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COLOUR ENHANCEMENT OF ORNAMENTAL FISHES USING NATURAL CAROTENOIDS DERIVED FROM PLANT ORIGIN

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Abstract

Ornamental fishes are known and priced for its colouration but due to intensive rearing the food on which they rear are devoid or not adequately fortified with the colour pigments i.e. carotenoids. As fish is not capable of synthesizing carotenoids so they are to be incorporated in the diet of cultured species. Carotenoids are costly ingredient so as to keep the price factor into consideration manufactures used to incorporate carotenoid in least quantity or not at all. There are two type of carotenoids artificial and natural depending on the source. Artificial carotenoids are having deleterious effect on aquatic environment so natural carontenoids are been preferred as they are more sustainable. They can be very well obtained from animal sources like Krill, shrimp and crab waste but it also can be extracted from plant origin like Marigold, Hibiscus, Chlorella, Spirulina, Yeast etc. which are cost effective and more environment friendly. Apart from colour pigmentation carotenoids are vitamin A precursors, marked affect on reproductive performance, an potent antioxidants and enhance immune system of ornamental fishes.

Introduction:

Ornamental fish keeping is one of the hobbies which are growing in tremendous pace due to its ease and health benefit. It acts as a stress buster for the people undergoing hypertension.

Ornamental fishes are priced for its striking vibrant colour. These fishes which found in wild are more colour than the fish reared in confined aquarium tanks. Hence wild fishes are getting a balanced diet from the aquatic water bodies in which they thrive in the form of plankton (both Phytoplankton and zooplankton). The plankton is the aquatic microscopic organism which drifts by the action of waves. The plankton is rich in carotenoids i.e. colour pigments .The fishes feed on this plankton which is more digestible and balance food. Due to this fishes naturally get colour pigments.

In confined rearing facilities are solely depends on the artificial food. The artificial food is the mixture of various ingredients which are rich in protein, carbohydrates, fats, vitamins and minerals. Artificial feed mostly lacks carotenoids or not incorporated adequately due to its cost effectiveness. The artificial diets which are in corporate adequately with carotenoids are charged exorbitantly.

Fish colour is a major price determining factor in Ornamental fish trade. Fish lacks the ability to synthesise carotenoids and solely depends on dietary source.(Johnson and An,1991) Fish farmers has keen desire to enhance the fish colour which he reared in his rearing improve health status and fetches premium price. Synthetic carotenoids are very costly and deleterious effect on the environment.

Carotenoids

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 from the diet (Schiedt, 1998). The majority of carotenoids are derived from a 40-carbon polyene chain, which could be considered as the backbone of the molecule. This chain may be terminated by cyclic end-groups (rings) and may be complemented with oxygen containing functional groups.

Types of Carotenoids in Fishes

In fishes various kind of carotenoids are found but dominant of them are tunaxanthin (yellow), lutein (greenish-yellow),carotene (orange), doradexanthins (yellow), zeaxanthin (yellow-orange), canthaxanthin (orange-red), astaxanthin (red), eichinenone (red) and taraxanthin (yellow). In *Salmonidae* fish the flesh colour is due to the accumulation of astaxanthin in muscle. Except for catfish, carotenoids in the integuments of fish exist in an esterified form. Though fishes cannot synthesize carotenoids *de novo* certain fishes have the capacity to convert one form of carotenoid into another carotenoid.

Based on this capacity fishes are classified into three types

1. **Red Carp type:** In this group lutein is converted into astaxanthin molecule.
2. **Sea bream type-** In this group lutein and carotene remains in the tissue and cannot be transferred in any form inside the bodies.

3. **Prawn type**- Beta carotein molecule can be converted into astaxanthin.

Natural carotenoids are based on two groups plant and animal based carotenoids.

a. **Animal carotenoids** –Animal based carotenoids are been extracted from the by products of crustacean s such as the Antarctic Krill (Euphausia superb), crayfish meal,shrimp meal, crab meal etc. Main constrain in utilizing animal based natural carotenoids is that they are limited in supply as there is downward trend in catch from marine resources and also expensive source of carotenoids.

b. **Plant carotenoids**- Plant based carotenoids are mainly from micro –algae i.e. Chlorella vulgaris, Hematococuss pluvialis, Dunaliella salina, Teodar, Yeast extract (*Phafia rhodozyme* and fermented product Xanthophyll.

