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SOURCES OF CAROTENOIDS AND THEIR USES AS ANIMAL FEED ADDITIVES – A REVIEW

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Abstract

Carotenoids are natural pigments, widely distributed in nature, synthesized by plants, algae, fungi, and phototrophic bacteria. Carotenoids have coloring power and antioxidant properties, being used as colorants for foods, cosmetics and feeds. Nowadays, a small part of industrial carotenoids is obtained from algae and plants, and the most part is chemically synthesized, but with high production cost. Taking into account consumer's demand for natural compounds, there is an increased trend for products containing natural ingredients including those used in animal feed. Products quality and animal health are influenced by carotenoids added in feed as important component in daily diet. This paper reviews aspects regarding the main sources of carotenoids used as feed additives for nutritional and coloring properties and their impact on animal and human health.

Key words*: carotenoids, feed additives, sources of carotenoids, pigmentation.*

INTRODUCTION

Carotenoids gained great interest lately for food and feeding, as their functions related to nutrition, health and wellbeing are well appreciated. As consumers become more and more aware of the importance of natural instead of synthetic and artificial ingredients, they tend to change preferences and replace products in their diets according to norms which no longer respond to good, tasty and affordable consumptions, yet to higher, long-term perspective standards (García-Chavarría and Lara-Flores, 2013; Carotenoids Market by Type, 2016; Mohiseni., 2017).
In this line, arguments related to the replacement of synthetic carotenoids with natural ones in case of animal feed additives lay in multiple health impact through environment and animal to human health (Kaur and Shah, 2017).
As such, many researches have been directed to in-depth knowledge on carotenoid sources, especially natural ones, for their use as animal feed additives, so as to benefit from their functions including skin and tissues pigmentation (especially when it comes to animals/birds grown in captivity), as growth promoters (in terms of mass production), for reproduction and survival goals, sensory properties and deposition of carotenoids crucial for consumers (egg yolks and other products). In fact, as studies show, there is a strong relationship between diets/foods rich in carotenoids and the risk reduction of various diseases. Carotenoids increase the immune response, are excellent antioxidants, scavenging and inactivating free radicals. This is why both vitamin A precursor carotenoids and the non-precursors ones play an important role in the protection and inhibition of serious diseases as cancer, atherosclerosis, cataracts, macular degeneration, multiple sclerosis, degenerative diseases, and cardiovascular diseases (Mezzomo et al., 2016).
On the other hand, there is no novelty feeding animals with carotenoids, these have always been part of their daily diet. Astaxanthin is the major carotenoid used for pigmentation of fish muscle, mainly salmons (Bhosale, 2004). Beta-carotene, lycopene, and lutein increase growth performance of animals and quality of pigmented products (Nelis and De Leenheer, 1991). Animals and humans are unable to synthesize carotenoids, they have to meet their daily nutritional needs from the products ingested (Priyadarshani and Rath, 2012). Carotenoids, as part of the nutrients in the feed, support animal health and animal products quality (Amaya et al., 2014). They have an

important role in molecular processes of cell membranes whose structure, properties and stability can be modified, leading to possible beneficial effects on human health (Zaheer, 2017).

Out of high production and marketability reasons, carotenoids are present in the animal kingdom, playing functions such as coloring (pets/ornamental birds and fish, mimicking), flavoring (scents and pollination in nature), reproduction (bird feathers and finding mates; development of embryos), resistance to bacterial and fungal diseases, immune responses (lutein connected to anti-inflammatory natural substance in poultry), as well as normal development of skin and mucosa.

The aim of this review is to highlight the various sources of carotenoids and their application in animal feed additives. This topic has rarely been described in a general matter, and as such we endeavor to provide an overview of published works on the subject.

DEFINITION, CHEMICAL STRUCTURE AND CLASSIFICATION OF CAROTENOIDS

Carotenoids are natural pigments, widely distributed in nature, responsible for the coloring of fruits, vegetables and flowers. Carotenoids take their name from carrot (*Daucus carota*), which accumulates high levels of these pigments in the root, being an exception among plants (Stange, 2015). They are used as food colorants, feed supplements, for cosmetic and pharmaceutical purposes.

