



Potential natural carotenoid sources for the colouration of ornamental fish: a review

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Abstract

Attractive colouration of ornamental fishes is an important quality criterion in the aquarium fish industry. Ornamental fish cannot synthesize colour-producing carotenoid pigments and therefore must rely on dietary carotenoids in natural or synthetic forms to achieve their colour pigmentations. The aim of this review is to compile and summarize recent investigations into different carotenoid sources used in ornamental fish feed formulations and to highlight the research gaps and investigation needs in the field of aquaculture. The natural carotenoid sources which have been widely used for enhancing colouration are non-photosynthetic organs of higher plants, microalgae, seaweeds, crustacean by-products, and red yeast. Consumers mostly prefer to use natural sources rather than synthetic sources. The problem faced by aquaculturists is stabilization of the gained skin colour after terminating feeding of the fish. Advanced investigations are needed to identify the stability of the colouration in the ornamental fish during their life cycle. Further, this review encourages the use of other available natural carotenoid sources in the ornamental fish industry in order to reduce the use of synthetic pigment products and invites research to be done on a genetic level in order to fully understand colour distribution patterns and sustainability of colouration gain.

Keywords Algae · Carotenoid pigments · Colouration · Crustacean waste · Non-photosynthetic organs in higher plants · Ornamental fish feed

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Introduction

Ornamental fish keeping is a popular and stress-relieving hobby with world-wide interest. Attention to aquarium fish production has steadily increased in the aquaculture industry. Attractive and eye-catching bright colour and physical appearance of ornamental fishes are vital criteria in determining consumer enthusiasm and market demand all over the world. It is therefore of crucial interest for fish farmers to achieve acceptable pigmentation of fish flesh integument by formulating fish feeds using different carotenoids either from natural or synthetic sources.

Colouration of ornamental fish species is primarily dependent on chromatophores, such as melanophores, xanthophores, erythrophores, iridophores, leucophores, and cyanophores, which contain different pigments (Das 2016). There are three major pigments responsible for the integument colouration in all vertebrates: melanins, pterins, and carotenoids (Grether et al. 2001; Steffen and McGraw 2007). Among those pigments, melanins and pterins can be only synthesized by animals, while carotenoid pigments cannot be synthesized by animals, and thus, dietary carotenoids in natural or synthetic forms must be relied on to achieve their colour pigmentations (Alonso-Alvarez et al. 2008; Vinkler and Albrecht 2010).

The most widely used primary source of colouration of fish skin is carotenoid. This is a group of lipophilic compounds, consisting of C-40 based isoprenoid pigments and encompassing more than 600 pigments. According to their chemical structure, carotenoids can be divided into two major groups, namely, carotenes and xanthophyll. In terms of structure, carotenes consist of hydrocarbon molecules without oxygen, while xanthophylls are oxygenated carotenoids and carry at least one oxygen atom (Olson and Krinsky 1995). β -Carotene, α -carotene, and lycopene are prominent members of the carotene group. Zeaxanthin, lutein, α - and β -cryptoxanthin, canthaxanthin, and astaxanthin are the essential xanthophylls, containing structural elements of hydroxy and keto groups (Stahl and Sies 2005). Carotenoids can be synthesized by plants, phytoplankton (microalgae), zooplankton, and crustaceans, while other organisms do not de novo biosynthesize carotenoids, so must either obtain them directly from the diet or modify the dietary carotenoid precursors through metabolic reactions (Maoka 2011). Carotenoid pigments can be deposited either directly within chromatophore cells of fishes or can be converted by cellular metabolism and imparted in an array of colours to the skin and other tissues (Chapman and Miles 2018).

Carotenoid is an essential nutrient (Craik 1985; Grung et al. 1993) which plays a vital role in growth, reproduction, and disease resistance in animals (de Carvalho and Caramujo 2017) and thus should be included in all aquatic diets. Dietary carotenoid pigments and other competing physiological features may transfer from maternal carotenoid-ornamented animals to offspring. Higher accumulation of yolk carotenoids increases offspring growth and survival rate, enhances offspring immunity, and reduces offspring oxidative stress (Blount et al. 2002; McGraw and Ardia 2003; Bazyar Lakeh et al. 2010). If a significant number of carotenoids are supplied by maternal fish to offspring growth and survival, a lower concentration of carotenoids will remain in the ornaments of maternal fish (Sefc et al. 2014).

Besides having strong effects on colouration and providing nutrition, carotenoids serve as antioxidants and radical scavengers to prevent oxidative stress (Grynbaum et al. 2005; Yeum et al. 2009). A few studies have shown that potential antioxidant effects of fish can be significantly improved when fish are fed a diet with carotenoids, particularly, astaxanthin compared to when they are fed a diet without carotenoids (Brambilla et al. 2009; Kalinowski et al. 2019; Nakano and Wiegertjes 2020). Antioxidant defence enhanced in the plasma, liver,

and kidney of the juvenile rainbow trout when taking dietary astaxanthin (Rahman et al. 2016). Moreover, increasing total antioxidant status by dietary astaxanthin together with sodium taurocholate reduces the lipid peroxidation in the fry of European seabass (Sallam 2017).

Nevertheless, the beneficial effects of astaxanthin have vastly been recorded on aquatic animals in terms of colouration, growth, reproduction, antioxidant activity, and immunity, and its adverse effects and potential toxicity impacts have not experimentally recorded in the previous studies when the animals fed a diet incorporated with the astaxanthin sourced either natural or synthetic. There are no adverse effects on Atlantic salmon fry after 11 weeks when supplemented astaxanthin up to 317mg/kg diet (Torrissen and Christiansen 1995). Similarly, the health status of the post-larvae of giant tiger prawn *P. monodon* was not affected after taking the diet containing astaxanthin up to 810 mg/kg (Merchie et al. 1998).

