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CAROTENOIDS IN SALMONID AQUAFEEDS: A REVIEW OF USE AND EFFECTS

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Abstract

This review aims to analyse the use and effects of carotenoids in salmonid aquafeeds. Species of the family Salmonidae are of great interest for aquaculture due to their many properties, like fast growth and nutritional quality. Some species from this family are among the most farmed fish in the world. Thus, there are many studies on different farming technologies, fish biology, market dynamics and others. However, there are always new challenges in the aquaculture of salmonids, being a continuously improving activity. Consumers are becoming more considerate about what they choose to buy and consume. Thus, some aspects emerge as great influencers in the buying decisions of consumers, such as animal welfare, in addition to usual influencing factors, like product freshness and colour. A category of ingredients used in the salmonid aquaculture are carotenoids. They are pigments supplemented to diets for colouring the fish flesh, making it more appealing to consumers. However, some other benefits can also be observed, especially on the health of fishes. Be it synthetic or natural, carotenoids have become a largely used ingredient in salmonid aquafeeds.

***Key words**: carotenoids, physiological effects, salmonids, synthetic.*

INTRODUCTION

Aquaculture is one of the fast growing sectors of the farming industry, supplying qualitative and safe food for consumers. The growth of the sector is especially influenced by the limitations on wild fisheries and by the continuous growth of the world population. According to FAO (2023), salmonids have 18% of the value of the total traded aquatic products in 2020, this being the case since 2013, with demand for salmon and trout exceeding that for other fish. The exports of salmon and trout amounted to 18.4% of total exported aquatic products in 2020, with a value of 27.6 billion USD (FAO, 2023). This was possible due to the high nutritional quality of the fish and favourable organoleptic properties, but also because of the advanced technology, logistical capabilities and marketing campaigns of the salmonid farming sub-sector.

High quality products are sought by consumers. Usually, the colour of the fish product is the first perceived immediate indicator of product quality (Shahidi & Brown, 1998), many times deciding the rejection or purchase of the product. For example, the flesh colour is considered the second most important quality indicator in salmonids, after freshness (García-Chavarría & Lara-Flores, 2013). Thus, the use of carotenoids as pigments in aquaculture is fairly common. Wild salmonids have access to feed that naturally contains carotenoids, like crustaceans, bioaccumulating carotenoids in their tissue, colouring it depending on the predominant pigment of the feed. Farmed salmonids are usually administered commercial feed, without pigments, presenting a white flesh, or only slightly coloured. The consumers often perceive the coloured flesh as a sign of health, and ultimately, as an indicator of a better taste in fish. In addition to direct monetary benefits, other benefits on fish health and growth have been observed when administering carotenoid supplemented feeds (García-Chavarría & Lara-Flores, 2013; Nakano & Wiegertjes, 2020). In salmonids, carotenoids are precursors of vitamin A, improve reproduction performances, have antioxidant functions and help the immune system (Garcia-Chavarria & Lara-Flores, 2013), in addition to providing muscular tissue colour. Nowadays, the use of carotenoids in aqua feeds has three main directions: the first is the coloration of the flesh of farmed fish, making it more appealing to consumers; the second is for the health and welfare of farmed fish; and the third is for the external appearance of ornamental fish.

Synthetic carotenoids are mostly used in aquaculture, due to high production compared to natural carotenoids (Li et al., 2011; Koller et al., 2014). However, natural carotenoids are much more effective regarding health benefits, and their market has the potential to grow, especially as consumers are better informed and aware of the after effects of synthetic products, but also of health and environmental impacts (Kaur & Shah, 2017; Novoveská et al., 2019). This review aims to provide a general description of carotenoids and address the effects of some widely used carotenoids in salmonid aquaculture, referring to health benefits, bioproductive performances, and flesh colour of fish.

MATERIALS AND METHODS

A comprehensive literature search was conducted to collect relevant studies and articles on carotenoids in salmonid aquafeeds. The following databases were utilized for the search: PubMed, Google Scholar, Web of Science, Scopus, ScienceDirect. The search strategy incorporated a combination of keywords related to the topic. The primary keywords used included "carotenoids", "salmonid", "aquafeeds", "astaxanthin", "canthaxanthin", "β-carotene", "lycopene".

