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Use of carotenoid supplementation for enhancement of pigmentation in ornamental fishes

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Abstract

Ornamental fish culture in the glass tank or aquarium is a very common practice around the globe mainly due to their cheerful and lively color. Pigmentation is one of the most significant eminence that attributes the fish for consumer acceptability. Carotenoids are liable for pigmentation of muscle in food fish and skin color in ornamental fish. Fish endures in the natural water have good sources of carotenoid pigments as compare to fish reared in the glass tanks. This creates differences between the both i.e. wild living animals are having bright body color than those of aquarium reared fishes. As fish cannot synthesize the carotenoids *de novo*, therefore it is consistently added in the ornamental fish diet. Although the fish lack of synthesizing the pigments, they have advantage of storing them in the integuments and tissues which will imitate in the skin or flesh color. Aquaculture industries involve remarkable attention in respect to color, as the quality of fish in the market is also determined by the flesh color. Therefore, pigmentation is an essential factor for farming fishes as well. Consequently, dietary application of pigmentation has considerable importance not only for ornamental fish; but also, for food fishes.

Keywords: carotenoids, fishes, color

Introduction

Ornamental fish culture, a fast-developing market not only in India but also all over the world which demands true innovations and implementation of advanced technologies to promote itself to the next level to compete in the international market [20]. The rearing of ornamental fishes are the hobby and real interest for many people around the globe due to their different color and attractive nature. Even though they are vibrant in the nature, when it culture in the glass or artificial aquarium tanks they mostly lose their color than that of its wild counterparts. This is due to the fact that natural environments have good sources of pigments, which needed for nurture the coloration in the animals. Pigments are responsible for the wide spectrum of colors in fishes which is an essential prerequisite for the quality as they fetch higher price in the commercial market. As fishes cannot synthesize their own coloring pigments *de novo*, the coloring agents which are synthesized by some plants, algae and microorganisms, need to be incorporated in the diet [28]. Therefore, the carotenoid sources to the fish must be imparted through feed. Varieties of coloring agents are being used in aquaculture industry to impart bright coloration in fishes, as their color plays a vital role for commercial acceptability. So as to boost the coloration of the artificial tank reared decorative animals' application of carotenoids are vital and it's getting used in decorative fish feed. Color is the primary feature associated with the acceptance or rejection of fish products by customers [45]. The colored fishes are often considered as quality fishes among the consumers. Certain fishes are not preferred to export in to the foreign countries due to faded color or lack of good coloration. Consumers from several foreign countries want to eat colored fish. Fishes like salmonids have excellent export value; however, its quality and market values are determined by the its flesh color. Various products have been introduced to alleviate this problem, but none has performed so effectively and consistently as carotenoid pigment. Varieties of carotenoids pigments are used in fish diet for color enhancement. The most promising carotenoids proved to be successful in enhancing color is astaxanthin that shows marked improvement in color among most species of brightly colored ornamental fishes like Tetras, Cichlids, Gouramis, Goldfish, Koi, Danios and many other species.

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Carotenoid pigments

Carotenoids are among the most common natural pigments responsible for many of the hues found in nature as well as a variety of functions. Carotenoids are a class of 800 natural fat-soluble pigments found principally in plants, algae, fungi, animals, photosynthetic bacteria and some non-photosynthetic bacteria. Only plants, bacteria, fungi and algae can synthesize carotenoids; animals cannot biosynthesize them thus, they must be obtained through the diet [43]. In the animal kingdom, carotenoids are the most widely occurring pigments after melanin. They play a critical role in the photosynthetic process and they carry out a protective function against damage by light and oxygen. Carotenoids additionally play different vital functions as pro-vitamin A, antioxidants, immunoregulators and which they are mobilized from muscle to ovaries which recommend a function in reproduction [4, 36, 45]. It has also observed that fishes with a high level of carotenoids are more resistant to bacterial and fungal diseases [45]. The majority of carotenoids are derived from a 40-carbon polyene chain, which could be considered as the backbone of the molecule.

Pigments in fishes

Fish 'skin' has chromatophores, a kind of cell that contains color pigments. These pigments utilize carotenoids to get forth shades of yellow (Xanthophylls), red and orange (Carotenoids), and brown and black (Melanin) color. Xanthophylls and carotenoids are the most important classes of pigments among fishes. Yellow and Red shades are the two colors most meritoriously influenced by color enhancing foods, which utilized by the chromatophores. However, protein and foods such as seaweed are effective with the chromatophores to produce intense blues, purples and bright greens in fishes. The main chromatophores found in fish influence coloration as follows:

Xanthophores: Yellow

Erythrophores: Red

Melanophores: Black / Brown

Leucophores / Iridophores: Reflective crystals [34]