European Food Safety Authority (2014) reviewed the toxicology of the active ingredient of canthaxanthin by covering acute toxicity, repeat-dose sub-chronic and chronic toxicity, carcinogenicity, mutagenicity, reproductive and developmental toxicity, and immunotoxicology, and it was declared that the safety and efficacy of synthetic astaxanthin at the dietary level of 908 mg/kg diet was well tolerated by salmonids and ornamental fish. The FEEDAP panel further made the recommendation concerning the use of astaxanthin up to authorized maximum dietary level (100mg/kg).

The aim of this review is to compile recent studies on different carotenoid sources utilized in ornamental fish feed formulation and to fill the gaps in the existing research. According to the published data, parts of higher plants, microalgae, seaweed, crustacean waste, and yeast are the major carotenoid sources utilized for the colouration of ornamental fishes as a whole. Finally, research gaps and standpoints are analysed in this review to promote advanced investigation into fish colouration.

Factors influencing ornamental fish colouration

Colouration 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 (Paula et al. 1998; Zaremba and Smoleński 2000; Buttle et al. 2001; Silverstein 2002; Gupta et al. 2007; Lall and Tibbetts 2009; Leal et al. 2011; Dong et al. 2014; Safari and Mehraban Sang Atash 2015; Lee et al. 2017; Jayant et al. 2018; Abd El-Gawad et al. 2019; Heuvel et al. 2019; Kong et al. 2020) (Fig. 1).

Potential carotenoid sources, types of pigmentations, and deposition ability are species-specific (Withers 1992; Maoka 2020). Wild fishes can obtain colour 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 (Choat and Clements 1998). Nevertheless, fish generally feed on a variety of food types and obtain carotenoids from their heterotrophic prey (de Carvalho and Caramujo 2017). Exceptionally, freshwater Cyprinidae fish can convert (3S,3'S)-astaxanthin from zeaxanthin by oxidative metabolic reaction (Schiedt 1998; Matsuno 2001).

Carotenoids provide a range of colouration from deep orange to red in the skin of many animals and plants. Astaxanthin and canthaxanthin are responsible for the red, orange, or yellow colour in animals and are the most abundant carotenoid pigments found in aquatic

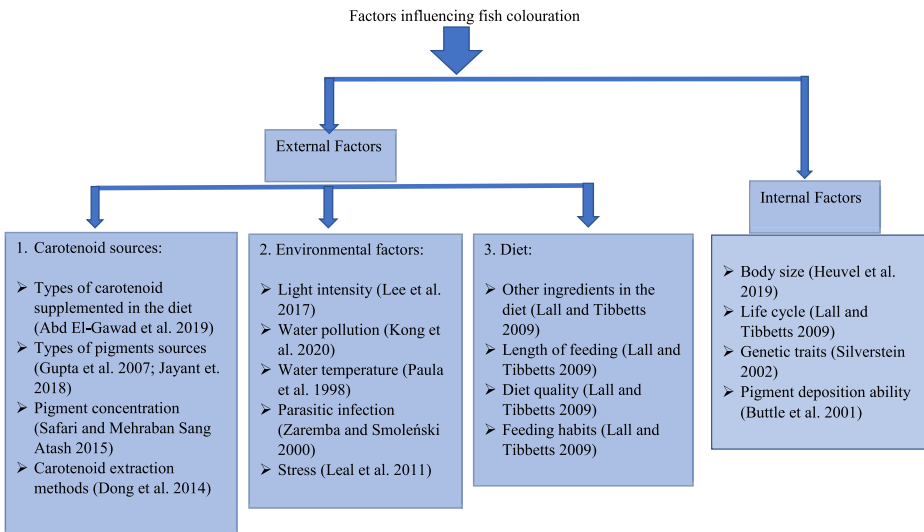


Fig. 1 Flow diagram illustrating the factors influencing fish colouration

animals. Among the carotenoid pigments, astaxanthin is primarily accumulated in both marine and freshwater fishes, while tunaxanthin is metabolized from astaxanthin via zeaxanthin in the order Perciformes and gives bright yellow colouration in the fins and skin of the fishes (Schiedt 1998; Matsuno 2001; Maoka 2011). In the exoskeleton of crustaceans, astaxanthin, a red colour pigment, exhibits diverse array of colours, such as blue, purple, and blue-black, resulted by binding with a multimeric protein called crustacyanin (CRCN) (Chayen et al. 2003). This complex directly effects on fitness and survival of many crustaceans through camouflage or mate selection (Wade et al. 2009; Ferrari et al. 2012). The colour expression predominantly in the outer layer of the hypodermis is regulated by CRCN genes (CRCN-A and CRCN-C) (Wade et al. 2012), and this can be coupled with a wider variety of (micro) habitats (Duarte et al. 2017).

There is no common pathway for the metabolism and transformation of carotenoids in all fish tissues (Chatzifotis et al. 2005). Carp and goldfish belonging to Cyprinidae can absorb zeaxanthin (yellow colour pigment) from their diets, accumulated and metabolized into astaxanthin (red colour pigment) through 4-ketozeaxanthin (Hata and Hata 1972; Simpson and Chichester 1981). In contrast, trout convert the astaxanthin into zeaxanthin pigment (Katsuyama et al. 1987). Some fishes, such as salmon and red sea bream (*Pagrus major*) cannot convert carotenoid pigments (Schiedt 1998; Matsuno 2001). Salmonids can absorb canthaxanthin and astaxanthin up to 20 times more efficiently than lutein and zeaxanthin, whereas channel catfish absorb lutein and zeaxanthin more efficiently than astaxanthin (Robinson 1998).