Most carotenoids are made up of eight condensed 5-carbon-containing isoprene molecules, joined in a head to tail pattern (Mattea, 2009; Domonkos, 2013). Structurally, carotenoids take the form of a polyene chain that functions as a chromophore, due to 9-11 conjugated double bonds and possibly terminating in rings, what determines their characteristic color in the yellow to red range (Vershinin, 1999). The presence of different number of conjugated double bounds leades to various stereoisomers abbreviated as E- and Z-isomers, depending on whether the double bonds are in the trans (E) or cis (Z) configuration (Vincente et al., 2017). Carotenoids are synthesized by all photosynthetic organisms and some non-photosynthetic bacteria and fungi (Ruiz-Sola, 2012). Due to length of the polyene tail, carotenoid compounds are involved in capturing energy from light, absorbing wavelengths, ranging from 400-550 nanometers (violet to green light), thus assisting in photosynthesis and causes the compounds to be deeply colored yellow, orange, or red (Vershinin, 1999; Zakynthinos, 2016).

There have been identified over 750 naturally occurring carotenoids, biosynthesized *de novo* by plants, algae, fungi and bacteria (Okada et al., 2008), among which only 50 have significant biological activity for animals and humans (Mezzomo et al., 2016).

Animals cannot synthesize carotenoids (Rock, 2009), obtaining them through dietary intake, with a few exceptions, represented by aphids and spiders which acquired the ability and genes from fungi (Moran, 2010; Nováková, 2012) or are produced by endosymbiotic bacteria in whiteflies (Sloan, 2012). Carotenoids are classified in Table 1.

Table 1. Classification of carotenoids

Classification criteria	Major classes	Structure	Examples	Attribute	References
Chemical structure	Carotenes	Hydrocarbons (constituted by carbon and hydrogen) that can be cyclized at one or both ends of the molecule	α-carotene β- carotene γ-carotene lycopene torulene	-color range from pale yellow, bright orange to deep red -maintains eye health -does not color the fish	Delgado-Vargas, 2000 Tinoi, 2005 Aizawa, 2007 Kaur and Shah, 2017
	Oxycarotenoids (xanthophylls)	Oxygenated hydrocarbon derivatives that contain at least one oxygen atom as hydroxyl groups, keto, epoxy, methoxy, or carboxylic acid	lutein, zeaxanthin, β-cryptoxanthin, astaxanthin, canthaxanthin, violaxanthin,neoxanthin, antheraxanthin	-generally yellow in color -antiallergic and anti-cancerous actions -can color the fish -colors egg yolks	Delgado-Vargas, 2000; Bhosale and Berstein, 2005; Kushwaha et al, 2014.

Classification criteria	Major classes	Structure	Examples	Attribute	References
Functionality	Primary	Xanthophylls	β-carotene violaxanthin neoxanthin	-structural and functional components of the cellular photosynthetic apparatus	Delgado-Vargas, 2000
	Secondary		α-carotene β-cryptoxanthin zeaxanthin antheraxanthin capsanthin capsorubin	-localized in fruits and flowers -encompasses carotenoids produced at large levels	Delgado-Vargas, 2000
Nutritionally	Precursors of vitamin A	Have at least one ring of β-ionone not replaced and side polyenic chain with at least 11 carbons	α-carotene β-carotene β-cryptoxanthin	-helps maintain eye health, healthy mucus membranes and immunity	Ambrosio et al., 2006; Premkumar, 2014
	Non provitamin A		lutein zeaxanthin lycopene		Premkumar, 2014

β-carotene is used as food colorant and food supplement having antioxidant capacity and provitamin A activity, in a concentration ranges from 2 to 50 ppm. Once converted to vitamin A, health benefits include maintenance of normal eye health, epithelial function, embryonic development, and immune system function. Currently, the application of carotenoids in food formulations is limited because of it poor water solubility, high melting point and chemical instability (Qian, 2012a; Piorkowski, 2014), promoted by heat, light, singlet oxygen, acid, iron and iodine, and free radical, because of conjugated polyene chain which is characteristic of carotenoids (Dutta, 2005; Boon, 2010). Good sources of beta-carotene are cantaloupe, mangoes, papaya, carrots, sweet potatoes, spinach, kale and pumpkin (Premkumar, 2014).

