6 Microalgae for Animal and Fish Feed

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CONTENTS

6.1 Introduction: Background and Driving Forces 177
6.2 Definition of Microalgae 178
6.3 Microalgal Biochemical and Nutritional Value 180
  6.3.1 Proteins 182
  6.3.2 Lipids 182
  6.3.3 Carbohydrates 183
  6.3.4 Pigments 184
  6.3.5 Vitamins 185
6.4 Industrial Applications of Microalgae in the Functional Aquafeed Industry 185
6.5 Industrial Applications of Microalgae in the Functional Terrestrial Feed Industry 189
6.6 Commercialized Formulated Aqua and Terrestrial Feed 191
6.7 Challenges and Future Perspectives 203
6.8 Summary 203
References 204

6.1 INTRODUCTION: BACKGROUND AND DRIVING FORCES

In applied phycology, the term *microalgae* refers to microscopic algae and photosynthetic bacteria, such as cyanobacteria. Both classes are considered a potential source of energy, fuel, food, and many other interesting commercial products. Microalgae are the primary producers found in several ecosystems, both marine and

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freshwater. Seventy-two thousand five hundred species and 16 classes have been listed. They have a unique biochemical composition and produce primary and secondary metabolites with interesting biological properties. In particular, these compounds have shown potential as antioxidant, anti-inflammatory, and anti-cancer agents. Microalgae have been used for centuries as human food or animal feed. However, large-scale industrial cultivation is relatively recent technology. Recent articles review state of the art biotechnological production and the use of microalgae. However, this chapter aims to provide an overview of microalgae properties and to examine its potential in use as a feed product.

6.2 DEFINITION OF MICROALGAE

The estimated number of microalgae species is between 30,000–1,000,000. While there is vast application potential due to their unique metabolism, chemical diversity, and ability to tolerate wastewater for culture, certain limitations and challenges remain for the commercial development of microalgae and microalgae-related products. The most complex and crucial issues are related to science, technology, legislation, administration, and marketing gaps.

The concept of *algae* is associated with a diversity of micro- and macro-organisms that can proliferate through photosynthesis (Patras et al., 2019). Both macro- and microalgae can grow in freshwater and marine environments. Microalgae include eukaryotic microalgae and prokaryotic cyanobacteria (Sathasivam et al., 2019), which are ubiquitous and can be found in the most diverse environments, including extreme ecosystems experiencing high or low temperatures, light intensity, pH, and salinity (Martínez-Francés and Escudero-Oñate, 2018).

Eucaryotic microalgae have traditionally been classified according to their pigments. The current classification systems consider other criteria, including the chemical nature of photosynthetic storage products, the organization of photosynthetic membranes, and other morphological features (Remize et al., 2021). Blue-green algae (*Cyanophyceae*), green algae (*Chlorophyceae*), *Bacillariophyceae* (including diatoms), and *Chrysophyceae* (including golden algae) have been described as the most abundant microalgal phyla (García et al., 2017).

Environmental diversity results in the synthesis and secretion of primary and secondary metabolites, which have industrial applications and principal health benefits. (De Morais et al., 2015). Microalgal biomass has the potential to be used as a feedstock for an alternative and innovative source of nutrients, in cosmetics, pharmaceuticals, and nutraceuticals (Rumin et al., 2020). It is important to note that the interest of all those markets in microalgae has been bolstered by the growing concerns concerning environmental sustainability and regulatory issues linked to synthetic chemicals found in food and feed and in beauty products and health care.

Microalgae cultivation can take place in three principal ways, namely photoautotrophic, heterotrophic, and mixotrophic. Most microalgae are designated as photoautotrophs once they can generate energy directly from light, CO_2, and water, typically through photosynthesis, producing organic compounds. Autotrophic microalgae use inorganic carbon forms, such as CO_2 or bicarbonate (HCO_3^-) (Borowitzka, 2018). Additionally, some microalgae species are heterotrophic using organic substrates, such as acetate or glucose, both as energy and carbon sources, in the absence of light (Perez-Garcia et al., 2015). According to the conditions, several of those organisms have a plastic metabolism that allows switching between auto- and heterotrophy. When the microalgae combine the two metabolisms, they are defined as mixotrophs (Pires, 2015). Those organisms can metabolize, simultaneously, both carbon forms in the presence of light.

