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Research Paper

An Evaluation on response of Goldfish (*Carassius auratus*) to Wolffia, a carotenoid source

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ABSTRACT: *Color is a major factor in determining the market value of ornamental fish. In captivity, the fish exhibit faded coloration and fails to attract buyers. Carassius auratus (Goldfish) was used as an experimental fish, and it was fed artificial feed that included the Wolffia plant. Wolffia can be used to enhance colour by providing a natural source of carotenoid. For 40 days, the experiment was carried out. The findings are promising, suggesting that the Wolffia plant powder diet could be used as a pigmenting source for ornamental fishes.*

KEYWORDS: *Pigmentation, Carotenoids, Wolffia plant powder, Carassius auratus*

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I. INTRODUCTION

Ornamental fishes are having high demand worldwide and are becoming a popular hobby. The ornamental fish industry has made a drastic growth to meet the rising demand in the market [1]. Ornamental fish trade has a significant role in providing income for economically backward people and women in India as it requires minimal capital investment [2]. The estimated global ornamental fish trade is more than 15 million [3]. Nearly 5000 ornamental fish units are present in India [4].

Ornamental fish trade is dominated by freshwater and brackish water fish species only 10 percent is constituted by marine fishes [5]. In India there are around 300 different types of freshwater ornamental fish that are marketed and sold under various trade names. Because to its numerous variations in colour, finnages, tail, form, size, and body structure, the goldfish (*Carassius auratus*) is regarded as the most well-liked and appealing pet fish [6]. China, Japan and Central Asia are the native homes of gold fish. Goldfish can get up to 6 pounds (2.7 kg) in weight and approximately 23 inches (58 cm) long. The goldfish must be coloured with a vivid orange and red tint in order to be accepted by consumers and to be sold for the best price [7].

Success in the ornamental fish trade heavily depends on the fish's brilliant colour. Ornamental fishes exhibit a broad variety of colours and colour patterns. One of the key elements that affects price is colour [8]. Like other animals, fish cannot produce carotenoid and depend on the carotenoid concentration in the diet to produce the colour. Thus, there is a direct connection between carotenoids and pigmentation in them [9]. Feeding is the most important aspect of ornamental fish culture the feed should contain all essential nutrients like vitamins and other dietary components which would result in rapid growth and improved health of the fishes [4]. As fish depends carotenoids for colouration, concentration of carotenoids in diet has a direct connection with the pigmentation of fishes [10]. Natural carotenoid is preferred over the synthetic carotenoid as synthetic carotenoid cause pollution [11].

Wolffia is duckweed of the Lemnaceae family which is also called as water meal [12]. *Wolffia arrhiza* is a native species of Europe and is widespread all over the world [13]. Duck weeds" have a protein content of 20–30% and are very nutritious .Interestingly, a variety of fish that consume plants prefer them as food [14]. Regarding its usefulness, *W. arrhiza* appears to be a highly promising choice. It has a very rapid growth rate, a protein content that is between 30 and 45 percent on a dry matter basis, and an essential amino acid profile that is identical to that of animal protein with a significantly higher concentration of lysine and metheonine than that of other plant proteins [15]. Addition of wolffia powder in fish feed can improve growth and coloration [16].

Comprehensive studies on the effect of Wolffia in the colouring pattern of gold fish at specific intervals were never conducted in the past. The present study is an attempt to compare the colouring pattern of Wolffia

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fed gold fish to a normal gold fish which fed similar feed without Wolffia. Regular recording and comparison of colouring pattern would help to notice the extend of colour change which would be useful for farmers in upcoming days.

II. MATERIALS AND METHODS

2.1 *Carassius auratus* (Gold fish)

Goldfish *Carassius auratus* is chosen as an experimental fish. The goldfish lives in fresh water. It's a popular aquarium fish. The body of *Carassius auratus* is elongated and stocky. Not everyone has the well-known bright gold skin tone. Colors in wild populations range from gold to olive green and even creamy white. It has a long dorsal fin with 15 to 21 rays and a hard serrated spine at the dorsal and anal fin origins.