Lycopene is an aliphatic carotenoid which dissolves in the oil, and that is why the presence of oils greatly increases its absorption by the digestive system (Story, 2010). Lycopene is abundant in tomatoes, watermelons, pink grapefruit, papaya and are the most effective singlet oxygen scavenger in vitro (Sies and Sthal, 1995). Epidemiological studies concluded that diets rich in high lycopene foods may reduce risk of cardiovascular diseases and different cancer types (Boon C., 2010).

Torulene has one β-ionone ring and a longer polyene chain than that of β-carotene; it has 13 double bonds and red color, in contrast to other carotenoids produced by the microorganisms. Torulene is found in red yeasts such as *Rhodotorula glutinis, Sporobolomyces ruberrimus.* Its structural characteristics make possible antitumoral and immune enhancing activities and could be used as food, feed and cosmetic additives (Zoz et al, 2015).

Lutein is a dihydroxy-carotene with an alcohol group containing hydroaromatic α structure (Kim, 2006) being in covalent interactions within fatty acids (Mezzomo, 2016). Lutein is found in leaves of green vegetables (spinach, kale, pumpkin, turnip greens) and is yellow-colored. Lutein gives color to chicken fat, egg yolk, and chicken feathers and also increase the efficacy of vaccination against infectious bronchitis virus in laying hens (McGraw, 2003).

Lutein and zeaxanthin are associated with eye health, because they are the only carotenoids found in the retina in macula lutea, being likely effective in age-related macular degeneration (Premkumar, 2014).

Astaxanthin is a dark-red organic pigment being the most valuable microalgal compounds. *Haematococcus pluvialis* strain is the richest source of astaxanthin (Mezzomo and Ferreira, 2016; Zaheer, 2017). This substance gives the pinkish-red hue to the salmonids (salmons and trout), shrimps, lobsters and crabs and has an important role in their immune-system and fertility. Nutritionally, astaxanthin is the most powerful antioxidant in the nature, with role to scavenge efficiently free radicals from the human body (Koller et al., 2014).

Similarly to lutein, it was proved to have a positive role in many human health problems, such as: UV-light protection, anti-inflammatory properties, support for eye health (helps diabetic retinopathy, macular degeneration, eye strain and fatigue, and seeing in fine detail), immune-boosting effects (improves the ability of protective white blood cells to surround and destroy infecting organisms, especially fungi), prevention of different types of cancer (colon and breast cancer) (Amaya et al., 2014).

Canthaxanthin is an orange-red oxycarotenoid found in crustaceans, fungi and algae, particularly used in color feeding. Canthaxanthin has an antioxidant activity and enhances junctional communication between cells directly or through the formation of 4-oxo-retinoic acid, which is also able to stimulate the retinoic acid receptor (Amaya et al., 2014).

SOURCES OF CAROTENOIDS FOR FEED ADDITIVES

As have been pointed out above, there has been a change lately in the perspective towards the ingredients of foods, what is considered healthy for humans and for the environment and what should constitute the food of animals and birds. Yet, researches are still conducted to efficiently obtain natural carotenoids and optimal feed formulas for animals/birds. (Kaur and Shah, 2017).

According to the new study by *reportsnreports.com* (2016), the carotenoid market is led by the feed segment. The demand for incorporation of carotenoids in animal feed is growing due to consumers preference for good quality and meat, meat products, and fish aspect. Carotenoids also increase the palatability of feed, aquaculture being currently the fastest growing sector. It is known that the farming of salmon and shrimp grew exponentially in the last years and the trend is ascendant, so the market of feed carotenoids, especially of astaxanthin, expands rapidly (Anonimous, 2010).

With the growth of world population and increased awareness and demand for better animal nutrition (in terms of quality and quantity), carotenoid additives seem to be the solution (García-Chavarría and Lara-Flores, 2013; Mohiseni, 2017).

According to global trends and forecasts (https://www.marketsandmarkets.com/Market-Reports/carotenoid-market-158421566.html), global carotenoids market is estimated to be valued at USD 1.53 Billion by 2021 and this is mainly due to the increasing number of health-conscious consumers and the rising demand for natural coloration. The quality of food is associated with color, flavor, texture, and odor, humans considering color as the most relevant aspect due to its appealing nature (Amaya et al., 2014).

If, today, carotenoids produced commercially are mostly by total synthesis to meet the demands, intention is to growingly substitute these with natural ones, if possible obtained by as mild as possible methods and processes (Mezzomo and Ferreira, 2016).

