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Evaluation of colour enhance potential of three natural plant pigment sources (African tulip tree flower, red paprika, pomegranate peel) in goldfish (*Carassius auratus*)

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Abstract
Attractive colouration is a decisive factor in determining the commercial value of ornamental fish. Gold fish (*Carassius auratus*) are in demand in world markets due to their attractive golden colour. The present study was designed to investigate the effect of three natural plant pigment sources (African tulip tree flower, red paprika, pomegranate peel) in the colour enhancement of goldfish, *Carassius auratus*. Commercially available feed was used as the base feed. The pigment sources of plant origin tested were African tulip tree flower (T1), red paprika (T2) and pomegranate peel (T3), added @ 5%. The control feed (T0) was without any such additive. The fishes were fed with experimental feed *ad libitum*. The experiment was conducted for a period of 60 days. The average body weight gain of the gold fish fed the test diets was measured once in every 15 days, over the period of study. The final mean weight of gold fish in treatment T0 is 4.65g, 5.16g in treatment T1, 5.21g in treatment T2 and 5.13g in treatment T3. Fish fed the 5% red paprika (5.21g) feed recorded higher growth than other treatments. Survival rate was good in all the treatments with mean survival value ranging from 86.6% to 100%. The poorest FCR was obtained in the red paprika (1.54) feed and best FCR was obtained in the pomegranate peel (1.28) feed. Highest SGR was observed in the pomegranate peel diet (1.42) followed by red paprika (1.21), control (1.20) and African tulip flower (1.18) feed. The pigment concentration obtained in goldfish were high in red paprika fed {6.37µg/g (T2)} fishes when compared to other treatments and control. The present investigation indicated that among the diets tested, the diet containing 5% of red paprika feed led to the maximum enhancement of colouration in goldfish.

Keywords: African tulip tree flower, red paprika, pomegranate peel, gold fish, FCR, SGR, growth, colour

1. Introduction
Aquarium keeping as a hobby in India is nearly 70 years old and it began with the British who ruled Indian until 1947 [9]. Ornamental fish culture is steadily gaining in importance as a major component in Indian fisheries scenario, alongside the culture of food fishes. India is one of the countries having a vast potential of ornamental fishes [11]. Ornamental fish culture as a vocation, is environmental friendly, socially acceptable and involves low investment, for adapting as a small scale enterprise with high returns [6]. Ornamental fish keeping trade is rapidly expanding and there is a growing demand for aquarium fishes in the domestic and international market. Globally, ornamental fish keeping is treated as an industry due to its high market demand and profitability. Today, ornamental fish trade is a multimillion dollar industry. More than 125 countries figure prominently in the global trade of ornamental fishes. It is estimated that approximately 150 million ornamental fish, both marine and fresh water, was sold each year worldwide and the trade and its associated aquarium accessories were worth over US$7 billion annually in the late 1980's. Thereafter, an annual increase of 14% was observed per year [5]. Over 1471 species of marine ornamental fish and more than 4000 species of fresh water ornamental fishes trading globally every year [2].
Goldfish is one of the oldest known aquarium fish. It is also considered to be the most popular ornamental fish due to its many variations such as colors, finnages, tail, shape and size and body structure.

Aquarists believe that a community aquarium tank is not a complete one, unless it is having a few varieties of gold fish. To achieve consumer acceptance and optimal price, the gold fish must be pigmented to have an orange and red color [17].

Colour is one of the major factors, which determines the price of aquarium fish in the world market. Fish which are brightly coloured in nature, often show faded colouration under intensive culture conditions. Fish, like other animals do not biosynthesize carotenoids and depend on dietary carotenoid content for colouration. A direct relationship exists between dietary carotenoids and pigmentation in them [12].