Compared with autotrophic microalgal growth, heterotrophic and mixotrophic cultivation are more productive in terms of biomass yield (Pires, 2015), as heterotrophic production, the cultivation mode achieving higher productivities. Besides higher productivity, heterotrophy reveals many other advantages. Heterotrophic cultivation occurs in closed fermenters, significantly reducing the crop area used and allowing strict parameter control (Hu et al., 2018), such as pH, temperature, oxygen levels, and nutrients. The contaminations and consequent culture collapse are, moreover, better avoided due to this strict control and sterilization. Therefore, heterotrophic cultivation is economically advantageous once the cost per produced biomass is significantly lower (Hu et al., 2018).

Chen (1996) and Borowitzka (1999) had long recognized that the cell density of a heterotrophic culture could vary between 20 and 100 $g.L^{-1}$ (Chen, 1996; Borowitzka, 1999). However, cell densities of 174.5 and 255 $g.L^{-1}$ had already been reported for *Chlorella vulgaris* (Barros et al., 2019) and *Chlorella protothecoides* (Ghidossi et al., 2017), respectively. Compared to autotrophic cultivation, heterotrophy had allowed enhancing *C. vulgaris* final biomass up to 137.4 times (Barros et al., 2019). However, the chlorophyl content had demonstrated to be lower when *C. vulgaris* was cultivated heterotrophically, increasing from 5 mg g^{-1} DW to 24 mg g^{-1} DW when the culture was exposed to autotrophic conditions (Barros et al., 2019). The same changes induced a 70% increase in protein content in this microalga (Barros et al., 2019). Therefore, the biochemical composition of the final biomass achieved depends on the trophic state of the cultures. The lipid content of a heterotrophic *C. protothecoides* culture was demonstrated to be 55.2% (Xu et al., 2006), compared to 37.5% in the same autotrophic species (Krzemińska et al., 2015). Similar patterns were reported for different microalgae: autotrophic *C. sorokiniana* presented 12–18% lipid content, compared to 24–31% in the heterotrophic biomass (Rosenberg et al., 2014); autotrophic *Chlorella minutissima* had shown 20.2% lipids, compared to 36.2% of heterotrophic biomass (Dubey et al., 2015).

Several limitations are associated besides hetero- and mixotrophic cultivation of microalgae, undoubtedly offering higher productivities and cell densities. The inability to produce light-induced metabolites, primarily related to antioxidant activities, represents a great constraint. Besides, there is also the indirect need for arable land to produce the organic carbon sources used during those cultivations (Borowitzka, 1999), suppressing one of the most significant microalgae cultivation advantages. It is also important to note that only a limited number of microalgae species can metabolize organic carbon sources (Perez-Garcia et al., 2015).

Generally speaking, selecting a suitable microalgae cultivation system depends on the species and added-value compounds intended, as well as their final application. Therefore, several parameters should be considered for cultivation, such as light efficiency, pH and temperature control, species/biocompounds productivity, hydrodynamic stress, the need for an axenic culture and harvesting, and ease of scale-up (Guedes and Malcata, 2012). The primary decision when cultivating microalgae is whether to use closed or open systems. The open systems require low investment and operational costs, and they are also easy to build and operate. However, contamination issues are more common and difficult to control and high cell densities are not reached due to self-shading effects (Wu et al., 2017). Most of the species commercialized and produced industrially on open systems, like *Spirulina* sp. and *Dunaliella salina*, grow in extreme environments, suitable for cultivation in open pounds avoiding the most common contaminations.

On the other hand, closed systems have shown to be more efficient than open ones, allowing better control of the culture parameters. Close bioreactors include tubular configurations, flat plate reactors, and fermenters (Perez-Garcia et al., 2015). The first two configurations are ideal for the autotrophic cultivation of microalgae and the fermenters are used for heterotrophic growth.

Despite a wide range of advantages of using closed systems for microalgae cultivations, some constraints also arise. Those systems are commonly associated with fouling and overheating, cleaning is more time- and resource-consuming and oxygen accumulation (in the autotrophic reactors) results in a decrease in culture growth. Additionally, the closed systems require relatively high investment and operational costs.

Thus, there are many opportunities to cultivate microalgae, which are dependent on the species aimed to produce and the application of the final biomass. Besides this, the investment and operational costs are key factors when selecting the trophic mode of cultivation and the suitable bioreactor.

6.3 MICROALGAL BIOCHEMICAL AND NUTRITIONAL VALUE

The bioactive compounds produced by microalgae and the cellular content are strain-specific and respond to biotic and abiotic factors, e.g., growth phase, light intensity, etc. (Lafarga, 2020). Therefore, these factors should be considered when cultivating microalgae to manipulate their cell composition and biomass productivity, cell metabolism pathway, and final bioactivity (Matos et al., 2017). Most metabolites accumulate intracellularly, even if, in the case of exometabolites, their excretion to the culture medium takes place.