Carotenoid sources

Carotenoids are widely distributed in all living nature from primitive bacteria (Archebacteria) to the highly developed flowering plants (Angiospermae) and from the unicellular organisms (protozoa) to mammals. More than 750 structurally defined carotenoids from nature, including

land plants, algae, bacteria including cyanobacteria and photosynthetic bacteria, archaea, fungus, and animals, have already been reported (Britton et al. 2004). These can be classified as higher plant carotenoids, algae carotenoids, crustacean carotenoids, and yeast carotenoid.

Higher plant carotenoids for fish colouration

There are three main groups of pigments for colouration in higher plants: carotenoids, flavonoids, and betalains (Tanaka et al. 2008) (Fig. 2).

Carotenoids are found in chloroplasts of higher plants, which are mixture of α - and β -carotene, lycopene, xanthophyll, lutein, cryptoxanthin, zeaxanthin, violaxanthin, and neoxanthin (Delgado-Vargas et al. 2000), and they are most widely distributed in photosynthetic and non-photosynthetic organs. They are biosynthesized and accumulated in the chloroplast of the photosynthetic organs and are involved in two major roles: photosynthesis and photo-protection (Young 1991; Maoka 2020). Carotenoids in non-photosynthetic organs of plants, such as fruits, pericarps, seeds, roots, and flowers, act as photo-protectors, antioxidants, colour attractants, and precursors of plant hormones (Voutilainen et al. 2006; Maoka 2020). The colour of the fruits and seeds in plants changes during ripening stage due to the formation of carotenoids.

Flavonoids, a group of secondary metabolites belonging to the class of phenylpropanoids, consist of two main groups, anthocyanins and anthoxantins, and have exhibited the widest range of colours, from pale yellow to red, purple, and blue in flowers, seeds, fruits, and vegetables (Khoo et al. 2017).

Betalains, nitrogen-containing compounds, are derived from tyrosine and display as brilliant colours in flowers: red, yellow, and purple (Delgado-Vargas et al. 2000).

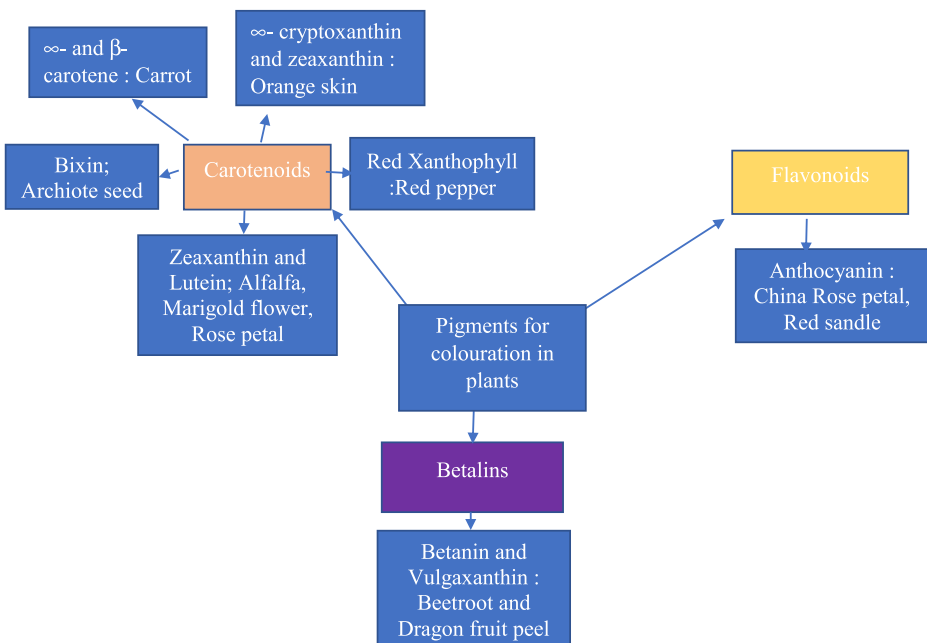


Fig. 2 Pigments and sources of higher plants for ornamental fish colouration

Natural carotenoids are identified in fruits, flowers, seeds, roots, and leaves of higher plants (Priyadarshani and Jansz 2014; Saini et al. 2015; Das 2016), and a considerable number of studies have emphasized the level of interest in utilization of plant sources of carotenoid pigments (Table 1).

Among the recorded carotenoid sources, carrot (*Daucus carota*) is the most common plant source used in the aquaculture industry for enhancing the colouration of ornamental fishes, such as zebra malawi cichlid, swordtail, banded cichlid, and goldfish. This plant source (carrot) consists of alpha and beta carotene deposited in skin, fin, and flesh of the fishes and provides their characteristic yellow and orange colour.

Goldfish, *Carassius auratus*, is the most popular reared freshwater ornamental fish, with the highest market value overall (Yanar et al. 2008). More varieties of carotenoid in higher plants have been applied to enhance the skin pigmentation of goldfish, and an increasing carotenoid level (49.56 µg/g) in the skin of goldfish was recorded by adding marigold flower, containing xanthophyll and lutein to the diet of goldfish (Sallam 2017).

