The use of carotenoid in aquaculture

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ABSTRACT

Aquaculture is a rapidly growing global industry, comprising cultivation of various freshwater and marine species of finfish, shellfish, mollusks and ornamental fish. By the year 2020, as much as 20% of the world production of fish will be based on aquaculture. Properly formulated feeds are significant part of successful aquaculture. Carotenoids play a major role in commercial aquaculture. Various biological and nutritional role of carotenoids in aquatic animals are documented along with the effect of biotic and abiotic factors on carotenoids transport and retention and final flesh pigmentation. Pigmentation is one of the important quality attributes of the aquatic animal for consumer acceptability. Carotenoids are responsible for pigmentation of muscle in food fish and skin color in ornamental fish. The functions, sources and importance in aquaculture are discuss in this review.

Key words: Carotenoids, pigmentation, aquatic animals, carotenoids function, carotenoid absorption, source of carotenoids.

Introduction

In the last few decades there has been remarkable growth of the aquaculture industry (9.2% per year since 1970) due mainly to a plateau in wild capture and limited stock potential (FAO, 2002), together with an increase in world population and global per capita fish consumption which has doubled over the past 50 years (Ahmed and Delgado, 2000). Besides these factors, aquaculture has also been promoted in view of the fact that it provides consumers with safe, nutritious and high quality food products.

High quality aquaculture products must fulfill several requirements well appreciated by consumers, including the adequate color, which is among the most important fish quality parameters in the market. Color is the first characteristic perceived and is a determinant selection criterion, directly related to the subsequent acceptance or rejection (Shahidi et al., 1998).

Pigments are responsible for the wide spectrum of colors 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 coloring pigments de novo, the coloring agents which are synthesized by some plants, algae and microorganisms, need to be incorporated in the diet (Johnson and An, 1991). Varieties of coloring agents are used in aquaculture industry to important criterion for fishes, since their color affect commercial acceptability.

One of the greatest challenges in the ornamental fish industry is to replicate the accurate natural color of the fish in the captive environment. Numerous operations that have been propagated, failed to successfully market fish due to faded color. Various products have been introduced to alleviate this problem, but none has performed so effectively and consistently as carotenoid pigment. Varieties of carotenoid pigments are used in fish diet for color enhancement. The most promising carotenoids proved to be successful in enhancing color is astaxanthin that shows marked improvement in color on most species of brightly colored ornamental fishes like Tetras, Cichlids, Gouramis, Goldfish, Koi, Danios and many other species.

Carotenoids In Aquaculture:

In the aquaculture industry, carotenoids have been included in diets of salmonids, crustaceans and other farmed fish, mainly as pigments to provide desirable coloration to these cultured organisms. Consumers subconsciously relate product color to nutritive value, healthiness, freshness and taste. Therefore, color is a decisive quality criterion that has to be maintained and optimized. Carotenoids may not only contribute in improving quality by enhancing color, but could also help to a better image in minds of consumers of aquaculture products, in view of increasing information available on carotenoids positive effect on human
health. Aside from their quality enhancing properties, carotenoids seem to improve certain production parameters of farmed species. However, most of the research up to date has focused on their role as pigment.

Traditionally, it has been held that coloration is associated to superior flavor, an opinion that still persists (Clydesdale, 1993; Sylvia et al., 1995; 1996). In fish, color is much more than a cosmetic effect; consumers associate natural coloration with healthy and high quality products. For instance, muscle pigmentation in farmed salmonids is regarded as the most important quality parameter after freshness (Koteng, 1992). Aside from flesh pigmentation, vivid skin coloration of cultured red and yellow skinned fish such as red porgy (Pagrus pagrus) (Basurco and Abellán, 1999), Japanese red sea bream (Pagrus major) (Lin et al., 1998), Australian snapper (Pagrus auratus) (Booth et al., 2004; Doolan et al., 2005) and yellowtail (Seriola quinqueradiata) (Miki et al., 1985) are well appreciated and lead to high market values. In ornamental fish, skin color, is as well an important characteristic affecting market price and playing a major role in the overall appraisal (Gouveia and Rema, 2005).