Nowadays, a variety of plant source like leaves, fruit skin and flowers were used to enhance the coloration in ornamental fishes. In intensive ornamental fish culture, a nutritionally balanced diet, containing all the essential nutrients as well as dietary supplements of carotenoids is required [1]. Different sources of carotenoid pigments like pure carotenoid pigments, animal sources and plant sources have been included in fish diets for this purpose. Red bell pepper which is abundant and rich in carotenoid pigment (*Capsicum annum*), was studied by Hancz *et al* [13] to evaluate the colour intensity enhancer in diet for the koi carp and gold fish. Red bell pepper is a rich source of vitamin A and C [9]. Pomegranate (*Punica granatum* L.) peel, a by-product of juice processing industries contains a series of bioactive compounds, minerals and fibers for a wide range of dietary requirements. Intelligent utilization of pomegranate peel powder (PoP) and peel extracts (Px) has been successfully utilized in various food preparations including meat and meat products, edible oils, bakery products and jellies [14]. African tulip tree has been the most abundant tree species in the U.S. Virgin Island since 1982. African tulip tree leaves are rich in N, P, K, and Ca. The flower of pomegranate is orange-red in color [16].

With these background the present study was taken up to evaluate the color enhancement potential of three indigenous pigment sources like African tulip tree flower (*Spathodea campanulata*), Red bell pepper (*Capsicum annuum*) and peel of Pomegranate (*Punica granatum*) respectively.

2. Material and methods

The duration of the study was 60 days; the fishes were procured locally and were transported in oxygenated polythene bags. 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, for a period of 60 days. The fishes were acclimatized to the culture conditions for a period of two weeks prior to commencement of the experiment. The study was carried out in indoors in circular cement cisterns each of capacity 300 liter. Each experimental diet was fed to fish in a set of three tanks each. Feeding was done adlibitum. Initial weight and length measurement were recorded individually for fish stocked in each tank. Completely randomized design employed for the study. Each diet was fed to feed to fish in a set of three tanks each. Feeding was done ad libitum twice a day. The water quality was maintained by periodic partial replenishment as required.

Sampling was done at fortnightly intervals to assess colour enhancement, fish growth and variation in water quality parameters over the experimental period. Standard procedures [7] 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 60 days of feeding trials.

2.1 Nutritional Evaluation of Experimental Diet:

Specific growth rate: SGR was calculated using the following formula.

$$SGR = \frac{Ln\ (W2)-Ln\ (W1)}{\text{Time intervals in days}} \times 100$$

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

$$FCR = \frac{\text{Total feed intake (g)}}{\text{Gain Net weight (g)}}$$

2.2 Spectrophotometric Analysis

The method used for pigment extraction from the red sword tail tissue was as described in Olson, 1979. One gram of entire gold fish body tissue (without head and alimentary canal) was taken in 10 ml screw capped clear glass vials and 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 added and left overnight at 0°C. When the chloroform formed a clear 1-2 cm layer above the caked residue, the optical density was read at 380, 450, 470 and 500 nm, in a spectrophotometer taking 0.3 ml aliquots of chloroform diluted to 3 ml with absolute ethanol. A blank prepared in a similar manner was used for comparison. The wavelength, at which maximum absorption, was recorded was used for the calculation.

$$\text{Total carotenoids} = \frac{\text{Absorption at wave length} \times \text{Dilution factor}}{0.25\times \text{ sample weight in g}}$$

Where, Dilution factor =10
Extinction co-efficient=0.25

2.3 Water Quality Analysis: The water quality parameters of the experimental units like temperature, pH, dissolved oxygen, total alkalinity, ammonia, nitrite and nitrate were analysed adopting the standard procedures [7].

3. Statistical Analysis: The experiment was designed in a completely randomized design with three replications for each treatment. The data were analyzed by a two-way classification with one observation per cell. Post Hoc test, Duncan's multiple range was applied for the comparison of mean.

4. Results

Final sampling was done after 60 days of feeding trial for the assessment of colour intensity, growth in length and weight, specific growth rate, % survival and FCR.

