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# Estimation of Color Enhancement Potential of Natural Pigment Sources on Marine Ornamental Clown Fishes

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**Abstract:** ***Pigmentation is the key value of the ornamental fish market suitability. Fish are colored in nature, often show faded coloration under intensive aquarium conditions. Carotenoids are responsible for pigmentation of skin and muscle color of any fish. Animals are not adept to biosynthesis carotenoids, so diet is their only source as only plants, fungi, bacteria and algae have the capacity for its synthesis. Diet is the major support of successful culture of ornamental fish in confined environment. In this contest an attempt has been made to evaluate the colour enhancement performance of five natural pigment sources like Carrot (Daucus carota), marigold petal (Tagetes erecta), China rose petal (Hibiscus rosasinensis), Rose petal (Rosa chinensis) and polycheates in two famed clown fishes (Amphiprion ocellaris and Amphiprion frenatus). The experiment revealed that both clown fishes were significant positive responded to the dietary supplements of polychaete diet and followed by H. rosasinensis and carrot diets. Other diets were documented an adequate level of outcomes.***

## 1. Introduction

The art of rearing and keeping fish in an aquarium is an age-old practice dawn of 21st century. Marine ornamental fishes are characterized by a wide range of color patterns and pigmentation are one of the key values of marketplace. The carotenoid present as fish result from the piments present in the diet. Since, the animals are not adept to biosynthesis, diet is their only source for its synthesis (Das and Biswas, 2016).

Clown fishes are most attractions and colorful in the international aquarium trade. Fish are colored in nature often show faded coloration under intensive aquarium conditions. Several synthetic carotenoids are available in the market, moreover they involve petrochemical solvents as well as complex organic solvents causing residual problems (Butnariu, 2016). Appropriately formulated diet is the major support of successful rearing of ornamental fish in confined environment. In this view an attempt has been made to color enhancement potential of five natural pigment sources in two species of common clown fishes (*Amphiprion ocellaris* and *Amphiprion frenatus)*.

## 2. Materials and Methods

A total number of 120 sub-adult clown fish species *A. ocellaris and A. frenatus* (each species 60 numbers) were procured from ornamental fish traders. All the fishes were prophylactically treated with a sodium chloride bath and acclimatized in laboratory condition. For the experiment, each species (10 fish per tank) six fiber glass tanks setup were made with the capacity 500 l. Each experiment tank provided constant aeration and 20% of the water was exchanged daily.

Five different experimental diets were prepared with natural pigment sources in addition to a controlled diet (commercial aquarium pellet). The pigment sources of carrot (*Daucus carota*), marigold petal (*Tagetes erecta*), China rose petal (*Hibiscus rosasinensis*) and rose petal (*Rosa chinensis*) were shade dried and powdered for test diet. The powdered frozen dried polychaete was prepared separately for the experiment. The pigment sources were thoroughly coated with the feeds added at the rate of 10g/100g in to the respective experiment feed.

The feeding trials were carried out for 60 days and the fishes were fed the experimental diets to apparent satiation twice a day. Both fish species were sampled randomly from each tank and photographed at fortnight intervals. The colorimetric analysis method was adapted for measuring the total carotenoid content of fish skin tissues (Butnariu, 2016). After completion of 60 days feeding trial, the final colour enhancement measurements data were tabulated.

## 3. Results

The experimental fish *A. ocellaris* and *A. frenatus* were accepted and consumed the test pellet diets, with the exception of two and three individuals that were died during the first week respectively. All other fishes survived and remained healthy from the beginning to end of trial period. The results of colour enhancement that in the beginning of experiment trials the *A. ocellaris* species fish were primarily light yellow in color. Later 30 days most of these fish had completely changed with distinct colors of their body surface. Fish fed with hibiscus supplemented diet were dark yellow orange coloration (Table.1). Fish fed with polychaetes in their diets became dark yellow orange in colour and had white head bar with vertical stripe just behind the eyes.

The carotenoid content of the muscle tissues of *A. ocellaris* ranged between 1.45 and 2.34 µg /g in the group of fishes fed without carotenoid supplement (control). The carotenoid content of diet with carrot developed in to 6.32 µg /g., the marigold feed skin colour concentration raised up to 5.64 µg /g. The values of carotenoid in hibiscus diet developed in to

6.46 µg /g (Table.2). Rose source supplemented diet increased to 5.15 µg /g. The maximum concentration of carotenoid contents was recorded in polychaete diet, ranged between 1.31 and 7.47 µg /g. Pigment content in diet prepared with Hibiscus and carrot mixtures were found as second prevailing (Table 2 & 3).