In crustaceans, such as shrimp, a bright and appropriate color is also associated with freshness and quality and the desired coloration must be preserved through storage, processing and cooking (Boonyaratpalin et al., 2001). In the sea urchin industry, based on the production of marketable gonads, the highest commercially valuable sea urchin gonads are bright yellow-orange (Shpigel et al., 2001).

Four main groups of pigments account for the coloration of mammals, birds, fish and invertebrates of economic importance. These are porphyrins, pteridines, melanins and carotenoids (Hudon, 1994). Porphyrins are of primary importance mainly in the coloration of avian eggshell (Kennedy and Vevers, 1976; Lang and Wells, 1987). Pteridines are responsible for many of the bright yellows and reds in fish, amphibians and reptiles (Nixon, 1985; Ziegler, 1965); these pigments are water soluble and are produced endogenously (Hudon, 1994). Melanin gives all the blacks, greys and browns to vertebrates and many invertebrates, and also several of their reds and yellows. Melanins are heterogeneous polymers made up of metabolites of tyrosine (Hudon, 1994). Carotenoid pigments, obtained by animals from their diets, give most of the bright red, yellow and orange colors well appreciated not only in aquaculture (Toyomizu et al., 2001).

**Carotenoids:**

Carotenoids are among the most common natural pigments responsible for many of the hues found in nature as well as a variety of functions. 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 (Schedl, 1998). In the animal kingdom, carotenoids are the most widely occurring pigments after melanin. They play a critical role in the photosynthetic process and 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 muscle to ovaries which suggest a function in reproduction (Shahidi et al., 1998; Nakano et al., 1999; Bell et al., 2000). It has also observed that fishes with a high level of carotenoids are more resistant to bacterial and fungal diseases (Shahidi et al., 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.

**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 colors are tunaxanthin (yellow), lutein (greenish-yellow), -carotene (orange), .. -doradexanthins (yellow), zeaxanthin (yellow-orange), canthaxanthin (orange-red), astaxanthin (red), eichinenone (red) and taraxanthin (yellow). Many fish accumulate carotenoids in their integuments and gonads. On the other hand, Salmonidae fish peculiarly accumulate 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.

Cyprinidae fish can synthesize (3S,3’S)-astaxanthin from zeaxanthin by oxidative metabolic conversion. On the other hand, Perciformes and Salmonidae fish cannot synthesize astaxanthin from other carotenoids (Matsuuro et al., 2001). Therefore, astaxanthin present in these fish originates from dietary crustacean zooplankton. Astaxanthin in these fish comprises three optical isomers. Perciformes and Salmonidae fish can convert astaxanthin to zeaxanthin (Matsuuo et al., 1985). Therefore, zeaxanthin in these fish also exist as three optical isomers (Maoka et al., 1986).

Tunaxanthin is widely distributed in fish belonging to Perciformes. The bright yellow color in the fins and skin of marine fish is due to the presence of tunaxanthin. Feeding experiments involving red sea bream and
yellow tail revealed that tunaxanthin was metabolized from astaxanthin via zeaxanthin (Matsuno et al., 2001). Carotenoids with a 3-oxo-end group such as -carotene-3,3-dione are key intermediates in the metabolic conversion (Matsuno et al., 1985).

Unique apocarotenoids were reported from integuments of the black bass (Micropterus salmoides) (Yamashita et al., 1996). They were assumed to be corresponding oxidative cleavage products of tunaxanthin, lutein and alloxanthin.

Since 2000, there are a few reports on new structures from fish. Carotenoids with a 3,6-dihydroxy-end group, salmoxanthin, deepoxysalmoxanthin (from the salmon (Oncorhynchus keta) (Tsushima et al., 2001), and gobiusxanthin (from the freshwater goby (Rhynogobius brunneus) were isolated (Tsushima et al., 2000). A series of carotenoids with 7,8-dihydro- and/or 7,8,7',8'-tetrahydro polyene chain were isolated from the integuments and eggs of the Japanese common catfish (Silurus asotus) (Tsushima et al., 2002). Recently, new carotenoids, 7,8,9,10-tetrahydro- -cryptoxanthin, 7,8-dihydrodioxanthin and (3,8S,6S,S)- -cryptoxanthin, were isolated from the integuments and gonads of the Japanese common catfish as minor carotenoids (Maoka and Akimoto, 2011).