Microalgae compounds can be grouped into proteins/enzymes, acids, pigments, and vitamins, derived from primary metabolism. Secondary bioactive compounds can also be synthesized (Levasseur et al., 2020). Figure 6.1 summarizes the nutritional components that can be acquired from microalgae biomass.

Even though the biochemical composition appears species-, and even strain-dependent, protein is typically the principal organic constituent (12–35%), usually followed by lipids (7–23%) and carbohydrates (5–23%) (Vieira et al., 2020). It is important to note that these proportions may change depending on the culturing conditions (Vieira et al., 2020).

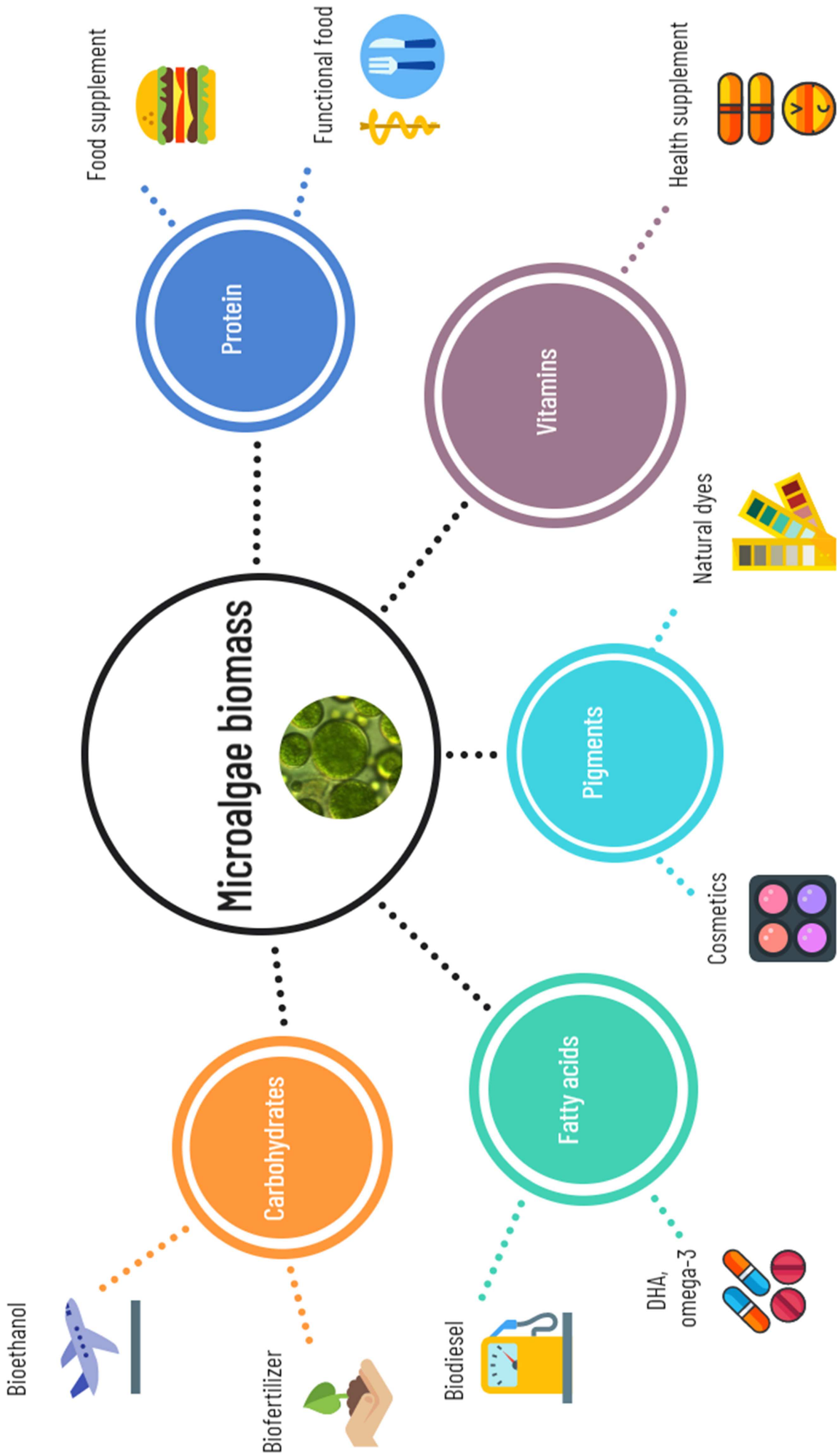

FIGURE 6.1 Algal biomass and their applications.