Carotenoids in fishes

Fishes contain various kinds of carotenoids, the dominant of which is peculiar to the species concerned. Carotenoids commonly occurring in fishes with their colors are tunaxanthin (yellow), lutein (greenish-yellow), beta-carotene (orange), alpha, beta-doradexanthins (yellow), zeaxanthin (yellow-orange), canthaxanthin (orange-red), astaxanthin (red), eichinenone (red) and taraxanthin (yellow). Many fish gather carotenoids in their integuments and gonads. Alternatively, Salmonidae fish peculiarly accumulate astaxanthin in muscle. Lutein pigment is common to freshwater fishes, but also found in many marine fishes. Tunaxanthin is extensively distributed in fish belonging to Perciformes. The brilliant yellow color in the fins and skin of marine fish is because of the presence of tunaxanthin. Feeding experiments involving red sea bream and yellow tail revealed that tunaxanthin was metabolized from astaxanthin via zeaxanthin [33]. Some carotenoids are specific to certain groups of fishes. Fish usually contain various carotenoids in smaller amounts, the proportion of which often differs between samples possibly due to their physiological and/or dietary conditions. Though fishes cannot synthesize carotenoids *de novo*, certain fishes have the capacity to convert one form of carotenoids in to another. Based on this

capacity, fishes are classified into three types:

1. Red cap type: In this group lutein is converted into astaxanthin molecules
2. Sea bream type: In this group of fishes lutein carotene remains in the tissues and cannot be transferred in any other form inside the bodies
3. Prawn type: The beta-carotene molecule can be converted in to astaxanthin molecule.

Sources of carotenoid

There are three major sources through which animal can achieve carotenoids i.e. from plant, animal and synthetic sources.

Plant based carotenoids

Plants also have potential as carotenoid sources. Plant based carotenoids are mainly derived from the micro algal pigment. The commercially available products of the astaxanthin rich yeast *Phafia rhodozyma* and fermentation product of *Xanthophyllomyces dendrorhous* is being used extensively. Feed ingredients like yellow corn, corn gluten meal and alfalfa are mainly used as sources of carotenoids in aquaculture feed formulation. Other carotenoids rich ingredients used are marigold (*Tagetes erecta*) meal and red peppers (*Capsicum sp.*) extract. Experiments with red pepper have given good results, although a lower efficacy was found in comparison to commercially available astaxanthin [8, 62]. Furthermore, paprika oleoresin pigments confer a less desirable coloration in comparison to canthaxanthin in rainbow trout [1]. In a study carried out with *Sparus aurata* fed a diet containing corn gluten meal, the coloration of the front head and operculum achieved the characteristic yellow found in their wild counterparts [42]. However, plant-based carotenoids are mainly derived from the micro algal pigment. For example, if the culture conditions such as nitrogen depletion, high light intensity and temperature are kept optimum, the algae, *Haematococcus pluvialis*, *Chlorella vulgaris*, *Dunaliella salina* and *Arthrospira maxima* can accumulate secondary carotenoids and their biomass is can be used as a coloring ingredient in aquaculture. The freshwater micro algae, *Haematococcus pluvialis* has been commercially exploited for aquaculture mainly due to its rapid growth and high astaxanthin content [13, 48, 49]. It is the primary source of pigmentation in tropical ornamental fish, responsible for various species related yellow, red and others colors. These are obtained through carotenoids containing organisms in the aquatic food chain. Koi carp and gold fish fed with Paprika @ 171 mg/kg shown bright red color in the skin [25]. Use of *Chlorella vulgaris*, *Haematococcus pluvialis* and *Arthrospira maxima* @ 80 mg/kg in koi carp and gold fish improved the color of both the species [23]. Gold fish fed with astaxanthin from red yeast @ 60 mg/kg increased the pigmentation in the flesh, scales, head and fin [61]. Rainbow trout fed with red chilli (*Capsicum annum*) oleoresin @ 120 mg/kg exhibited no significant colour loss during processing [17]. Dietary incorporation of *Haematococcus pluvialis* at 50 mg/kg in Red porgy developed pink-coloured skin [53]. Dietary incorporation of alfalfa @ 15mg/kg improved pigmentation in the tissues [62]. Japanese ornamental koi carp fed with *Spirulina platensis* @ 75.0 g/ kg improved pigmentation [50]. Dietary incorporation of *Spirulina platensis* meal @ 7.5 mg/kg improved better pigmentation [52]. Dietary incorporation of Paprika and *H. pluvialis* @ 100 mg/kg extract exhibited better skin pigmentation in Olive flounder [41].

Animal based natural carotenoids

Animal sources commercially utilized by aquaculture industry as feed additive for color enhancement are mainly by-products from shell fishes and some microorganisms which are abundant in carotenoids. Shell fishes such as shrimp, krill, crabs, lobsters, etc. are used as potential carotenoid sources [47, 60]. They also rich in mineral salts (15-35%), proteins (25-50%) and chitin (25-35%) [31]. Amongst the microorganisms, yeast (*Phaffia rhodozyma*) is a main astaxanthin source [45]. *Phaffia rhodozyma* also functions as a good source of proteins and lipids [27]. The inclusion of this carotenoid source, aside from its positive effect on fish pigmentation, enhances liver function and defensive potential against oxidative stress [36]. Dietary incorporation of astaxanthin from the shrimp *Pleisonika sp.* @ 33 mg/ Kg showed skin coloration acquired by the Red porgy was similar to that of wild reared [9]. Red porgy fed with Shrimp shell meal @ 40 mg/ Kg changed skin color from dark grey to red pink silver [30]. Dietary incorporation of Shrimp shell meal @ 16 % in feed improved carotenoid accretion in the skin tissue of Red porgy [29]. Dietary incorporation of marine and freshwater crab meals at rate of 10% and 20% in feed improved fillet quality as TBARS values from the raw fillets shows clear delay in the oxidation of lipids along with improved skin color of Red porgy [21].

