tagestes erecta*) and Ixora (*Ixoracoccinea*) in the sword tail, *Xiphophorushelleri*. Attempts have been made to see the colour enhancement by feeding different pigmented flower sources at appropriate levels besides observing the growth and survival of the species.

## Material and Methods

The duration of the study was 90 days; the fishes were procured locally and were transported in oxygenated polythene bags of 20 l capacity. The experiment was conducted in indoor Wet lab of Department of fish nutrition and feed Technology of Kerala University of Fisheries and Ocean Studies, Panangad. The study was carried out in fiber glass tanks of 45 l capacity. The fishes were acclimatized to the culture conditions for a period of two weeks prior to commencement of the experiment. During this period, the fishes were maintained in an oval, flat bottom fiber glass tank of 500 l capacity and were fed with commercially available pelleted feed. The tank was filled to half of its volume with freshwater and gentle aeration was provided. No water exchange was done during this period. After 14 days, the fishes were weighed individually and randomly distributed to the treatment tanks at the rate of 10 numbers per tank.

Two experimental diets were prepared containing pigment sources of different plant origin in addition to a control diet. One of the pigment enhancement sources, Marigold flower was purchased from local market and the other Ixora was collected from KUFOS Campus. All the samples were dried in the shade and powdered by using grinder prior to preparation of the test diet. Similar ingredients were used for the preparation of all the three diets. The control diet was prepared initially without any added pigment sources. Pigment source was added at the rate of 5% to the respective test diet. The test diets were designated as "M" for Marigold flower incorporated diet and "I" which had Ixora flower. The control diet was designated as "C". All the ingredients were mixed thoroughly and were made into dough. The dough was pelletized using hand pelletizer and dried until the moisture content was reduced to less than 10%. The overall protein content of feed was maintained as 38%.

The experiment was carried out in triplicate. Feeding was done to*ad-libitum* twice a day. 75% of the water in the experimental tank was exchanged daily and the aeration was provided round the clock. Sampling was done at fortnightly intervals to assesscolour enhancement, fish growth and variation in water quality parameters over the experimental period. Standard procedures[12] were followed for assessment of water quality parameters. On completion of the experiment, all surviving fishes were collected for recording the length and weight. Colorimetric analysis was carried out to assess the colour enhancement after completion of 90 days of feeding trials.

**Nutritional Evaluation of Experimental Diet: Specific growth rate:** SGR was calculated using the following formula.

$$\text{SGR}\,(\%) = \frac{\ln(\text{W2}) - \ln(\text{W1})}{\text{Time interval in days}} \times 100$$

**Survival rate:** The survival rate of fishes is expressed in terms of percentage. This was calculated as follows:

$$\text{Survival}(\%) = \frac{\text{Final Number}}{\text{Initial Number}} \times 100$$

**Feed conversion ratio (FCR):** FCR was calculated by dividing the total feed intake by net gain weight

$$\text{FCR} = \frac{\text{Total feed Intake(g)}}{\text{Net Biomass gain(g)}}$$

**Spectrophotometeric Analysis:** For the sample preparation, one gram of entire red sword-tail body tissue, excluding head and alimentary canal was taken in 10 ml screw capped clear glass vials to which 2.5 g of anhydrous sodium sulphate was added.

The sample was gently mashed with a glass rod against the side of the vial. 5 ml of chloroform was then added and kept overnight at $0^0$C for separation of chloroform. 0.3 ml of aliquots of separated chloroform was taken and diluted with 3 ml of absolute ethanol and the optical density was read at 380, 450, 470 and 500 nm, in a spectrophotometer. A blank was prepared in a similar manner without the body tissue for comparison. The wavelength, at which maximum absorption recorded,was used for the calculation.

**Water Quality Analysis:** The water quality parameters of the experimental units like temperature, pH, dissolved oxygen, total alkalinity, ammonia, nitriteand nitrate were analysed adopting the standard procedures[12].

**Statistical Analysis:** Complete randomized design with three replications for each treatment was followed for the study. The average fortnightly gain in length, weight and water quality parameters for each treatment was subjected to a oneway ANOVA using factorial CRD.

