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## A review on the application and effect of carotenoids with respect to canthaxanthin in the culture of fishes and crustaceans

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### Abstract

Fisheries sector serves as a substantial source of income and employment for a huge number of individuals around the world and it comparatively remains a crucial source of food and nutrition. Culture of aquatic species for food and ornamental purpose has been on increasing trend. High-performance feeds are formulated for the growth and progress of the cultured animals, usually supplemented with carotenoids of natural and synthetic origin. The market acceptability of ornamental fishes depends on pigmentation, one of the considerable attributes for determining quality. Carotenoids are a group of fat-soluble pigments broadly distributed in nature and are well recognized for their bright red, yellow, and orange colours. Studies on these carotenoids over decades have shown that these carotenoids are beneficial for the fish and crustacean aquaculture and also in the ornamental trade by imparting pigmentation. Canthaxanthin falls under the category of red keto-carotenoid and is one of the most important xanthophylls in the business perspective due to its extensive application in the field, next to astaxanthin. A large portion of the commercial canthaxanthin available in the market are chemically synthesized as the naturally available canthaxanthin are found in very lower concentrations. In addition, to a lesser extent, they are also implicated in the growth performances, digestion and as an anti-oxidant. This review updates the ongoing advancement on canthaxanthin on the dietary utilisation, storage, pigmentation and other enhanced growth performances on the aquaculture of food fish, crustacean and ornamental fishes.

**Keywords:** Carotenoids, canthaxanthin, food fishes, ornamental fishes, crustaceans

### Introduction

The population across the whole world has seen a tremendous increase in numbers and experts have estimated that the numbers would reach around 9 billion by the year 2050 from the present population of 7.7 billion in the year 2018 <sup>[1]</sup>. There is an increased demand and consumption of fish in the recent past, as the nutritional status of the fishes were continuously reported and created awareness among the public. Fisheries sector serves as a substantial source of income and employment for a huge number of individuals around the world and it comparatively remains a crucial source of food and nutrition. The fisheries sector has two sources of satisfying the needs of the people, that is capture fisheries and aquaculture. The marine and inland fish capture contributes almost above 50 percent of the global fish demand, the aquaculture industry contributes to the rest of global fish consumption <sup>[2]</sup>. Even though the world capture fisheries production increases annually, a stable and nearly constant production from it across the years have indicated that the we have reached the peak. While on the other side, the aquaculture production tends to be dynamic and on the increasing trend. The trade of ornamental fishes crossed over 2 billion live fishes and the worldwide ornamental industry was estimated to be about US\$ 15 billion <sup>[3]</sup>. Though the majority of ornamental fishes traded in the global market were from freshwater, there is an increasing trend in the proportion of the marine fishes. India's contribution to world ornamental fish trade is <1%, but many potential resources are still untapped.

Carotenoids are colouring pigments that were able to enhance the coloration of the fishes. Commonly used carotenoids in the aquafeed industry are astaxanthin and canthaxanthin come

under the xanthophylls. These xanthophylls apart from enhancing the colour of the fishes, they also play a substantial role in growth, immunity, digestibility, stress activities of the farmed species. For an interminable time, astaxanthin has been the major choice of use for the colouring pigment. Synthetic canthaxanthin have proven to deliver similar results as that of astaxanthin in the recent decades. Thus, the intention of this review is to give an overview of the canthaxanthin pigment with respect to the other carotenoid pigments and its effect in the aquaculture industry.

### Pigment incorporation in aquafeed formulation

The most important factor to be considered in the farming of food fishes and ornamental fishes is the feed that is given to the cultured animals. It becomes so irresistible that it almost contributes 60% of the total production cost in the culture of fishes. The usage of raw materials effectively in diets relies on the capacity to formulate the diets to endure digestible energy and nutrient requirements. Formulation issues, explicitly the supply of species-specific feeds to necessitate the nutritional requirements of various developmental phases of the farmed species, remain an essential topic for both commercial and farm-made feed production sectors. "Pigment" in connection with animal nutrition is generally a term for designating carotenoid compounds existing in raw materials, feedstuffs, or component that has a colouring effect on the tissues of the animals (flesh, exoskeleton, muscle, fat, skin) or on derived products like egg yolk. This method of incorporation of food colouration is an indirect method that forms a major component in the modern animal production [4]. Colour plays an important primary attribute anticipated and directly related to the taking or the dismissal of the products in the market [5]. Unfortunately, the fish cannot synthesize their own colouring pigments [6]. Hence various colouring agents synthesized by some algae, plants, yeasts, microorganisms and synthetic ones are incorporated in the diets of the farmed species.