Synthetic carotenoids

For carotenoid synthesis, one need to construct the polyene chain, meaning the hydrocarbon skeleton conjugated by alternating double and single bonds. For the specific location of the double and single bonds, different reactions have been developed along time, the first to be obtained being the β-carotene (Amaya et al., 2014).

Out of economic and technological reasons, although various carotenoids have been synthesized at laboratory level, few of them went to commercial scale, among which are the following: astaxanthin, astaxanthin-dimethyl-di-succinate, as well as β-carotene, β-apo-8'-carotenal, also canthaxanthin, citranaxanthin, and the ethyl ester of β-apo-8'-carotenoic acid. For commercial use in animal nutrition, they are usually formulated as 10% concentrated products as a powder or beadlet (Amaya et al., 2014).

Currently, carotenoids synthetic production is the most common manufacturing method. Over 95% of the feed market uses synthetic astaxanthin, mainly from major producers BASF (Germany) (Lucantin pink) and Hoffman-La Roche (Switzerland) (Carrophyll pink), which contains only 25% of the biologically active stereoisomer found naturally. For replacing, only in aquaculture feed additive, the synthetic pigment with natural astaxanthin produced by *Haematococcus*, the biomass of this microalga should be increased to 10 000 ton per year, the price of astaxanthin being too high (Lorenz, 2000; Minyuk, 2008).

The addition of synthetic carotenoids to the diet of laying hens and broilers is a common practice, for improving yolk color (Santos-Bocanegra, 2004). Canthaxanthin is the preferred synthetic red xanthophyll in poultry farming available as Carophyll® Red (DSM Nutritional Products, Switzerland) or Lucantin® Red (BASF, Germany). The preferred yellow xanthophyll is β-apo-8´-carotenoic acid ethyl ester available as Carophyll® Yellow and Lucantin® Yellow (Marounek, 2015).

Synthetic carotenoids are technologically feasible and cost-effective. These are standardized and more stable, i.e. apo-carotene-ester concentration in feed samples showed very little variation from expected values, whereas it was 30% below expectations for marigold extracts.

It has been determined that 1 ppm of apo-carotene-ester is equivalent to about 2 ppm of lutein-zeaxanthin from marigold. Canthaxanthin is the red carotenoid which dominates pigmentation of broilers, whereas paprika xanthophylls which require two to three times the amount compared to canthaxanthin, receives little attention.

The stability of various red carotenoids products in premixes was checked after three months of storage and recovery varied considerably: 66% to 92% for canthaxanthin, 76% for citranaxanthin, and 39% for capsanthin from paprika, which shows that carotenoids are very unstable on their isomeric forms. Another property is the transfer efficiency from feed to egg, which is better for the synthesized ones. Apo-carotene-ester has a 55% transfer efficiency to egg yolk, as compared to only 17% for marigold carotenoids and canthaxanthin an average deposition rate of about 40% (www.poultryworld.net/Broilers/Nutrition/2012/8/The-effect-of-carotenoids-on-yolk-and-skin-pigmentation-WP010752W/).

The consumer's demand for "non-synthetic" colorants has driven the industry to identify and develop natural sources of carotenoids to replace chemical synthesis (Olesen, 2010).

Natural sources of carotenoids

Plants

The majority of naturally occurring carotenoids are found in vegetables and fruits, dark green leaves of plants and seeds, flowers and roots (Amaya et al., 2014). Carotenoids found in plants differ in concentration depending on plant varieties, degree of ripeness, time of harvest, growing and storage conditions, etc. (Zakynthinos et al., 2016).

Feed ingredients such as corn, maize, tomato, green beans and cabbage, pumpkins, prunes or red pepper all are sources of carotenoids with benefits for animals (Amaya et al., 2014; Zakynthinos et al., 2016; Mezzomo and Ferreira, 2016,).

Red pepper or paprika (*Capsicum annuum*) is reported to be a rich, abundant and inexpensive source of keto-carotenoids (capsanthin). It has been studied as a fish feed additive but with a lower efficacy in comparison to commercially available astaxanthin and a less desirable coloration in comparison to canthaxanthin in rainbow trout (Akhtar et al., 1999). In addition to pigmentation, paprika increased growth rate and improved the reproductive performance in yellow tail. Good results have also been reported for laying hens' yolk pigmentation (Yanar et al., 2016).

