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Pigment Perfection of Marine Ornamental Fish *Amphiprion Ocellaris* in Captivity under Laboratory Condition

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ABSTRACT

Aquarium fishes are well-liked by everyone because of their magnificent coloration and graceful movement. Fish that are colored in nature often get faded under intensive culture conditions where the chance of revitalizing the carotenoid sources is tine. To study the skin color change the present study, experiments (*Amphiprion ocellaris)* culture were made for 60 days in six tanks as five groups used mainly algae. Five different types of formulating feeds were prepared using four different natural carotenoid sources *viz.,* Damask rose (*Rosa damascena*), Hibiscus (*Rosa-sinensis*), red algae (*Gelidiella acerosa*) Brown algae (*Cystoseria trinodis*) collected from natural sources and commercially available Kashmir saffron (*Crocus sativus)* also used. Control feed (Feed without pigment extracts). One set of control and four set of experimental groups were maintained for colour enhancement analysis. All fishes were fed three a day with one control with five different experimental feeds. From this study, it is concluded that the natural colour enhancer feeds can be prepared at a lower cost using low coast available sources for pigments enhancement in ornamental fishes.

Keywords: *Amphiprion ocellaris;* Pigment perfection; Carotenoid sources; Fish colour enhacement

INTRODUCTION

Marine ornamental fishes are characterized by a wide range of colors and color patterns success in the ornamental fish employment. The majority of marine ornamental fish are small size, having attractive colors. Clown fishes are one of the most popular attractions in the international marine aquarium fish trade. Changes in color hue or pattern are vital for the adaptation of aquatic animals to their environment. A slow color change is related to the hormone subject to variation pigment quality, while a fast color change is related hormone regulation [1]. False clownfish, *Amphiprion ocellaris*, is a most important ornamental fish in the aquarium industry because of its body coloration, swimming performance and its symbiotic relationship with anemones. Many different varieties of natural and synthetic pigments are available to enhance the skin coloration of ornamental fishes. Carotenoids are the primary sources of pigmentation on skin of fishes. In a natural environment, fishes meet their requirements by ingesting natural foods. The sources and concentration of carotinoids play an important role in the pigmentation of fish. Various synthetic carotenoids, such as β- carotene, astaxanthin as well as natural source has been used as dietary supplements to enhance the pigmentation of ornamental fish. Enhancement of coloration can be achieved by administrating pigment enriched feed which will ultimately improve the quality and cost of the fish.

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The commercial value of clown fish makes the ornamental fish growers to explore methods of enhancing skin coloration. The efforts are made to discover natural products for production of synthetic carotenoids as because of concerns about the use of synthetic additive and their high cost [2]. To reduce this problem the present study was made to evaluate the enhancement of pigmentation and growth performance on *Amphiprion ocellaris* fed with different types of natural carotenoids supplemented diets. Ramamoorthy, et al. was reported the color enhancement on marine ornamental fish, *A. ocellaris* using natural carotenoid sources. In this present study, was carried out to make a trail in color enhancement on clownfish, *Amphiprion ocellaris* using carotenoid sources, which were isolated from two species of flower hibiscus and rose, two species of seaweed red seaweed and brown seaweed and Kashmir saffron (*Crocus sativus)*.

MATERIALS AND METHODS

Collection of Ornamental Fish

A. ocellaris was collected from marine ornamental fish traders and were acclimated to laboratory condition in an aquarium tanks. The fishes were conditioning by treating taken for colors enhance assessment. For each species six experimental tanks setup were made in the laboratory [3]. Among the six feeds one was control (commercial aquarium pellets feed) and the other five feed were using the same ingredients as the control diet but with the addition of carotenoids pigments from different natural sources.

Collection of Samples

Seaweeds (brown and red) were collected from Mandapam regions (Lat. 09°17.417'N; Long. 079°08.558'E), Tamil Nadu, India. Brown seaweed, Cystoseria trinodisis dark-brown pigment. Red seaweed, Gelidiella acerosais reddish in colour. It contains variety of pigments, including cholorophyll a, phycobiliproteins, red phycoerythrin, carotenes, lutein and zeaxanthin. Kashmiri saffron (*Crocus sativus*) generally appear red to red violet in colour. For the present study, saffron and rose were collected from local market. Hibiscus was collected from local areas.