Farming of salmonids depends mostly on the market demand and good organoleptic grades are required. The pink colour flesh fetches higher demand in the market. Hence the salmon industries are focusing mainly on achieving efficient strategies to enhance the market demand by enhancing the colour of the fish, as it is known that the colour of the fish plays a major role that they earn higher price in the market. Therefore, farmed salmonids are often fed during the grow out period with carotenoid-enriched diets. Many studies have been carried out on carotenoid pigments on food fishes like Atlantic salmon *Salmo salar* L. [7], red porgy *Pagrus pagrus* [8], Australian snapper *Pagrus auratus* [9], rainbow trout *Oncorhynchus mykiss* [10, 11], channel catfish *Ictalurus punctatus* [12], large yellow croaker [13]. Among many physical attributes in regard to ornamental fish trade, the most important quality parameters that greatly governs the market value of an ornamental fish is the colouration [14]. Under the captive culture environment, the ornamental industry is facing the greatest challenge to imitate the natural colour of the fish. The fishes usually fed with a normal diet fetch lower price as the colour of the fish fades away due to the lack of the colouring pigment in the diet. Many studies on ornamental fishes with colouration has been reported on Korean rose bitterling fish *Rhodeus uyekii* [15], goldfish *Carassius auratus* L. [16], charachins *Hypessobrycon callistus* [17], koi carp *Cyprinus carpio* L. [18, 19], and clown fish *Amphiprion ocellaris* [20].

### Carotenoids

Carotenoids are a group of fat-soluble pigments derived mainly from the biosynthesis of algae and plants, are widely distributed in nature. They are also produced by several archaea, yeasts, fungi, and eubacteria and found in the whole animal kingdom through the food chain by selective absorption of the pigments. These diverse groups are hydrocarbons with 40 carbon atoms, comprises over 700 compounds. Carotenoids play a major substantial role in the photosynthesis of the plants. They are popularly found among the widely distributed pigments and are well recognized for their bright yellow, orange and red colours. Due to the complex derivatizations of these isoprenoid skeleton structures (C<sub>40</sub>), a large number of structures are formed which are responsible for their vast physical, chemical and biological properties, including the formation of the colours. These colouring pigments are often used in the animal feed industry for their colouring properties [21].

Besides their broad use as colorants, they are also used for the fortification of food, as some of the carotenoids have the capacity to act as provitamin A and impart health benefit by reducing the risk of degenerative diseases [22], antioxidant properties [23] and hypolipidemic activities [24]. In commercial industries, two types of carotenoids are used either natural concentrates like extracts or synthetic colours. These carotenoids are analysed by absorption spectroscopy, as they can absorb particular UV and visible spectrum by producing colours [21].

### Effects of carotenoids in the body of aquatic organisms

Dietary inclusion of carotenoids has been practiced to provide desirable coloration to the salmonids and other farmed fishes, many crustaceans and ornamental fishes. Most common carotenoids found in the fish are astaxanthin (pink-red), canthaxanthin (orange-red), lutein (greenish-yellow), zeaxanthin (yellow-orange),  $\beta$ -carotene (orange), tunaxanthin (yellow), taraxanthin (yellow), and  $\alpha$  /  $\beta$  doradexanthins (yellow). Astaxanthin is commonly found in salmonids and red pigmented fishes. Though lutein is present in plenty of marine species, they are more common in freshwater fishes. Tunaxanthin is found in yellowtail and are commonly seen in Scombridae, Carangidae, and Percidae family [25].

Some authors have mentioned traditionally that the coloration of the fish is associated with the superior flavour [26]. There is also a perception among the consumers that the colours of the fish are associated with healthy animals and of higher quality. The importance of colouration in the fishes are reported in salmonids [27-29], *Pagrus major* [30], brown trout [31]. Carotenoids are carried in the chromatophores in the fish. Apart from the above mentioned, the colour of the fish also plays major roles in communication, speciation, sexual attraction and ecological interactions [25]. The change of possession of astaxanthin pigment from the fish flesh to the skin on maturation has also been reported [32]. Most fishes have their own pigment in the body like astaxanthin, whereas yellow pigments like lutein and tunaxanthin are acquired. The yellowtail and Red sea bream often convert the dietary astaxanthin into tunaxanthin and then deposit in their skin which is the reason for their yellow colour.

