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Carotenoids as a Dietary Supplement for Enhancing Pigmentation and Colouration in Ornamental Fishes: A Review

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Due to the rising demand for aquarium fish, the ornamental fish-keeping business has significantly increased its contribution to fisheries. The sector requires little investment and is socially and environmentally acceptable. Fish's skin colour, which is influenced by their diet and nutrition, is a major determinant of their market value. Fish have vivid colouring because they consume plants and animals that are naturally rich in pigment. Dietary carotenoids and coloration are intimately connected. Carotenoids are the major natural pigments, responsible for yellow to red coloration and are synthesized mainly by plants, algae and some photosynthetic bacteria. The core component of a successful culture of ornamental fish in a limited environment is properly formulated feed. Fish

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cannot produce carotenoids by own. Hence, diverse sources of carotenoid pigments, including pure carotenoid pigments, animal sources, and plant sources, have been added to fish diets to increase the pigmentation in ornamental fish. The significance of different sources of carotenoids as a dietary supplement for enhancing the pigmentation and coloration in aquaculture has been highlighted in this review paper.

Keywords: Carotenoid; pigmentation; coloration; ornamental fish.

1. INTRODUCTION

The trade in ornamental fish is a multibillion dollar business involving more than 125 countries [1]. Along with the development of food fishes, ornamental fish culture is progressively becoming a more significant part of Indian fisheries. When used as a small-scale business with good profits, ornamental fish cultivation is environmentally beneficial, socially acceptable, and requires little investment [2]. The ornamental fish keeping industry is increasing quickly, both domestically and internationally and hence there is a rising demand for aquarium fish [3]. Like any other industry, the expansion of the ornamental fish trade is reliant on market conditions, infrastructure, and best management practices. The Indian ornamental fish industry is a potential source of wealth due to a profitable export market and significant domestic demand. However, despite having a wide variety of ornamental fish, India is lagging behind in exporting ornamental fish, with a total contribution to global exports of only Rs. 8.4 crores (0.32%) [4]. The growth, coloration, and overall health of fish depend on the diet, its components, and the feeding schedule. Thus, dietary supplements and their administration affect how well an aquaculture system can be sustained and maintained as well as how well income can be produced, thereby also minimizing significant economic losses. The skin pigmentation of fish, which is influenced by their diet and nutrition, is one of the crucial qualities that increase their market value [5].

The vast array of colors and patterns that fish develop is typically correlated with their lifestyle. Fish often have darker dorsal fins and lighter ventral or side fins. Some fish, like the goldfish *Carassius*, have consistent coloration, with dazzling color all over their body. The inhabitants of the bottom are frequently highly detailed and vividly colored above and pallid below. It's possible to notice color variations in a single fish. The *Ostracion*, also known as the trunkfish, has a green body, an orange tail, a yellow belly, and blue stripes on its body. Living in weeds, pipe fish, sea horses, and anglers frequently display coloring and patterning that resembles weeds. Mahasheer (*Tor tor*) has dark grey back sides that are golden or reddish, and an abdomen that is silver. Paired fins, however, are reddish or yellowish. In fish, the color differences between the sexes are fairly obvious. *Lebistes*, a little million fish, with males of varied colors and females of one coloration. The Y-chromosome plays a genetic role in the variety of hues in males. Lack of pigment produces transparency in pelagic, free-swimming juveniles of many species, which is another significant characteristic. Similar to cavefish, which live in complete darkness, they lack pigment and are colorless [6].

Dietary carotenoids and pigmentation in fish are directly related. Since fish cannot synthesize their carotenoids, they must get them from the food they eat. Many experts have observed that adding any acceptable pigment (at the required concentration) to the food can enhance fish color. As a result, the ornamental fish produced were of higher quality than those found in a wild habitat [5]. A nutritionally balanced diet with all the essential components and dietary supplementation of carotenoids is necessary for intensive ornamental fish culture. Fish diets have been modified to include a variety of sources of carotenoid pigments, including pure carotenoid pigments, animal sources, and plant sources [3]. Carotenoids also form complexes of carotenoproteins and carotenolipoproteins when they attach to proteins or lipoproteins. Biological pigments that may exhibit visible spectral colors from red and orange to yellow, green, blue, and violet are produced by carotenoids and their complexes. The most potent carotenoids evaluated for fish coloring are lutein, zeaxanthin, and astaxanthin, which are also produced synthetically and sold commercially. Astaxanthin or canthaxanthin carotenoids, which are quite costly, are typically used to improve fish meals. Therefore, there is an increasing need to identify less expensive carotenoid alternatives. Fish size or age can affect carotenoid deposition. It is generally accepted that fish that are getting close to sexual maturity ingest carotenoids better [7]. Carotenoid is a crucial component for animal growth, reproduction, and disease resistance. In carotenoid-adorned animals, dietary carotenoid pigments and other physiological features that compete with them may be handed on from mother to offspring. Increased yolk carotene accumulation increases offspring development and survival, improves offspring immunity, and reduces offspring oxidative stress. If mother fish provide a significant amount of carotenoids to offspring growth and survival, a lower concentration of carotenoids will stay in maternal fish decorations [8].