Diagram 1

2.2 Wolffia (*Wolffia arrhiza*)

Wolffia arrhiza is a flowering plant that goes by the common names spotless water meal and rootless duckweed. It is a member of the Araceae family, which includes Arum and Pistla. It is an aquatic plant that grows in calm bodies of water such as ponds. The front green part of the plant is a sphere about 1 mm wide with a flat top that floats at the water's surface. It has a few rows of stomata that run parallel to each other. There is no such thing as a root. The plant produces a small flower with one stamen and one pistil.

Diagram 2

III. METHODOLOGY

3.1 Collection and maintenance of experimental fish

For the present experiment, gold fish *Carassius auratus*, a red variety obtained for the fish hatchery and was kept starver for two days and then acclimatized to the experimental conditions. During this period, the fish were fed with the control diet.

3.2 Feed preparation

In the present study, tow diets (C1 and D1) were prepared (Figure 2). The common ingredients such as fish meal powder, rice bran powder, groundnut oil cake, tapioca powder, and vitamin were used for the diets (Figure 1). The control diet (C1) was prepared with the above mentioned feed ingredients and it was devoid of carotenoid supplements. The experimental diet (DI) were prepared by the addition of carotenoid feed supplement such as powdered Wolffia plant and were added separately with the common ingredients. The dried dietary ingredients were weighed according to the formulation and mixed well by adding sufficient quantities of water. Then the diets were sun dried and powdered. The powered diets were stored separately in an air-tight container for further use.

3.3 Experimentation

To test the efficiency of Wolffia plant on *C. auratus* indoor culture experiment was carried out for a period of 40 days. During the experimentation, two tanks were prepared. The control tank and the experimental tank and then, 10 fishes were stocked in each aquarium. The fishes in the control tank were fed with control diet and the fishes in the experiment tank were fed with experiment diet, twice a day. Every day morning the unfed feed remains was collected and 50% water exchange was made. The water quality parameters were maintained by aerators (Figure 3).

During the study period, fishes were collected from each tank, at frequent intervals (10, 20, 30 and 40), and the skin tissues were dissected out in aseptic condition and were used for further analysis.

INGREDIENTS	CONTROL DIET (C1)	EXPERIMENTAL DIET (D1)DIET
FISH MEAL POWDER(gm)	20gm	20gm
RICE BRAN POWER(gm)	45gm	45gm
GROUNDNUT OIL CAKE(gm)	20gm	20gm
TAPIOCA POWDER(gm)	5gm	5gm
VITAMINS AND MINERALS	2gm	2gm
WOLFFIA(gm)	---	9gm

Figure 1. List of ingredients for Carotenoid based food preparation

Figure 2. Powdered Control diet and experimental diet

IV. QUANTITATIVE METHOD FOR THE ESTIMATION OF CAROTENOID

The pigment extraction method from goldfish tissue was described by [17]. In 10ml screw-capped clear glass vials, one gram of entire goldfish (without head and alimentary canal) was placed, and 2.5gm of anhydrous sodium sulphate was added.

The method gently meshed gently meshed against the side of the vials with a glass rod, and then 5ml of chloroform was added and left overnight at zero degrees Celsius. The optical density was measured at 500nm in a calorimeter using 0.3ml aliquots of chloroform diluted to 3ml with absolute ethanol when the chloroform forms a clear 1-2cm layer above the caked residue. For comparison, a blank prepared in a similar manner was used. The maximum absorption wavelength was used in the calculation.

Figure 3. Experimental tank and Control tank with goldfish

V. FORMULAS USED FOR CALCULATIONS

TOTAL CAROTENOID CONTENT = [ABSORPTION AT MAXIMUM WAVE LENGTH] X10
0.25 × SAMPLE WEIGHT (gm)}

WHERE, 10 = DILUTION FACTOR

0.25 = EXTINCTION COEFFICIENT

VI. RESULT

The present study was carried out to determine whether carotenoids of Wolffia powder could enhance pigmentation in *Carassius auratus*. Wolffia powder was found to be an effective colour enhancer at a cheaper price (Figure 4-9). The results clearly showed that the carotenoid content reached to 13.24µg/g wet weight for experimental fish and 6.38µg/g wet weight for control fish in the 40th day of experimentation (Table 1).