6.3.1 Proteins

Microalgae have been recognized as a protein source since the 1950s (Venkataraman, 1997). Depending on the species and culturing factors, the protein content ranges from 42% to over 70% on a dry weight basis, reported for specific cyanobacteria species (Barkia et al., 2019).

In terms of quality, microalgae have a rich and varied amino acid composition, containing all the essential amino acids (EEAs) humans are incapable of synthesizing (Barkia et al., 2019). In addition, this group of organisms contains non-essential amino acids (NEAAs), such as arginine, aspartic, proline, glutamic acid, glycine, cysteine. NEAAs of *C. vulga*ris and *Haematococcus pluvialis* appear around 51.03 and 48.50% of the total amino acid profile, respectively. Biological properties in immunity, gene expression regulation, redox homeostasis, and cell communication that can be used in the nutritional, cosmetics, and pharmaceuticals fields have been reported for those compounds (Jacob-Lopes et al., 2019).

6.3.2 Lipids

Particular attention has been given recently to microalgae lipids. These compounds can make up to 74% of microalgae's total biochemical composition (Sathasivam et al., 2019). Polyunsaturated fatty acids (PUFAs) have recently been the subject of attention because of their health benefits. Based on 12 to 24 carbon atom fatty acids, these molecules include the n-3 and n-6 polyunsaturated fatty acid families. Among PUFAs, the bioactivity of docosahexaenoic acid (DHA, 22:6) and eicosapentaenoic acid (EPA, 20:5) is usually highlighted. For example, brain function, more specifically, short- and long-term memory, can be improved by DHA. This molecule also has a positive effect on cognitive decline, depression, bipolar symptoms, and mood swings. EPA and/or DHA have also been associated with a preventive role in cardiovascular diseases (CVDs), and cancer. Generally speaking, the bioactive lipid properties are expected to be of central interest in developing novel ingredients or compounds for various sectors, including the feed and food industry, with high commercial value (Calder, 2006).

Fish such as salmon, tuna, mackerel, sardines, anchovies, herring, or pollock is a good source of EPA and DHA for human consumption. However, marine fish cannot give the global demand for n-3 fatty acids due to their limited stocks and some undesirable contaminants, encouraging industries to find novel alternative sources (Oliver et al., 2020). In this sense, the feasibility of using microalgae as an alternative has been long-explored (Remize et al., 2021). However, it is essential to note that the content of n-3 PUFAs varies within species according to environmental factors, given that it is naturally relatively low (Sahu et al., 2013). Thus, cell EPA and DHA productivity improvement strategies have been investigated, including manipulating culturing conditions, partial or complete deprivation of nitrogen, or using two-phase culturing approaches (Wang et al., 2019). Acting directly on the microalgae metabolism and applying the heterotrophic mode is another way to stimulate lipid production (Remize et al., 2021). Thus, under optimal cultivation conditions, several species, especially those belonging to the genera *Chlorella*, *Nannochloropsis*, and *Dunaliella* are described as showing exceptionally high amounts of lipids in their cell mass.

Other essential fatty acids such as alpha-linolenic acid (ALA), gamma-linolenic acid (GLA), linoleic acid (LA), and arachidonic acid (ARA) are produced by microalgae. They can be applied by the feed and food markets. They have also proven to be active on healing and wound repair, as well as having anti-microbial properties (Choopani et al., 2016).

6.3.3 Carbohydrates

Besides lipids, microalgal biomass can be a source of carbohydrates with great industrial applications. They are represented by poly- or oligosaccharides present in vacuoles and cell walls. Cellulose, and amylose are some main polysaccharides present in microalgae, which also excrete exopolysaccharides (EPS). These carbohydrates have different biological roles linked to 1) energy reservation, 2) formation of the cell wall and 3) cell communication.

Tetraselmis sp., *Isochrysis* sp., *Porphyridium cruentum*, *Porphyridium purpureum*, *Chlorella* sp. and *Rhodella reticulata* are the main genera and species used for the production of polysaccharides. More particularly, *Chlorella* sp. is described as having high carbohydrate content, with *C. vulgaris* able to accumulate 37–55% dry weight (Illman et al., 2000). *Chlamydomonas reinhardtii* and *Scenedesmus obliquus* have been indicated as suitable for biofuel feedstock due to their 45–60% carbohydrate content. With appropriate cultivation conditions, such as under three-day nitrogen starvation, the production of microalgal carbohydrates can be improved. The biomass concentration and carbohydrate content of *S. obliquus* reached 4.96 $g.L^{-1}$ and 51.8%, respectively under this stress condition (Ho et al., 2012).