Table 1 Content of catenoids in selected natural sources

Animal source	Content (mg/Kg)	Plant Source	Content (mg/Kg)
Crab meal	75-1300	Marigold (Petal meal)	7000
Crayfish meal	30-800	Chlorella	4000
Shrimp meal	100-150	Yeast (*Chaffier rhodoyza*)	1000
Shrimp oil	25-125	Sea weed	390-900
		Corn gluten	290
		Alfa alfa	280

Source- Gupta etal. 2007

Carotenoid absorption and transportation

Carotenoids are hydrophobic compound and hence not easily solubilise in aqueous environment of gastro intestinal tract of fishes hence for digestion absorption and transport process are associated with lipids. (Castenmiller and West, 1998). Intestinal absorption of carotenoids involves steps, including disruption of the matrix, dispersion in lipid emulsions and solubilisation into mixed bile salt micelles, before being carried to the enterocyte brush border were the absorption takes place (Furr and Clark, 1997; Tyssandier *et al.*, 2001). In salmonids, approximately 35% of

dietary astaxanthin is absorbed (Torrissen *et al.*, 1989; Storebakken and No,1992; Ytrestøyl *et al.*, 2005) mainly along the proximal intestine (Torrisen, 1986; Al-Khalifa and Simpson, 1988;Torrisen, 1989; Hardy *et al.*, 1990; White *et al.*, 2002), taking approximately 18 to 30 hours (March *et al.*, 1990; Choubert *et al.*,1994). In comparison with other fish nutrients, absorption of carotenoids is considered slow.

Many authors suggest that the intestinal absorption from micelles is a passive diffusion process (Choubert *et al.*, 1994; Parker, 1996; Castemiller and West, 1998; Van den Berg, 1999). Carotenoids are absorbed without prior metabolic conversion, except for xanthophylls esters, hydrolyzed before absorption, by a nonspecific bile salt dependent lipase, since no esters are found in plasma or white muscle of salmonids (Schiedt, 1998; White *et al.*, 2003). Hence, astaxanthin esters found in the skin of salmonids are a result of reesterification of free carotenoids with endogenous fatty acids (Foss *et al.*, 1987).

Carotenoid metabolism and deposition

Carotenoid metabolism take place in the organs where their metabolites are found (Storebakken and No 1992), such as the liver (Hardy *et al.*, 1990; Metusalach *et al.*, 1996) or in the intestine (Aas *et al.*,1999). In salmonids, approximately 50% of dietary astaxanthin absorbed may be metabolized (Torrissen *et al.*, 1989; Storebakken and No, 1992; Ytrestøyl *et al.*, 2005).

Classification based on carotenoids metabolic capacity of fish (Tanaka, 1978)

1. Type of fish cannot oxidize the ionone ring and, therefore, the specific oxygenated derivatives have to be included in their diet.
2. Type of fish, such as gold fish (*Carassius auratus*) and the fancy red carp (*Cyprinus carpio*) are able to oxidize 4 and 4´ positions of the ionone ring, hence being able to convert zeaxanthin and lutein to astaxanthin (Matsuno and Tsushima, 2001).

Function Of Carotenoids:

In fish, carotenoids have similar functions as those found in other animal species:

1. **Vitamin A precursors** (Schiedt *et al.*, 1985; Guillou *et al.*, 1992; Christiasen *et al.*, 1994; White *et al.*, 2003);
2. **Marked affect on reproductive performance** (Craik, 1985; Christiansen and Torrissen, 1996;

Verakunpiriya *et al.*, 1997; Chou and Chie, 2001; Vassallo-Agius *et al.*, 2001);

3. **An potent antioxidants** (Bjerkeng and Johnsen, 1995; Shimidzu *et al.*, 1996; Nakano *et al.*, 1999; Bell *et al.*, 2000);
4. **Enhance immune system** (Nakano *et al.*, 1995; Amar *et al.*, 2003); and affect liver structure (Segner *et al.*, 1989; Page *et al.*, 2005).