Synthetic Source

Plant and animal-based carotenoid sources in feed application contains a mixture of different pigments. On the other hand, synthetic carotenoids contain only a specific pigment; but problem is the storage as they are very sensitive to heat, light and air. However, stable forms of synthetic pigments are available in the market which is emulsified with ascobyl palmitate [26], ethoxyquin or coated with gelatin and maize starch. Astaxanthin (Naturouse®) at the rate of 100 mg/Kg increased bright reddish color in both dorsal and ventral areas of Red porgy [10]. Astaxanthin @ 4.7-32.8 mg/Kg received higher dark color in tropical spiny lobster than that of control fishes [3]. Red porgy fed with 25 or 50 mg/Kg of astaxanthin exhibited reddish hue pigmentation in animals [53]. Dietary incorporation of astaxanthin at a rate of 39 mg/Kg gave higher pigment retention in the skin of Australian snapper [18]. Japanese ornamental carp fed with Synthetic carotenoid (Carophyll) @ 1.5 g/kg improved pigmentation [50]. Dietary incorporation of xanthophylls (Wisdem Golden Y20) @ 75 mg/Kg exhibited 1.10–1.20 times greater yellowness color in ventral skins and 1.25–1.35 times greater in dorsal skins of Yellow croaker [63]. Giant tiger prawn fed with astaxanthin @ 100 mg/kg improved pigmentation in animals when reared under both black and white substrates [58].

Importance of carotenoids

Apart from the pigmentation, carotenoids have many vital roles which are necessary for the biological systems. They are precursor to vitamin A [15, 24, 40, 59], improve the reproductive performance [12], powerful antioxidants [6, 35, 36], boost immune system against bacterial and fungal disease protection [2, 4, 45], increase survival [37, 55], promotes the growth [4, 14, 22] and have positive impacts on the structure of liver [39, 44].

Effect of carotenoids on growth of fishes

There is a controversy on the role of carotenoids in fish growth, several studies reporting a positive influence whereas others did not find any effect. In Atlantic salmon fry and

juveniles, the addition of synthetic astaxanthin and canthaxanthin not only enhanced growth but also survival [15, 16, 57]. For example, an improved growth of Atlantic salmon was found by supplementing commercial start feeding diets with astaxanthin or canthaxanthin and no significant differences were found between the astaxanthin and canthaxanthin supplemented diet [57]. Similar results are found for the red tilapia (*Oreochromis niloticus*) [7] and kuruma shrimp (*Penaeus japonicus*) [11]. Supplementation of carotene and canthaxanthin to the diets of major Indian carps resulted in a better survival and growth compared to conventional diets used without carotenoids [22]. The interaction between astaxanthin and vitamin A supplementation on growth and survival in first feeding fry of Atlantic salmon [15]. The experimental diets were based on a semi-purified diet based on vitamin and carotenoid free casein and gelatin as protein sources [46]. The results obtained from this 135-day feeding study clearly showed a significantly improved growth and survival on supplementation of astaxanthin to the experimental diet and vitamin A supplementation alone did not support growth and survival. The results also show a provitamin a function of astaxanthin but this alone is not able to explain the effect of astaxanthin supplementation. The dietary inclusion of carotenoid pigments did not affect the growth and feeding efficiency of goldfish juveniles [5]. Gold fish fed with 6 % carrot showed higher specific growth rate than control [54]. Koi carp fed with 1% carrot peel powder was more effective in enhancing the growth and colouration [32]. Incorporation of 180ppm of marigold oleoresin in diet was found better to enhance the growth and coloration in *C. carpio* [51]. Three different kinds of natural carotenoid sources, *Phaffia rhodozyma*, *Paracoccus sp.*, and *Haematococcus pluvialis*, at a level of 45 mg kg-1 total carotenoids did not significantly differ in the growth and feed utilization efficiency [38]. A gelatine based diet enriched with both marigold petal powder and protein ingredients might give a better result of growth and pigmentation of Swordtail, *Xiphophorus helleri* [19].

Conclusion

Due to environmental degradation, residue and other pollution related problems recent aquaculture practices give more importance to organic farming as the synthetic supply of feed additive is restricted in the animal diet. Moreover, the incorporation of synthetic carotenoids in the feed may raise feed cost. The natural sources of plant and animals contain ample sources of carotenoids. Consequently, the natural sources of plant and animal derived carotenoids have to be abundantly explored for the commercial aqua feed formulation. The research attempts must be more focused with special reference to natural carotenoids and incorporation in aqua feed and productivity has to reach the feed formulation stage. The dosage at which animal can reveal to noticeable color patterns in the skin and muscle should be well explored and standardized.

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