Marigold flower (*Tagetes erecta*) is rich in lutein and zeaxanthin and it is efficient for poultry skin and egg coloration. Lutein from marigold is also used as a yellow coloration for cultured marine fish yellow tail and red sea bream (Maoka T, 2011). As consumers associate color to nutritive value, healthiness, freshness and taste, this may count as an interesting dietary alternative (García-Chavarría and Lara-Flores, 2013).

Medicinal plants are known to be sources of natural antioxidants used in treatments for human and animal diseases. Because of their fair price and strong effects, medicinal plants are rapidly becoming a safe alternative for antibiotics and chemical drugs in aquaculture. Furthermore, these can help increase growth as they maintain healthy intestinal microflora (Mohiseni, 2017).

Some valuable natural supplements for pigmentation of chicken eggs, broilers and fish are presented in the following Table 2.

Table 2. Natural animal feed supplements

Natural sources	Main carotenoids	Role as feed additive	References
Carrots	β-Carotene α-Carotene	Increases yolk color parameters and carotenoid contents Coloration of skin and flesh of food fish	Isler, 1967 Hammershoj et al, 2010 Weerakkody et al., 2016
Alfalfa (*Medicago sativa*)	Lutein Zeaxanthin β-apo-8'-carotenal β-apo-8'-carotenoic acid Lycopene	Pigmentation of broilers Improve egg quality	Amaya et al., 2014 Varzaru et al., 2015
Marigold flower (*Tagetes erecta*)	Lutein Zeaxanthin	Poultry skin and egg coloration	Rajput et al, 2012 García-Chavarría and Lara-Flores, 2013
Yellow maize	Lutein Cryptoxanthin, Zeaxanthin	Boosts carotenoids in eggs and improve egg yolk color	Breithaupt, 2007
Natural sources	Main carotenoids	Role as feed additive	References
Saffron (*Crocus sativus)*	Crocetin β-Carotene Zeaxanthin	Oxidative stability of shell eggs and liquid yolks	Botsoglou et al, 2005
Seeds of *Bixa orellana*	Bixin and decomposition products	Animal nutrition	Amaya et al., 2014
Paprika (red pepper) (*Capsicum annuum*)	Capsanthin Capsorubin	Pigmentation and weight gain of rainbow trout Pigmentation of salmonids Pigmentation of Pacific white shrimps Yolk pigmentation in laying hens Weight gain in broilers	Yanar, 2016 Talebi et al., 2013 Arredondo-Figueroa et al., 2003 Vicente et al., 2007 Galib et al., 2011

There are some problems regarding carotenoids production from plant origin, due to seasonal and geographic variability that cannot be controlled. A better option, regarding yields and costs, is the microbial production of carotenoids on agro-industrial wastes which are cheap substrates (Mata-Gómez, 2014).

Crustacea and marine animals

Marine animals accumulate various carotenoids from foods (bacteria, algae, other animals) and modify them through metabolic reactions (oxidation, reduction, translation of double bonds, oxidative cleavage of double bonds, cleavage of epoxy bonds). Many of these carotenoids are metabolites of β-carotene, fucoxanthin, peridinin, diatoxanthin, alloxanthin, and astaxanthin (Maoka, 2011).

Astaxanthin is distributed in both marine and fresh water fish. *Cyprinidae* fishes synthesize (3S,3′S)-astaxanthin from zeaxanthin by oxidative metabolic conversion. *Perciformes* and *Salmonidae* fish cannot synthesize astaxanthin from other carotenoids (Matsuno, 2001). Astaxanthin present in these fishes originates from crustacean sources (Atlantic krill, crayfish meal, crab meal) used in aquaculture feed formulation as additive. Crustacean by-products represent an "attractive ingredient for industrialization, since around 70% of the raw weights of the catch are processing discards" reducing at the same time an environmental problem (Shahidi et al., 1998). Lobsters, crabs and shrimps, together with their processing waste are sources of astaxanthin (Mezzomo et al., 2016).

Supply of marine animal based natural carotenoids are limited because of declining trend in catches of crustaceans from marine resources. These sources of carotenoids are very expensive and thus aquaculture feed production involves high production costs (García-Chavarría and Lara-Flores, 2013).

