Application of carotenoids on coloration of aquatic animals

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Abstract
Ornamental fish culture in the glass tank is a very common custom around the globe mainly due to their bright coloration and natural stress reliever for the heart patients. Carotenoids are the major compound which imparts coloration to the aquatic animals. Fish live in the natural water have good sources of carotenoid pigments as compare to fish reared in the glass tanks. This makes differences between both that the wild living animals are nurtured with bright body color than that of aquarium reared fishes. As fish cannot synthesize this de novo, it is a regular additive in the ornamental fish diet. Despite the fish lack of synthesizing the pigments, they have advantage of storing them in the integuments and tissues which will reflect in the skin or flesh color. Aquaculture industries require remarkable attention in respect to colour, as the quality of fish in the market is also determined by the flesh colour. Therefore, pigmentation is a necessary factor for farming fishes as well. As a result, dietary application of pigmentation has considerable importance not only in ornamental fish; but also in farming fishes. The dietary importance of carotenoids, types, sources with respect to colour of the aquatic organisms is discussed in this article.

Keywords: carotenoids, types, source, colour, aquatic animals

Introduction
The rearing of ornamental fishes are the hobby and real interest for many people around the globe due to their different color and attractive in nature. Despite they are colourful in the nature, when it culture in the glass or artificial tanks they will lose their color than that of wild ones. This is due to the fact that natural environments have good sources of pigments, which needed for nurture the coloration to the animals. But the culture animals have no access to get this as tanks we use artificial waters lack of this source. The higher plants and microorganisms can synthesize carotenoids de novo and it is not possible by the aquatic animals. Therefore, the carotenoid sources to the fish must be imparted to through feed. In order to improve the coloration to the artificial tank reared ornamental animals application of carotenoids are important and it is being used in ornamental fish feed.

Colour is the primary feature associated with the acceptance or rejection of fish products by customers (Shahidi et al., 1998) [1]. The coloured fishes are often considered as quality fishes among the consumers. Certain fishes are not exported to the foreign countries due to faded colour or lack of good colouration. Consumers from many foreign countries wish to eat coloured fish. Fishes like salmonids have excellent export value; however, its quality and market values are determined by the colour. The aquaculture products may be well utilized by clients if meet their expectations. According to Howell et al. (1991) [3], the application of carotenoids is important to tiger shrimp as its deficiency in the diet leading to the discoloration called “Blue disease”. Therefore, the application of dietary carotenoids has equal opportunity in the diet of farming fishes as well.

Carotenoid pigments
Four main groups of pigments such as porphyrins, pteridines, melanins and carotenoids are incorporated in the feed of mammals, birds, fish and invertebrates for the enhancement of coloration (Hudson, 1994) [3]. Porphyrins are particularly important for the coloration of eggshell in birds (Lang and Wells, 1987) [4]. Pteridines are reason for brilliant yellow and red colours in fish, amphibians and reptiles (Nixon, 1985) [5]. Melanin is responsible for the blacks, greys, browns, reds and yellows (Hudon, 1994) [3]. Carotenoids are responsible for the bright red, yellow and orange colours for many vertebrates and invertebrates (Toyomizu et al., 2001) [6]. It is very important to note that except carotenoid pigments, all three pigments can be made endogenously by cells of the organisms.

Carotenoids is comes under fat soluble natural pigments produced from the backbone molecule of 40-carbon polyene chain, which contains cyclic end-groups and oxygen containing functional groups. The color property of carotenoids is due to the presence of conjugated double bonds in the hydrogen backbone (Bendich and Olson, 1989) [7]. Carotenoids found almost in algae, fungi, bacteria, plants and animals. In animals, carotenoids are the pigments found maximum next to melanin pigment. There are different types of carotenoids according to the colour they can administer to the animals. They include astaxanthin (red), tunaxanthin (Yellow), lutein (Greenish-yellow), beta-carotene (orange), alpha and beta-doradexathins (Yellow), canthaxanthin (orange-red), xeaxanthin (yellow-orange), eichinenone (red) and taraxanthin (yellow) (Saito and Regier, 1971) [8]. But, the animals colour varies due to what dominant carotenoid is supplied and expressed; which are species specific.