Carotenoid content of fish fed the test diets

The spectrophotometric analysis of pigment content of sword tail recorded the highest value of 6.37µg/g wet weight in red paprika feed (T_2) and lowest value of 4.65µg/g wet weight for the control group (T_0) and followed by 6.09µg/g and 6.05µg/g is the pomegranate peel (T_3), African tulip tree flower (T_1) respectively (Fig.1).

Growth Response

Weight

In culture period the final weight recorded were 4.65g in treatment T_0, 5.16g in T_1 treatment, 5.21g in treatment T_2 and 5.13g in treatment T_3. The lowest weight gain recorded in treatment T_0 (control) (Fig. 2).

Length

At the end of the growth trials, the highest length of 6.53 cm was recorded in T_3 treatment while the lowest value 6.03cm was recorded in T_0 Treatment. T_1 and T_2 diet led to terminal length of 6.43cm and 6.36cm respectively (Fig. 3).

Survival%

The average percentage survival recorded in the treatments are 96.66% in treatment T_0, 93.33% in treatment T_1, 100% in treatment T_2 and 86.66% in treatmentT_3. The highest survival rate was recorded from treatment T_2 100% and the lowest survival rate was recorded from treatments T_3 86.66% (Fig. 4).

SGR%

The highest specific growth rate recorded of 1.42% in the treatment T_3 (Pomegranate peel feed). The values in T_0, T_1 and T_2 treatment were 1.20%, 1.18% and 1.21% respectively (Fig. 5).

FCR

The highest Feed conversion ratio recorded was 1.54 for the treatment T_2 (Red paprika feed) and lowest of 1.28 for the treatment T_3 (Pomegranate peel feed) (Fig. 6).

The average temperature in all the treatments varied between 25.7 ^{0}C to 29.4 ^{0}C. The lowest value of pH 6.9 was measured in treatment T_1, T_2 on the 15th day and T_0 on the 30th day. The highest value of P^H 7.9 was measured in treatment T_2. The dissolved oxygen content of the experimental units varied between 4.5 to 6.5mg/l all through the experimental period. The total alkalinity of the water in the experimental containers showed values which ranged between 69 to 110.5mg CaCo3 /l throughout the period of investigation. During the experiment, the ammonia values varied between 0 to 0.5mg/l, while nitrite values varied from 0 to 1.5 mg/l. Nitrate values ranged between 0 to 2.5 mg/l throughout the period of investigation.

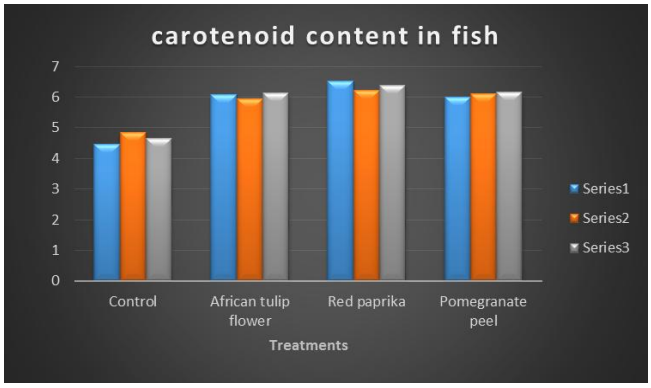

Fig 1: Carotenoid content in fish fed the test diet (µg/g wet weight)