This review's objectives are to summarize researches on the various carotenoid sources used in the composition of feed for ornamental fish and to close knowledge gaps to encourage further research into fish coloration.

2. PIGMENTATION IN ORNAMENTAL FISHES

One of the key elements that affect ornamental fish prices on the global market is color [9]. Since fish consume plants and animals that contain natural sources of pigment, bright coloration is seen in fish taken from their natural habitat. However, when kept in captivity for an extended period in a pond or aquarium, the skin color fades, which is detrimental to the marketing of fish. Many scientists have reported that adding any suitable pigment (at the required concentration) to the diet can enhance fish color [10]. As a result, the ornamental fish produced were of higher quality than those found in a wild habitat [11]. The vibrant colors of ornamental fish are caused by biological pigments that are found within unique skin cells called chromatophores present below the epidermis. Pigments are transformed by cellular metabolism into other compounds that give skin its colour [5]. The neural crest cells (NCC) are the source of chromatophore cells in vertebrates. After going through an epithelial-to-mesenchymal transition, these cells migrate throughout the embryo, colonizing various tissues and organs where they settle and differentiate [12].

The two most significant types of fish pigments are xanthophylls and carotenoids [13]. Yellow and red tones are two colors that are most favorably influenced by meals that enhance color, as used by chromatophores. However, fish can produce vivid blues, purples, and greens when protein and foods like seaweed are combined with chromatophores. The following factors affect fish coloring due to their major chromatophores: yellow xanthophores, red erythrophores, black and brown melanophores, reflective crystals called leucophores, and iridophores [14]. The location of these colors is determined by genetics. The number and spatial arrangement of several types of chromatophores present in both the epidermis and the dermis determine the species-specific pattern of skin pigmentation [15].

Due to fish specific genome duplication fish have a much greater range of pigmentation patterns than other vertebrates [16]. Additionally, the skin's pigmentation pattern can alter throughout a lifetime due to factors like nutrition, UV exposure, ambient light, and social interactions, as well as during metamorphosis and the reproductive cycle [17]. According to research on flatfish species, there is a vulnerable time called the pigmentation window during pre- and pro-metamorphosis when many outside influences might interfere with the natural development of pigmentation [18].

3. AN OVERVIEW OF CAROTENOID

Carotenoids are a class of lipid-soluble pigments that are extensively found in nature and are primarily produced by plants and algae. By means of selective absorption of the pigments, they are also created by a number of archaea, yeasts, fungi, and eubacteria and found throughout the entire animal world. These diverse groups are hydrocarbons with 40 carbon atoms, comprises over 700 compounds. During plant photosynthesis, carotenoids are a crucial component. They are frequently found among the pigments that are commonly used, and they are well known for their vivid red, orange, and yellow colours. A great number of structures are created as a result of the intricate derivatizations of these isoprenoid skeleton structures (C40), and these structures are in turn responsible for their wide range of physical, chemical, and biological properties, including the creation of the colors. Because of their coloring abilities, these colouring pigments are frequently utilised in the animal feed business. These carotenoids may absorb specific wavelengths of the UV and visible spectrum and produce colors by absorption spectroscopy [19].

3.1 Structure of Carotenoid

Isopentenyl diphosphate polymers with 40 carbon atoms make up carotenoids. Chemically speaking, they originate from a fundamental structure made up of two groups of four head-to-tail isoprenic units arranged in a linear sequence of eight isoprenic units [20]. Fig. 1 shows the structure of carotenes, which are chains of carbon atoms connected by alternating single and double bonds and ending in ionone rings. The color of carotenoids is caused by the alternating single and double bonds. The carotenes are made up only of carbon and hydrogen atoms; when oxygen is added, hydroxyl groups are created, which results in xanthophylls [21].

3.2 Types of Carotenoids

a) Carotenes, which are oxygen-free carotenoids and include

- lycopenes (found in tomatoes) and
- carotenes (found in carrots).