Carotenoid Absorption And Transport:

Carotenoids are hydrophobic compounds that are not easily solubilized in the aqueous environment of the gastrointestinal tract of fish; therefore, digestion, absorption and transport processes are associated to lipids (Castenmiller and West, 1998).

The intestinal absorption of carotenoids involves several 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; Torrissen, 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. Furthermore, many authors suggest that the intestinal absorption from micelles is a passive diffusion process (Choubert et al., 1994; Parker, 1996; Castenmiller 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 re-esterification of free carotenoids with endogenous fatty acids (Foss et al., 1987). Studies in salmonids species regarding absorption have been assessed through monitoring carotenoid levels (Choubert et al., 1994; Kiessling et al., 1995; Gobantes et al., 1997; White et al., 2002, 2003). These levels are affected by metabolism, absorption and excretion processes (Castenmiller and West, 1998). Therefore, this approach is informative but does not quantify carotenoid uptake by the intestine (White et al., 2003).

Salmonids preferentially absorb more polar carotenoids, particularly astaxanthin rather than canthaxanthin, zeaxanthin or carotenes (Schiedt et al., 1985; Guilhou et al., 1992; Foss et al., 1984). The unesterified or esterified carotenoids forms also seem to influence absorption. Many studies have led to contradictory results, with authors claiming that the free form is better absorbed than the ester form (Schiedt et al., 1985; Foss et al., 1987; Storebakken et al., 1987; Choubert and Heinrich, 1993), while other report that both forms are equally absorbed (Barbosa et al., 1999; Bowen et al., 2002). Japanese red sea bream seems to absorb synthetic astaxanthin dipalmitate more efficiently than unesterified astaxanthin as reflected by skin pigmentation results (Ito et al., 1986).

In regards to transport, due to the hydrophobic nature of carotenoids these cannot circulate freely in plasma, and must be associated to plasma lipoproteins (Aas et al., 1999). Fish carotenoids are mostly transported to peripheral tissues by high density lipoproteins (HDL) (Nakamura et al., 1985) and to a limited extent (5-7%) by low density lipoproteins (LDL) (Ando et al., 1985). In rainbow trout and other Oncorhynchus species, astaxanthin and canthaxanthin were found to be present in all serum lipoprotein fractions (Choubert et al., 1994; Choubert and Heinrich, 1993). In mature female fish, significant amounts of carotenoids also bind to vitellogenin, a female specific serum lipoprotein (Ando et al., 1985). During sexual maturation of Oncorhynchus keta, HDL and vitellogenin (VtG) were associated with carotenoid transport during redistribution of carotenoids from muscle to the integument, and from muscle to ovaries, respectively (Ando and Hatano, 1988). Albumin, an abundant soluble protein in the body of all vertebrates and a major transport protein for fatty acids and hydrophobic compounds (Sheridan, 1988; Peters, 1996), is also suggested to play an important role in blood transport of carotenoids of Atlantic salmon (Aas et al., 1999).
Metabolism And Deposition:

In fish, reductive and oxidative metabolic transformations play an important role (Schiedt, 1998). Carotenoid metabolism is suggested to 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 (Torrisen et al., 1989; Storebakken and No, 1992; Ytrestøylet et al., 2005). Early studies established a classification based on carotenoids metabolic capacity of fish (Tanaka, 1978): A first type of fish cannot oxidize the ionone ring and, therefore, the specific oxygenated derivatives have to be included in their diet. A second 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).