Improvement of colour using carotrenoids

Incorporation of carotenoids in diet improves the skin colour of fish which ultimately increases its market value of ornamental fishes. Coloration of gold fish and Koi carp is improved by addition of carotenoids in their diet as these fishes are capable to metabolize zeaxanthin to astaxanthin**.** As per the findings of Hata and Hata, 1975, Gold fish lacks the ability to metabolize lutein and have limited ability to convert ß carotene to astaxanthin. Tiger barb (*Barbaus tetrazona*) has been reported to increase the skin pigmentation by incorporation of shrimp meal, marigold petal and annatto seed extract. Blue green alga has also been used as a source of pigmentation to koi carp (Choubert,1979). The optimum level of astaxanthin for intense colouration in Gold fish was found to be 36-37mg/Kg diet and also improves the survival rate (Paripatnanot et al., 1999)

When red velvet sword tail (*Xiphophorus helleri*),rainbow fish (*Pseudomugil furcatus*) and topaz cichlid (*Cichlasoma myrnae*) were fed with the diet incorporated with carotenoid rich strain of *Spirulina platensis @* 1.5- 2% and 1% of *Haematococcus pluvialis* for 3 weeks results in intense colouration.(Ako et al, 2000).

Varieties of carotenoids are being developed from synthetically or naturally occurring products for ultimate use in aquaculture. Carotenoids derived from natural sources contain mixture of variety of carotenoids Alpha carotene, Beta carotene, zeaxanthin, lutein, cryptoxanthin etc. Whereas synthetic processes provides only specific carotenoids like Beta carotenoids. Synthetic carotenoids are been derived from petro chemicals and other complex organic solvents leads to residual problem in aquatic environment, too expensive and also shows deteriorating the environment if used in excess.

Table2 : List of some authors reported the plant origin carotenoids for enhancement of colour in fishes

Source of Plant origin Carotenoid	Dose with feeding duration of	Name of the experimental fish with size	Reference
Hibiscus petals	5mg/Kg and 8 week duration	Gold fish *Carassius auratus* **Weight-1.55 to 2.20g**	Sinha, A., and Asimi, O. A. 2007.
Marigold	15g/100g and 28 day duration	Red sword tail, *Xiphophorus helleri Weight*- 0.6 g	Ezhil, et al., 2008
Spirulina platensis + *Vitamin C*	30g/kg+ 200mg/Kg and 120 day duration	*Carassius auratus 45 day* **old 0.27g weight**	Vasudhevan and. James 2011.
Rose Petals	4% of feed and 45 days	Rosy Barb (*Puntius conchonius*). **Av. wt. 1.5 g**	Pailan, *etal.*, 2012.
Spirulina platensis	10% of feed and	*Puntius sophore* **Av wt. 1.47g**	Pratibha *etal*.,2012
Marigold Flower and Beetroot Meals	10% duration 60 days	Snow trout, *Schizothorax richardsonii.* Fingerlings Av. Wt.- 9.19±0.29 g	Jha, *etal.*, 2012
Hibiscus petals and Spirulina	5% and 40 day duration	Gold fish *Carassius auratus*. **Weight 3.0 ± 0.5g**	Somanath,*etal.*,2013..
Spirulina plantensis	30g/Kg duration 60 days	Blue Gourami *Trichogaster trichopterus Weight- 2g and length 2.5cm*	Hamlin etal.,.(2013)
Azolla filiculoides	50g/Kg duration 30days	Gold fish *Carassius auratus Av.Wt.-1.13g*	Vasudhevan etal.,2013
Marigold	5% duration 90 days	Red sword tail, *Xiphophorus helleri* Length-2.4 to 2.73 cm	Golandaj etal.,.(2015).

Conclusion

Natural carotenoids are cost effective and more sustainable colouring pigmentation of fishes as compare to synthetic pigmentation/ carotenoids. The natural pigments (carotenoids) are more affordable and can be obtained from locally available ingredients for resource poor ornamental fish farmers. It improves the colour pigmentation and health status of ornamental fishes without disturbing the aquatic environment**.**

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