Carotenes have a yellowish hue, but they don't give fish their colour

b) Xanthophylls, also known as oxy-carotenoids (oxygen-containing carotenoids; xanthophylls is the scientific name for yellow leaves). Unlike carotenes, these molecules include one or more oxygen atoms. These substances have the ability to alter the color of egg yolks as well as the skin or fillet of fish. Followings are a few examples of xanthophylls

- **Astraxanthin** (found in prawns and trout, used to colour trout and salmon fillet; trade names Carophyll pink by DSM or Lucanthin pink by BASF);
- **Canthaxanthin** (found in cantharelle mushrooms; used to colour eggs; trade name Carophyll red by DSM);
- **Capsanthin** (found in red pepper, the capsicum);
- **Lutein** (luteus meaning yellow in mays or corn, *Spirulina*, alfalfa and grass meal)
- **Zeaxanthin** (in *Zea mays*, red mais or maize, *Spirulina*, alfalfa and grass meal [23].

3.3 Properties of Carotenoids

Carotenoids are antioxidants which mean that they shield fatty acids and cell membranes from free radicals along with vitamins C and E. At the same time, it was discovered that astaxanthin's effects were more intense than those of other carotenoids, vitamins C, and E (astaxanthin is a 500-fold stronger antioxidant than vitamin E). Fish that consume carotenoids are more resistant to bacterial, viral, and fungal infections (beta-carotene stimulates the release of lysozyme, a bactericidal natural enzyme generated by leukocytes). The intensity of phagocytosis, one of the body's defense systems against infections, is also increased by carotenoids. All stages of reproduction (maturation, germ cell production, fertilization, embryo development, larval and fry growth) have been shown to benefit from carotenoids [24].

3.4 Distribution of Carotenoids

Carotenoids often only exist in trace amounts in living things, but their total abundance in nature has been calculated to be greater than 100 million tonnes. Most animals are unable to naturally generate carotenoids. On the other hand, photosynthesis-capable species like plants can create carotenoids from scratch. Salmonids are unable to produce ASX (3,3′-dihydroxy--carotene-4,4′-dione), a kind of ketocarotenoids. The content of carotenoids in the meal affects how much carotenoids are deposited in fish muscle [25]. According to reports, the ASX concentration in wild salmonid muscle varies from 3 to 38 mg/kg [26]. In light of this, salmonid flesh appears to be a healthy dietary supply of ASX [27].

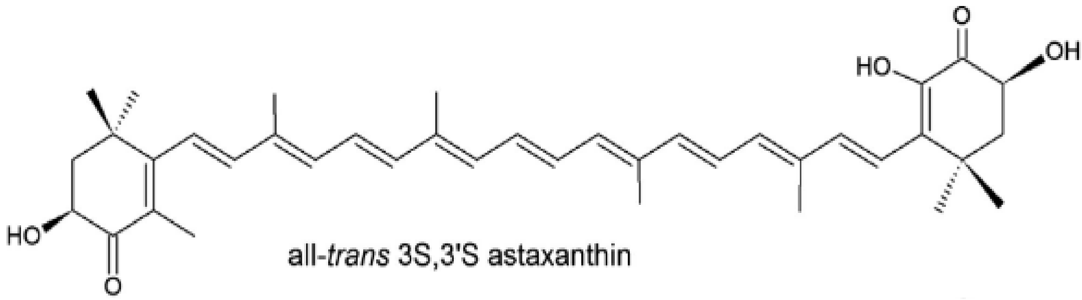

Fig. 1. Structure of carotenoid [22]

4. CAROTENOIDS IN FISH COLOURATION

The carotenoids that give fish their distinctive colors include tunaxanthin (yellow), lutein (greenish-yellow), beta-carotene (orange), alpha, beta-doradexanthins (yellow), zeaxanthin (yellow-orange), canthaxanthin (orange-red), astaxanthin (red), eichinenone (red), and taraxanthin (yellow). Most commercial color formulas contain astaxanthin, a red carotenoid that can bring out red colour. The coloration of ornamental fishes is influenced by many internal and external factors, such as the type of dietary carotenoid supplemented in the diet, pigment source, concentration, length of carotenoid feeding, other dietary ingredients present in the diet, carotenoid extraction methods, body size and weight, life cycle, genetic, metabolism of carotenoids, environmental factors, and stress [28]. Potential carotenoid sources, types of pigmentations, and deposition ability are species-specific [29]. Wild fishes can obtain color pigments by eating planktonic copepods and species of zooplankton when the fishes are drifting and swimming in the water column. Wild herbivorous fishes consume their pigments directly from autotrophs [30]. Nevertheless, fish generally feed on a variety of food types and obtain carotenoids from their heterotrophic prey.