Significance of pigmentation in aquaculture
Pigments are vital source for animal diet to impart wide variety of colours as they cannot synthesize by de novo.
Carotenoids are supplemented in the diets of cultivable and ornamental fish to produce a fascinating colouration (Scheidt, 1998) [9]. It is also linked to quality standard as coloured fishes fetches highest market price. It is the fact that customers are willing to bargain for paying the demanded money if the fish colour is faded. To overcome this drawback, sorts of products were introduced to aquaculture trade; but none has executed so efficaciously as pigment source. To enrich colour, researchers established to apply a wide variety of carotenoids pigments in feed of many decorative and other alternative fish species. In order to duplicate the original colour of the fish as like found in the wild atmosphere, ornamental traders use carotenoids. At present, the ranges of colouring pigments are utilized in the aquaculture trade, due to the fact that they have effect on business acceptability. Figure 1 depicts the color of fish receives natural carotenoids in the diet.

**Fig 1:** Carotenoids beautify fish colourful

From ancient days, it is believed that colour is related to quality and advanced taste of the organisms; that belief still continuous (Clydesdale, 1993) [10]. Muscle pigmentation is considered as important factors which tell the freshness and quality of animals and result in high market prices (Koteng, 1992) [11]. Skin colour is yet a fundamental determinant factor affecting the overall evaluation in the ornamental and food fish trades (Gouveia and Rema, 2005) [12]. In shrimp, the desired coloration in the flesh is related to freshness and quality (Boonyaratpalin et al., 2001) [13]. Similarly, gonads of the echinoderm with vibrant yellow-orange supporting with best commercially value (Shipgel et al., 2004) [14]. Carotenoids in the feed of salmon, trout and char enrich coloration of integument and flesh (Spinelli et al., 1974; Coral et al., 1997) [15, 16]. Figure 2 shown the flesh of salmon fed with carotenoids in the feed.

**Fig 2:** Salmon fed with carotenoids in diet nurture pink coloured flesh (Source: https://rayfiftydotcom) [17]

### Types of carotenoids
According to Katayama et al. (1973) [18] aquatic animals are grouped into three categories (Red carp type, Sea bream type and prawn type) based on their *de novo* biosynthesis. Goldfish, red carp and fancy red carp comes under first category. This group convert lutein, zeaxanthin or intermediates to astaxanthin. However, β-carotene is not the most important precursor for astaxanthin biosynthesis and they can store astaxanthin from the diet. Crustaceans include prawns, crabs and lobsters are covered under second category; they convert β-carotene and zeaxanthin to astaxanthin. Sea bream typed fish comes under third category; they have no capacity to convert β-carotene, lutein or zeaxanthin to astaxanthin. But, they can convert the dietary carotenoids into tissue carotenoids i.e. transfer of carotenoids from feed to tissue.

### Sources of carotenoids
There are three major sources through which animal can obtain carotenoids. They are plant, animal and synthetic sources.

#### Plant source
Plant sources such as corn gluten meal, yellow corn, alfalfa; marigold (*Tagetes erecta*) meal, red peppers (*Capsicum sp.*) extract and paprika oleoresin are rich in carotenoids and used as dietary source in the fish feed (Yanar et al., 1997; Robaina et al., 1997; Akhtar et al., 1999) [19, 20, 21]. In addition, algae such as *Chlorella vulgaris*, *Dunaliella salina*, *Arthospira maxima* and *Haematococcus pluvialis* are used in aquaculture as carotenoid source (Sommer et al., 1991; Choubert and Heinrich, 1993; Ben-Amotz et al., 1982; Ben-Amotz and Avron, 1983) [22, 23, 24, 25]. Earlier studies shows that dose of carotene @ 125 ppm from plant source furnish admirable coloration and Choubert (1979) [26] reported in rainbow trout higher doses ranging from 125 to 300 ppm further enriching coloration. Lists of some studies conducted with the feeding plant sources and their inference related to changes in the color of aquatic animals are represented in the table 1.

#### Animal Source
Animal sources commercially utilized by aquaculture industry as feed additive are mainly by products from shell fishes and some microorganisms abundant in carotenoids. Shell fishes such as shrimp, krill, crabs, lobsters, etc. are used as potential carotenoid sources (Wilkie, 1972; Simpson and Haard, 1985) [27, 28]. They also rich in mineral salts (15-35%), proteins (25-50%) and chitin (25-35%) (Lee and Peniston, 1982) [29]. Among the microorganisms, yeast (*Phaffia rhodozyma*) is a main astaxanthin source (Shahidi et al., 1998) [1]. But, it exists as optical isomer, which is different from normal configuration found in other sources (Andrewes and Starr, 1976) [30] and at the same time rainbow trout this configuration exists in flesh even after its deposition (Foss et al., 1984) [31]. According to Johnson et al. (1980) [32], *P. rhodozyma* is also rich in lipids and proteins; its supplementation in feed improves the function of liver and protects cells from oxidative damage in animals (Nakano et al. 1999) [33]. The dietary carotenoid animal sources in fish with reference to changes in the color of aquatic animals shown in the table 2.