Salmonid species are capable of reducing, but not oxidizing dietary carotenoids to their own tissue-specific molecules. These reductive metabolic reactions involve the stepwise removal of the keto group at 4 and 4’ positions of the ionone ring (Matsuno and Tsushima, 2001). The skin of this group of fish presents predominantly astaxanthin esters, when fed astaxanthin either free or esterified (Bjerkeng et al., 2000). In a study carried out with Arctic charr, aside from mono- and di-ester astaxanthin, small amounts of unesterified astaxanthin and yellow xanthophylls (iodaxanthin, tunaxanthin, lutein and zeaxanthin) were found, all of them expected metabolites of astaxanthin (Bjerkeng et al., 2000). When canthaxanthin was included in salmonid diets, -carotene prevailed in the skin, followed by echinone and finally canthaxanthin; in Arctic charr skin the presence of isocryptoxanthin was also reported (Bjerkeng et al., 1990; Metusalach et al., 1996). All these carotenoids are reductive metabolites of canthaxanthin, -carotene being the end product.

Yellowtail, an extensively cultured species in Japan that is characterized for exhibiting bright yellow bands near the lateral line, also seems to reduce ingested carotenoids (Miki et al., 1985). The yellow color observed in the integuments is conformed predominantly by tunaxanthin, although astaxanthin is the prevalent carotenoid in their natural diet. These observations suggest that astaxanthin must be reductively converted into tunaxanthin by the removal of the keto group at 4 and 4’ positions of the ionone ring, and the conversion of -ring to -ring (Miki et al., 1985). When adult yellowtails were fed a lutein diet, an increase of skin tunaxanthin was also observed (Schiedt, 1998). Therefore, both astaxanthin and lutein could be tunaxanthin precursors, following the pathways. In black bass (Micropterus salmoides) tunaxanthin is also the predominant carotenoid in the integuments; no astaxanthin is found even though its diet is based on crustaceans, which is rich in astaxanthin (Yamashita and Matsuno, 1992). Striped jack (Caranx delicatissimus) presents more than 90% of tunaxanthin, lutein and zeaxanthin in the integuments, and when this fish is fed Spirulina maxima the content of these carotenoids increases (Shahidi et al., 1998). In both striped jack and black bass a reductive metabolic pathway from astaxanthin or zeaxanthin to tunaxanthin is suggested.

In relation to bright pink red skinned fish, such as Japanese red sea bream, astaxanthin esters are the predominant carotenoids deposited in their integuments, and in minor amounts, tunaxanthin (Tanaka et al., 1976; Allahpichay et al., 1984; Nakazoe et al., 1984). The inclusion of natural or synthetic dietary astaxanthin returned the characteristic skin color, so well appreciated and lost under farmed conditions. Japanese red sea bream fed diets supplemented with -carotene and canthaxanthin, showed a decrease in skin carotenoid level, however when fed zeaxanthin or lutein a certain increase was shown, although not comparable to levels achieved when fed an esterified astaxanthin source (Nakazoe et al., 1984). Japanese red sea bream is capable of reducing but not oxidizing dietary carotenoids. The increase in skin carotenoid concentration observed with zeaxanthin and lutein supplementation could be due to the transfer of these carotenoids unchanged or perhaps due to a reductive metabolic process of both lutein and zeaxanthin to tunaxanthin; in yellowtail this transformation has also been suggested (Miki et al., 1985).

Function Of Carotenoids:

In fish, carotenoids have similar functions as those found in other animal species: they are vitamin A precursors (Schiedt et al., 1985; Guillou et al., 1992; Christiansen et al., 1994; White et al., 2003); markedly affect reproduction performance (Craik, 1985; Christiansen and Torrissen, 1996; Verakunpiriya et al., 1997; Chou and Chie, 2001; Vassallo-Agius et al., 2001); are potent antioxidants (Bjerkeng and Johnsen, 1995; Shimizu et al., 1996; Nakano et al., 1999; Bell et al., 2000); enhance immune system (Nakano et al., 1995; Amar et al., 2003); and affect liver structure (Segner et al., 1989; Page et al., 2005). Although some authors claim that the biological functions of carotenoids in fish are still speculative (Choubert et al., 2005), other consider these compounds as important micronutrients that fish are not able to synthesize and, therefore, must be included in the diet (Baker et al., 2002).
Vitamin A precursors:

It is well-known that carotenoids have an unsubstituted -end group, such as -carotene, -carotene, and the -cryptoxanthin precursor of vitamin A in animals. Furthermore, canthaxanthin was also converted to retinol in *Salmonidae* fish. 3-Hydroxy carotenoids: lutein, zeaxanthin and astaxanthin, were also reported to be precursors of 3,4-dehydroretinol (vitamin A2) in some fish (Matsuno, 1991).