RESULTS AND DISCUSSIONS

Carotenoid structure, formation mechanisms and sources

Carotenoids are pigments produced by plants, algae, fungi or bacteria. They produce the yellow, orange or red coloration in plants, as well as in some animal organisms. Due to the colours produced, numerous scientists have started to study these pigments at the beginning of the 19th century (Bendich & Olson, 1989). Thus, by 1971, 273 distinct carotenoids had been identified (Isler et al., 1971), the number reaching 563 in 1987 (Straub & Pfander, 1987). Presently, more than 750 carotenoids have been identified (Nakano & Wiegertjes, 2020), with new examples discovered and described annually.

Carotenoids are liposoluble terpenoid compounds, with 40 carbon atoms in the base structure and many conjugated double bonds. Polyenes present conjugation processes that result in special optical properties (Hermann et al., 1973; Götze, 2019). The number of carbon atoms can vary due to various processes. The base structure can be modified by cyclization at one or both ends of the molecule, by hydrogenation, or by the addition of oxygen groups (Britton, 1995). Thus, from the perspective of the chemical structure, there are two distinguishable groups: carotenes, which are carotenoid hydrocarbons that can be cyclized at one or both ends of the molecule, and xanthophylls, which contain oxygen groups.

Two isopropene isomers are required for carotenoid biosynthesis, namely isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The two isomers can be produced by two pathways, in different cellular compartments of plants (Vranova et al., 2012). The first pathway, of mevalonic acid (MVA), was discovered in the 60s in the biosynthesis of cholesterol from isoprenoids (Katsuki & Bloch, 1967; Lynen, 1967). This pathway involves the formation of IPP, which is then converted to DMAPP by the activity of IPP isomerase. IPP is formed by the decarboxylation of the compound mevalonate diphosphate, which, in turn, is obtained from mevalonic acid (MVA), through various reactions. Acetyl coenzyme A, formed from the synthesis of free acetates, the oxidation of sugars or the condensation of pyruvic acid or fatty acids, is the precursor of MVA (Merhan, 2017). The process takes place in the cytosol. By condensing IPP and DMAPP, geranyl diphosphate (previously named geranyl pyrophosphate) is obtained, and later, by condensing with IPP, farnesyl diphosphate (previously named farnesyl pyrophosphate) is obtained. The condensation of IPP and farnesyl diphosphate produces geranylgeranyl diphosphate (previously named geranylgeranyl pyrophosphate), which is the main precursor of carotenoids. So, carotenoids are formed by the condensation of two molecules of geranylgeranyl diphosphate (Iriti & Faoro, 2009; Nisar et al., 2015).

The second pathway was discovered in the 90s (Rohmer et al., 1993; Rohmer et al., 1996; Arigoni et al., 1997; Rohmer et al., 1999). Methylerythritol 4-phosphate (MEP) involves the simultaneous production of the two compounds (IPP and DMAPP) in plastids (Pulido et al., 2012), starting from the condensation of glyceraldehyde 3-phosphate and pyruvate. Thus, after obtaining geranylgeranyl diphosphate, an intermediate compound called prephytoene diphosphate is produced through its condensation, which then forms the first carotenoid, called phytoene (Altman et al., 1972; Merhan, 2017). By desaturation, phytofluene, the second carotenoid, is obtained. Through a chain of dehydrogenations, lycopene results from phytofluen, which has 13 double bonds. β-carotene, α-carotene and γ-carotene result from lycopene, from which other carotenoids are formed through various reactions. The MAV pathway is only present in plants, but the MEP pathway has also been discovered in protozoa from the phylum Apicomplexa and numerous bacteria, many of which pathogenic (Phillips et al., 2008).