## Result and Discussion

Final sampling was done after 90 days of feeding trialfor the assessment of colour intensity, growth in length and weight, specific growth rate, % survival and FCR.

The survival rate varied from 97-98.66% among the different treatment groups. Control group had the lowest survival of 97%, while the highest was recorded in treatment "I" (98.66%). However, these treatments were significantly similar to each other.

Specific growth rate varied among the different treatments from 1.07 to 1.17%. Control diet gave the lowest specific growth rate of 1.07%. The fishes fed on diet "I" and "M" had the highest specific growth ratesthough were significantly homogenous to each other.

The feed conversion ratio also differed among the treatment groups. The Control diet "C" had the highest feed conversion ratio of 1.78, while fishes fed on diet "M" had the lowest FCR (1.57).

Colour intensity was found to be maximum in fishes fed on "M" diet (8.48µg/g) followed by "I" diet (7.25µg/g). The least colour intensity was observed for Control group (4.27µg/g).

The average temperature in all the replicates varied between $25.8^0$C to $27.4^0$C. The average pH values in all the treatments varied from 6.56 to 7.66. The dissolved oxygen content of the experimental units varied between 4.7 to 6.1mg/l all through the experimental period. The total alkalinity of the water in the experimental containers showed values which ranged between 81 to 115mg $CaCo_3$ /l throughout the period of investigation. During the experiment, the ammonia values varied between 0 to 1.16 mg/l, while nitrite values varied from 0 to 1.83 mg/l. Nitrate values ranged between 0 to 3.3 mg/l throughout the period of investigation.

**Table-1**
**Growthparameter of *Xiphophorus helleri* fed diets containing pigments of plant origin**

| Sl. No. | Growth Parameters | C | M | I |
|---|---|---|---|---|
| 1. | Initial Length (cm) | 2.73 | 2.56 | 2.4 |
| 2. | Final length(cm) | 5.13 | 5.63 | 5.5 |
| 3. | Weight gain(gm) | $1.17^a$ | $1.46^b$ | $1.40^b$ |
| 4. | Food Conversion Ratio (FCR) | 1.78 | 1.57 | 1.67 |
| 5. | Specific growth rate (%) | $1.07^a$ | $1.17^b$ | $1.17^b$ |
| 6. | Survival% | $97^a$ | $98^a$ | $98.66^a$ |

**Table-2**
**One way ANOVA Table of fortnightly data of water quality parameters comparing different Days and Treatments**

| | Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| pH | Days | 1.852 | 6 | .309 | 12.231 | .000 |
| | Treatments | .176 | 2 | .088 | 3.478 | .040 |
| | Days × Treatments | .429 | 12 | .036 | 1.416 | .197 |
| Oxygen | Days | 2.960 | 6 | .493 | 3.670 | .005 |
| | Treatments | .182 | 2 | .091 | .677 | .514 |
| | Days × Treatments | 1.593 | 12 | .133 | .987 | .476 |
| Temperature | Days | 6.851 | 6 | 1.142 | 9.721 | .000 |
| | Treatments | 1.297 | 2 | .649 | 5.523 | .007 |
| | Days × Treatments | 1.636 | 12 | .136 | 1.161 | .341 |
| Alkalinity | Days | 2510.095 | 6 | 418.349 | 5.560 | .000 |
| | Treatments | 188.984 | 2 | 94.492 | 1.256 | .295 |
| | Days × Treatments | 1381.905 | 12 | 115.159 | 1.531 | .151 |
| Ammonia | Days | .413 | 6 | .069 | 2.245 | .057 |
| | Treatments | .128 | 2 | .064 | 2.092 | .136 |
| | Days × Treatments | .726 | 12 | .060 | 1.974 | .052 |
| Nitrite | Days | .019 | 6 | .003 | .770 | .598 |
| | Treatments | .017 | 2 | .008 | 2.093 | .136 |
| | Days × Treatments | .028 | 12 | .002 | .584 | .842 |
| Nitrate | Days | 11.262 | 6 | 1.877 | 10.759 | .000 |
| | Treatments | .669 | 2 | .335 | 1.918 | .160 |
| | Days × Treatments | 5.961 | 12 | .497 | 2.848 | .006 |