In addition to the colour enhancement of ornamental fishes, other parameters, such as sensory, immunity, feed conservation ratio, growth performance, social behaviour, and survival rate of ornamental fish, have also been studied with the mixing of carotenoids from higher plants in their diets (Wang et al. 2006; Baron et al. 2008; Ezhil et al. 2008; Dananjaya et al. 2017). Clear hierarchies with high level of aggregation for the flame-red gourami *Colisa lalia* assessed with a diet mixing beetroot juice powder for 12 weeks. The results showed that there were no significant differences in social interaction in group of fishes (Baron et al. 2008). In another study by using marigold petal meal as a diet for the red swordtail, *Xiphophorus helleri*, no considerable progress in growth rate was recorded (Ezhil et al. 2008). Moreover, diet containing purified natural bixin from achiote seeds shows lower specific growth rate and higher feed conversion ratio in goldfish compared to the controlled fish (Dananjaya et al. 2017).

Increasing interest in skin colouration of ornamental fishes has arisen as a result of using some other pigments, such as flavonoids and betalains (Mathew and Gopakumar 1970; Kurnia et al. 2019). It was reported that the incorporation of red sandal (*Pterocarpus santalinus*) containing flavonoid pigments into the diet of tilapia (*Tilapia mossambica*) enhanced pink colour (Mathew and Gopakumar 1970). Similarly, skin colour of koi carp (*Cyprinus carpio*) was improved by adding red dragon fruit peel (*Hylocereus polyrhizus*), which contains betalains as natural pigment (Kurnia et al. 2019).

In addition, these natural carotenoid plant sources, such as achiote seeds, red pepper, and marigold flower, were found to intensify the skin colouration of species other than ornamental fishes, such as salmonids, rainbow trout, and *Catla catla* (Ingle de la Mora et al. 2006; Yanar et al. 2007; Weerakkody and Cumararatunga 2016). Similarly, prominent diet composition for some marine cultured ornamental fishes was prepared with carotenoid from higher plants for colour enhancement (Ramamoorthy et al. 2010; Sevdan Yılmaz 2011). Skin colour of *Amphiprion ocellaris* considerably increased by consuming diets containing carrot powder (Ramamoorthy et al. 2010). Similarly, red pepper meal was used as an alternative natural carotenoid source for juvenile blue streak hap (*Labidochromis caeruleus*) and ensured good pigmentation (Sevdan Yılmaz 2011).

In contrast, skin pigmentation of ornamental fishes did not increase efficiently due to the consumption of diets containing anthocyanins and betalain pigments. For example, Baron et al. (2008) examined the effect of skin colouration in dwarf gourami by formulating their diets by adding of betalain-based pigments (betanin and vulgaxanthin) in the form of beetroot

Table 1 Carotenoids of non-photosynthetic organs in higher plants for ornamental fish colouration

Non-photosynthetic organs	Types of carotenoids	Carotenoids amount in organs	Type of ornamental fish	Saturation level of carotenoid content in ornamental fish	Change in Skin colour	References
Fruits/seeds						
Seed of Amatto plant (<i>Bixa orellana</i>)	Bixin	1.1% (w/w) purified bixin/achiote seeds	Goldfish (<i>Carassius auratus</i>)	671.18% at 60 day in skin (diet 0.60 g/kg)	Increase	Dananjaya et al. (2017)
Red pepper Fruit, <i>Capiscium annuum</i>	Red xanthophylls (capsanthin, capsorubin)	70 mg kg ⁻¹ in 5% (Yanar et al. 2007) 120 ppm/kg diet (Tsuchima et al. 1998)	Cichlid (<i>Cichlasoma severum</i>) Goldfish (<i>Carassius auratus</i> L.)	0.454±0.94 mg g ⁻¹ in skin and flesh 1.44mg/individual	Increase Increase	Kop et al. (2010) Tsuchima et al. (1998)
Carrot (<i>Daucus carota</i>)	β-carotene and α-carotene	β-carotene 25 mg/100 g	Zebra Malawi Cichlid (<i>Pseudotropheus zebra</i>) Red swordtail (<i>Xiphophorus helleri</i>) Cichlid (<i>Cichlasoma severum</i>) Goldfish <i>C. auratus</i>	----- ----- 0.416±0.20 mg g ⁻¹ 2.55±0.043 μgg ⁻¹	Increase Increase Increase Increase	Pirnia and Shadi (2015) Wagde et al. (2018) (Kop et al. 2010) Jasmin and Somanath (2016)
Flowers						
Marigold flower meal <i>Tagetes erecta</i>	Xanthophyll, lutein	70 mg kg ⁻¹ carotenoids in 1.8% (Yanar et al. 2007) 34 xanthophyll units/g	Red Swordtail, (<i>Xiphophorus helleri</i>) Tiger barbs (<i>Puntigrus tetrazona</i>)	28.48 ± 0.38 μg/g	Increase	Ezihl et al. (2008)
Petals of China rose (<i>Hibiscus rosa-sinensis</i>)		2.19% (Ramamoorthy et al. 2010)	Goldfish (<i>Carassius auratus</i>) Goldfish (<i>Carassius auratus</i> L.)	0.626±0.036 μgg ⁻¹ in skin 0.44±0.036μgg ⁻¹ in muscle 4.01 mg g ⁻¹ 102 mg/kg	Increase Increase Increase	Boonyaratpalin and Lovell (1977) Somanath and Jasmin (2013) Sinha and Asimi (2007)

Table 1 (continued)