#### Synthetic source
Plant and animal based carotenoid sources in feed application comprises 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 ascosyl palmitate (Ito et al., 1986) [34], ethoxyquin or coated with gelatin and maize starch. Application of different synthetic carotenoid source in aquaculture and ornamental industry with reference to the colouration of the aquatic animals are represented in the table 3.

Utilization of carotenoids in animals

Carotenoids are the fat soluble nature. So, most of the fishes has less carotenoid utilization in gastrointestinal tract (Castenmiller and West, 1998) [35]. The animal capacity to utilize the carotenoids from the feed is less. Also, for the better utilization of carotenoids by the animals requires dietary lipids for facilitating the formation of micelles to assimilate the nutrient (Choubert et al., 1994; Van den Berg, 1999) [36, 37]. The absorption of carotenoids doesn’t require any metabolic conversion (Schiendt, 1998) [9]. But this is reverse for xanthophyll esters since they are hydrolysed by nonspecific bile salt dependent lipase (White et al., 2003) [38]. Salmonoids absorb only 35% of the dietary astaxanthin (Torrissen et al., 1989) [39] and rest may be stored in the skin or flesh. Carotenoids are deposited in the fish skin and flesh is due to as a result of slow metabolism, less utilization (Castenmiller and West, 1998) [35] and re-esterification of available carotenoids by endogenous fatty acids (Foss et al., 1987) [40].

The utilization of carotenoid is varied from species to species or we can say that species specific, supported by Guillou et al. (1992) [41] in salmonoids as they absorb esterified forms of carotenoids. However, some evidence shows they absorb free form of carotenoids better than ester form (Storebakken et al., 1987; Choubert and Heinrich, 1993) [42, 23]. Many fish acquire carotenoids in their skins and reproductive organs. As an alternative, salmonid species specifically collect astaxanthin in the muscle. Carotenoids accumulated in the skins as esterified form, except cat fish that they store as free form. Fishes have no capacity to produce carotenoids de novo (Baker et al., 2002) [43], but, they have capacity to transform one type of pigments into any other type of pigments.

Factors affecting the utilization of carotenoids in fishes

There are several factors affect the utilization of carotenoids in fish (Leng and Li, 2006) [44]; mainly biotic factors such as vitamin E, lipid, and vitamin A content of diets and abiotic factors such as temperature and salinity. Besides this, property of nutrients and the diet palatability make a difference in fish coloration or quality (Leng and Li, 2006) [41, 49, 50, 38]. The increase information about the dietary carotenoids for skin and muscle should be well explored and standardized. Which animal can reflect to noticing a variation in the structure of liver (Segner et al., 1989, Page et al., 2005) [59, 60].

Table 1: Dietary plant carotenoid sources in fish with respect to changes in the colouration

<table>
<thead>
<tr>
<th>S. No</th>
<th>Source</th>
<th>Study animal</th>
<th>Inclusion level</th>
<th>Experiment duration</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dunaliella salina, Astaxanthin and Alflfa meal</td>
<td>Crayfish</td>
<td>100 μg/kg</td>
<td>55 days</td>
<td>Fish displayed improved body colouration than that of control group.</td>
<td>Harpaz et al., 1998 [61]</td>
</tr>
<tr>
<td>2</td>
<td>Paprika</td>
<td>Koi carp and Goldfish</td>
<td>171 mg/kg</td>
<td>2 months</td>
<td>Koi carp and goldfish fed with paprika shown bright red color in the skin.</td>
<td>Hancez et al., 2003 [62]</td>
</tr>
<tr>
<td>3</td>
<td>Chlorella vulgaris, Haematococcus pluvialis and Arthospira maxima</td>
<td>Koi carp and Gold fish</td>
<td>80 mg/kg</td>
<td>10 weeks</td>
<td>Color was improved in both species.</td>
<td>Gouveia et al., 2003 [63]</td>
</tr>
</tbody>
</table>
Table 2: Application of the dietary animal carotenoids sources in fish with reference to changes in the color