Gross and Budowski (1966) reported that astaxanthin, canthaxanthin and isozeaxanthin in addition to -carotene were precursors for vitamin A in both guppies (*Lebistes reticulatus*) and platies (*Xiphophorus variatus*). Astaxanthin, canthaxanthin and zeaxanthin were precursors of both A1 and A2 in rainbow trout (*Oncorhynchus mykiss*), but the rate of incorporation was highly dependent on fish size and age, and the vitamin A status of the fish. Schiedt et al. (1985) reported that astaxanthin, canthaxanthin and zeaxanthin were transformed into vitamin A in the intestinal wall and the liver. In the freshwater fishes *Saccobranchus fossilis* and *Clarias batrachus*, lutein is reported to be the precursor of vitamin A2 (Goswami and Bhattacharjee, 1982), and in tilapia (*Tilapia nilotica*) astaxanthin, zeaxanthin, lutein and tunaxanthin were directly converted into vitamin A2 (Katsuyama and Matsuno, 1988).

Communication:

Many animals accumulate carotenoids in their integuments. Integumentary carotenoids may contribute to photoprotection, camouflage and signaling such as breeding color.

Fishes change their hues in response to background coloration and also display color responses during excitement and courtship (Fujii, 1969). The color pattern can be viewed as compromises between the need to communicate with other members of the species and the need to avoid being eaten (Moyle and Cech, 1982). The internal control of color changes is complex and involves both hormones and nerves where the initiation comes from visual cues. It has been shown that carotenoids are integral constituents of chromatophores and xanthophores and are functional in the photoreponses of fish. Goodwin (1952) suggested this to be the major role of carotenoids in fish, and lack of sufficient pigments may have negative effect on their general performance.

Growth:

There is a controversy on the role of carotenoids in fish growth, several studies reporting a positive influence whereas others did not find any effect. In Atlantic salmon fry and juveniles the inclusion of synthetic astaxanthin and canthaxanthin not only enhanced growth but also survival (Torrissen, 1984; Christiansen et al., 1994; Christiansen and Torrissen, 1996). For example an improved growth of Atlantic salmon was found by supplementing commercial start feeding diets with astaxanthin or canthaxanthin and no significant differences were found between the astaxanthin and canthaxanthin supplemented diet (Torrissen, 1984).

Corresponding results are found for the red tilapia (*Oreochromis niloticus*) (Boonyaratpalin and Unprasert, 1989) and kuruma shrimp (*Penaeus japonicus*) (Chien and Jeng, 1992). Supplementation of -carotene and canthaxanthin to the diets of major Indian carps resulted in a better survival and growth compared to conventional diets used without carotenoids (Goswami, 1993) and Negre-Sandargues et al. (1993) found a higher survival rate for *Penaeus japonicus* receiving astaxanthin-canthaxanthin supplementation (50/50), but no differences were observed in growth.

Christiansen et al. (1994) investigated the interaction between astaxanthin and vitamin A supplementation on growth and survival in first feeding fry of Atlantic salmon. The experimental diets were based on a semipurified diet based on vitamin and carotenoid free casein and gelatin as protein sources developed by Shearer et al. (1993). The results from this 135 day feeding study clearly showed a significantly improved growth and survival on supplementation of astaxanthin to the experimental diet and vitamin A supplementation alone did not support growth and survival. The vitamin A source used was a mixture of retinol palmitate and retinol acetate. The bioavailability of the two forms is known and one or both might be poorly utilized by start feeding salmon. The results also show a provitamin A function of astaxanthin but this alone is not able to explain the effect of astaxanthin supplementation.