Two significant carotenoids found in the meat of salmonids, including Atlantic salmon (*Salmo salar*) and rainbow trout (*Salmo gairdneri*), are astaxanthin and canthaxanthin[31]. These two carotenoids are the main commercially available carotenoid source for aqua feed formulation and are supplemented in feeds to create the required color of the meat to match consumer preferences. Many fish store carotenoids in their gonads and integuments. Fish in the Salmonidae family, on the other hand, oddly store astaxanthin in their muscles. Freshwater fish frequently have lutein pigment, although many marine species also have a widespread distribution of tunaxanthin found in Perciformes fish. The presence of tunaxanthin is what gives marine fish their vivid yellow fins and skin. Tunaxanthin was found to be metabolized from astaxanthin via zeaxanthin in feeding studies on red sea bream and yellow tail. Certain fish species only contain certain carotenoids. Fish typically include a variety of carotenoids in modest concentrations, while the proportions of these carotenoids vary widely between samples, probably as a result of their physiological and/or dietary circumstances [32]. The fish body converts some carotenoids, including beta-carotene, astaxanthin, zeaxanthin and canthaxanthin into vitamin A, which is necessary for differentiation of embryonic cells, reproduction, vision and good skin [24]. Freshwater cyprinidae fish can convert astaxanthin from zeaxanthin by oxidative metabolic reactions [33]. Fishes are divided into three kinds based on the ability to convert one form to other:

i. **Red carp type**: Lutein is converted into astaxanthin molecules in this group.
ii. **Sea bream type**: Lutein carotene remains 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 [21].

5. ABSORPTION AND TRASPORTATION OF CAROTENOIDS IN FISHES

Age and physiological state, kind of feed consumed, and living environment all have an impact on fish's ability to absorb and distribute carotenoids [34]. Carotenoids are hydrophobic, making it challenging for them to dissolve in the aqueous environment of the digestive system. Carotenoids are connected to lipids so that they may be transported. Carotenoids are absorbed in the enterocyte brush border of the intestine through a variety of processes, such as matrix rupture, lipid emulsion dispersion, and solubilization into mixed bile salt micelles. Additionally, carotenoids absorb significantly more slowly than other fish components. For instance, in Salmonids, it takes between 18 to 30 hours for the proximal intestine to absorb about 35% of astaxanthin [35]. The intestinal absorption of micelles also involves the passive diffusion process [20]. The changes in carotenoids' assimilation by fish and simultaneous color enhancement are influenced by variances in their chemical structure, interactions with one another, and interactions with other substances in the meal. Additionally, it is reliant on the particular species and living circumstances. The most readily absorbed carotenoids employed in fish nutrition are astaxanthin, canthaxanthin, and beta-carotene. Because a carotenoid has numerous chemical isomers, there are variations in the bioavailability of each one. The position of the carotenoids within the cell affects their ability to be assimilated as well. They can be dispersed in lipid droplets (fruits), attached to proteins of the photosynthetic system, or embedded in cell membranes. This explains why fruit-based beta-carotene is more absorbed than beta-carotene found in leaves. Additionally, the bioavailability of raw materials containing carotenoids is significantly impacted by processing. Beta-carotene availability changes with how carrots are processed:

• Raw carrot contains < 1%
• Raw carrots heavily grinded contains about 25%
• Cooked carrots lightly grinded contains about 5%
• Cooked carrots heavily grinded contains about 57% carotenoids.

This explains why fish-fed color-enhancing diets exhibit a range of coloring intensities and rates. As a result, different fish within a flock can have different colorations [24].

6. METABOLISM AND DEPOSITION OF CAROTENOIDS IN FISHES

The metabolism of carotenoids in tissues and their subsequent transformations in fish do not share any similar pathways [36]. Organs such as the liver and gut, where carotenoids' metabolites are found, are thought to be the sites of carotenoids' metabolism **[**37]**.** Fish may be categorized based on their capacity to metabolize carotenoids. Due to its inability to perform ionone oxidation, one type of fish needs the addition of specific oxygenated derivatives to its diet, whereas the other type, such as goldfish or fancy red carp, can oxidise the 4 and 4' positions of the ionone ring and has the potential to transform zeaxanthin and lutein into astaxanthin [38]. In fishes, the metabolism of carotenoids in tissues and their subsequent conversions do not follow any universal pathways [36].

7. EFFECTS OF DIFFERENT SOURCES OF CAROTENOIDS IN FISH PIGMENTATION

To improve color performance, carotenoid supplementation is necessary. An abundance of studies on the addition of carotenoids to fish diets have been conducted and involved the use of different carotenoid sources and inclusion levels [39-44]. The addition of carotenoids to the diets of ornamental fish, such as goldfish and koi carp [44] neon serpae tetra [45] and guppies [46], has received less research attention [7].

Carotenoids can be obtained by fishes from three main sources: plants, animals, and synthetic sources.

7.1 Effects of Plant Based Carotenoids in Fish Pigmentation

Plants have the potential to be sources of carotenoids. Green plants' leaves contain carotenoids, which act as auxiliary pigments during photosynthesis. They capture solar energy and transfer it to chlorophyll, the main pigment involved in photosynthetic processes.