<table>
<thead>
<tr>
<th>S. No</th>
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<th>Inclusion level</th>
<th>Experiment duration</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Astaxanthin from the shrimp <em>Pleonotus sp.</em></td>
<td>Red porgy</td>
<td>33 mg/ Kg</td>
<td>4 months</td>
<td>Result shows skin coloration acquired by the fish was similar to that of wild reared.</td>
<td>Cejas et al., 2003 [72]</td>
</tr>
<tr>
<td>2</td>
<td>Shrimp shell meal</td>
<td>Red porgy</td>
<td>40 mg/ Kg</td>
<td>105 days</td>
<td>Skin color was changed from dark grey to red pink silver.</td>
<td>Kalinowski et al., 2005 [73]</td>
</tr>
<tr>
<td>3</td>
<td>Shrimp shell meal</td>
<td>Red porgy</td>
<td>16% in feed</td>
<td>180 days</td>
<td>Carotenoid accretion in the skin tissue was improved.</td>
<td>Kalinowski et al., 2007 [74]</td>
</tr>
<tr>
<td>4</td>
<td>Marine and freshwater crab meals</td>
<td>Red porgy</td>
<td>10% and 20% in feed</td>
<td>193 days</td>
<td>Fillet quality improved as TBARS values form the raw fillets shows clear delay in the oxidation of lipids along with improved skin colour was noticed.</td>
<td>García-Romero et al., 2014 [75]</td>
</tr>
</tbody>
</table>

Table 3: Application of the synthetic carotenoids sources in fish with reference to changes in the color

<table>
<thead>
<tr>
<th>S. No</th>
<th>Source</th>
<th>Studied species</th>
<th>Inclusion level</th>
<th>Study duration</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Astaxanthin (Naturorse®)</td>
<td>Red porgy</td>
<td>100 mg/Kg</td>
<td>10 weeks</td>
<td>Diets fed with carotenoids presented bright reddish color in both dorsal and ventral areas.</td>
<td>Chatzifotis et al., 2005 [76]</td>
</tr>
<tr>
<td>2</td>
<td>Astaxanthin</td>
<td>Tropical spiny lobster</td>
<td>4.7-32.8 mg/Kg</td>
<td>12-weeks</td>
<td>Prawns received higher dietary carotenoid attained dark color than that of control fishes.</td>
<td>Barclay et al., 2006 [77]</td>
</tr>
<tr>
<td>3</td>
<td>Astaxanthin</td>
<td>Red porgy</td>
<td>25 or 50 mg/Kg</td>
<td>4 months</td>
<td>Astaxanthin supplementation in diet exhibited reddish hue pigmentation in animals.</td>
<td>Tejera et al., 2007 [66]</td>
</tr>
<tr>
<td>4</td>
<td>Astaxanthin</td>
<td>Australian snapper</td>
<td>39 mg/Kg</td>
<td>9 weeks</td>
<td>Fish received astaxanthin in diet gave higher pigment retention in the skin.</td>
<td>Doolan et al., 2008 [78]</td>
</tr>
<tr>
<td>5</td>
<td>Synthetic carotenoids (Carophyll)</td>
<td>Japanese ornamental carp</td>
<td>1.5 g/Kg</td>
<td>99 days</td>
<td>Pigmentation was improved when fish received carotenoid in diet.</td>
<td>Sun et al., 2012 [69]</td>
</tr>
<tr>
<td>6</td>
<td>xanthophylls (WisdemGolden Y20)</td>
<td>Yellow croaker</td>
<td>75 mg/Kg</td>
<td>9 weeks</td>
<td>Fish fed with xanthophylls in diet exhibited 1.10–1.20 times greater yellowness color in ventral skins and 1.25–1.35 times greater in dorsal skins respectively.</td>
<td>Yi et al., 2014 [79]</td>
</tr>
<tr>
<td>7</td>
<td>Astaxanthin</td>
<td>Giant tiger prawn</td>
<td>100 mg/kg</td>
<td>6 weeks</td>
<td>Astaxanthin supplementation in diet improved pigmentation in animals when reared under both black and white substrates.</td>
<td>Wade et al., 2015 [80]</td>
</tr>
</tbody>
</table>

References


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