Microbial sources of carotenoids

Interest in microbial sources of carotenoids was renewed due to consumers opinions against synthetic additives. Types of carotenogenic microorganisms, like bacteria and fungi, can be stimulated to produce cost-effective carotenoids in response to environmental stress (light, temperature), chemical compounds or modification of metabolic pathway using recombinant DNA technology (Bhosale, 2004). Advances have been made through genetic manipulation of some non-carotenogenic microbes such as *Escherichia coli*, *Saccharomyces cerevisiae*, *Candida utilis* in terms of carotenoids production (Das et al., 2007).

Several bacteria have biotechnological potential for the production of carotenoids. Some thermophilic halophilic bacteria such as *Halococcus morrhuae* and *Halobacterium salinarum* develop red and orange colonies, bacterioruberin synthetized by *H. salinarum* being the most found carotenoid (Asker and Ohta, 1999). *Haloferax alexandrines* has good industrial perspective for the production of canthaxanthin (Asker and Ohta, 2002) and the marine bacterium *Flavobacterium* sp. produces zeaxanthin, being considered one of the best microbial sources of zeaxanthin (Masetto et al., 2001). Other bacteria with ability to synthesize carotenoids are *Agrobacterium aurantiacum, Mycobacterium brevicaie*, *Mycobacterium lacticola*, *Rhodobacter sphaeroides*, *Rhodococcus maris*, *Streptomyces chrestomyceticus* (Dannert, 2000).

Some examples of carotenoid-producing microorganisms are presented in Table 3. Practical obstacles still need to be overcome for their commercial exploitation, though developments in recent years have proven that, in both farmed fish and poultry, carotenoids like astaxanthin, canthaxanthin, β-carotene, zeaxanthin and lycopene from bacterial sources impart color to skin, flesh and eggs (Bhosale, 2004).

Table 3. Microorganisms sources of carotenoids and their application as feed additives

Bacteria and Yeasts	Carotenoids/Activity	Application as animal feed additives	References
Mycobacterium lacticola	Astaxanthin/ antioxidant, photo- protectant	Fish feeds	Kushwaha et al., 2014
*Paracoccus carotinifaciens (*commercially sold as Panaferd*)*	Astaxanthin/ pigmentation	Coloration of farm raised salmon and trout	Bories, 2007
Spongiococcum excentricum	Lutein/antioxidant	Poultry feeds	Kushwaha et al., 2014
Flavobacterium sp.	Zeaxanthin/ pigmentation	Additive in poultry feed to increase yellow color of animal's skin and egg yolk Fish feed	Alcantara and Sanchez, 1999 Masetto et al., 2001 Bhosale, 2004
Haloferax alexandrines	Canthaxanthin/ pigmentation	Feed in salmon farming to guarantee the flesh color of fish	Asker and Ohta, 2002
Dietzia natronolimnaea HS-1	Canthaxanthin/ pigmentation, antioxidant	Egg yolk pigmentation	Esfahani-Mashhour et al., 2009 Gharibzahedi et al., 2012
Phaffia rhodozyma	Astaxanthin/ pigmentation	Pigmentation of salmon, trout, and red sea bream (*Pagrus* sp.) Pigment source for egg yolk of laying hens	Maoka, 2011 Kushwaha et al., 2014
Blakeslea trispora	β-carotene/growth performance	Chickens for fattening Shrimp feed Feed for dairy cows	Sales et al., 2008
Rhodotorula spp.	β-carotene Torulene Torularhodin/ antioxidant, precursor of vitamin A	Nutrition of laying hens	Bhosale P., 2004 Kushwaha et al., 2014
Xanthophyllomyces dendrorhous	Astaxanthin	Feed supplement for salmons, crabs, shrimps, chickens and egg production	Bhosale P, 2004 Kushwaha et al., 2014
Mushrooms			
Cantharellus cinnabarinus	Canthaxanthin	Poultry feeds and fish feeds	Kushwaha et al., 2014

Microbial sources are an environmental-friendly method for the production of carotenoids, which meet the increasing demand of these natural products (Das, 2007).

Microalgae, rich source of carbohydrates, protein, enzymes and fiber, have been a major source of food for humans in Asian countries. They have been naturally taken over, researched and commercialized both for nutritional supplements for humans and as animal feed additives to replace synthetic components (Priyadarshani and Rath, 2012). Microalgae play an important role in the high-level nutrition for both aquaculture and farm animals. The most famous sources of microalgae, which accumulate carotenoids in their biomass, are *Chlorella, Chlamydomonas, Dunaliella, Muriellopsis* and *Haematococcus* spp, offering economical alternatives to chemical synthesis (Bhosale and Berstein, 2005).