Reproduction and fertility:

Aquatic animals also accumulate carotenoids in their gonads. Carotenoids are assumed to be essential for reproduction in aquatic animals. Astaxanthin supplementation in cultured salmon and red sea bream increased ovary development, fertilization, hatching and larval growth (Torrissen and Christiansen, 1995). In the case of the sea urchin, supplementation with -carotene, which was metabolized to echinenone, also increased reproduction and the survival of larvae (Tsushima et al., 1997).
Tveranger (1986) did not detect any effect of astaxanthin supplementation on fertility of rainbow trout, however, improved egg buoyancy was observed from red sea bream (Chrysophyrys major) broodstock fed diets containing -carotene, canthaxanthin and astaxanthin the night before spawing. Hatching was not affected, but the number of oil globules was reduced.

Heteropneustes fossilis showed atrophied gonads with damaged germinal epithelium when fed a carotenoid free diet (Goswami, 1988). Senger et al. (1989) reported an improved liver histology in Oreochromis niloticus and Colisa labiosa fed high astaxanthin levels (71-132g/kg) and low level (32mg/kg) in the diet. Particularly the parenchymal and intracellular organization was better developed. In tilapia the glycogen storage was enhanced and the cell volume slightly increased, although the biochemical mechanism is unknown.

Marine pelagic cold water fish spawn large numbers of small eggs without visible carotenoid depositions, while demersal fish and viviparous fish often have eggs containing high levels of carotenoids. Pelagic eggs have, in general, a short period of development from spawing until the progeny start exogenous feeding which satisfies their carotenoid requirement. The lack of visible fish pigmentation in transparent eggs may be an adaptation to minimize predation pressure. Abnormal skin pigmentation of halibut and turbot is a large problem in first years. This deficiency syndrome seems to be reduced by enriching the live food with carotenoid containing algae. We have shown that vitamin A esters have a limited availability for Atlantic salmon fry (Christiansen et al., 1994) and pilot studies indicate that marine fish larvae require carotenoids or preferably astaxanthin.

Sources Of Carotenoids:

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 -carotene, -carotene, zeaxanthin, lutein, cryptoxanthin, etc. whereas synthetic processes provide only specific carotenoids like -carotene. Contrary to this, synthetic processes involve petro-chemical solvents, leading to residue problems. Further, synthetic carotenoids are expensive and it has limitation to be used in aquaculture feed formulation depending upon 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.

Animal based natural carotenoids:

The commercial natural astaxanthin production utilizes by products of crustacean such as the Atlantic krill, crayfish meal, crab meal, etc., and some microorganisms. These are rich sources of carotenoid astaxanthan and are used in aquaculture feed formulation as additive.

Among microorganisms, yeast Phaffia rhodozyma is probably the most important as it contains astaxanthin as its main carotenoid (Andrewes and Star, 1976), constituting approximately 85% of total pigments (Shahidi et al., 1998). The optical isomer of astaxanthin predominates in red yeast, opposite to the normal configuration found in other sources (Andrewes and Starr, 1976). The optical isomers are utilized to the same degree and the optical configuration is maintained after deposition in the flesh of rainbow trout (Foss et al., 1984). Johnson et al. (1980) reported that Phaffia rhodozyma also serves as a good source of proteins and lipids. The inclusion of this carotenoid source, aside from its positive effect on fish pigmentation, enhances liver function and defensive potential against oxidative stress (Nakano et al., 1995, 1999).

Crustacean processing discards (shrimp, krill and crabs) are also potential carotenoid sources. Crustaceans discards constitute and attractive ingredient for industrialization, since around 70% of the raw weights of the catch are processing discards (Wilkie, 1972; Simpson and Haard, 1985) with high carotenoid content and its use reduces the environmental problem caused by the large amounts of wastes (Torrissen and Naevdal, 1984; Shahidi and Synowiecki, 1991; Shahidi, 1995). Astaxanthin is the predominant carotenoid (Shahidi et al., 1994; Higuera-Ciapara et al., 2006) in crustacean by-products that also include significant proportions of mineral salts (15-35%), proteins (25-50%) and chitin (25-35%) (Lee and Peniston, 1982). Crustacean by-products have been successfully used for coloration of integument and flesh in feeds of fish with high economic importance (Satio and Regier, 1971; Spinelli et al., 1974; Torrissen and Naevdal, 1984; Coral et al., 1997). However, certain disadvantages of this carotenoid source such as its variability in pigment concentration and its high ash and chitin content which reduces its digestibility for fish, severely limit the rate of inclusion in diet formulations.