Non-photosynthetic organs	Types of carotenoids	Carotenoids amount in organs	Type of ornamental fish	Saturation level of carotenoid content in ornamental fish	Change in Skin colour	References
Alfalfa flower (<i>Medicago sativa</i>)	Xanthophylls, such as lutein and zeaxanthin (Scott et al. 1968)	400–500 mg total carotenoids/kg	Goldfish (<i>Carassius auratus</i> L.)	-----	Colour was not improved	Yanar et al. (2008)
Turmeric (<i>Curcuma longa</i>)	Curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Naghetini 2006)		Thick-lipped gourami (<i>Trichogaster labiosa</i>)			Silva Nascimento et al. (2019)
Leaves Spinach (<i>Spinacia oleracea</i>)		45 mg/50 g (Mukherjee et al. 2009)	Fantail guppy, (<i>Poecilia reticulata</i>)	478.8 ± 4.596 (ppm) astaxanthin content in caudal fin		Mukherjee et al. (2009)
		β-carotene 30 mg/100 g	Swordtail (<i>Xiphophorus hellerii</i>)	-----	Increase	Wagde et al. (2018)

juice powder and anthocyanin-based pigments (cyanidin-3-glucoside) in Overseal Carantho powder. However, the authors identified poor pigmenting efficiency in the skin of dwarf gourami.

Carotenoid in algae for fish feed formulation

Algae can be divided into two groups, namely, microalgae and macroalgae (seaweed), which closely resemble the higher plants with respect to the type, distribution, and location of their carotenoids (Shahidi et al. 1998). Algae are characterized by a wide variety of carotenoids such as β -carotene, lutein, violaxanthin, zeaxanthin, and neoxanthin (Takaichi 2011) (Fig. 3). The carotenoid zeaxanthin is present in much higher concentrations in algae than in higher plants. Therefore, in recent years, industrial interest towards the production of natural carotenoids using algae has considerably increased, as they offer cost, scale, time, and yield advantages over terrestrial plants (Poojary et al. 2016).

Microalgae and seaweeds serve as a unique, sustainable, and alternative source of carotenoids in aquaculture (Guedes et al. 2011; Boominathan and Mahesh 2015; Safafar et al. 2015). Several research studies focused on skin colour patterns and intensities of pigments achieved by incorporating algae into the diets of ornamental fish (Table 2).

Microalgae, such as *Chlorella vulgaris*, *Haematococcus pluvialis*, *Phormidium valderianum*, *Arthrospira maxima*, *Spirulina platensis*, *Nostoc ellipsosporum*, *Navicula minima*, *Porphyridium cruentum*, *Leptolyngbya valderiana*, and *L. tenuis*, have been widely used as a carotenoid source in the form of live and formulated feed for enhancing skin pigmentation and prominent colouration in ornamental fishes (Gouveia et al. 2003; Gouveia and Rema 2005; Hekimoglu et al. 2017). For the main market criterion, *Chlorella* sp. and *Spirulina* sp. are frequently incorporated into ornamental fish feeds for healthy appearance and colour enhancement (Gouveia and Rema 2005; Zařková et al. 2011; Sergejevová and Masojřdek 2012). *Spirulina platensis* is more efficient in the pigmentation of ornamental fishes than other algae plants (Table 2).

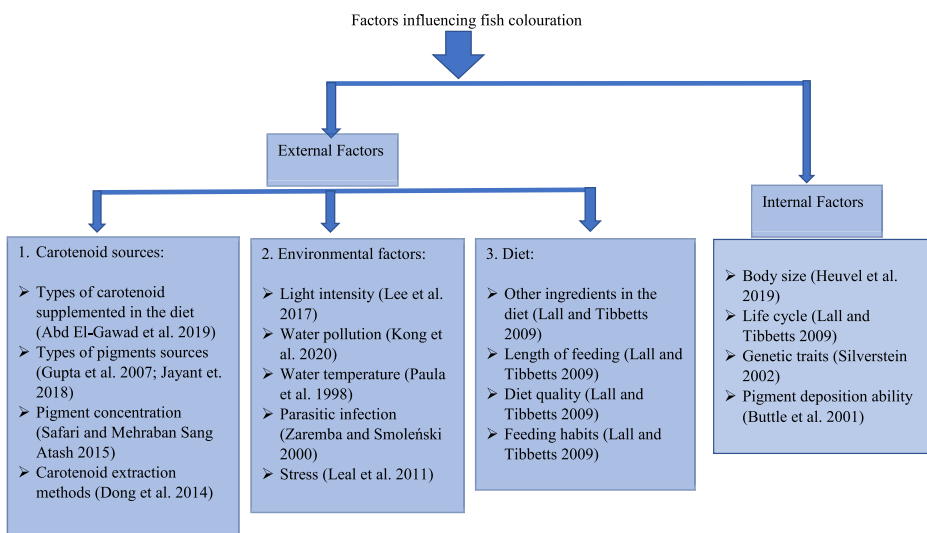


Fig. 3 Carotenoids in algae (Dring 1991; Grama et al. 2016)