Carotenoids, even at levels of parts per million, are strong dyes, especially canthaxanthin, astaxanthin and lutein from *Chlorella,* which have been used as pigments and included as ingredients in feed for salmonid fish, trout, and poultry to enhance the reddish color of fish and egg yolk yellowish color (Plaza et al., 2009). Broiler chickens fed with algae have yellow skin and shanks, and the egg yolk is darker indicating higher carotenoids content (Becker, 2004). Microalgae like *Chlorella vulgaris* and *Haematococcus pluvialis* synthetize large amount of astaxanthin (Yin, 2013; Kim, 2016) and *Dunaliella salina*, *Spirulina maxima and S. platensis* are biotechnologically relevant, as sources of natural pigments (β-carotene, α-carotene, β-cryptoxanthin and zeaxanthin, respectively) for the culture of black tiger prawns (*Penaeus monodon*), salmonid fish and ornamental fish (Priyadarshani and Rath, 2012). Zeaxanthin from *Spirulina* is used as a red coloration for goldfish and ornamental carp. Astaxanthin became the most important carotenoid in salmons and rainbow trout with the development of salmonid farming. Astaxanthin can be incorporated in feeds up to 100 mg/ kg and canthaxanthin up to 25 mg/kg (high accumulation in humans can be toxic). Animal feed is the largest field of commercial application of microalgae carotenoids due to the importance of astaxanthin and canthaxanthin in aquaculture (Britton, 2004).

Bellow given is Table 4, including the sources of carotenoids from microalgae with application as feed additives.

Table 4. Microalgae sources of carotenoids

Algae and cyanobacteria	Carotenoid	Application as feed additives	References
Haematococcus pluvialis (commercially sold as NaturRose)	Astaxanthin	Animal feed additives Aquaculture Used for culture of prawns, salmonid fish and for ornamental and tropical fish	Priyadarshani and Rath, 2012 Mezzomo and Ferreira, 2016 García-Chavarría, Lara-Flores, 2013 Bhosale, 2004 Amaya et al., 2014
Dunaliella sp.	β-carotene	Animal feed additives Used as natural food coloring agent in aquaculture feed industry	Priyadarshani and Rath, 2012 Mezzomo and Ferreira, 2016 García-Chavarría, Lara-Flores, 2013 Bhosale, 2004 Amaya et al., 2014
Chlorella vulgaris	Lutein Astaxanthin Fucoxanthin	Used in diets of rainbow trout yielding both muscle and skin pigmentation effects Animal feed additives	Priyadarshani and Rath, 2012 Mezzomo and Ferreira, 2016 García-Chavarría M., Lara-Flores M, 2013
Chlorella protothecoides	Lutein	The reddish color of salmonid fish and yellowish color of egg yolk	Guedes et al., 2011 Mezzomo and Ferreira, 2016
Algae and cyanobacteria	Carotenoid	Application as feed additives	References
Chlorella zofingiensis	Lutein	Enhances the reddish color of salmonid	Guedes et al., 2011
Muriellopsis sp.	Lutein	Poultry farming and/or aquaculture	Blanco et al., 2006
Scenedesmus obliquus	Lutein	Fish feed	Mezzomo and Ferreira, 2016
Tetraselmis sp	Lutein	Aquaculture and larval feeds	Priyadarshani and Rath, 2012
Nannochloropsis oculata	Astaxanthin	Aquaculture & larval feeds Hen supplements - increases egg nutritional value	Priyadarshani and Rath, 2012 Zaheer, 2017 Gładkowski et al, 2011
Spirulina sp	*β*-carotene	Animal feed additives	Indira Priyadarshani and Biswajit Rath, 2012

The market demand for pigments natural sources has promoted large-scale cultivation of microalgae for biosynthesis of such compounds (well-established are β-carotene and astaxanthin), so significant decreases in production costs are expected in coming years.

The European legislation specifies that before their use in EU, carotenoids have to be subjected to an authorization process.