All vegetables and fruits that have colour contain carotenoids (Mezzomo & Ferreira 2016). β-carotene is the predominant carotenoid in nature (Olson & Krinsky, 1995), being found in large concentrations in pumpkins, carrots, nuts, and other vegetables and fruits (Arima & Rodríguez-Amaya, 1990; Godoy & Rodríguez-Amaya, 1998). Lycopene is especially found in red fruits and vegetables, such as tomatoes or watermelons (Park et al., 2020). Lutein and zeaxanthin are found in green leafy vegetables, especially spinach, parsley or broccoli (Chenard et al., 2005; Bunea et al., 2008). Some species of microalgae, bacteria or yeasts produce astaxanthin (Yuan et al., 2002). As primary producers, algae end up in the diet of primary consumers, generally crustaceans, molluscs and even fish. Along the food chain, the astaxanthin produced by the algae is propagated into the body of secondary and tertiary consumers. Thus, the meat of some species of salmon becomes reddish-pink coloured, the lobster has a red colour, and the flamingo bird that has access to molluscs and crustaceans has pink plumage (Fox, 1955).

Carotenoids from natural sources in salmonid aquaculture

Wild salmonids present coloured flesh due to the dietary inclusion of some carotenoid-rich feeds, such as crustaceans or algae. Thus, salmonid farms have tried and sometimes succeeded to obtain the natural coloured flesh through the supplementation of commercial feeds with carotenoids. A distinctive orange-red or pink colour was the initial purpose of using carotenoids in salmonid feeds. Usually, colour is measured visually by using the DSM Salmo fan cards, the Roche colour scale or colorimetrically, with a colorimeter. According to Smith et al. (1992), an optimal colour is correspondent to the values of 13-14 on the Roche scale. The most commonly used synthetic carotenoids in salmonid aquaculture are astaxanthin (pink-red color) and canthaxanthin (orange-red colour) (Akhtar et al., 1999), astaxanthin being the major carotenoid accumulated in the body of salmonids (de Carvalho & Caramujo, 2017).

The higher production price of synthetic carotenoids (Regulation 2018/848 of the European Parliament and of the Council) has made a venue for the use of carotenoids derived from natural sources. Low-cost sources of carotenoids have been studied as dietary supplements for salmonids, such as crustaceans, algae, different vegetables, and others. As salmonids are carnivorous, the supplementation of carotenoids from vegetal sources must be low, to avoid adverse effects on bioproductive performances.

Shekarabi et al. (2020) determined that a dietary supplementation with less than 1% black mulberry (*Morus nigra*) juice powder can significantly enhance the colour of rainbow trout, especially the yellowness and redness of fillet. When using cyanobacteria in the diet of rainbow trout (*Oncorhynchuss mykiss*), Pulcini et al. (2021) obtained an undesirable colour, but the authors noticed a pink pigmentation when the diet had *Procambarus clarkii* meal included. Red pepper can also produce a marketable colour of rainbow trout (Diler et al., 2005; Büyükçapar et al., 2007; Yanar et al., 2016). For a farmed trout to be considered marketable, it should contain minimum 3-4 µg/g total carotenoids (Torrissen et al., 1989). Table 1 presents the effects of different sources of natural carotenoids on the colour of rainbow trout. Although the results presented in the table show an improvement in the colour of rainbow trout, it is important to mention that most experiments had a positive control with synthetic astaxanthin, which performed better in terms of colour and total carotenoid content in the majority of cases.

Table 1. Effects regarding colour of different natural carotenoid sources included in the diets of salmonids