According to Grether et al. there are two types of algae: microalgae and macroalgae (seaweed), which mimic higher plants in terms of the type, distribution, and position of their carotenoids [47]. According to Shahidi et al. a wide range of carotenoids, including carotene, lutein, violaxanthin, zeaxanthin, and neoxanthin, distinguish algae [48]. Compared to higher plants, algae contain substantially larger amounts of the carotenoid zeaxanthin. Because algae have advantages over terrestrial plants in terms of cost, scale, time, and yield, industrial interest in producing natural carotenoids from algae has significantly increased in recent years [49]. In aquaculture, microalgae and seaweeds offer a distinctive, long-lasting, and different supply of carotenoids [50-52]. Several studies examined the skin pigmentation patterns and intensities produced by algae in ornamental fish diets. *Phormidium valderianum, Arthrospira maxima, Spirulina platensis, Nostoc ellipsosporum, Navicula minima, Porphyridium cruentum, Leptolyngbya valderiana,* and *L. tenuis* have all been utilized frequently as a carotenoid source in live and formulated feed for enhancing skin pigmentation and prominent coloration in ornamental fishes. The microalgal pigment is the principal source of plant-based carotenoids. *Chlorella* sp. and *Spirulina* sp. are widely added to ornamental fish meals for the primary market requirement to increase color and promote a healthy appearance [53-54]. The algae *Haematococcus pluvialis, Chlorella vulgaris, Dunaliella salina*, and *Arthospira maxima* can accumulate secondary carotenoids and their biomass can be used as a coloring ingredient in aquaculture if the culture conditions such as nitrogen depletion, high light intensity, and temperature are kept optimal. Due to its rapid growth, the freshwater microalga *Haematococcus pluvialis* has been used commercially for aquaculture. Red porgy's skin turned pink after eating *Haematococcus pluvialis* at a dosage of 50 mg/kg. *Spirulina* is multicellular, filamentous blue-green algae, a member of the phylum Cyanophyceae. *Spirulina* is one of the most potent natural sources of nutrition for both aquatic and terrestrial species [55]. *Spirulina* is utilized as a feed component for the coloration of prawns, marine fish, and ornamental fish [56]. The total carotenoid contents and fish colouration increased when 55g/kg of *Spirulina* powder diet is used for Oscar fish [57]. Fish meal replaced with 50% by BSFL (black soldier fly larva) and supplemented with astaxanthin and *Spirulina* increased the survival rate and color intensity of discus fish but not the groth performance [58]. Japanese fish farmers identified five significant advantages of *Spirulina* use, including improved growth rates, higher survival rates, higher quality, and improved color of cultured fish, as well as a decrease in medical needs and waste in the effluent (wastewater treatment). Fish-fed *Spirulina* had better flesh texture, firmer meat, and brighter skin colors, as well as better flavor [59]. Adding *Spirulina* to diets improved the color of ornamental fish such as sea bream, mackerel, yellowtail, and carp. Fish with a strong texture and distinct hues may sell for more money. *Spirulina* may have antiviral capabilities in addition to reducing the toxicity of medicines [60]. According to Boonyaratpalin & Unprasert (1989), *Spirulina* affects the red tilapia's pigmentation [61]. Similar to ornamental fish, adding *Spirulina* to the diet increased pigmentation [62], however Choubert found no pigmentation in rainbow trout-fed spirulina-containing diets [63]. Japanese ornamental koi carp's color was improved when fed *Spirulina platensis* @ 75.0 g/kg. Better pigmentation was improved by dietary integration of Spirulina platensis meal @ 7.5 mg/kg. Swordtail (*Xiphorus helleri*), topaz cichlids (*Cichlasoma myrnae*), and rainbow fish (*Pseudomugil furcatus)* all exhibit considerably improved color intensity when fed a diet containing a 1.5–2% carotenoids enriched strain of *Spirulina platensis* with *Haematococcus pluvialis* over three weeks [20]. It is generally accepted that fish that are getting close to sexual maturity ingest carotenoids better. This hypothesis was tested by giving fish that was three months old at the start of the experiment and post-larvae various amounts of carotenoid-supplemented diet [7]. Gouveia et al. demonstrated that microalgal biomass, particularly *C. vulgaris*, may help to improve the skin color of koi and goldfish by comparing various microalgal biomass carotenoid sources (*Chlorella vulgaris, H. pluvialis,* and *Spirulina maxima*) with synthetic astaxanthin (Carophyll Pink) [10]. Experiment on fancy carp revealed that *Phafia* diet supplementation can enhance the red skin color [64].