Skin colour is the most important characteristics in the ornamental industry. The colour of the skin is the major factor that affects the market value of the ornamental fishes and it

plays a significant role in the overall assessment [33]. In goldfish, the astaxanthin is readily metabolized from the zeaxanthin (yellow pigment) which is the reason for their peculiar pink-red colouration of skin [34]. Some ornamental species like goldfish and koi are efficient of acquiring astaxanthin in the feeds by metabolizing zeaxanthin. Though majority of the colouring pigments for the ornamental fishes are derived from live feeds, they are also supplied through carotenoid-rich feed ingredients. Astaxanthin shows a marked enhancement in the colour of most widely used ornamental fishes like goldfish, koi, gourami, tetras, cichlids, zebra fish, etc. [25, 35]. Supplementing dietary canthaxanthin, bixin and  $\beta$ -carotene are also commonly incorporated in ornamental fish feeds.

Majority of the crustaceans and their tissues are featured by the presence of numerous carotenoids present [36]. The existence of carotenoids helps in higher pigmentation, growth enhancement, higher survival, improved resistance to stress and enhanced reproductive potential [37-39]. Carotenoids play an important role in tolerating environments that are polluted, by observing lower levels of vitamins and carotenoids in the hepatopancreas of crayfish when exposed to polluted environment [40]. The appearance of bright and relevant colour in crustaceans (shrimps) are correlated with the quality and freshness. The exoskeleton of lobsters, shrimps, and crabs contain different colours. Though the red colour is due to astaxanthin, the exoskeleton is dark greenish to brown shell colour as the molecules of the crustaceans are wrapped up in protein chains. When these protein molecules are denatured on heating, the exoskeleton changes its colour to red due to the presence of astaxanthin [41].

Though the carotenoids are not essentially required for the aquatic animals, they tend to be useful for their health [42]. Apart from coloration properties of the carotenoids, they involve in some of the important biological functions. The antioxidative properties for the dampening of singlet oxygen molecule ( $^1O_2$ ) and curb lipid peroxidation [43]. Lysozyme and integrated proteins in fish are determined by the availability of carotenoid pigments in birds and fish [44, 45].

### Canthaxanthin and its history in aquaculture

Canthaxanthin falls under the category of red keto-carotenoid [46]. Most of the commercially available canthaxanthin are chemically synthesized as the naturally available canthaxanthin are found in very lower concentrations [47]. They are more commonly as an additive used in poultry feeds for yielding red colour in yolks and skin [46]. The use of canthaxanthin in United states has been limited to below 30 mg/0.74 L for the liquid food and at a level lower than 30 mg/0.45 kg canthaxanthin to be used in solid or semi-solid foods [48], while the European Union suggests the use in feedstuff at a highest level of 25 mg/kg of final feedstuff [49].

Salmonids mainly absorb astaxanthin and canthaxanthin and deposit them in the muscles during the earlier stages and during sexual maturation, they move them to the gonads and skin [50]. On comparing the deposition of the pigments in the flesh, the combination of both the carotenoid gave a higher deposition result than the deposition of using either a single carotenoid. He also stated that the deposition of the carotenoid increases with the increase in the weight of the fish. The absorption and deposition of astaxanthin in the flesh of rainbow trout was observed to be higher than the canthaxanthin [51]. The reasons for the differences in the deposition of the carotenoid in the flesh may be due to the

variation in the metabolic turnover of a particular carotenoid, differences in the digestibility of the carotenoid and may be due to the preferred absorption and deposition of that particular carotenoid i.e. preference to astaxanthin than the canthaxanthin.

Canthaxanthin is one of the most crucial xanthophylls in the business perspective due to its extensive application in the field. Commercial marketing of canthaxanthin was started in the 1960s and was used predominantly in the salmon and trout diets. As astaxanthin is more efficient than the canthaxanthin, the former took over the market in the 1980s by almost completely replacing the latter. However, it is still in use in many countries as an additive along with astaxanthin. Apart from colouring agents, the canthaxanthin finds its appliance in the field of cosmetics, human skin applications, feed additive for aquatic organisms, etc. They have been found to be useful in the polymorphous light eruption treatment and idiopathic photo dermatosis. The market for canthaxanthin globally is approximately US\$100 million [47].