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 carotenoids:

Plants also have potential as carotenoid sources. Feed ingredients such as yellow corn, corn gluten meal and alfalfa are also used as sources of carotenoids in aquaculture feed formulation. Other carotenoids rich ingredients used are marigold (Tagetes erecta) meal and red peppers (Capsicum sp.) extract.

Experiments with red pepper have given good results, although a lower efficacy was found in comparison to commercially available astaxanthin (Carter et al., 1994; Yanar et al., 1997). Furthermore, paprika oleoresin pigments confer a less desirable coloration in comparison to canthaxanthin in rainbow trout (Akhtar et al., 1999). In a study carried out with Sparus aurata fed a diet containing corn gluten meal, the coloration of the front head and operculum achieved the characteristic yellow found in their wild counterparts (Robaina et al., 1997). It would be interesting to further investigate other probable plant-based carotenoid sources for fish. Marigold, rich in lutein, might be an interesting dietary alternative given its efficacy with egg and skin coloration of poultry.

However, plant based carotenoids are mainly derived from the micro algal pigment. For example 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 coloring ingredient in aquaculture.

The freshwater micro algae, Haematococcus pluvialis has been commercially exploited for aquaculture primarily due to its rapid growth and high astaxanthin content (Somm er et al., 1991, 1992; Choubert and Heinrich, 1993). It is the primary source of pigmentation in ornamental or tropical fish, responsible for various species related yellow, red and others colors. These are obtained through carotenoids containing organisms in the aquatic food chain.

The biflagellate algae, Dunaliella salina is a source of -carotene and used as natural food coloring agent in aquaculture feed industry. Under appropriate culture conditions, some strains of Dunaliella salina were reported to accumulate up to 10% carotenoids consisting mostly of -carotene (Ben-Amotz et al., 1982; Ben-Amotz and Avron, 1983; Borowitza and Borowitzka, 1983). The bioavailability of -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 -carotene from Dunaliella is currently a substantial and growing industry. It has been reported that 125 ppm -carotene from 6.25g agro based feed mix/kg diet gave excellent pigmentation and higher doses 200 ppm and 300 ppm further improved pigmentation. Arthospira maxima have also been used in rainbow trout culture for color enhancement purposes (Choubert, 1979).

The microalgae Chlorella vulgaris has become a potent pigment source, which imparts yellow/blue hues. The biomass of this algae had already been proved to be useful in the diets of rainbow trout yielding both muscle and skin pigmentation effects (Gouveia et al., 1996,1997, 1998) and in gilthead sea bream for skin pigmentation (Gouveia et al., 2002). It has also been reported that it contains carotenoid pigments in concentrations of up to 0.4%, of which 80% were potential red hue inducing pigments (Gouveia et al., 1996).

Conclusion:

Carotenoids are widespread and important pigment classes in the organisms as well as contributing characteristic quality criterion for marketing and consumer demands of aquaculture products. Appearance of an animal product, especially color plays an important role on the marketing. Color, nutritional value, healthy appearance, freshness and sensory test components are the subconscious elements to chose the product. Choosing a product is effected by the educational condition, environment and customs as well.

Traditional aquaculture is being replaced with the modern manner. This situation needs to harvest more amount of product in higher quality. This has been accompanied by some problems regarding the product. Fish in the nature have special skin and flesh color. In the diets of fish for which pigmentation is important, synthetic and natural carotenoid source are included in order to eliminate the problem.

In view of the deteriorating effects on the environment due to use of synthetic pigments, the researchers are emphasizing the need for natural pigment coloring agents which will act as an alternative to synthetic chemicals. As the aquaculture feed industry seeks as 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 aquaculture feed industries to promote their products as natural with a distinct shift away from synthetic ingredients and colorants.

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