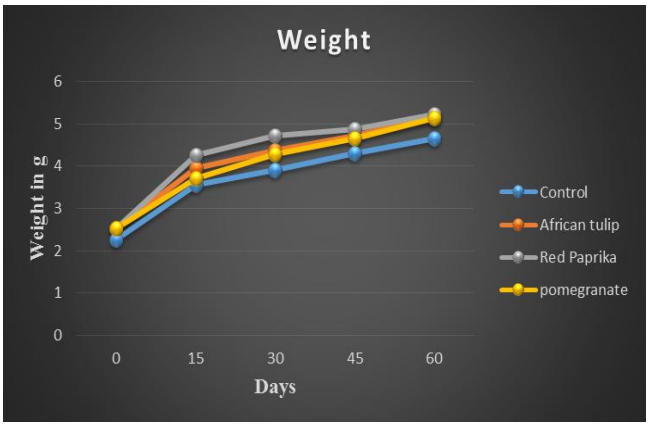

Fig 2: Average gain in total Weight (g) goldfish in different treatments

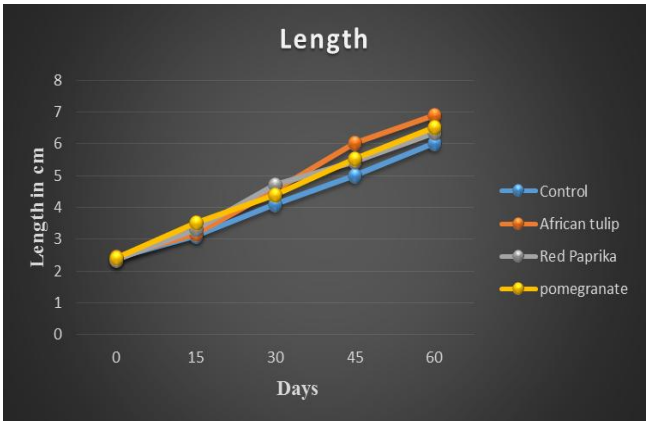

Fig 3: Average gain in total Length (cm) goldfish in different treatments

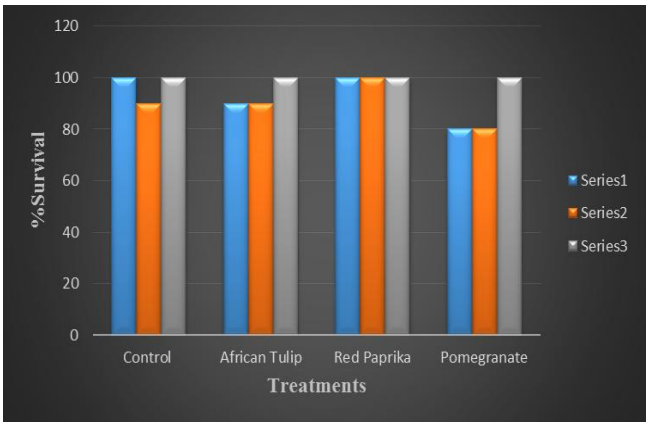

Fig 4: Percentage survival obtained in different treatments

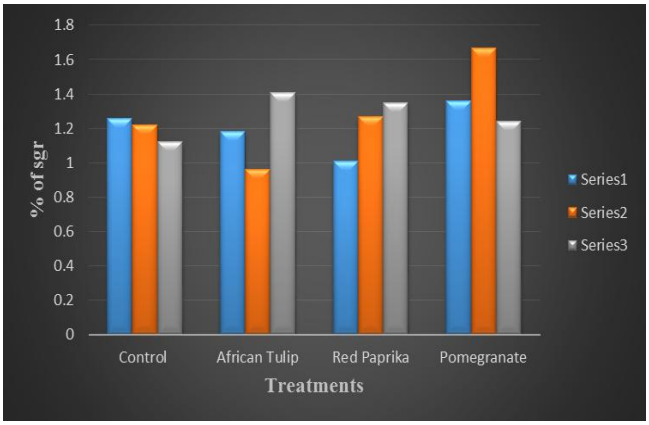

Fig 5: Specific growth rate (%) obtained in different treatments

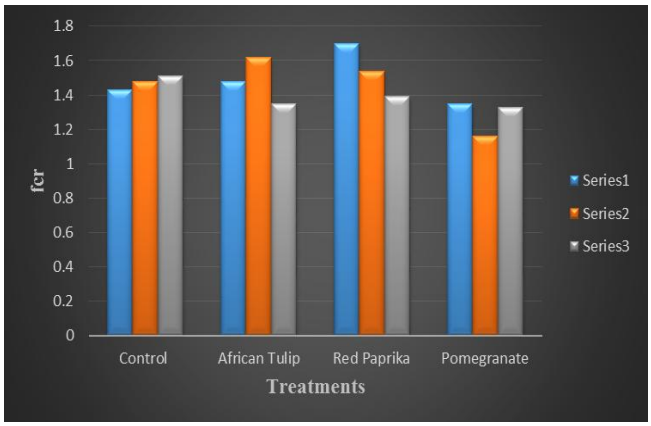

Fig 6: Feed conversion ratio obtained in different treatments

5. Discussion

Ornamental fishes can be defined as attractive colourful fishes of peaceful nature that are kept as pets in confined spaces of an aquarium or a garden pool as visually exciting objects. The art of keeping aquarium is ancient and dates back to around 800 B.C in China. Ornamental fish keeping is one of the most popular hobbies the world over. This popularity is attributed to a number of aspects [17].

However, the studies in this area are limited and hence the present study was carried out to determine the colour enhancement potential of three indigenous materials of plant origin pigment enhancing agents in gold fish.

In the present study a rapid increase in growth of gold fish was observed from 15 days onwards till the end of the experiment in fish fed the in all treatment groups compare to the control. Natural plant pigment source *Spirulina platensis* influences the growth of *Puntius sophore* [10]. It was revealed that 10% Spirulina additive feed resulted in better growth. Incorporation of 180ppm of marigold in basal diet was found to significantly enhance the growth and coloration in koi carp [19].

Survival is an important aspect in fish production. The survival of fish depends on physico-chemical characters of water, availability and type of feed etc. Survival of fishes was high in all the cases ranging from 86.6% to 100%. Inclusion of the pigment sources did not significantly influence survival rates.