EXPERIMENTAL PERIOD	10th day
EXPERIMENTAL FISH (D1)	3.64
CONTROL FISH (C1)	2.92

Figure 4. Carotenoid content and carotenoid extract of goldfish on 10th day

EXPERIMENTAL PERIOD	20th day
EXPERIMENTAL FISH (D1)	5.04
CONTROL FISH (C1)	3.25

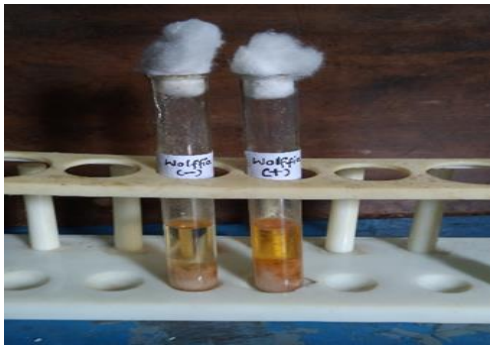

Figure 5. Carotenoid content and carotenoid extract of goldfish on 20th day

EXPERIMENTAL PERIOD	30th day
EXPERIMENTAL FISH (D1)	8.92
CONTROL FISH(C1)	4.16

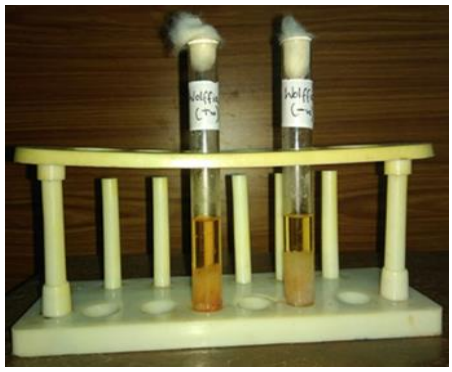

Figure 6. Carotenoid content and carotenoid extract of goldfish on 30th day

EXPERIMENTAL PERIOD	40th day
EXPERIMENTAL FISH (D1)	13.24
CONTROL FISH (C1)	6.38

Figure 7. Carotenoid content and carotenoid extract of goldfish on 40th day

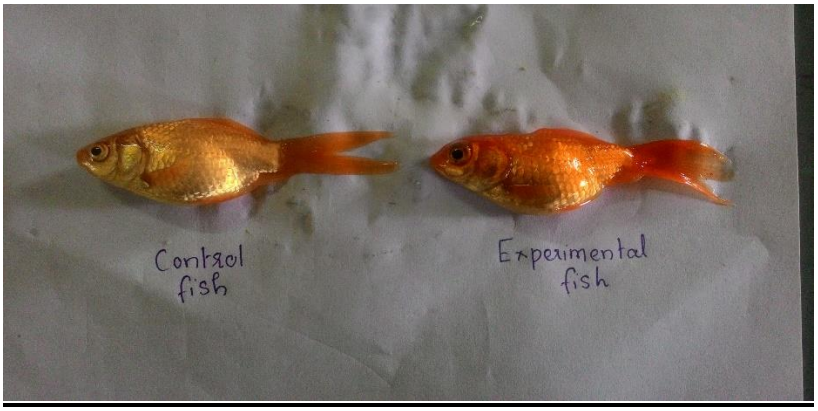

Figure 8. Comparison of fishes

Table 1: Carotenoid content of *Carassius auratus* fed with control diet and experimental diet

Sl. No	Experimental Period	Experimental Fish (D1)	Control Fish (C1)
1	1st Day	2.88	2.88
2	10th Day	3.64	2.92
3	20th Day	5.04	3.25
4	30th Day	8.92	4.16
5	40th Day	13.24	6.38