The polysaccharides derived from *Porphyridium* sp. are described as having many techno-functional and biological properties (Delattre et al., 2016). *Porphyridium cruentum* is a fast and flexibly grown eukaryotic marine microalga. In addition, its cells are encapsulated in a mucilaginous sheath, representing a cell's structural polysaccharide. Some of these polysaccharides are excreted in this medium in the form of exopolysaccharides (EPSs). The exact physiological function of EPS remains ambiguous, even if the literature reports it as the prevention against desiccation and protection against many environmental conditions such as pH, temperature, salinity, and irradiance (Ramus, 1972). Many factors such as culture media, mode of cultivation (batch, continuous), illumination, and salinity impact the soluble EPSs (Ramus, 1972).

The most studied red microalgae exopolysaccharides were produced and extracted from *Rhodella reticulata*, *Porphyridium* sp., *P. aerugineum*, and *P. cruentum*. *Porphyridium cruentum* is described as producing a high amount of exopolysaccharides (0.1–0.7 $g.L^{-1}$), such as *Arthrospira platensis* (0.37 $g.L^{-1}$), *Botryococcus braunii* (0.25–1 $g.L^{-1}$) and *Dunaliella salina* (0.94 $g.L^{-1}$) (Trabelsi et al., 2013; Casadevall et al., 1985; Mishra and Jha, 2009).

6.3.4 Pigments

Microalgae appear macroscopically in different colors due to the presence of pigments, which absorb visible light and have a fundamental role in photosynthetic cell metabolism. The main classes of pigments found in microalgae include chlorophylls (0.5–1.0% DW), carotenoids (0.1–0.2% DW, but some species can achieve up to 14%), and phycobiliproteins (up to 8% DW) (D'Alessandro and Filho, 2016). For several years, pigments have acquired crucial importance in various fields, namely the pharmaceutical, medical, and food fields (Santiago-Santos et al., 2004), because of their health benefits, including antioxidant, anti-cancer, and anti-inflammatory properties. They are also used in cosmetics because they can replace artificial colors (Rodrigues et al., 2015).

Chlorophylls are green pigments with polycyclic planar structures esterified by a phytol side chain. According to their structural features and wavelength absorption, different chlorophylls (a, b, c, d, e) have been identified in microalgae. Chlorophyll a is present in all photoautotrophic algae and the only found in Cyanobacteria and Rhodophyta. Chlorophyll b is found, generally, in green algae (Stengel et al., 2011). Chlorophylls c, d and e can be found in diverse marine microalgae and freshwater diatoms. When isolated, the fraction containing chlorophyll represents 0.5–1.0% DW (Vieira et al., 2020).

Carotenoids are another class of pigments found in microalgae in abundance, ranging from red and brown to orange and yellow colors. Their biological properties related to redox homeostasis, anti-cancer, regulation of triglycerides and HDL (high-density lipoprotein) (Tanaka et al., 2012; Berthon et al., 2017) allow this group of compounds to accumulate several industrial applications in cosmetics, food and feed.

Carotenoids are classified as α, β, ε, and γ or oxygen-containing compounds, named xanthophylls (Guedes and Malcata, 2012). The last group involves lutein, violaxanthin, spirilloxanthin, neoxanthin, and fucoxanthin. Their distribution patterns vary depending on the species, with more than 600 different carotenoids identified.

Chlorophyceae class represents the primary source of carotenoids within the microalgae group of organisms. Those organisms can produce carotenes (β-carotene, lycopene) and xanthophylls (astaxanthin, violaxanthin, antheraxanthin, zeaxanthin, neoxanthin and lutein, among others). Other pigments such as fucoxanthin, diatoxanthin and diadinoxanthin are produced by different microalgae phyla (Berthon et al., 2017). *Dunaliella salina*, *Dunaliella bardawil*, *Dunaliella tertiolecta,* and *Scenedesmus almeriensis* demonstrated their capability in producing a significant proportion of β-carotene when compared to the other pigments (Berthon et al., 2017).