Table 2 Carotenoid of algae for ornamental fish colouration

Micro-/macroalgae	Types of carotenoid	Total carotenoid amount in algae	Ornamental fish	Carotenoid content in fish	References
<i>Spirulina platensis</i> (blue-green algae)	51 pigments under the groups; carotenes, xanthophylls, and chlorophylls (Sommella et al. 2018)	0.952 µg/g dry algae	Blue gourami <i>Trichogaster trichopterus</i>	In skin: 13.6259 µg/g for 4g/kg of basal diet In muscle: 5.8315 µg/g for 4 g/kg powder of spirulina	Alagappan et al. (2004)
			Red swordtail, <i>Xiphophorus helleri</i>	0.378±0.026 % in fin, 0.189 ±0.002 % in skin, 0.082 ±0.005 % in muscle	James et al. (2006)
			Japanese ornamental carp (koi, <i>Cyprinus carpio</i>)	6.13 ± 1.11 mg kg ⁻¹ in white scale; 4.45 ± 0.14 mg kg ⁻¹ in black scale; 7.85 ± 1.40 mg kg ⁻¹ in red scale 3.89 ± 0.28 mg kg ⁻¹ in white skin; 2.38 ± 0.87 mg kg ⁻¹ in black skin; 3.42 ± 0.35 mg kg ⁻¹ in red skin	Sun et al. (2012)
			Yellow tail cichlid <i>Pseudotropheus acei</i>	Red/green tonality (a) of skin from the body was 0.90 ± 0.02 in 10% spirulina meal	Güroy et al. (2012)
			Red velvet swordtails (<i>Xiphophorus helleri</i>), rainbowfish (<i>Pseudomugil furcatus</i>), and topaz cichlids (<i>Cichlasoma myrnae</i>) kissing gouramis (<i>Helostoma temminckii</i>), 24 K mollies (<i>Pachouli latipinna</i>), and rosy barbs (<i>Barbus chunianensis</i>)	Panel judged the intensity of colour, which was increased	Ako et al. (1999)
			Golden gourami (<i>Trichogaster trichopterus</i>), Wag-swordtail (<i>Xiphophorus helleri</i>), orange molly (<i>Poecilia latipinna</i>), and pink zebra (<i>Danio rerio</i>)	Colour increased by a factor of: 1.86 in golden gourami 1.33 in Wag swordtail 1.66 in orange molly 2 in pink zebra	Mukherjee et al. (2015)
Algal mixture <i>Leptolyngbya valderiana</i> , <i>L. tenuis</i> , <i>Arthrospira maxima</i> , <i>Nostoc ellipsosporum</i> and <i>Navicula minima</i> (40:20:25:5:10, respectively)	Echinemone, β-carotene, lycopene, lutein, and zeaxanthin (Lopes et al. 2020)				
<i>Spirulina platensis</i> and <i>Haematococcus pluvialis</i>					

Table 2 (continued)

Micro-/macroalgae	Types of carotenoid	Total carotenoid amount in algae	Ornamental fish	Carotenoid content in fish	References
Red algae <i>Porphyridium cruentum</i>	β -carotene, zeaxanthin, and chlorophyll (Grabowski et al. 2000)		Cichlids (<i>Cichlasoma severum</i>)	Less colour increase bright orange in reproductive phase 0.22 ± 0.2 (mg g ⁻¹) 1.8 ± 0.15 % in muscle	Kop and Dumaz (2008)
<i>Nostoc ellipsosporum</i> (Cyanophyceae) and <i>Navicula minima</i> (Bacillariophyceae)			Goldfish (<i>Carassius auratus</i>)		Khatoon et al. (2010)
<i>Chlorella vulgaris</i> , <i>Haematococcus pluvialis</i> , and <i>Arthrospira maxima</i>			Varieties of koi carp (<i>Cyprinus carpio</i>), namely Kawari (red), Showa (black and red), and Bekko (black and white) and goldfish (<i>Carassius auratus</i>)	<i>C. vulgaris</i> biomass yielded the best colouring overall Kawari - 0.16 ± 0.01 (mg/kg) DM Goldfish - 43.4 ± 8.2 (mg/kg) DM	Gouveia et al. (2003)
Cyanobacteria <i>Phormidium valderianum</i>			<i>Hemigrammus caudovittatus</i>	Carotenoid content (1.81-fold)	Mukherjee et al. (2013)
Green microalgae <i>Haematococcus pluvialis</i>			Angel fish (<i>Pterophyllum scalare</i>)	Stronger colouration	Kouba et al. (2013)

Table 3 Carotenoid in crustacean waste for ornamental fish colouration

Source of carotenoid	Ornamental fish	Highest total carotenoid yield	References
Shrimp shell meal diet (SM)	Red porgy (<i>Pagrus pagrus</i>) skin	Skin of fish after 180 days Redness 9.04 ± 1.10 Yellowness 11.19 ± 2.38 for 16% shrimp shell meal	Kalinowski et al. (2007)
Shrimp shell meal	Red porgy (<i>Pagrus pagrus</i>) skin	Modified from a dark grey to a red pink silver colour, with 40 mg of astaxanthin/kg diet	Kalinowski et al. (2005)
Shrimp waste (<i>Pleisonika</i> sp.)	Red porgy (<i>Pagrus pagrus</i>) skin	In carcass, 12.6 ± 4.4 ($\mu\text{g/g}$) for 31.3 mg total carotenoids/kg of diet	Cejas et al. (2003)
Shrimp waste	Goldfish	Effectively enhanced the skin colour within 10 days	Weeratunge and Perera (2016)

Studies have investigated the influence of algae incorporated into the diet on growth and carcass composition and reproductive performance in some ornamental fishes, such as *Labeo rohita*, *Catla catla* (Nandeeshya et al. 2001), and Mekong giant catfish (Tongsiri et al. 2010). Together with the increase in the skin pigmentation, there is a reported decrease in the mortality rate in different varieties of fishes such as tetra, cichlids, Nile tilapia, and rainbow trout as a result of supplementing the diet with *Ulva*, *Cystoseira*, or *Chlorella* (Deventer and Heckman 1996; Gouveia et al. 1997; Kut Güroy et al. 2007). Based on the findings by Kut Güroy et al. (2007), lower mortality rate was recorded, when incorporated *Cystoseira* and *Ulva* meals into Juvenile tilapia (*Oreochromis niloticus*) diets. Moreover, growth performance of juvenile *Oreochromis niloticus* tends to increase with an increase in both algae meal concentrations.