Specific growth rate were significantly higher in sword tail fish fed with diet containing 15% beetroot meal than on the control diet [18]. Orange chromide fishes fed with 60 ppm marigold oleoresin incorporated diets showed significantly higher specific growth rate [15]. In the present study the highest SGR was observed in the pomegranate peel diet (1.42) followed by red paprika (1.21), control (1.20) and African tulip flower (1.18) feed.

In the present investigation, mean of FCR obtained with different diets ranged from 1.54(T_2) -1.28(T_3). Best FCR was obtained in the pomegranate peel (1.28) feed and lowest FCR was obtained in the red paprika feed (1. 54). Values obtained for the T_1 diet the control were a 1.48 and 1.47 respectively. FCR values decreases with increasing protein level [3]. 180ppm marigold oleoresin fed fishes showed lower feed conversion ratio (FCR) of dietary supplementation of marigold oleoresin in feed of koi carp [19].

Coloration is one essential sales factor deciding the market value of ornamental fish. Conducted experiments on the effect of paprika on the red colour intensity of goldfish and ornamental koi carp [13]. In the present study, paprika had good effect on gold fish than koi carp. The red colour intensity was higher in gold fish than in koi carp. The difference in red colour intensity between the control and paprika groups was clearly visible to the naked eye in goldfish after 1 month and in koi carp after 2 months of feeding paprika incorporated feed. Studies on the pigmentation of gold fish by feeding with marigold (*Tagetes erecta*) incorporated feed [4]. The diets containing 0, 100, 200 and 300 mg of carotenoid/kg diet from marigold meal. The results showed that a level of 200mg carotenoids from marigold meal increased skin pigmentation in gold fish. Also, this study demonstrated that this level of incorporation of marigold meal led to maximum carotenoid accumulation in the skin of goldfish.

6. Conclusion

Hence the pigmentation inducing three natural plant pigment sources (African tulip tree flower, red paprika, pomegranate peel) can be incorporated in the diet of *Carassius auratus* at 5% of the dietary level without any negative influence on growth and survival of the fishes.

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