Salmonid species	Dietary carotenoid source	Administered quantity	Trial duration	Effect	Source
Oncorhynchuss mykiss	*Arthrospira platensis*	0, 2.5, 5, 7.5, 10% S. platensis per kg of feed	10 weeks	The 10% S. platensis diet produced the highest carotenoid content in skin and flesh. Authors recommend an addition of 7.5% S. platensis for pigmentation, without effects on growth.	Teimouri et al. (2013)
Oncorhynchuss mykiss	Bee pollen extract	25 and 50 mg carotenoids content per kg of feed	8 weeks	Dietary intake and growth performance were not affected. Commercial scale colour was not obtained.	Sánchez et al. (2019)
Oncorhynchuss mykiss	Red pepper	4.4, 6.6, 8.8% in feed	60 days	Desired coloration obtained. Negative effects on growth in the 2 higher dose treatments	Büyükçapar et al. (2007)
Oncorhynchuss mykiss	Marigold flower	1.2, 2.4, 3.6% in feed	60 days	Yellow colour obtained. Negative effects on growth in the 2 higher dose treatments	Büyükçapar et al. (2007)
Oncorhynchuss mykiss	*Haematococcus pluvialis*	20, 40, 60, 80 mg carotenoids per kg of feed	100 days	The treatments with 20, 60 and 80 mg carotenoids per kg of feed achieved marketable colour.	Sommer et al. (1992)
Oncorhynchuss mykiss	*Chaceon quinquedens*	20% meal; 0.1 and 0.2 mg carotenoid per g of feed	4-23 weeks	The 0.2 mg carotenoid per g of feed produced highly pigmented fish.	Kuo et al. (1976)
Oncorhynchuss mykiss	Black mulberry (*Morus nigra*)	0.25, 0.5, 0.75% in feed	8 weeks	Increased fillet yellowness and redness. Better growth performance parameters.	Shekarabi et al. (2020)
Oncorhynchuss mykiss	Hot or sweet red pepper	0.5, 2, 4.4% dried peppers in the feed	80 days	The 4.4% diet for both peppers produced an adequate accumulation of carotenoids in the flesh and the desired colour.	Yanar et al. (2016)
Oncorhynchuss mykiss	Shrimp waste meal and red pepper meal	60 mg per kg of feed	100 days	Both sources produced a desirable colour, with panellists perceiving a better taste.	Diler & Gokoglu (2004)
Salmo salar	*Calanus* spp. oil	20, 60 mg astaxanthin per kg of feed	181 days	Marketable colouration was not obtained.	Hynes et al. (2009)
Oncorhynchuss mykiss	*Haematococcus pluvialis*	75 mg astaxanthin per kg of feed	6 weeks	No differences in colour between fish fed treatment diets and fish fed control diet with synthetic astaxanthin.	Choubert et al. (2006)
Oncorhynchuss mykiss	Red pepper and shrimp by-products	30, 60 ppm	90 days	The red pepper treatments produced a colour close to the optimal one according to the Roche color scale.	Diler et al (2005)

In some cases, the growth of salmonids does not suffer with the addition of carotenoid sources in the feed. Growth did not very among Atlantic salmon (*Salmo salar*) groups fed diets containing synthetic astaxanthin and *Calanus* oil (Hynes et al., 2009). Teimouri et al. (2013)

did not notice a significant change in the final weight, weight gain, specific growth rate and FCR of rainbow trout fed diets with *Arthrospira platensis* compared to the control. However, the same authors noted that the diet with 10% *A. platensis* addition did produce a significantly higher final weight ($p<0.05$) than the treatment with astaxanthin. Furthermore, the FCR was also reduced to 1.03 in the treatment with 10% *A. platensis*, compared to 1.13 in the control and 1.1 in the astaxanthin treatment (Teimouri et al., 2013). Sanchez et al. (2019) did not determine significant changes in growth performances when adding bee pollen extract in the diet of rainbow trout. Even though not significant, they mention that an addition of bee pollen extract providing 50 mg of carotenoids per kg of feed does provide better results in terms of final weight, total weight gain and specific growth rate for rainbow trout.

According to Kaleshtari et al. (2019), the replacement of synthetic astaxanthin with carrot powder increased the weight gain and decreased the FCR in rainbow trout, at a 75% replacement, the differences being significant ($p<0.05$). The maximum concentration used was 0.1 g/kg of feed.

Thus, some studies observe the effect of different natural supplements rich in carotenoids on salmonids, while others observe the effect of a specific carotenoid, be it natural or synthetic. Overall, the main studied carotenoids are synthetic and natural astaxanthin, synthetic canthaxanthin, natural β-carotene and lycopene. When using natural supplements, in addition to the main carotenoid, other carotenoids are also present, such as lutein, zeaxanthin, α-carotene, and others, which might produce synergistic effects.