Apart from ornamental fish species, most farmed species, such as mollusc, salmonids, rainbow trout, red porgy, rohu labeo, and Prawn, display increased pigmentation of the skin after consuming algae in their diets, which enhances their attractive appearance in order to satisfy customer demand (O'Connor and Heasman 1997; Neori et al. 1998).

Crustacean waste as carotenoid sources

Crustacean carapace and ecdysial exoskeleton contain significant amounts of free astaxanthin and lutein (Matsuno 2001). Free astaxanthin can esterify by the attachment of one or two fatty acids, which may result in mono- or di-esters, respectively (de Carvalho and Caramujo 2017). Astaxanthin binds with protein and exists as carotenoproteins, such as crustacyanin, in the exoskeleton of crustaceans, and exhibits purple, blue, black, and yellow colours (Wade et al. 2009; Ferrari et al. 2012). This pigment protects light (photooxidation) and other detrimental oxidation reactions in fish and crustaceans (Miki 1991). The immune systems of fish and other animals are also enhanced by the presence of astaxanthin (Maoka 2020).

Crustaceans can rapidly produce astaxanthin from dietary phytoplankton, mainly β -carotene and some zeaxanthin after absorption of precursors (Goodwin and Goodwin 1980; Matsuno 2001), and a small amount of accumulation of astaxanthin precursors is observed in their body tissues (Dall et al. 1995; Van Nieuwerburgh et al. 2005).

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 styliifera*, and *Aristeus alcocki* constituting 64–98% of the total carotenoids in these animals (Sachindra et al. 2005).

The carotenoid content in the waste of shrimp and crab was found between 119 and 148 $\mu\text{g/g}$, depending on the species (Higuera-Ciapara et al. 2006; Sachindra et al. 2006), and crustacean by-products contain less than 1000 $\mu\text{g/g}$ of astaxanthin. Therefore, it would suggest the need for a large quantity of crustacean waste in the formulation of fish feed to achieve the optimum skin colouration (Higuera-Ciapara et al. 2006).

However, despite the potential utilization of shrimp, krill, crab, and langostilla by-products to induce pigmentation of cultured fish has been tested (Hinostroza et al. 1997), few researchers have attempted to formulate diets using crustacean's wastes to improve the ornamental fish colouration (Table 3).

Some studies were carried out to improve the growth rate and feed efficiency of fish by replacing the fish meal with the shrimp waste meal (Kono et al. 1987; Nwanna et al. 2004; Oliveira Cavalheiro et al. 2007; DIOP et al. 2013; Lu and Ku 2013). Kono et al. (1987) recognized that the growth rate of red sea bream, Japanese eel, and yellowtail fishes was enhanced by supplementing the diet incorporated with 10% chitin which was obtained from Kyowa Yushi Co., Ltd. In the diet of African catfish *Clarias gariepinus*, shrimp head silage meal preserved for 14 days at a pH range of 3.6–4.7 replaced for the fish meal (Nwanna et al. 2004). The effect of the formulated diet was assessed on feed digestibility, weight gain, feed conversion ratio, protein efficiency ratio, and specific growth rate of the catfish for 84 days feeding trial. Authors identified that the quantity of these parameters was significantly ($p < 0.05$) higher in the fish fed with 20% of ensiled shrimp head meal than that of fish fed with other formulated diets. Similarly, Oliveira Cavalheiro et al. (2007) reported that the shrimp head silage powder could replace with fish flour as an ingredient in tilapia feed with economic advantages and without sacrificing the quality of the feed. Tilapia fry gained more weight by taking diet incorporation of shrimp waste product compared to the control diet (DIOP et al. 2013). Similarly, increasing trend was observed in weight gain and feed conversion rate of the juvenile cobia (*Rachycentron canadum*) when replacing fish meal with shrimp waste meal from 0 to 25%.

Shrimp waste may also be used as a source of chitin (Sakai 1999) and as alternative protein sources or attractants in diets for aquaculture species (Goytortúa-Bores et al. 2006; Tibbetts et al. 2006; Kader et al. 2012). Tibbetts et al. (2006) assessed the apparent digestibility coefficients (ADCs) of protein and the digestible energy (DE) content in the three crustacean by-product meals (whole krill, crab, and shrimp) when these ingredients included at 30% in the diet for Atlantic cod. Protein ADCs were found to be high in whole krill (96%) and crab (89%) meals and low in shrimp meal (67%). Kader et al. (2012) developed a non-fish meal practical diet for juvenile red sea bream *Pagrus major* supplemented with dehulled soybean meal and crude attractants, such as 10% fish soluble (FS), 5% krill meal (KM), and 5% squid meal (SM). The authors suggested to utilize the ingredients to improve the nutrient composition, mainly amino acid concentration, and to increase the palatability of the diets in order to increase the feed intake of the juvenile red sea bream.

Shrimp waste meal has been investigated to enhance the sensory properties (general appearance, taste, and colour) of the flesh of brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri* Rich. and *Oncorhynchus mykiss*) (Choubert and Luquet 1983; Diler and Gokoglu 2004).

Yeast as a carotenoid source

Red yeast is used for colour pigmentation in goldfish. It contains astaxanthin, a carotenoid pigment. Xu et al. (2006) evaluated the addition of red yeast, *Xanthophyllomyces dendrorhous*,

in the diets of goldfish, *Carassius auratus*. Colouration was increased significantly by supplementing their diet with *Rhodotorula sanneii* astaxanthin (60–80 mg/kg).