Another carotenoid that is industrially exploited not only in the cosmetic field, but also in aquaculture, is astaxanthin. However, the production cost of this pigment, mainly produced by *Haematococcus* sp., is very high (Canales-Gómez et al., 2010). In aquaculture feed, it has been used to culture salmon, shrimp, ornamental fish and sea bream. 3 $g.kg^{-1}$ feed of *Haematococcus pluvialis* administration effectively enhanced the oxidative status and other biochemical parameters in rainbow trout (Sheikhzadeh et al., 2012).

Despite also being produced by *Chlorella zofingiensis*, *Chlorococcum* sp., *Scenedesmus* sp. and yeast *Xanthophyllomyces dendrorhous* (Yuan et al., 2011), *Haematococcus pluvialis* is the main source of this pigment once it can synthesize up to 81% astaxanthin out of its total carotenoids (Rammuni et al., 2019).

Phycobiliproteins represent the last class of pigments found in red algae, cyanobacteria, cryptophytes, and glaucocystrophytes. According to their absorption spectra, they are classified into four major subgroups: 1) red phycoerytrin, 2) magenta phycoerythrocyanin, 3) blue phycocyanin, and 4) light blue allophycocyanin. In the medical field, they are used as markers in flow cytometry, microscopy, and DNA tests linked to their highly sensitive fluorescent properties. The natural blue pigment phycocyanin, the major microalgae phycobiliprotein, is used for multiple applications in the pharmaceutical field due to its bioactive properties such as antioxidant, anti-inflammatory, and anti-cancer activities. The cosmetic industry also seeks it out for application into lipsticks, eyeliners, and so on (Bingula et al., 2016). Moreover, since 2013, the Food and Drug Administration has accepted its use in food matrices as a coloring additive.

6.3.5 Vitamins

Microalgae represent a source of vitamins which is more complete than terrestrial plants. These microorganisms produce vitamin A, B1, B2, B6, B12, C, E, K, niacin, nicotinate, biotin, and folic acid. The concentrations of vitamin A, B_1, B_2, E, and niacin can achieve those found in vegetables by some microalgae genus, such as *Arthrospira* sp., *Chlorella* sp., and *Scenedesmus* sp. (Del Mondo et al., 2020). *Dunaliella* is also known to highly accumulate vitamins B_2, B_{12}, B_9, B_3, C, and E. More specifically, Hernández-Carmona et al., (2009) reported that *Eisenia arborea*, a species of brown microalgae, contains 3.44 $mg.g^{-1}$ of vitamin C, which is similar to that of mandarin oranges.

Therefore, microalgae are essential sources of macro- and micro-nutrients with interesting market possibilities in nutraceuticals and feed. However, due to food safety regulations, especially in Europe, few of them are currently marketed.

6.4 INDUSTRIAL APPLICATIONS OF MICROALGAE IN THE FUNCTIONAL AQUAFEED INDUSTRY

Up to the present day, microalgae have diverse industrial applications, including food formulation, cosmetics, health products, and fertilizers. Due to their high nutritional and functional value, microalgae have a high potential for application in the feed industry, being incorporated as a feed supplement, enhancing animal performance. Moreover, the increased capacity of microalgae to produce natural anti-microbial compounds promoted the use of some species for immunostimulant applications. Moreover, microalgae cultivation has already been tested for wastewater treatment and biofuel production (Rumin et al., 2020).

The increasing global population and increasing demand for protein will promote feed ingredient soybean production. (FAO, 2018). This process will involve using large areas of arable land, causing the destruction of several terrestrial ecosystems. In addition, several marine ecosystems are being destroyed due to anthropogenic activities, diminishing their natural resources and decreasing the fish and seafood stocks available for human consumption. Nowadays, aquaculture has been demonstrated to be the best way to mitigate lack of natural resources and obtain edible protein. The aquafeed market has been growing faster than other food sectors (Napier et al., 2019) and it is projected to reach 73.15 million tonnes of feeds by 2025, representing a growth of 8–10% (FAO 2018). Feed inputs for aquaculture production represent 40–75% of the global production costs, representing the most relevant driver in this market (FAO, 2018). The increasing demand for meat by a rising population will become particularly dramatic in the coming decades, because dedicated soybean food crops, the conventional feedstuff for animal feeding, will need to occupy an increasing fraction of arable land (FAO, 2018). Aquaculture is currently the world's most efficient producer of edible protein, and continues to grow faster than any other major food sector in the world in response to the rapidly increasing global demand for fish and seafood (Napier et al., 2019). Feed inputs for aquaculture production represent 40–75% of aquaculture production costs and are a key market driver for aquaculture production (FAO, 2018). The aquafeed market is expected to grow 8–10% per annum and is the production of compound feeds is projected to reach 73.15 million tons in 2025 (FAO, 2018).