The astaxanthin-rich yeast *Phafia rhodozyma*'s commercially available productsand *Xanthophyllomyces dendrorhous'*s fermentation products are both widely used. *Phaffia rhodozyma,* which contains around 85% astaxanthin, is important as a source of pigment in industrial aquaculture. Despite having a lower efficacy than commercially available astaxanthin, red pepper experiments have produced positive outcomes. Red yeast is used for color pigmentation in goldfish. It contains astaxanthin, a carotenoid pigment. Xu et al. evaluated the addition of red yeast, *Xanthophyllomyces dendrorhousin* the diets of goldfish, *Carassius auratus* [65]. Coloration was increased significantly by supplementing their diet with *Rhodotorula sanneii* astaxanthin (60–80 mg/kg) and *R. paludigena* [66]. Additionally, compared to rainbow trout canthaxanthin, paprika oleoresin pigments impart a less appealing coloration [33]. Olive flounder skin pigmentation improved when paprika and *H. pluvialis* extract were consumed in food [21]. Ebeneezar et al. performed an experiment on clown fish, *Amphiprion ocellaris* feeding five different diets and found that paprika is the best diet for skin pigmentation [67].

The front head and operculum of *Sparus aurata* were colored to resemble their wild counterparts' distinctive yellow in a study that used animals fed a diet comprising maize gluten meal [68]. When processed, rainbow fish fed with 120 mg/kg of red chilli (*Capsicum annuum*) oleoresin showed no discernible color loss [69]. In addition to improving fish color, the introduction of this carotenoid source improves liver function and oxidative stress resistance [70]. Alfalfa @ 15 mg/kg added to the diet improved tissue pigmentation [71].

Higher plant parts that naturally contain carotenoids include fruits, flowers, seeds, roots, and leaves [72]. Numerous researches have shown the amount of interest in using plant sources of carotenoid pigments. Red bell pepper can enhance the color intensity of the koi carp and goldfish when added in the diet [73,74].

Pomegranate (*Punica granatum L.)* peel, a waste product from the juice industry, includes a variety of bioactive chemicals, minerals, and fibers for a variety of nutritional needs. Pomegranate peel extracts (Px) and powder (PoP) have been successfully used in a variety of food preparations, including meat and meat products, edible oils, and bread. *Lantana camara*, a member of the Verbenaceae family and sometimes known as Sleeper weed or wild red sage, has been studied as a potential natural source of pigment that could be utilized to combat bacteria with multiple antibiotic resistances. It has many medical qualities, including antioxidant, anti-microbial, anti-fungicidal, and anti-insecticidal properties, as well as bioactive components like carotenoid (carotene). Natural mixed carotenes have been isolated and purified from the fruits of the oil palm tree to create the spray-dried, water-dispersible form of carotenes known as CarosolTM 3%. Natural alpha-, beta-, gamma carotene, lycopene, and other carotenoids are present in CarosolTM 3%. It is a special combination that occurs frequently in fruits and vegetables in their natural proportions. Worldwide producers of food colorants have employed CarominTM to create color emulsions. In addition to being a natural food coloring, CarominTM possesses vitamin A action and offers human cells protection against free radical damage [75]. In both male and female fish of the banded gourami, studies revealed that papaya containing diet can increase the carotenoid level as well as muscle and skin pigmentation [20]. Tomato containing diet riched in natural carotenoid, when incorporated in gold fish showed np satisfactory results [76]. Woad extract incorporated in yellow tail cichlid also showed no significant results on coloration [77].

