# Use of natural carotenoids for pigmentation in fishes

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## Abstract

Pigmentation is one of the important quality attributes of the fish for consumer acceptability. Carotenoids are responsible for pigmentation of muscle in food fish and skin colour in ornamental fish. As fish is not capable of synthesizing carotenoids *de novo* there is a need to incorporate carotenoids in the diet of cultured species. Since synthetic carotenoids are known to have deteriorating effects on the environment, there is a great demand for inclusion of natural carotenoids in *aqua* feed to achieve bright coloration in fish. The possible use of naturally available carotenoid rich ingredients such as microalgal pigments (*Chlorella vulgaris* Beijer, *Haematococcus pluvialis, Dunaliella salina* (Dun.) Teodor), yeast extract (*Phafia rhodozyma, Xanthophyllomyces dendrorhous)*, marigold, capsicum, etc. has been discussed in this article.

**Keywords:** Carotenoids, Pigmentation, Fish, Astaxanthin, *Haematococcus pluvialis*, *Dunaliella salina*, *Chlorella vulgaris*, Marigold, Capsicum, Yeast extract.

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## Introduction

Pigments are responsible for the wide spectrum of colours 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 colouring pigments *de novo*, the colouring agents which are synthesized by some plants, algae and microorganisms, need to be incorporated in their diet[1, 2]. Varieties of colouring agents are used in *aqua* industry to impart colour for the muscle and skin of fishes. Thus, pigmentation is an important criterion for fishes, since their colour affect commercial acceptability.

One of the greatest challenges in the ornamental fish industry is to replicate the accurate natural colour of the fish in the captive environment. Numerous operations that have been propagated, failed to successfully market fish due to faded colours. 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 colour enhancement. The most promising carotenoids proved to be successful in enhancing colour is astaxanthin that shows marked improvement in colour on most species of brightly coloured ornamental fishes like, Tetras, Cichlids, Gouramis, Goldfish, Koi, Danios and many other species.

Carotenoids are a class of 800 natural fat-soluble pigments found principally in plants, algae, photosynthetic bacteria and some non-photosynthetic bacteria, and they play a critical role in the photosynthetic process. They also occur in yeast and moulds where they carry out a protective function against damage by light and oxygen. Carotenoids also play other important functions as pro-vitamin-A, antioxidants, immunoregulators and they are mobilized from muscles to ovaries in salmonids, which suggest a function in reproduction[3-5]. It has also been observed that fishes with a high level of carotenoids are more resistant to bacterial and fungal diseases[3].

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. The hydrocarbon carotenoids are known as carotenes, while oxygenated derivatives of these hydrocarbons are known as xanthophylls. Beta-carotene, the principal carotenoid in carrots, is a familiar carotene, while lutein, the major yellow pigment of marigold petals, is a common xanthophyll.

## 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 colours are tunaxanthin (yellow), lutein (greenish-yellow), beta-carotene (orange), alpha, beta-doradexanthins (yellow), zeaxanthin

Swordtail (*Xiphophorus helleri*)

Red mosaic variety of guppy (*Poecilia reticulate*)

(yellow-orange), canthaxanthin (orange-red), astaxanthin (red), eichinenone (red) and taraxanthin (yellow). Among these, dominant carotenoid is astaxanthin, which is common in red fishes. The pink coloration of salmon flesh is mainly due to astaxanthin. Lutein pigment is common to freshwater fishes, but also, widely found in many marine species. Tunaxanthin is common in scombrina, carangina and percina fishes, but abundant in yellow-coloured fishes, like yellow tail (*Seriola quinqueradiata*). 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 carotenoid into another carotenoid. Based on this capacity, fishes are classified into three types:

(i) *Red carp type* : In this group lutein is converted into astaxanthin molecules.

(ii) *Sea bream type* : In this group of fishes lutein and carotene remain in the tissues and cannot be transferred in any other form inside the bodies.

(iii) *Prawn type* : The beta-carotene molecule can be converted in to astaxanthin molecule.

## Improvement of colour in fishes

Dietary supply of carotenoids can improve the skin colour as well as market value of ornamental fishes. The pigmentation of Goldfish and koi is improved by addition of carotenoids and these fishes are found to be capable of metabolizing zeaxanthin to astaxanthin. However, Goldfish lack the ability to metabolize lutein and have limited ability to convert β-carotene to astaxanthin[6]. Skin pigmentation in tiger barb, *Barbus tetrazona,* has been reported to increase, when fed with diet containing carotenoids from shrimp meal, marigold petal and annatto seed extract. The blue green alga has also been used as a source of pigmentation for Koi carp[7].