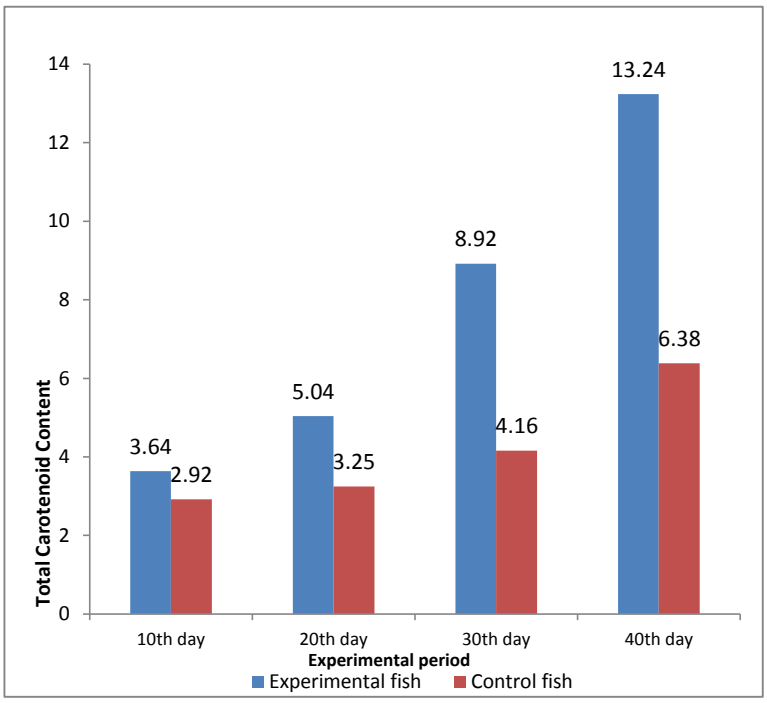

Figure 9. Graph showing carotenoid content of *Carassius auratus* fed with experimental and control diets

VII. DISCUSSION

In recent years, the ornamental fish sector has gained importance both as a hobby and as a business. It is widely known that one of the most crucial variables affecting a fish's ability to reach potential growth, reproduction, coloration, and longevity is its optimal diet. In fish production, feed is the one item with the highest cost. As a result, the usage of natural and locally available feed ingredients is essential for the growth and sustainability of ornamental aquaculture. *Wolffia arrhiza* (duckweed) is a tiny aquatic plant rich in protein that can be used as a food source for fish.

Since there are only a few informational studies on the effect of *Wollfia arrizha* as a feed on goldfish. Its effect on coloration is an unexplored area, a study on "the effect of, *Wolffia arrizha* a carotenoid source of pigmentation on goldfish (*Carassius auratus*), was initiated and the colour change induced by Wolffia was recorded at regular intervals for a period of 40 days. The results of the study show that Wolffia powder was found to be an effective colour enhancer at a cheap cost.

Huang, Bin et al., [18] also reported that by supplementing with Wollfia powder, the growth and body colour of goldfish got improved. The present study clearly indicated that the carotenoid content reached to 13.24µg/g wet for the wolffia fed fish and 6.38 µg/g wet weights for the control fish on the final day of experimentation. From the present study, it is clearly understood that Wolffia arrizha can be used as an alternate carotenoid source for fish feed formulation. As it is economically cheap and easily available, it is promising aquafeed stuff for the future.

VIII. CONCLUSION

In the present study, *Wolffia arrhiza* is taken as a Natural carotenoid source for the pigmentation in *Carassius auratus* which is taken as the experimental fish. The results clearly indicated that the fishes who fed with Wolffia plant powder shows high colouration as compared to the control diet. It can hence, be concluded that this could be used as a food colourant and also as a feed additive for the colouration of other ornamental fishes. In view of the deteriorating effect on the environment due to use of synthetic pigments, the researchers are emphasizing the need for natural pigment colouring agents which will act as an alternative to synthetic chemicals. As the aqua feed industry seeks a natural, environmental friendly source of pigment to improve colouration and to enhance commercial acceptability, there is a great potential for use of natural based carotenoids for pigmentation in aquaculture. It paves the way to many aqua feed industries to promote their products as natural with a distinct shift away from synthetic ingredients and colourations.

The main impediment to commercial exploitation of the source materials was technological limitations. The success of any pigment produced through fermentation is determined by market acceptance, regulatory approval, and the size of the capital investment required bringing the product to market. The colouration of pet fishes will fade if they are not provided with proper nutrients and carotenoids. The current work may also be of interest to ornamental fish feed manufacturers who may choose natural plant based carotenoids enriched feed for replacement of synthetic carotenoids for better colouration in ornamental fishes.

A comprehensive study of ornamental fish nutrition and colour enrichment is underwhelming. According to the findings of the study, carotenoids are an essential component of the commercial ornamental fish industry. It will also help to promote the ornamental fish industry, as well as the colour enhancer feed industry and job creation.

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