Extraction of Pigment

About 1 kg of brown seaweed and 1 kg of red seaweed was collected separately and brought to the laboratory in plastic bags with seawater. The samples were washed thoroughly with seawater to remove epiphytes followed by tap water and distilled water so as to remove the salts and other extraneous materials and then shade dried for 15 days to 20 days and ground in an electric mixer for 2 hrs [4]. Fresh rose and hibiscus was collected and washed with distilled water then shade dried for 5 days to 7 days and ground in an electric mixer for 1 hrs. Finally, the powdered samples of seaweeds and saffron were packed and stored in refrigerator (-4°C) for further use.

The prepare five natural carotenoid source such as rose petals, hibiscus, red seaweeds, brown seaweeds and saffron were collected, air dried in the dark room to prevent denaturing of carotenoids.

Growth Parameters

At the beginning of the experiment, the total wet weight and length of the fish in each group was determined by weighing in an electronic balance. All fish in each group were weighed at the end of the experiment.

Absolute growth rate (g)=Final weight-Initial weight

Specific growth rate (%)=((In Final weight-In Initial weight-In Initial weight)/Rearing period (days)) × 100

Weight gain (mg/g)=((Final weight-Initial weight)/Initial weight) × 100

SR (%)=(Final number of fish/Initial number of fish) × 100

Carotenoid Estimation

The carotinoid content of the fish diet was extracted by a method similar to the used by Torrissen and Naevdal. 10 mg samples of each diet were ground and transferred to 10 ml pre-weighed glass tubes with 0.2 ml chloroform. Samples were shaken for 15 min at 1,400 rpm at 25°C in chloroform [5]. Solutions were centrifuged at 5,000 rpm for five minutes, the supernatant was removed and absorbance was measured at a wavelength between 250 nm and 900 nm using a spectrophotometer. Total carotenoid concentration in the diet was determined spectrophotographically in chloroform using extinction coefficients (EI%, 1 cm).

The values were converted from E1% to. The amount of carotenoid in each sample was calculated on a sample dry weight. Total pigment content was calculated as µg carotenoid/g diet, using the formula:

$$\text{Total carotenoid content} = \frac{A \times FW}{\varepsilon \times 1 \times SW}$$

Where,

A=Absorbtion at maximum wavelength

FW=Molecular weight

ε=Extinction coeffiecient

1=Length of cuvette (cm)

SW=Sample weight

RESULTS

Growth Rate in *A. ocellaris* Fed with Different Experimental Diets

The experimental fish *A. ocellaris* in all treatment groups, gained significant weight during the experimental period of 60 days. Average weight gain alone was greater the standard deviation from the initial average mean of approximately

4.50 ± 0.66 g (control) to an average mean 5.55 ± 0.89 g (saffron) during 60 days of experiment [6]. Fish in all treatment groups, gained significant weight during the experimental period of 60 days. Average weight gain alone was greater in the group of fish which was fed with 8.52 ± 0.11 g (saffron) supplement diet. Minimum weight gain was notice in the group of fishes which was fed without any supplement diet (control) (**Table 1 and Figure 1**).

Table 1: Growth rate in *A. ocellaris* fed with different diets.

Treatment	Initial weight 0 days (g)	Weight 15th day (g)	Weight 30th day (g)	Weight 45th day (g)	Weight 60th day (g)
Control	4.50 ± 0.66	4.62 ± 0.42	4.89 ± 0.91	5.01 ± 0.22	5.11 ± 0.75
Rose	4.83 ± 0.74	4.99 ± 0.25	5.25 ± 0.56	5.48 ± 0.37	5.73 ± 0.02
Hibiscus	5.28 ± 0.94	5.66 ± 0.73	5.94 ± 0.19	6.00 ± 0.28	6.37 ± 0.91
Red seaweed	5.00 ± 0.38	5.81 ± 0.44	6.30 ± 0.78	7.01 ± 0.59	7.71 ± 0.34
Brown seaweed	4.91 ± 0.40	5.75 ± 0.99	6.35 ± 0.01	6.95 ± 0.11	7.80 ± 0.39
Saffron	5.55 ± 0.89	6.70 ± 0.69	7.58 ± 0.25	7.98 ± 0.23	8.52 ± 0.11

Figure 1: Colour enhancement experimental set-up of *A. ocellaris*. 0 (Control), (Hibiscus), (Rose), (Red seaweed), (Brown seaweed) and (Saffron).