Astaxanthin

Astaxanthin, a fat-soluble xantophyll, is the most used carotenoid in salmonid feeds. Nowadays, it is well known that astaxanthin, natural or synthetic, induces the pink-red coloration of fish flesh, bioaccumulating in the tissues. The European Commission classifies natural astaxanthin as a food dye (Roche, 1987). It can be found in algae, yeasts and crustaceans (Abati et al., 2014). The main source of natural astaxanthin is the microalga *Haematoccocus pluvialis*, with 3-5% astaxanthin in dry weight (Orosa et al., 2005; Oslan et al., 2021). Synthetic astaxanthin is mass produced, relatively cost effective, and utilized in many salmonid feeds, while natural astaxanthin is more expensive and more difficult to obtain. However, there are some other differences between natural and synthetic astaxanthin (Villaro et al., 2023), mainly that natural astaxanthin is more stable and more bioavailable, being 20 times more efficient in eliminating free radicals than synthetic astaxanthin (Capelli et al., 2013; Capelli et al., 2019). Structurally, astaxanthin has three optical isomers with all trans configuration of the chain, and the distribution of these isomers differs between natural and synthetic astaxanthin (Moretti et al., 2006).

Astaxanthin acts against reactive oxygen species, it has anti-lipid peroxidation properties, it maintains membrane integrity, helps in reproduction and has roles in the immune system, improving the general health status of fish (Shastak & Pelletier, 2023). It is one of the most used carotenoids in laboratory experiments on salmonids, with many studies highlighting its positive effects on their growth performances and health status. However, flesh colouring remains an important effect of astaxanthin, being the original factor of interest studied. Some studies concentrate on the pigmentation of fish fed dietary astaxanthin under different temperatures, for different life stages of the fish, with different concentrations and sources, and in different time periods (Storebakken & No, 1992; Kurnia et al., 2015; Nickell & Bromage, 1998; Ytrestøyl et al., 2005; Choubert et al., 2006; Zhang et al., 2012). The general consensus is that astaxanthin has great pigmentation properties under various conditions, when the feeding is frequent (Wathne et al., 1998).

In addition, recent studies focus more on the health benefits that the carotenoid has on salmonids. Zhao et al. (2022), in a study comparing the effects of natural and synthetic astaxanthin administered to rainbow trout, determined that synthetic astaxanthin produced the best growth, best pigmentation, best flesh quality, and the trout had the highest level of aspartic and glutamic acids (umami amino acids), while natural astaxanthin achieved the best immunity and antioxidant properties. Compared to rainbow trout fed a negative control, without astaxanthin, all the fish fed experimental diets had a better pigmentation and a higher astaxanthin content in the muscle, a higher final body weight, weight gain rate and specific growth ratio ($p<0.05$). The level of synthetic astaxanthin was 0.1% in the feed, while natural astaxanthin was supplemented to the feed in a higher percentage (1%). The trial lasted for 56 days, and commercial sized rainbow trout was used (251.04±0.91 g). The intestinal morphology of rainbow trout was also improved by astaxanthin diets.

In a trial conducted for 12 weeks on rainbow trout weighing 309 g (initial weight), the fish were fed a normal diet and an experimental diet with a supplementation of 100 mg of synthetic astaxanthin per kg of feed. The study observed that the synthetic carotenoid modulated the effect of the oxidative pentose phosphate pathway (ox-PPP), and positively influenced hepatic health (Kalinowski et al., 2023). In a previous study, the same collective of authors observed that, under stress conditions (hyperoxya), rainbow trout (309±10 g initial weight) fed a diet supplemented with 100 mg synthetic astaxanthin per kg, performed better in terms of reducing oxidative stress. The dietary astaxanthin is believed to have increased glutathione reductase activity. The trial was conducted for 13 weeks, the hyperoxya being induced for 8 hours per day in the last week of experiment (Kalinowski et al., 2019).

In a study where rainbow trout were challenged with paraquat, the authors observed that synthetic astaxanthin improved growth rates and reduced oxidative stress (Hassanzadeh et al., 2022). The upregulation of antioxidant related genes was also observed in rainbow trout fed with astaxanthin-supplemented diets challenged with diazinon, together with better growth performances (Shabanzadeh et al., 2023).