In the aquaculture sector, carrot is the most frequently used plant material for improving the coloration of ornamental fishes, including goldfish, swordtail, banded cichlids, and zebra malawi cichlids. This plant source (carrot) is what gives fish their distinctive yellow and orange color by depositing alpha and beta carotene in their skin, fins, and meat. Plants' fruits and seeds change color when they ripen as a result of the production of carotenoids. The most widely kept freshwater ornamental fish is the goldfish, which has the largest market value overall [78]. The skin pigmentation of goldfish has been improved by using more carotenoid kinds found in higher plants, and an increased carotenoid level (49.56 g/g) in the skin of goldfish has been seen by adding marigold flowers, which contain xanthophyll and lutein, to the diet of goldfish [79]. But no appreciable improvement in growth rate was shown in a different study utilizing marigold petal meal as a diet for the red swordtail, *Xiphophorus helleri* [80]. The inclusion of carotenoids from higher plants in ornamental fish diets has been shown to improve a variety of parameters in addition to color enhancement, including sensory perception, immunity, feed conservation ratio, growth performance, social behavior, and survival rate [45]. For the flame-red gourami, there are clear hierarchies with a high level of aggregation. *Colisa lalia* was evaluated using a 12-week diet that included powdered beetroot juice. According to the findings of Sallam et al. (2017) there were no appreciable variations in the social behavior of the fish group [81]. A study on fantail guppy revealed that turmeric powder diet (45mg/50g) can enhance the coloration of muscle and caudal fin of the fish [82]. Additionally, goldfish fed a diet containing pure natural bixin from achiote seeds exhibit lower specific growth rates and higher feed conversion ratios than fish fed a control diet [83]. 200-250 mg/kg of the achiote seeds can increase the blood carotenoid levels in rainbow trout [84]. The skin coloration of species other than ornamental fishes, such as salmonids, rainbow trout, and *Catla catla*, was also found to be intensified by these natural carotenoid plant sources, including achiote seeds, red pepper, and marigold flowers [85]. An experiment was conducted on sword tail by feeding 5% marigold petal meal, shoe petal meal and ixora petal meal showed that highest amount of color enhancement in marigold peta meal followed by ixora and shoe flower petal meal [77]. Gamit et al. evaluated that among the natural beta caritenoid sources carrot, rose, marigold and palash, rose feed can provide better growth performance and coloration in molly fish [86]. *Basella* leaves ethanol (1.0g/kg) extract showed more intense color then *Mucuna pruriens* seed methanol (0.25 g/kg) and *Tribulus terrestris* seed ethanol (0.5 g/kg), when feed to *Botia rostrata* for 1 month [87]. Similar to this, a carotenoid from higher plants was used to construct a dominant diet composition for several marine-bred ornamental fishes to increase their color. Consuming meals containing carrot powder significantly improved the skin color of *Amphiprion ocellaris* [88].

7.2 Effects of Animal based Carotenoids in Fish Pigmentation

Animal byproducts from shellfish and various microorganisms that are rich in carotenoids are the main animal sources that the aquaculture sector commercially uses as a feed supplement for color improvement. Potential sources of carotenoids include shellfish like prawns, krill, crabs, and lobsters [52,69]. They are also abundant in proteins (25–50%), chitin (25–35%), and mineral salts (15–35%) [39]. Red porgy skin tissue carotenoid accretion was promoted by dietary integration of prawn shell meal @ 16% in feed [38]. As measured by TBARS levels from raw fillets, the dietary integration of marine and freshwater crab meals at rates of 10% and 20% in feed improved fillet quality. Pure astaxanthin and its esters have been isolated as major pigments from *Penaeus monodon, Penaeus indicus, Penaeus semisulcatus, Litopenaeus vannamei, Pandalus borealis, Penaeus japonicus, Farfantepenaeus paulensis, Parapenaeopsis stylifera*, and *Aristeus alcocki* constituting 64-98% of the total carotenoids in these animals [89]. According to the species, the carotenoid content of prawn and crab waste ranges from 119 to 148 g/g, while astaxanthin concentrations in crustacean by-products are fewer than 1000 g/g. Therefore, it would imply that a significant amount of crustacean waste is required when formulating fish feed to achieve the best skin coloring [90]. Few researchers have attempted to create diets using crustacean wastes to improve the coloration of ornamental fish, even though the potential use of prawns, krill, crab, and langostella by-products to induce pigmentation of cultured fish has been tested [91]. According to certain research, substituting prawn waste meal for a fish meal can increase the development rate and feed efficiency of fish [91-95]. Red sea bream, Japanese eel, and yellowtail fishes' growth rates were found to be accelerated by Kono et al. when they added 10% chitin from Kyowa Yushi Co., Ltd. to their diets [96]. Shrimp head silage meal stored for 14 days at a pH range of 3.6-4.7 was substituted for fishmeal in the diet of the African catfish *Clarias gariepinus* [92]. During an 84-day feeding trial, the catfish's specific growth rate, feed digestibility, weight gain, feed conversion ratio, and protein efficiency ratio were all evaluated in the designed diet. The amount of these parameters was found to be considerably (p 0.05) higher in fish fed 20% ensiled prawn head meal compared to fish fed other designed diets. Similar to this, [93] stated that prawn head silage powder could be used in place of fish flour as an element in tilapia feed with benefits to both the feed's quality and its economics. According to studies [97,98], prawn waste meal can improve the sensory qualities (general appearance, taste, and color) of the flesh of brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri* Rich. and *Oncorhynchus mykiss*) [33].