Research gaps and investigation needs

Natural vs synthetic carotenoids

Since a requirement of aquaculturists and ornamental fish keepers is to achieve more attractive pigmentation of the fish's skin in as short a time as possible, research on the colour pigmentation of ornamental fish has mainly focused on economic factors. For this reason, researchers have investigated carotenoid sources derived either from the natural biological environment (higher plants; Crustaceans; algae; yeast) or as pure synthetic pigments (astaxanthin; β carotene; Lucantin Pink).

In the production of synthetic pigments, carotenoids can be quickly produced using inexpensive chemicals and without extraction costs. In addition, synthetic pigments are widely produced in order to satisfy market demand. For example, the effect of astaxanthin on the pigmentation of goldfish (*Carassius auratus*) was observed and showed a significant increase in skin pigmentation and survival of fish fed with diets containing astaxanthin at 36–37 mg/kg (Paripatananont et al. 1999). Further, the market share for these synthetic carotenoids has increased, and some consumers find the appropriate pigments more convenient and affordable to purchase. However, although there are some benefits in incorporating synthetic pigments into diets directly, many consumers prefer to use natural sources rather than synthetic sources, owing to increasing health awareness, toxicity of additives, ill effects of the synthetic products, limited synthetic pigments, and cost in the market.

There are many uncertainties and limitations surrounding the production of synthetic carotenoids; it involves petrochemical solvents as well as complex organic solvents, which cause residual problems. The European Commission banned the use of antioxidant ethoxyquin (EQ) as a feed additive in 2017. Moreover, synthetic products contain only specific carotenoids, such as beta carotene and astaxanthin. Although astaxanthin is considerably being synthesized by the manufacturers of DSM in the Netherlands, BASF in France, and NHU in China, the total market value for synthesizing astaxanthin is above \$200M per year (Li et al. 2011). Further, the global market price for producing astaxanthin was valued as USD 447 million/280 metric tons in 2014, and it went up to USD 600 million in 2018 (Panis and Carreon 2016; Jannel et al. 2020). However, the production cost of natural astaxanthin from *Haematococcus* with conceptually designed facility in China is estimated to be \$718/kg astaxanthin, which is considerably much lower than the current industries (Li et al. 2011).

From the consumer point of view, the use of natural pigments can bring greater benefits than purified pigments can bring. This is due to the presence of additional proteins, carbohydrates, and fatty acids in the crude extracts, thereby fulfilling nutritional requirements. In addition, alternatives to natural carotenoid sources have also been investigated because of public interest in their health properties and low-cost availability. Also, some studies have reported that the effects of the natural carotenoid pigments are similar to the effects of the synthetic carotenoid sources. For example, the level of carotenoid obtained from natural sources was found to be adequate for the desired colouration with acceptable threshold level for marketing (Ingle de la Mora et al. 2006; Yanar et al. 2008).

Therefore, carotenoid pigments can be utilized from natural available sources to achieve the aim of the aquaculturist.

Stability of the fish colouration

The most important problem for ornamental fish keepers and aquaculturists is stabilization of the gained skin colour after terminating feeding of fish. Dananjaya et al. recorded unstable skin colouration of goldfish at 90 days of rearing.

There is a conventional method of stimulating the skin colouration easily (Eslamloo et al. 2015), that of rearing ornamental fish in a tank or pond containing carotenoid-rich algae, where the fish can achieve the required skin colouration. Although this method is simple, it cannot guarantee the stability of colour and sometimes creates water quality problems.

The skin colour in fishes could be changed by altering surrounding conditions such as light intensity, temperature, handling stress, and dietary conditions (Gouveia et al. 2003). Further research is needed to fully explore the stability of fish colouration

Quantifying the colour intensity

Quantification of the colour intensity of ornamental fishes was achieved by judging, carried out by a panel of experts (Weeratunge and Perera 2016). However, despite the easy and quick response of the panel, each had different individual observations, which affected the final results.

There are other methods such as chemical extraction (Miki et al. 1985; Katsuyama et al. 1987; Matsuno 2001) and fibre optic reflectance spectrophotometry (Wallat et al. 2005) and computer-assisted image analysis and software application of Expertomica Fishgui to evaluate the colour intensity of fish (Hancz et al. 2003; Urban et al. 2013). Notably, there is a simple method that can be performed by direct colour comparison using the colour card developed by F. Hoffmann-La Roche (Basel, Switzerland) for salmonids. The salmoFan™ colour measurement tools provide two colour scales, one intended for the measurement of the colour of fish slices and the other for the colour measurement of fish fillets (Choubert et al. 1997).

Uneven accumulation of carotenoids in fish skin

Accumulation of carotenoid pigments in skin, flesh, and fins is species-specific. For example, Christiansen and Wallace (1988) and Bjerkeng et al. (1992) have shown that caudal parts of fish may contain more carotenoids than the back and neck part of the fillet. Further, Bjerkeng et al. (1992) reported longitudinal variation in carotenoid content and red colour of the fish flesh, which indicates that higher carotenoid deposits occur in the caudal area than in anterior part of the body of salmonid fishes. This can occur due to the rapid accumulation of carotenoids close to the backbone. Similar results have been reported by McCallum et al. (1987), where colour increment in dorsal and midline cut area has been higher than in other areas.

Future suggestions

Some efforts are needed to focus on identifying the types of carotenoid pigments and suitable carotenoid extraction methods in natural products. Further, this review encourages the use of other available natural carotenoid sources in the ornamental fish industry in order to reduce the use of synthetic pigment products. Additionally, advanced investigation is required into the stabilization of ornamental fish colouration during their lifecycle. The current analysis invites further research to

be done on a genetic level in order to fully understand the colour distribution pattern and sustainability of gained colouration.

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