*All the water quality parameters shows significant difference at 0.05 level of significance ($P<0.05$)

**Table-3**
**Multiple comparisons for Testing the Homogeneity of Water Quality between different Days ( Post HocTest using Tukey's HSD)**

| Days | pH | Oxygen | Temperature | Alkalinity | Ammonia | Nitrite | Nitrate |
|---|---|---|---|---|---|---|---|
| 00 | $7.0222^{bc}$ | $5.2211^{a}$ | $26.6111^{bc}$ | $103.3333^{c}$ | $.0000^{a}$ | $.0000^{a}$ | $.0000^{a}$ |
| 15 | $6.7556^{a}$ | $5.0478^{a}$ | $26.9778^{c}$ | $97.0000^{abc}$ | $.0567^{ab}$ | $.0467^{a}$ | $.4067^{ab}$ |
| 30 | $7.0111^{bc}$ | $5.4300^{ab}$ | $26.0111^{a}$ | $93.8889^{abc}$ | $.1078^{ab}$ | $.0000^{a}$ | $1.2556^{c}$ |
| 45 | $7.1444^{cd}$ | $5.1200^{a}$ | $26.7556^{bc}$ | $84.6667^{a}$ | $.0956^{ab}$ | $.0222^{a}$ | $.7111b^{c}$ |
| 60 | $7.3222^{d}$ | $5.0578^{a}$ | $26.3333^{ab}$ | $90.6667^{ab}$ | $.2633^{b}$ | $.0444^{a}$ | $.0000^{a}$ |
| 75 | $7.1000^{cd}$ | $5.2422^{ab}$ | $26.0556^{a}$ | $100.6667^{bc}$ | $.0467^{ab}$ | $.0222^{a}$ | $.0800^{a}$ |
| 90 | $6.8667^{ab}$ | $5.6989^{b}$ | $26.4111^{ab}$ | $87.6667^{a}$ | $.0222^{ab}$ | $.0222^{a}$ | $.3822^{ab}$ |

The mean with same superscript belongs to the homogeneous subgroup

**Table-4**
**Multiple comparisons for Testing the Homogeneity of Water Quality between different Treatments ( Post Hoc Test using Tukey's HSD)**

| Treatments | pH | Oxygen | Temperature | Alkalinity | Ammonia | Nitrite | Nitrate |
|---|---|---|---|---|---|---|---|
| C | $7.1000^{b}$ | $5.2733^{a}$ | $26.4810^{ab}$ | $96.0476^{a}$ | $a.0286^{a}$ | $0.0000^{a}$ | $0.5505^{a}$ |
| M | $7.0238^{ab}$ | $5.3176^{a}$ | $26.6095^{b}$ | $94.0952^{a}$ | $0.0862^{a}$ | $0.0290^{a}$ | $.03233^{a}$ |
| I | $6.9714^{a}$ | $5.1881^{a}$ | $26.2619^{a}$ | $91.8095^{a}$ | $0.1390^{a}$ | $0.0.386^{a}$ | $0.3414^{a}$ |

The mean with same superscript belongs to the homogeneous subgroup

**Table-5**
**One way ANOVA Table of fortnightly Data of Spectrophotometric analysis comparing different Treatments and Colour**

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Treatments | 84.401 | 2 | 42.201 | 382.151* | .000 |
| Replication | .079 | 2 | .040 | .360 | .703 |
| Treatments * Replication | .200 | 4 | .050 | .454 | .769 |
| Total | 1288.670 | 27 | | | |

*All the water quality parameters shows significant difference at 0.05 level of significance (P<0.05)

**Table-6**
**Multiple comparisons for Testing the Homogeneity of colour intensity between different Treatment (Post HocTest using Tukey's HSD)**

| Treatments | C | M | I |
|---|---|---|---|
| Mean value | $4.2756^{a}$ | $8.4878^{c}$ | $7.2533^{b}$ |