Carotenoid Concentrat on Analyzed in Fresh Muscles of the*A. ocellaris*

The total carotenoid content of the muscles tissue of A. ocellaris ranged from 1.30 ± 0.22 to 2.25 ± 0.32 mg/kg in the group of fishes fed without carotenoid supplement (control). The carotenoid content of the feed with rose 1.54 ± 0.49 to 4.95 ± 0.09 mg/kg in 0-60 days observation.

Red seaweed feed fluctuated between 1.78 ± 0.51 to 6.23 ± 0.25 mg/kg during experimental days and brown seaweed observed 1.82 ± 0.34 to 7.01 ± 0.67 mg/kg as the same experimental period [7]. Finally the highest colour observation was carried out saffron 1.99 ± 0.29 to 7.98 ± 0.34 mg/kg during the treatment (**Figure 2**).

Figure 2: Growth and skin colour enhanced in *A. ocellaris* fed with carotenoid supplement diets. 0 (Control), (Hibiscus), (Rose), (Red Seaweed), (Brown Seaweed) and (Saffron).

DISCUSSION

The carotenoid are the primary sources of pigmentation in ornamental tropical fish, responsible for various colours like yellow, red and other related colours. In captive conditions, gradually, the fish show fade colouration and fail to get attracted by the buyers. Many different varieties of natural and synthetic pigments are available to enhance the skin colouration of ornamental fish. Normally, these are obtained through organisms rich in carotenoids content tin aquatic food chain. However, commercial feed incrediats such as yellow corn, corn gluten meal and alfalfa are used sources of carotenoids such as zeaxanthin and lutein [8]. The present study shows that various sources of dietary carotenoids did not affect significantly to the growth and survival of fish. This result agrees with the study of Bell, et al., who reported no effect of dietary supplement of astaxanthin 70 mg/kg on the growth of Atlantic salmon (*Salmo solar*) reared for 22 weeks. Sinha, et al. observed an increased value of carotenoid in skin color 4.01 ug/g and the growth rate of fishes fed with China

rose. In the present experiment the maximum carotenoid was found in the beet root fed fishes it could be directly related to the enhanced level of carotenoid content in the particular ingredients. Numerous studies have reported that carotenoid pigments play a major role to enhance the skin coloration. Forsherg and Guttormsen noticed beet root and mango peel as color developing agents in fish. Banani, et al. reported the growth and pigmentation in fantail guppy, *Poecilia reticulata* was more while they diet with carotenoid enriched feed [9,10]. Sajjad, et al. investigated the effects of tomato (*Solanum lycopersicum*), carrot (*Daucus carota)* and red bell pepper (*Capsicum annuum)* as a natural pigment sources and astaxanthin as synthetic pigment source on the skin colour of guppy fish, *Poecilia reticulata.* Mehmet and Halil studied the effect of tomato powders as a dietary supplement on skin pigmentation of goldfish, *Carassius auratus* [11].

This reported agrees with the study of the carotenoid content of the muscle tissues of *A. frenatus*. The carotenoid content of the feed with carrot varied from 1.64 mg/kg to 6.42 mg/kg. Carotenoid content in the marigold feed fluctuated between 1.57 mg/kg and 5.24 mg/kg. The values of carotenoid in the hibiscus added feed varied between 1.63 mg/kg and 6.49 mg/kg. Rose source supplemented feed it varied from 1.54 mg/kg to 5.85 mg/kg. The carotenoid content in the polychaete feed ranged between 1.71 mg/kg and 7.48 mg/kg [12].

In the present study the clownfish positively responded to the dietary supplement of saffron during 60 days of diet supplementation. The beet root supplemented feed enhanced brown orange color of the fish skin (increased the skin value). Thus the present experiment indicated that the colour and growth enhancement of marine clown fish *A. ocellariscan* be successfully achieved using saffron supplemented feed than any other feed.

CONCLUSION

Pigments area vital necessity for the colour enhancement and health of fishes in the ornamental region while the high cost of commercially available pigments is one of the major problems. Ornamental aquarists and farmers can get the benefits of pigments by using natural sources. There is a need to standardize the dose of locally available natural sources for more production of pigments supplementation to the aquaculture industry.

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