In Atlantic salmon fry (1.75 g weight), dietary synthetic astaxanthin supplementation between 36 and 190 mg kg^{-1} diet significantly increased growth ($p<0.05$), improved survival rate and lipid levels (Christiansen & Torrissen, 1996). Astaxanthin also functions as a vitamin A precursor in Atlantic salmon (Christiansen et al., 1995; Christiansen & Torrissen, 1996), and it is more absorbable when cholesterol (2%) is also supplemented in the diet (Chimsung et al., 2014). A different study concluded that the addition of synthetic astaxanthin and canthaxanthin in the diets of Atlantic salmon does not produce significant differences in growth performance and feed utilization (Baker et al., 2002). The fish used in the trial had an initial weight of 408 g. Another finding of the same study was that the deposition of the pigment in the flesh did not present significant differences, being related linearly to the carotenoid concentration of the feed (Baker et al., 2002).

Canthaxanthin

Canthaxanthin was a widely used keto-carotenoid in aquaculture, with potent antioxidant properties. However, it lost ground to astaxanthin, the latter producing better results. Canthaxanthin occurs naturally in bacteria, algae, and mushrooms and it can also be chemically synthetized. Its main roles involve free radical scavenging, immune-modulating activities and it also helps gap junction communication (Esatbeyoglu & Rimbach, 2017). It produces an orange-reddish colour, and it is approved for use as a feed additive for trout, salmon and other farmed animals in EU, with the specific upper limit of 80 mg kg^{-1} in the feed for salmonids, either alone or with other carotenoids, or 100 mg kg^{-1} when it is administered as a mixture with astaxanthin (European Commission, 2002).

Canthaxanthin is sometimes used in complementarity to astaxanthin to obtain a desirable flesh colour in salmonids. According to Garner et al. (2010), when used in a mixture, astaxanthin and canthaxanthin are better deposited in the flesh of Chinook salmon (*Oncrohynchus tshawytscha*) than when used separately. However, other synergistic effects are questionable. For example, when the oxidative stress biomarkers were studied in the liver and kidney of rainbow trout fed astaxanthin and canthaxanthin mixed and separate diets for 8 weeks, no synergistic effects were observed (Elia et al., 2019). In contrast, Choubert (2010) observed that an increase in the canthaxanthin ratio decreased the total carotenoid retention in the muscle, concluding that diets supplemented only with astaxanthin would provide more reliable results, and that a mix of the two carotenoids may provide non-beneficial results regarding carotenoid retention in the muscle and colour. In a trial on rainbow trout and Atlantic salmon using diets supplemented with astaxanthin and canthaxanthin, Page & Davies (2006) observed that rainbow trout has higher apparent digestibility coefficients and pigment retention efficiency than Atlantic salmon. They also observed that astaxanthin was deposited in higher concentrations in rainbow trout, while canthaxanthin was deposited better in Atlantic salmon, suggesting that carotenoid deposition is dependent on species. The higher deposition of astaxanthin compared to canthaxanthin in rainbow trout was also noted by Tzanova et al. (2022), who analyzed the content of the two xanthophylls in the gonads and liver of the fish.

Toan et al. (2021) observed that canthaxanthin- and α-tocopherol-loaded liposomes included in the diet of rainbow trout produced a significantly better growth, and also a more intense colour after two months of trial. In addition, they also observed that, after three months, the difference in colour faded, suggesting a saturation in canthaxanthin accumulation.

Overall, canthaxanthin is mainly used in salmonid feeds together with astaxanthin. When used alone, or in combination with astaxanthin, it generally produces good results in terms of oxidative stress reduction and flesh coloration, but astaxanthin is often preferred.

β-carotene

β-carotene is a carotenoid found in plants, algae, bacteria and some fungi. It is widely found in nature, being one of the most stable carotenoids. Fish are presented with many β-carotene sources in the waters and have a diverse set of β-carotene oxygenases, which help cleave carotenoids for further synthesis of vitamin A, and possibly retinoic acid. Thus, Atlantic salmon has 5 β-carotene oxygenases, while mammals have only 2 (Helgeland et al., 2014).