Recent studies have shown that animal feed supplemented with microalgae, either as a live feed or as an additive, present higher quality and performance, enhance antiviral and antibacterial protection, and improve the immune system, and consequently the disease resistance stress (Remize et al., 2021). Moreover, microalgae-enriched feed has been proven to contribute positively to animal physiology, improving protein turnover, gut function, and stress tolerance (Rehberg-Haas et al., 2015) which helps to achieve a final product with high quality and performance. Despite all advantages of microalgae as livestock feed, it is essential to define a feeding objective to adapt the use of different microalgae according to their biochemical characterization, such as protein, carbohydrates, lipids, vitamins, and pigments composition.

Astaxanthin is one of the pigments used in the aquaculture industry, and its benefits are well documented. Lim et al., (2018) showed that this pigment enhances the immune system, increasing the resistance to infectious diseases in farmed fish. Moreover, due to its high antioxidant activity, astaxanthin could improve the reproductive performance of aquatic animals, increasing the quality of eggs, the growth rate of larvae, and their survival (Lim et al., 2018). Recently, Jaseera and co-workers noted that 2% *Aurantiochytrium* sp. incorporation can significantly improve the growth, survival, nutritional quality of giant tiger prawn, *Penaeus monodon* postlarvae, and increase the tolerance to stress (Jaseera et al., 2021).

Fish-derived fishmeal (FM) and fish oil (FO) used in aquafeeds have long been identified as non-sustainable resources. The use of grain and oilseed crops, such as soy or corn, to substitute FM and FO have been debated in the past. However, it faces critical concerns once terrestrial plant ingredients appear to have low digestibility, anti-nutritional factors, and a deficient amino acid profile, as well as lacking the bioactive long-chain omega-3 EPA and DHA (Borowitzka, 2018, Li et al., 2009).

The use of microalgae blends in fish feed can be found in the literature from the perspective of allowing fish to benefit from a combination of the biochemical compositions of several microalgae species. A blend of *Nannochloropsis* sp. and *Isochrysis* sp. had proven to be a good substitute of 15% fishmeal of juvenile Atlantic cod, *Gadus morhua*, not interfering with fish survival and feed conversion ratio (Walker and Berlinsky, 2011). Sarker et al., (2020) combined two commercially available microalgae, *Nannochloropsis oculata* and *Schizochytrium* sp., to a fish-free feed for Nile tilapia (*Oreochromis niloticus*) that would promote its health performance. Compared to the control diet, Nile tilapia fed with microalgae showed higher growth, weight gain, and specific growth rate. Higher fillet lipid and DHA content, the highest degree of *in vitro* protein hydrolysis and protein digestibility were also obtained on fish fed with the experimental diet (Sarker et al., 2020).

Several studies exploring the possibility of substituting fishmeal with *Arthrospira platensis*, *Chlorella* sp., *Scenedesmus* sp., *Nannofrustulum* sp. and *Tetraselmis suecica* for diverse fish species are reported in the literature (Shah et al., 2018). A 5% replacement of fishmeal with *Spirulina pacifica* significantly increased weight gain, protein efficiency ratio and feed intake of Parrot Fish, *Oplegnathus fasciatus* (Kim et al., 2013). The same study reported that a 15% supplementation resulted in higher muscle protein and lower whole-body lipid (Kim et al., 2013). Hajiahmadian et al., (2012) reported that *Spirulina platensis* substitution of fishmeal up to 20% led to weight gain and increased specific growth rate and feed conversion ratio of golden barb fish, *Puntius gelius* (Hajiahmadian et al., 2012). The growth of silver seabream, *Rhabdosargus sarba*, was also not affected by a substitution up to 50% *Spirulina* sp. (El-Sayed, 1994). Neither were red tilapia fingerlings, *Oreochromis* sp., by 30% substitution with *Arthrospira maxima* (Rincón et al., 2012). Juvenile Nile tilapia presented a higher feed conversion ratio when 50% fishmeal was substituted by *Spirulina* sp. (Hussein et al., 2013), despite Velasquez and co-workers having found that 30% inclusion is the optimal level of replacement (Velasquez et al., 2016).