### Sources of canthaxanthin

Canthaxanthin ( $C_{40}H_{52}O_2$ ) was detected first as a major colouring pigment in the comestible mushroom (*Cantharellus cinnabarinus*), hence the name canthaxanthin. Though it occurs naturally, they are frequently found in fishes (golden mullet, carps, wrasse and seabreams), crustaceans, bacteria and some green algae. Canthaxanthin was reported as an intermediate product in the metabolism from  $\beta$ -carotene to astaxanthin [52]. One of the promising sources of canthaxanthin from the microorganisms is the bacterium *Dietzia natronolimnaea* HS-1 [53]. This bacterium has the ability to produce upto 9.6 mg  $l^{-1}$  canthaxanthin [54]. Canthaxanthin production was stimulated in *Chlorella emersonii* by nitrogen limitation [55]. Another strain of a bacteria *Bradyrhizobium* sp. was found as the producer of canthaxanthin and their full carotenoid gene cluster was sequenced. *Haloferax alexandrines* an extremely halophilic archaea also found to produce canthaxanthin. HPLC-MS extraction revealed that the isolates of a gram-positive bacteria producing pink colony namely *Gordonia jacobea* is canthaxanthin. The carotenoid content in these isolates are low and does not meet the industrial application. However, mutation was carried out and found to be 6 times more accumulation than the wild strain [52]. In the European Union, Canada and the United states, non-esterified and synthetically commercially available canthaxanthin are used along with the other pigment astaxanthin in salmon feeds.

### Canthaxanthin on growth performances and digestion

The studies related to dietary supplementation of carotenoids with shrimps and the functioning of the carotenoids could enhance the utilisation of the nutrients thus leading to enhanced growth performances apart from acting as an origin of pigmentation [39]. But there were many opposing views in the previous studies in relation to carotenoids on growth enhancement and survival. Recent study showed that the dietary supplementation had improved growth and survival of Lake Kurumoi rainbowfish [6]. Similar reports on enhancement of growth and survival on supplementation of astaxanthin and canthaxanthin was in reported in Atlantic salmon fry and juveniles [56]. Astaxanthin – canthaxanthin supplementation for kuruma prawn resulted in higher survival rate but no significant differences were found on growth [57]. Apparent digestibility coefficients give an idea on how well a

particular nutrient is digested and how far the digested material affects the outcome of the research. The ADC values of carotenoids (Astaxanthin, Canthaxanthin and lutein) ranged 77 to 79% [6]. Results of other studies like 100 mg/kg of dietary canthaxanthin in rainbow trout has ADC value of 61.4% [58] and 130 mg/kg of dietary canthaxanthin in rainbow trout has ADC values ranging from 54% and 64% [59]. Studies on ADC values on supplementation of astaxanthin and canthaxanthin increased the utilisation of protein and lipid as indicated by their higher ADC values [10].

### Canthaxanthin as an antioxidant

Usually carotenoids neutralise the peroxy radicals that leads to oxidative damage of the lipoproteins and cell membranes [46]. In trout, enhancement of total antioxidant ability was observed by the dietary administration of either astaxanthin or canthaxanthin or their combination [60, 61]. Short term treatment of canthaxanthin in liver or kidney of *Oncorhynchus mykiss* was found to be a weak inducer of the xenobiotic-metabolizing enzyme [62]. Canthaxanthin in particular, suppress the free radicals in unsaturated fatty acids, thus acting as a radical scavenger. Although Glutathione peroxidase (GPx) activity increases in rainbow trout on addition of canthaxanthin and astaxanthin – canthaxanthin diets, it was not accompanied by increased SOD levels [61].

### Conclusion

The focus on the role of individual additives are becoming more and more debatable as many researchers tend to specify the unique use of that particular additive in relation to improved and enhanced growth of the aquatic animals. The role of carotenoids in the nutrient utilisation is one among the additive that has recently received more attention as it is now clear that the carotenoids are important for the growth performances and survival of many farmed species. Though the carotenoid astaxanthin has an important role in the aquafeed industry across the world, canthaxanthin to a lesser extent also plays a major role in the supplementation as both of them are widely recognised as safe and more efficacious pigments. Apart from the use for pigmentation of aquatic animals, they also find application in the dairy foods, meats, cosmetics, beverages, cereal products and pharmaceuticals. The development of new strains of microbes yielding the carotenoid pigments are an important breakthrough in the development of carotenoid research. More strategies are required to improve the fermentation process of microbes as the production could be boosted up to the industrial scale, so that the biological products of carotenoid could become competitive for the synthetically prepared ones.

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