Amar et al. (2000) note that β-carotene does not influence the growth of rainbow trout, but some immune response parameters are improved, namely total plasma immunoglobulin and serum complement activity. The 45 g fish were administered feeds with 40, 200 and 400 mg β-carotene/kg of feed for 12 weeks. Contradictory results were obtained by Kelestemur & Çoban (2015) in a similar experiment, but with lower doses of β-carotene in the fish body, 30 and 70 mg β-carotene/kg of feed, where the authors obtained a significantly better ($p<0.05$) weight gain, survival rate and specific growth rate for 60 g rainbow trout, and a significantly lower FCR after 12 weeks of trial. Caspian brown trout (*Salmo caspius*) presented significantly better serum lysozyme activity when fed a diet with 100 mg carrot powder supplemented per kg (Farahani et al., 2021).

Ghtobi et al. (2011) conducted a trial to compare the effects of β-carotene and astaxanthin in the diet of 196 g rainbow trout. The supplementations consisted of 50 and 80 ppm for each carotenoid. Growth parameters and food utilization parameters were not affected after the 8-week trial. Dietary β-carotene produced a lower score on the SalmoFan scale, but none of the treatments produced a colour acceptable for commercializing the fish. The authors mentioned that the astaxanthin supplement was 6 times more expensive than the β-carotene supplement.

Thus, although β-carotene is cheaper than astaxanthin and has similar effects, the latter is preferred for better pigmentation properties.

Lycopene

Lycopene is the main carotenoid in tomatoes and other red fruits and vegetables. It relieves oxidative stress and helps the immune system in some fish species (Dawood et al., 2020).

Yonar (2012) observed that rainbow trout (168±24 g) fed with a diet of 10 mg lycopene per kg of fish bodyweight performed better when challenged with oxytetracycline in terms of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase activity (GSH-Px), also increasing the glutathione level. The trials were conducted for 14 days.

Sahin et al. (2014a) also observed a significant increase ($p<0.001$) in SOD, CAT and GSH-Px activities in rainbow trout (18±0.5 g) fed diets supplemented with 200 and 400 mg/kg of feed, under low and high stocking densities. The authors noted a significant increase in the levels of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and heme-oxygenase 1(HO-1) in the fish muscle, and a significant decrease in malondialdehyde and heat shock protein 70 in the muscle, concluding with the beneficial effects of lycopene against lipid peroxidation and oxidative stress. However, in a similar study conducted by the same authors, a significant decrease in growth performance parameters was observed for rainbow trout fed lycopene-supplemented diets in high stocking densities (Sahin et al., 2014b).

Wang et al. (2019), observed that rainbow trout (29.4±0.1 g) fed diets supplemented with lycopene improved significantly ($p<0.05$) the FCR and protein efficiency ratio, and also improved the antioxidant capacity. As a downside, the authors observed that intestinal amylase activity suffered when lycopene was added. In a similar study, Zhang et al. (2019) observed that the addition of lycopene decreased plasma MDA levels, and improved overall growth. Sheikhzadeh (2013) also noted that carotenoids from red peppers and tomatoes, including lycopene, improve growth performance and lysozyme activity of rainbow trout.

Thus, lycopene has beneficial effects on the health of fish, but the colouring properties are deficient.

CONCLUSIONS

Carotenoids are becoming a very important ingredient in salmonid aquafeeds, not only for the pigmentation of the flesh, but also for the overall welfare of the fish. In most cases, salmonids fed diets supplemented with carotenoids have a better growth performance. Synthetic astaxanthin remains the most widely used carotenoid, due to its absorption and colouration properties, and ease of production. However, natural carotenoids are gaining more interest, as they are much more efficient in improving the health status of the fish, have a favourable market perspective from consumers and may incur a lower cost. Currently, the main disadvantage of carotenoids from natural sources is the lesser effect on flesh colour compared to synthetic astaxanthin and, in some cases, the higher price of production.

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