In Goldfish, *Carassius auratus*, the optimum level of astaxanthin for intense coloration was found to be 36-37mg/kg diets and the supplementation significantly improved the survival rate[8]. In red velvet sword tail (*Xiphophorus helleri*), rainbow fish (*Pseudomugil furcatus*) and topaz cichlids (*Cichlasoma myrnae*) the intensity of coloration significantly improved when fed a diet containing 1.5-2% of a carotenoid rich strain of *Spirulina platensis* and 1% of *Haematococcus pluvialis* for 3 weeks[9].

A variety of carotenoids both synthetic and naturally occurring products are available or are being developed for use in aquaculture. Carotenoids derived from natural sources contain mixture of several carotenoids like α-carotene, β-carotene, zeaxanthin, lutein, cryptoxanthin, etc. whereas synthetic processes provides only specific carotenoids like β-carotene. Contrary to this, synthetic processes involve petro-chemical solvents and other complex organic solvents, leading to residue problems. Further, synthetic carotenoids are expensive and it has limitation to be used in *aqua* feed formulation depending upon the species. If used in excess synthetic carotenoids lead to deteriorating effect on the environment.

Natural carotenoids are categorized into two groups as plant and animal based carotenoids. The contents of these sources are given in Table 1.

**Table 1 : Contents of carotenoids from 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 | 100-130 | Yeast (*Phaffia rhodomyza*) | 1000 |
| Shrimp oil | 25-125 | Sea weed | 390-900 |
| | | Corn gluten | 290 |
| | | Alfa alfa | 280 |

**Animal based natural carotenoids**

The commercial natural astaxanthin production utilizes by-products of crustacean such as the Antarctic krill (*Euphausia superba*), crayfish meal, shrimp meal, crab meal, etc. These are rich sources of carotenoid astaxanthin and are used in *aqua* feed formulation as additive. However, animal based natural carotenoids are limited in supply as there is declining trend in catches of crustaceans like shrimp, crabs, crayfishes, etc. from marine landing resources. Besides, they are very expensive sources of carotenoids and thus aquaculture feed production becomes costlier.

**Plant based natural carotenoids**

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 widely. Feed ingredients such as yellow corn, corn gluten meal and alfa alfa are also used as sources of carotenoids in *aqua* feed formulation. Other carotenoids rich ingredients used are marigold meal (lutein) and red peppers (***Capsicum*** sp.) extract. 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 *Arthospira maxima* will accumulate secondary carotenoids and their biomass can be used as a colouring ingredient in aquaculture.

The freshwater micro alga, *H. pluvialis* has been commercially exploited for aquaculture primarily due to its rapid growth and high astaxanthin content[10-13]. It is the primary source of pigmentation in ornamental or tropical fish, responsible for various species-related yellow, red and other colours. These are obtained through carotenoids containing organisms in the aquatic food chain. *H. pluvialis* together with the yeast *X. dendrorhous* is considered the best microbial source of astaxanthin[1].

The biflagellate alga, *D. salina* is a source of β-carotene and used as natural food colouring agent in *aqua* feed industry. Under appropriate culture conditions, some strains of *D. salina* were reported to accumulate up to 10% carotenoids consisting mostly of β-carotene[14-16]. The bioavailability of β-carotene is greater when used with vegetable oil. It is an inexpensive and best source of natural mixed carotenoids. The discovery of commercial production of natural β-carotene from *Dunaliella* is currently a substantial and growing industry. It has been reported that 125 ppm β-carotene from 6.25g agro based feed mix/kg diet gave excellent pigmentation and higher doses 200 ppm and 300 ppm further improved pigmentation. *A. maxima* has also been used in rainbow trout culture for colour enhancement purposes[7].

The micro alga, *C. vulgaris* has become a potent pigment source, which imparts yellow/blue hues. The biomass of this alga had already been proved to be useful in the diets of rainbow trout yielding both muscle and skin pigmentation effects[17-19] and in gilthead sea bream for skin pigmentation[20]. It has also been reported that it contains carotenoid pigments in concentrations of up to 0.4% (dry wt), of which 80% were potential red hue inducing pigments[21].

## Conclusion

In view of the deteriorating effects on the environment due to use of synthetic pigments, the researchers are emphasizing the need for natural pigment colouring agents which will act as an alternative to synthetic chemicals. As the *aqua* feed industry seeks a natural, environment friendly source of pigment to improve coloration and to enhance commercial acceptability, there is a great

potential for use of natural plant based carotenoids for pigmentation in aquaculture. It paves the way to many *aqua* feed industries to promote their products as natural with a distinct shift away from synthetic ingredients and colorants.

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