**Table 1:** Visual assessment of skin color of *A. ocellaris* and *A. frenatus* species

| Species | *A. ocellaris* | | *A. frenatus* | |
|---|---|---|---|---|
| Pigment | Initial color | Final color | Initial color | Final color |
| Control | Light yellow | Yellow orange | Light red | Light red |
| Carrot | Light yellow | Light yellow orange | Light red | Moderate red |
| Marigold | Light yellow | Light yellow orange | Light red | Dark red |
| Hibiscus | Light yellow | Dark yellow orange | Light red | Dark red |
| Rose | Light yellow | Light yellow orange | Light red | Light red-orange |
| Polychaete | Light yellow | Dark yellow orange | Light red | Dark orange-red |

**Table 2:** Carotenoid concentration of fresh skin muscle of *A. ocellaris* species (Mean ± SD).

| Treatment | 0 day (A) | 15th day | 30th day | 45th day | 60th day (B) | Net Concentration A (-) B |
|---|---|---|---|---|---|---|
| Control | 1.453 ± 0.446 | - | - | - | 2.343 ± 0.156 | 0.89 µg /g |
| Carrot | 1.624 ± 0.437 | 2.54 ± 0.292 | 4.176 ± 0.593 | 5.69 ± 0.642 | 6.320 ± 0.532 | 4.69 µg /g |
| Marigold | 1.534 ± 0.337 | 2.23 ± 0.286 | 3.34 ± 0.348 | 4.36 ± 0.344 | 5.645 ± 0.632 | 4.11 µg /g |
| Hibiscus | 1.523 ± 0.315 | 2.49 ± 0.178 | 4.36 ± 0.386 | 5.88 ± 0.434 | 6.463 ± 0.635 | 4.93 µg /g |
| Rose | 1.486 ± 0.322 | 2.32 ± 0.272 | 3.87 ± 0.367 | 4.85 ± 0.372 | 5.152 ± 0.934 | 3.66 µg /g |
| Polychaetas | 1.315 ± 0.184 | 2.87 ± 0.154 | 4.637 ± 0.65 | 6.39 ± 0.424 | 7.476 ± 0.783 | 6.16 µg /g |

**Table 3:** Carotenoid concentration in fresh skin muscle of *A. frenatus* species (Mean ± SD)

| Treatment | 0 day (A) | 15th day | 30th day | 45th day | 60th day (B) | Net Concentration A (-) B |
|---|---|---|---|---|---|---|
| Control | 1.573 ± 0.329 | - | - | - | 2.443 ± 0.253 | 0.87 µg /g |
| Carrot | 1.644 ± 0.278 | 2.94 ± 0.462 | 4.576 ± 0.523 | 5.52 ± 0.642 | 6.420 ± 0.332 | 4.77 µg /g |
| Marigold | 1.574 ± 0.245 | 2.77 ± 0.563 | 3.24 ± 0.345 | 4.65 ± 0.344 | 5.247 ± 0.432 | 3.67 µg /g |
| Rose | 1.546 ± 0.522 | 2.67 ± 0.465 | 3.77 ± 0.362 | 4.93 ± 0.372 | 5.853 ± 0.734 | 4.30 µg /g |
| Hibiscus | 1.634 ± 0.615 | 2.78 ± 0.376 | 4.97 ± 0.383 | 5.54 ± 0.434 | 6.497 ± 0.935 | 4.86 µg /g |
| Polychaete | 1.715 ± 0.784 | 2.97 ± 0.754 | 5.73 ± 0.543 | 7.45 ± 0.424 | 7.486 ± 0.583 | 5.77 µg /g |

The species of *A. frenatus*, at the initiation of trial the skin colour were registered in light red. After 30 days most of these fishes have completely transformed with distinct colour patterns of their body patterns. After 60 days, the fishes fed without any supplemented diet (control) showed light red coloration. The fishes fed with hibiscus supplemented diet were dark reddish coloration. Fish fed with polychaete in their diets became dark orange-red in color had the most vibrant colors and had white head bar or vertical stripe just behind the eyes. Other diets were showed insignificant visual changes in fish skin colours. The carotenoid content of the muscle tissues of *A. frenatus* ranged from 1.53 to 2.44 µg /g in the group of fishes fed without carotenoid supplement (control). The carotenoid content carrot diet varied from 1.64 to 6.42 µg /g. Carotenoid content in the marigold diet increased in to 5.24 µg /g. The values of hibiscus added diet varied between 1.63 and 6.49 µg /g (Table.3). Rose source supplemented feed it varied from 1.54 to 5.85 µg /g. The polychaete diet enhanced the rate of 7.48 µg /g. The maximum concentration of skin carotenoid content was recorded in the polychaete diet fed fishes, followed by hibiscus and carrot diets. The fish fed with control diet were noticed in discrete types of pigments and have no significant changes in their skin coloration. The net concentration of skin carotenoid development varied between 0.87 and 6.16 µg /g in the experiment fishes.