The mean with same superscript belongs to the homogeneous subgroup

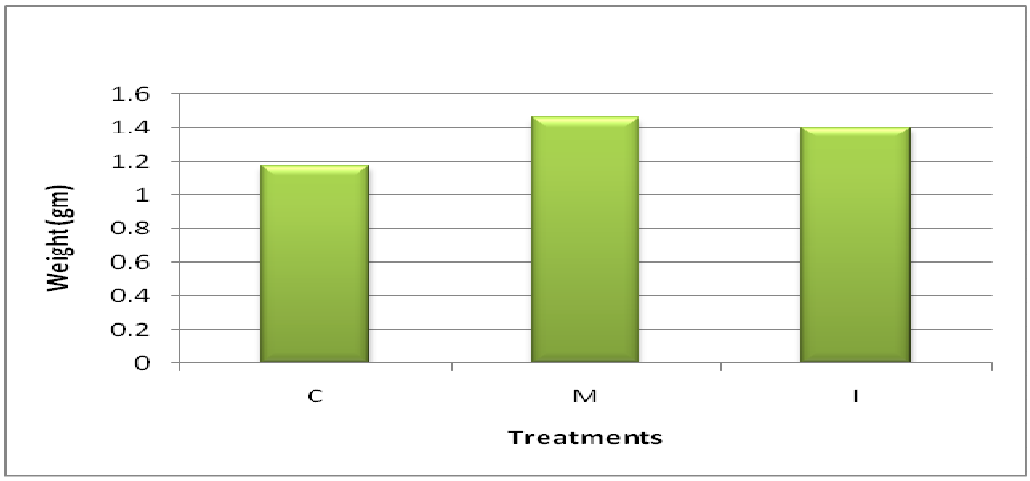


**Figure-1**
**Weight gain (gm) obtained in different treatments**

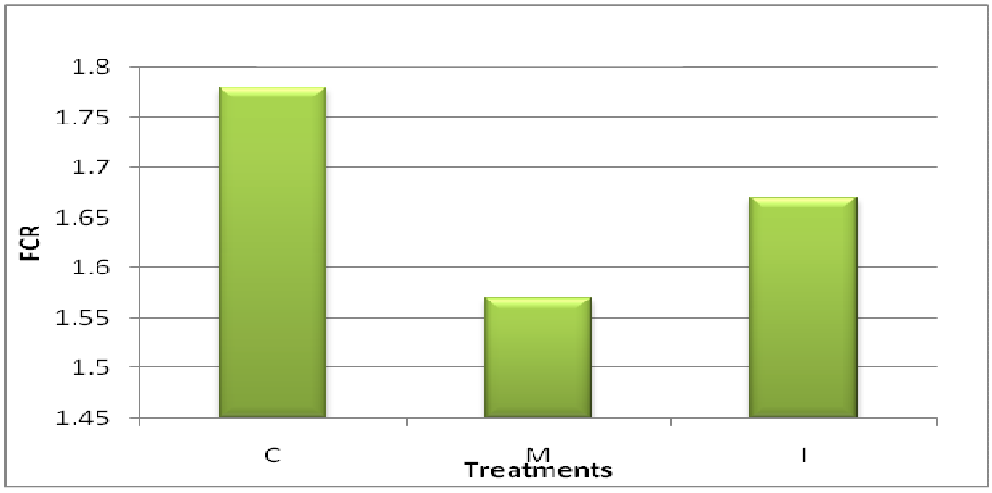


**Figure-2**
**Feed conversion ratio obtained in different treatments**

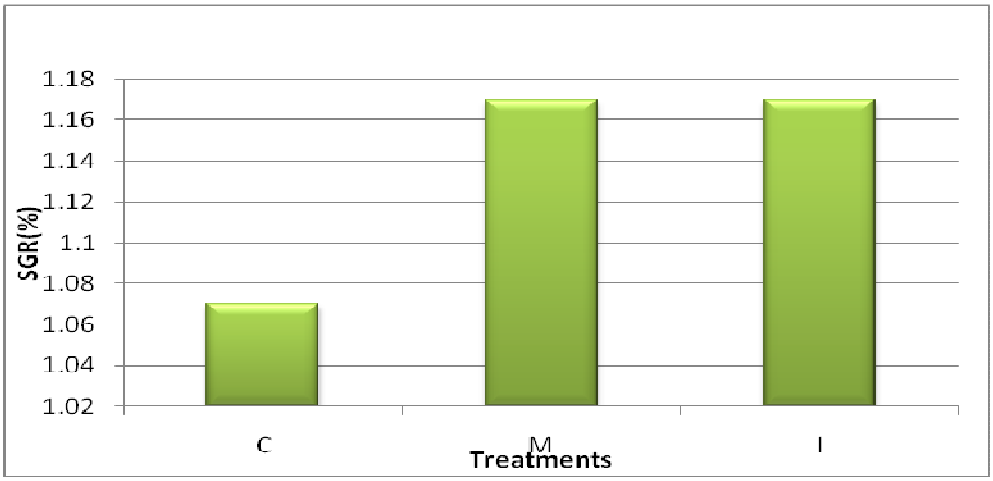


**Figure-3**
**Specific growth rate obtained in different treatments**

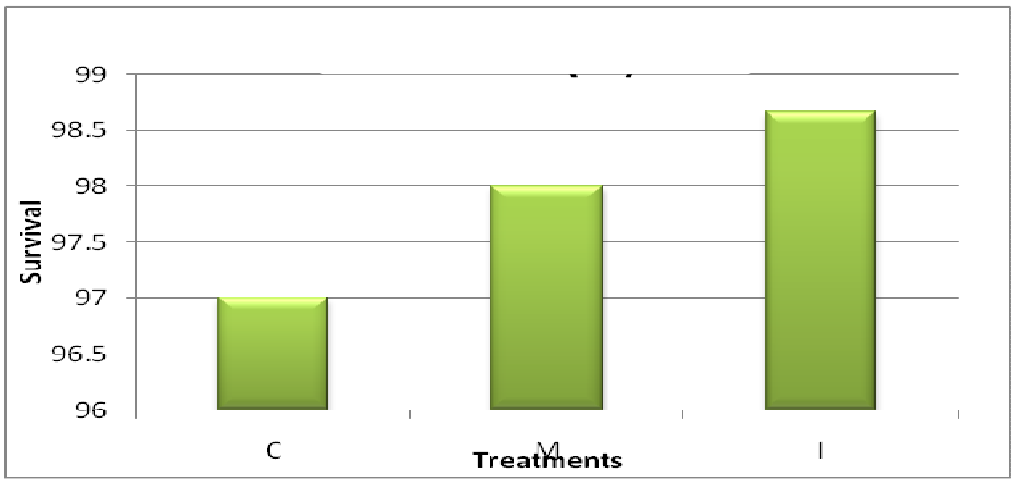


**Figure-4**
**Survival obtained in different treatments**

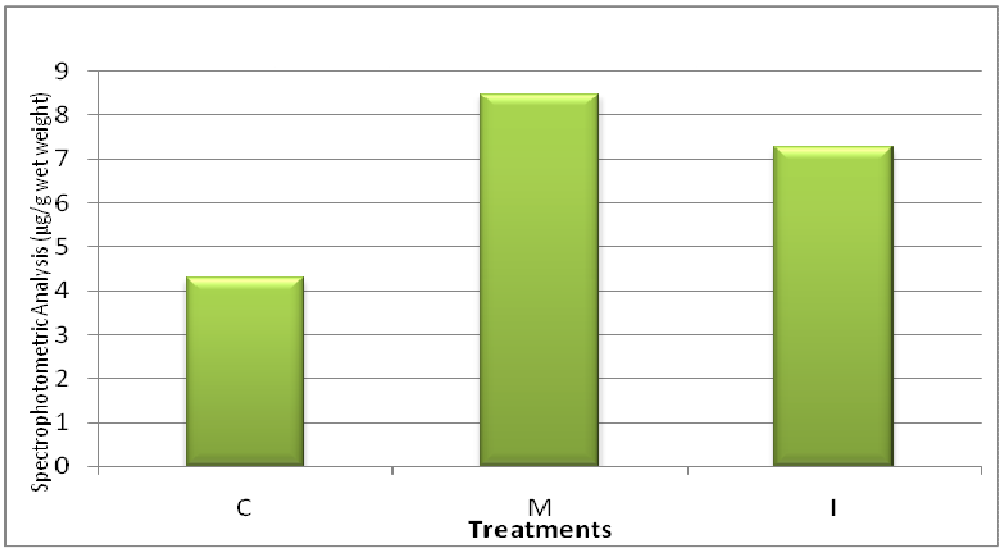


**Figure-5**
**Spectrophotometric Analysis (µg/g wet weight)**

## Discussion

The art of rearing and keeping fish in an aquarium is an age old practice. Most of the fishes are small in size with attractive colour patterns and are adaptable to confinement. The aesthetic as well as the immense commercial value has led to the rapid growth of the industry world over. Colour, a major factor determining the price of the aquarium fish can be improved through dietary enrichment using natural or synthetic pigments. Synthetic carotenoids being expensive and having a negative influence on the environment the natural pigment inducers are in demand. However, the studies in this area are limited and hence the present study was carried out to determine the potential of Marigold and Ixora as pigment enhancing agents.

The present study was carried out for a period of 90 days to determine whether carotenoids of plant source could induce pigmentation to make sword tail more colourful through dietary enrichment. The results were encouragingand clearly proved the effectiveness of the