A general overview of the current legal situation of the different carotenoids used as feed additives in compound feed in European

Union is shown in Table 5 (Vincent et al., 2017), with added data regarding what carotenoids are totally synthesized and which are extracted or biosynthesized come from (Pérez-Gálvez, 2003; Baldo, 2011; Amaya et al., 2014; Rodriguez-Amaya, 2015; Mezzomo and Ferreira, 2016).

Table 5. Carotenoids used in the feed industry as feed additives in the European Union

Carotenoids feed additive	Target animal	Total synthesized	Extracted	Biosynthesized	EURL-FA evaluation report
Astaxanthin	Fish (salmon, trout)		Crustaceans Pink shrimp (*Penaeus paulensis*) processing waste	*Phaffia rhodozyma* *Haematococcus pluvialis* *Chlorella vulgaris*	EURL-FA Feed Additives (2014)
Astaxanthin	Fish Ornamental fish Crustaceans	X			EURL-FA Feed Additives (2010a)
Astaxanthin dimethyldisuccinate[1]	Salmon and trout	X			EURL-FA Feed Additives (2007a)
β-apo-8'-carotenoic acid ethyl ester (apoester)[2]	Poultry	X			EURL-FA Feed Additives (2011c)
Astaxanthin, adonirubin, canthaxanthin	Salmon and trout			Red carotenoid-rich *Paracoccus carotinifaciens*	EURL-FA Feed Additives (2007b)
Canthaxanthin[2]	Chickens for fattening and minor poultry species for fattening; Laying poultry and poultry reared for laying; Ornamental fish and ornamental birds; Ornamental breeder hens	X		*Chlorella vulgaris*	EURL-FA Feed Additives (2011a)
Capsanthin[3]	Poultry		Red peppers		EURL-FA Feed Additives (2012b)
Citranaxanthin[2]	Poultry	X			EURL-FA Feed Additives (2016)
Lutein[3]	Poultry		Marigold (*Tagetes erecta and Tagetes patula*) Rose fruit (*Rosa canina*) Carrot		EURL-FA Feed Additives (2012a)
β-carotene (solid form)	All animal species	X		*Chlorella vulgaris* *Dunaliella salina* *Arthrospira maxima*	EURL-FA Feed Additives (2010b)
Zeaxanthin[3]	Poultry		Marigold (*Tagetes erecta* and *Tagetes patula*) Paprika (*Capsicum annuum*)		EURL-FA Feed Additives (2012a)

[1]formulated in an organic matrix
[2]formulation containing the active substance and other ingredients
[3]formulation containing a natural source of the active substance and other ingredients
EURL-FA - EU Reference Laboratory for Feed Additives

In the EU, carotenoids used as feed additives are mainly authorised under the category „sensory additives", as colorants (Vincente et al., 2017). The addition of natural and synthetic carotenoids in layer feeds in the European Union is currently limited to 80 ppm (mg/kg), excepted canthaxanthin, whose limit is 8 ppm, because excessive exposure in humans can lead to the development of precipitated crystals in the retina (Baker, 2001).

CONCLUSIONS

Beyond the properties related to health, various natural sources of carotenoids have been and still are investigated as they represent renewable raw materials, are profitable in terms of culture/cultivation costs, as well as for their replicability in terms of processes, and they act synergistically – complementing one-another in diets.

Even though the high demand of carotenoids has long been met by synthetic technology, because of by-products with undesirable effects on consumption, production of carotenoids from natural sources, improvement of bioproduction techniques and development of extraction methods, became the focus of extensive research.

In order to reach industrial production, more optimization studies and also validation methods are required to scale up the processes.

A most important part in animal nutrition is represented by feed additives, including especially carotenoids, which are mainly sensory additives used in the coloring of fish, birds and food of animal origin.

Many natural sources with high potential for animal feed additives have already been identified and studied, some of these being highlighted in this article.

According to market reports, demand for carotenoids in animal feed is increasing due to the increasing preference for good quality and appearance of meat, meat products, and fish. Global carotenoids market is expected to reach USD 1.53 Billion by 2021 and, due to the increasing number of health-conscious consumers and the rising demand for natural coloration, the use of natural carotenoids will contribute to the enhancement of the market growth.

The potential health benefits of microalgae represents the main drive behind the new developments in their usage in feed and foods. Even so, carotenoid production from algae is not as cost effective as the synthetic counterpart.

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