7.3 Synthetic Carotenoids

Synthetic carotene was one of the dietary supplements that allowed carotenoids and must obtain their color from their diet. The carotenoid sources from plants and animals used in feed contain a variety of colors. However, since synthetic carotenoids are extremely sensitive to heat, light, and air, storage is a problem because they only contain a single pigment. There are stable forms of synthetic pigments on the market that are coated with gelatin and maize starch, emulsified with ascobyl palmitate [99], or ethoxyquin. Red porgy's bright crimson color was intensified in both the dorsal and ventral regions by the addition of astaxanthin (Naturose®) at a dose of 100 mg/Kg [36]. Tropical spiny lobsters that were given astaxanthin at 4.7-32.8 mg/Kg had darker colors than control fishes [100]. Animals given red porgy containing 25 or 50 mg/Kg of astaxanthin had reddish hue pigmentation [101]. Higher pigment retention was seen in the skin of Australian snapper when astaxanthin was ingested at a rate of 39 mg/Kg [102]. Japanese ornamental carp's coloration was improved when given 1.5 g/kg of synthetic carotenoid (carophyll) [103]. Dietary incorporation of xanthophylls (Wisdem Golden Y20) at 75 mg/Kg caused the ventral and dorsal skins of Yellow Croaker to exhibit 1.10–1.20 times more yellowness color, respectively. When animals were raised on both black and white substrates, giant tiger prawns treated with astaxanthin @ 100 mg/kg increased pigmentation in the animals [21].

In 1964, Hoffman La Roche (Basel, Switzerland) began producing synthetic canthaxanthin for commercial use as a food and feed colorant under the brand name "Carophyll Red". Under the brand name Lucanthin® Red, the other producer, BASF group, also developed synthetic canthaxanthin. Hoffman La Roche has also manufactured free astaxanthin under the brand name "Carophyll Pink" in addition to this synthetic canthaxanthin. In salmonid cultivation, more than 6000kg of carotenoid pigments were used, costing approximately USD 1,000 per kg of the synthesised material. Carophyll Pink is currently being added by several commercial aquafeed producers to their dry-pelleted diets [31]. Researchers used Carophyll Pink and Carophyll Red to enhance the meat of Atlantic salmon and rainbow trout in several tests [65].

Studies on salmonids have shown that astaxanthin appears to be better absorbed and deposited than canthaxanthin [104]. Hatlen (1995) used Carophyll Pink in various concentrations to examine the coloring of Arctic charr [41]. When Harpaz et al. (1998) compared the effects of three different carotenoid sources on the growth and pigmentation of crayfish (dry algal cells from *Dunaliella salina,* Carophyll Pink, and alfalfa meal), they discovered that crayfish fed diets supplemented with carotenoids had better color. Carotenoids had no impact on the crayfish's growth or survival [105].

7.4 Natural vs Artificial Carotenoid

Maintaining nutrition and pigmentation in fish kept in aquariums under confined conditions is a challenge [106]. Fish fed with desired pigment at the proper dosage enhances fish coloration and generates fish of an ornamental quality that may be on par with or better than fish obtained from the wild. An attempt was made to study the ornamental fish *Poeciliia sphenops*, which has commercial interest, in order to evaluate and contrast the efficacy of diet formulations (D1, D2, and D3) containing carotenoids necessary for healthy and disease-resistant fish with 100% survivability, good growth performance, and improved skin and flesh pigmentation [5]. However, synthetic carotenoids have a few drawbacks. Natural carotene may be more useful than manufactured carotene [13]. To start, these processes can only produce specific carotenoids, like beta carotene. In addition, they use complex organic solvents and petrochemical solvents, which can leave behind lingering issues. Additionally, it is expensive to use synthetic carotenoids in many aqua feeds. Contrary to popular belief, natural sources include a variety of carotenoids, including zeaxanthin, alpha and beta carotene, and astaxanthin. Only red xanthophylls (capsanthin, capsorubin) are present in some plants, such as paprika (*Capsicum annuum*), and they have pigmentation effectiveness of canthaxanthin that ranges from about half to a third. Research institutions and multinational feed corporations have concluded after conducting scientific investigations on *Haematococcus* microalgae that it is a secure and efficient natural substitute for coloring aquatic animals. To test the koi's capacity to absorb xanthophylls from *Haematococcus* algae, koi breeders conducted fish trials. They discovered that koi fed astaxanthin from *Haematococcus* algal meal had a dark red coloration, whereas the control group that had no astaxanthin had a pale orange skin coloration [107]. Based on the unpublished results, a group of fish breeders from Reef Propagations Inc., Aquarium Centre, Inc. of Randallstown, Maryland, and fish farms in Hawaii added or combined NatuRose, which is a natural source of *H. pluvialis* into the meals for ornamental fish. Freshwater and marine ornamental fish, including tetras, could be observed to have significantly improved color and pigmentation [90].

8. CONCLUSION

Extensive research must be done on the natural sources of carotenoids obtained from plants and animals for commercial aqua feed formulation. The research endeavors need to be more narrowly focused, with a focus on natural carotenoids and their inclusion in aqua feed, and productivity needs to advance to the stage of feed formulation. It is important to thoroughly investigate the dosage at which an animal can exhibit distinct color patterns in its skin and muscle.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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