tested plant sources as pigment enhancers. The maximum carotenoid content was observed in treatment groups fed with Marigold diet (M) followed by Ixora diet (I) at 5% dietary inclusion levelby spectrophotometric analysis. The present investigation agreedwith Ezhil*et al.* (2008)[13] who observed enhanced pigmentation in *Xiphophorus helleri* redvariety when fed on Marigold diet. Similarly, Joseph *et al.*(2011)[14] reported the influence of Ixora on colouration in *Xiphophorus helleri* orangevariety through dietary incorporation.

In the present study, the growth parameters were also influenced by the dietary inclusion of these pigment enhancers of plant origin. The weight gain and specific growth rate were found to be higher in treatment groups fed with Marigold based diet followed by Ixora incorporated diet. Similar observation on the influence of Marigold and Ixora on growth are also reported by various other authors in *Xiphophorus helleri* redvariety (Ezhil et al., 2008)[13], in *Xiphophorus helleri* orange variety (Joseph*et al*., 2011)[14]. Likewise, the growth rate of rainbow trout was positively influenced by dietary supplementation of Marigold flower mealat 3.2%.

The present work conforms with the study of Ahilan*et al.* (2008)[9] who reported the effect of 3 botanical additives (Coriander, Mint and Amaranth leaves) on the growth and body colouration of gold fish. The Amaranth and Mint enhanced the growth and colouration in adult goldfish even at dietary inclusion level of 1%.Similar to the present study, the specific growth rate and skin colouration were improved in *Silurus glanis* when fed with carotenoid rich micro algal biomass[15].