## 4. Discussion

The carotenoids are the primary source of pigmentation in ornamental tropical fish, responsible for various colours like yellow, red and other related colours (Das, 2016). In general, these are obtained through organisms rich in carotenoid content in wild aquatic food chain. Dietary factors nutrients and chemical compounds that the fish eats which directly or indirectly influence color development (Paulo and Gouveia, 2005).

Present observation, the maximum fish skin carotenoid contents were found in polychaete fed fishes. The carotenoid content in the muscle tissues of *A. ocellaris* was improved and it measured in high net concentration in polychaete diet, the maxmum range of 6.19 µg /g. was recorded. Similarly, the carotenoid content of *A. frenatus* also recoded 5.77 µg /g. Hibiscus revealed second prevailing diet next to polychaete mixture diet. Other diet sources also showed substantial improvement in skin colour and concentration of carotenoid concentrations. The control diet was noticed in discrete types of pigments development and fading existent skin colouration were observed during the investigation. The visual assessment of skin colorations revealed that the increased the skin value and developing the unique original coloure patterns of both *A. ocellaris* and *A. frenatus* species.

Sinha *et al.* (2007), observed an increased value of carotenoid in skin (4.01µg /g) of fishes fed with China rose. It was supported by Ezhil *et al.*, (2008) who reported the total carotenoids found to be an effective colour enhancer at a cheaper price. Alagappan *et al.*, (2004) have also recorded the higher carotenoid level in the fish blue gourami (*Trichogaster trichopterus*) with 4 g spirulina/ kg feed. Ramamoorthy *et al.*, (2010) revealed that the enhancement of fish skin coloration can be done by administrating

pigment enriched feed and improve the quality of ornamental fishes. He also identified, the carrot was found to be an effective and inexpensive source of fish colour enhancer.

Present trail, the frozen dried polychaete diet registered highest concentration carotenoids in the fish skin (7.47 µg /g). Similarly, improved in colour quality was noticed in fishes fed with polychaetes by Murugesan *et al*., (2011). The studies on aquarium fishes indicating that fed with live feeds are highly advisable for vivid skin colour, but mass production of live feeds does not satisfy the demand of ornamental fish breeders and consumers (James and Sampath, 2004). The live and frozen foods, some are better than others for enhancing the coloration of aquarium fish. Crustaceans like brine shrimp are also showed great way to enhance coloration, as are frozen or freeze-dried foods like Mysis shrimp, krill and other crustaceans.

Various studies have proved that the fish can be pigmented by including processing plant sources (Ahilan and Prince Jeyaseelan, 2001). The Nile tilapia (*Oreochromi sniloticus*) fed with *spirulina* sp. have enhanced the color efficiently than the other sources such as marigold petal meal, shrimp head meal and Turmeric (Alagappan *et al.,* 2004). The beetroot (*Beta vulgaris*) and mango peel were evidenced as colour developing agents in fish (Forsberg and Guttormsen, 2006). Mircoalgae such as *Chlorella vulgaris* identified an effective counterpart in pigmentation of most important gold fish (*Carassius auratus*) species by Ezhil *et al*., (2008). *Enhancement of pigmentation* was observed in Green swordtail (*Xiphophorus helleri*) when fed with formulated feed containing Pot marigold (*Calendula officinalis*) concluding as boosting pigments source of carotenoids by Schiedt, (1998). Three botanical additives such as Coriandrum, Mentha and Amaranth leaves reported significant body colouration of gold fish by Ahilan *et al.* (2008)

## 5. Summary

Since, the attractive coloration defines the profitable value of aquarium fishes. The present investigation has proved that the enhancement of coloration can be achieved by administrating pigment enriched natural diets and ultimately improve the quality of fish skin. Particularly, polychaete, hibiscus and carrot diets were showed exceptional colour enhancement results. The aqua feed manufacture seeks an environment friendly natural source of pigment to enhance coloration and to elevate commercial acceptability. It paves the way-to many feed manufacturing to promote their products as natural with a distinct shift away from synthetic ingredients and colorants.

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## Author Contributions

This work was carried out in collaboration among all authors. K. Ramamoorthy designed and wrote the protocol. B. Manikandarajan**,** G. Sankar, managed the analyses of the study and wrote the first draft of the manuscript. S.P. Jeyapriya managed the literature collection.

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