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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 lives 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) [2], 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 (Hudon, 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 coloration (Schiedt, 1998) [9]. It is also linked to quality standard as coloured fishes fitches 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 (Shpigel *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.

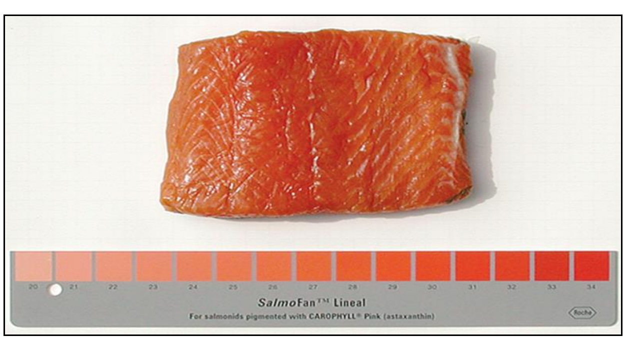

Fig 2: Salmon fed with carotenoids in diet nurture pink coloured flesh (Source: https://rayfiftydotcom) [17]

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 ascobyl 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 (Schiedt, 1998) [9]. But this is reverse for xanthophyll esters since they are hydrolysed by nonspecific bile salt dependent lipase (White *et al*., 2003) [38]. Salmonids 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 salmonids 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, salmonidae fish 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) [44]. Torrissen *et* al., 1990 [45] recommended that fat content of 4.1-23.0% in the fish diet increase the utilization of astaxanthin. It is very important to note that coloration effect not necessarily linked with the high digestion rate; it depends on high pigment absorption (Daniel, 2016) [46]. The study made in *Xiphophorus helleri* shown the functional roles of lipid on carotenoid utilization. It established that suitable quantity of lipid in the diet can increase the carotenoids absorption also this study found that astaxanthin pigmentation promoted by the addition of lipids and if the amount of lipid reaches 20% or beyond then it started decreased (Han, 2001) [47]. According to Bell *et al.* (2000) [48], the flesh quality of lipid can be improved when astaxanthin supplemented along with vitamin E in the diet. The utilization of carotenoids can also improve by the addition of sodium taurocholate in the diet (Daniel, 2016) [46].

Other biological importance

Apart from the pigmentation, carotenoids have many vital roles which are necessary for the biological systems. They are precursor to vitamin A (Guillou *et al*., 1992; Christiasen *et al*., 1994; Parker, 1996; White *et al*., 2003) [41, 49, 50, 38], improve the reproductive performance (Chou and Chien, 2001) [51]; powerful antioxidants (Bjerkeng and Johnsen, 1995, Nakano *et al*., 1999, Miki, 1991) [52, 33, 53]; boost immune system against bacterial and fungal disease protection (Shahidi *et al*., 1998, Bell *et al*., 2000, Amar *et al*., 2003) [1, 48, 54]; increase survival (Nègre-Sadargues *et al*., 1993, Tizkar, 2013) [55, 56]; promotes the growth (Goswami, 1993; Christiansen *et al*., 1995; Bell *et al*., 2000) [57, 58, 48] and have positive impacts on the structure of liver (Segner *et al*., 1989, Page *et al*., 2005) [59, 60].

Concluding remarks

Recent aquaculture practices give more importance to organic farming as the synthetic supply of feed additive is restricted in the animal diet due to environmental degradation, residue and other pollution related problems. Besides, the incorporation of synthetic carotenoids in the feed may raise feed cost. The natural sources of plant and animals contain abundant sources of carotenoids. Therefore, the natural sources of plant and animal derived carotenoids have to be copiously 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 output has to reach the feed formulation stage. The factors such as digestion, absorption and metabolism in the animals have to be well studied through dietary carotenoids. The dosage at which animal can reflect to noticeable colour patterns in the skin and muscle should be well explored and standardized. The increase information about the dietary carotenoids for aquatic animals over these conditions must be fulfilled for the sustainable production of aquatic commodities.

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

S. No	Source	Study animal	Inclusion level	Experiment duration	Results	References
1	*Dunaliella salina*, Astaxanthin and Alfalfa meal	Crayfish	100 µg/kg	55 days	Fish displayed improved body colouration than that of control group.	Harpaz *et al*., 1998 [61]
2	Paprika	Koi carp and Goldfish	171 mg/kg	2 months	Koi carp and goldfish fed with paprika shown bright red color in the skin.	Hancz *et al*., 2003 [62]
3	*Chlorella vulgaris*, *Haematococcus pluvialis* and *Arthrospira maxima*	Koi carp and Gold fish	80 mg/kg	10 weeks	Color was improved in both species.	Gouveia *et al*., 2003 [63]

4	Astaxanthin from red yeast	Goldfish	60 mg/kg	60 days	The concentration of pigment was increased in the flesh (31.0%), scales (45.5%), head (22.6%) and fin (21.2%) respectively.	Xu *et al.*, 2006 [64]
5	Carotenoid from red chilli (*Capsicum annuum*) oleoresin	Rainbow trout	120 mg/kg	6 weeks	Carotenoid from red chilli oleoresin received fishes exhibited no significant colour loss during processing.	De la Mora *et al.*, 2006 [65]
6	Astaxanthin from *Haematococcus pluvialis*	Red porgy	50 mg/kg	4 months	Acquired pink-coloured skin.	Tejera *et al.*, 2007 [66]
7	Alfalfa	Goldfish	15 mg/kg	60 days	Improved pigmentation was observed in the tissues.	Yanar *et al.*, 2008 [67]
8	Lucantin Pink (Synthetic astaxanthin), Beetroot juice powder and Overseal Carantho powder	Lame-red dwarf gourami	100 mg/kg	12 weeks	Synthetic astaxanthin gave better red coloration, but betalain and anthocyanin did not improve the skin color.	Baron *et al.*, 2008 [68]
9	*Spirulina platensis*	Japanese ornamental koi carp	75.0 g/ kg	99 days	Pigmentation was modified by adding *S. platensis* in diet.	Sun *et al.*, 2012 [69]
10	*Spirulina platensis* meal	Rainbow trout	7.5 mg/kg	10 weeks	Fish received *S. platensis* meal acquired better pigmentation.	Teimouri *et al.*, 2013 [70]
11	Paprika and *H. pluvialis* extract	Olive flounder	100 mg/kg	8 weeks	Exhibited better skin pigmentation.	Pham *et al.*, 2014 [71]

Table 2: Application of the dietary animal carotenoid sources in fish with reference to changes in the color

S. No	Source	Study animal	Inclusion level	Experiment duration	Results	References
1	Astaxanthin from the shrimp *Pleisonika sp.*	Red porgy	33 mg/ Kg	4 months	Result shows skin coloration acquired by the fish was similar to that of wild reared.	Cejas *et al.*, 2003 [72]
2	Shrimp shell meal	Red porgy	40 mg/ Kg	105 days	Skin color was changed from dark grey to red pink silver.	Kalinowski *et al.*, 2005 [73]
3	Shrimp shell meal	Red porgy	16% in feed	180 days	Carotenoid accretion in the skin tissue was improved.	Kalinowski *et al.*, 2007 [74]
4	Marine and freshwater crab meals	Red porgy	10% and 20% in feed	193 days	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.	García-Romero *et al.*, 2014 [75]

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

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

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