In the present investigation, the FCR varied from 1.78 to 1.57. The inclusion of pigment sources improved the FCR compared to the Control diet. It can be assumed that the feeds which had pigment sources of plant origin were utilized efficiently by the fishes.

Survival is an important factor is fish production and depends on the availability and type of feed, physico-chemical conditions of water etc. During the present study, the water quality parameters were well within the ideal range suitable for the culture of warm water species[16] and therefore was not thought to have influenced the result of the study. Inclusion of colour enhancers of plant origin had no influence on this parameter. Likewise, Harpaz and Padowicz (2007)[17] reported that paparikahad no influence on the survival of dwarf cichlids when the former was used as pigment enhancing agent in the diet. On the other hand, Arulvasu*et al.* (2013)[18] observed that *Rosa rubiginosa* had a negative influence on the survival of

*Xiphophorus helleri* when used for enhancing the pigmentation in fishes through diet.

Hence, both the pigmentation inducing Marigold and Ixora can be incorporated in the diet of *Xiphophorushelleri* at 5% of the dietary level without any negative influence on growth and survival of the fishes.

## Conclusion

The present work throws insight to the possibility of low-cost pigment enhancement programmes in ornamental fishes. Similar applications of natural products ensure sustainability of the industry with negligible impacts on the environment.

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