Twenty percent *Desmodesmus* sp. was included as a fishmeal substitute in Atlantic salmon, *Salmo salar*, feed without compromising the animal growth indexes (Kiron et al., 2016). Sørensen et al., (2016) verified they were able to use *Phaeodactyum tricornutum* as a substitute for 6% of fishmeal in Atlantic salmon, not compromising the nutrient digestibility and growth performance (Sørensen et al., 2016). *T. suecica* was described as able to replace 20% of fish protein, not interfering with the animal growth and quality of European juvenile sea bass meat (Tulli et al., 2012). Also, the complete substitution of fishmeal by *Chlorella* sp. resulted in an increase in the final weight, improvement in total cholesterol, LDL, triglyceride levels, as well as the reproductive performance of zebrafish, *Danio rerio* (Carneiro et al., 2020).

Aquaculture of mollusks is currently the market utilizing most of the feed-destined produced microalgae. The most recent numbers go back to the 1990s, but it was calculated that in 1999, 62% of the aquaculture-predetermined microalgae were used for mollusks. While 21% were used for crustaceans, only 16% were applied to fish aquaculture. Due to their richness in n-3 PUFAs, *Isochrysis* affinis *lutea* (T-iso), *Pavlova lutheri,* and *Chaetoceros* sp. represent the classic microalgae used in shellfish hatcheries (Packer et al., 2016). When released into the cultivation tanks, microalgae are quickly and efficiently filtered from the water. n-3 PUFAs are also crucial for cultivating crustaceans, which can feed directly on microalgae (Spolaore et al., 2006).

Other microalgae had been included in aquafeed due to their immunostimulant properties. 6–8% *Chlorella vulgaris* feed supplementation enhanced prophenoloxidase activity and total hemocyte counts and resistance of giant freshwater prawn *Macrobrachium rosenbergii* postlarvae against *Aeromonas hydrophila* infection (Maliwat et al., 2017). Other *Chlorella* sp. supplementation in the diet of rainbow trout *Oncorhynchus mykiss* fingerlings improved the animal growth and physiological parameters and stimulated the resistance to bacterial infection (Quico et al., 2021). *Labeo rohita* fingerlings demonstrated to be immunostimulated by 0.5 g *Euglena virilis* kg^{-1} dry diet, with increased levels of superoxide anion production, lysozyme, serum bactericidal activity, serum protein, and albumin, as well as increased resistance to *A. hydrophila* (Das et al., 2009). Watanuki et al., (2006) demonstrated that 5–10% of *Spirulina platensis* has the same effect on carp, *Cyprinus carpio* (Watanuki et al., 2006).

As illustrated, microalgae can also be used as probiotics. *Chaetoceros* sp., *Pavlova* sp., and *Isochrysis* sp. have been shown to improve pearl oyster resistance to bacterial pathogens when added to their diets (Shah et al., 2018). Inulin, galactooligosaccharides, xylooligosaccharides, agarose-derived oligosaccharides, neoagaro-oligosaccharides, alginate-derived oligosaccharides, arabinoxylans, galactans, and β-glucans are a few examples of microalgal compounds with prebiotic activities. The proliferation of leukocytes as monocyte-macrophages and neutrophils, as well as phagocytic activity and secretion of immune mediators (e.g., cytokines), are associated with this class of bioactive compounds (Vetvicka et al., 2021). Paramylon is a linear β-1,3 polymer of glucose initially isolated from *Euglena gracilis* and represents an immunostimulant intensively used in aquaculture. These prebiotic compounds are incorporated as feed supplements, enhancing the immune performance of species such as mussels and Atlantic salmon (Kiron et al., 2016).

Beta-glucan derived from yeast, mainly *Saccharomyces cerevisiae*, has to date been the most successful prebiotic on the market. Some other products can also be found on the market, such as WellMune™, by Biothera Corporation (Eagan, MN, USA), BetaGlucans, by BioTec Pharmacon (Tromsø, Norway), and Macrogard™ by Immunocorp (Werkendam, Netherlands). Paramylon, available commercially as Algamune™ by Algal Scientific Corporation (Plymouth, MI, USA). This β-1,3-glucan yield can go up to 90% DW when *Euglena gracilis* is grown heterotrophically (Barsanti et al., 2001). Despite many compounds already being commercially available, further research in this topic appears to be necessary. The probability of finding compounds with prebiotic activities into marine microalgae species is enormous. However, their complex polymer-derived structures are a puzzle for researchers.

Direct utilization of microalgae as feed is also practiced in aquaculture. Heterotrophic protists and small zooplankton, such as *Brachionus* sp. or *Artemia salina*, are critical players in supplying the microalgae nutrients and functionality to higher trophic levels (Camacho et al., 2019). The heterotrophic dinoflagellate *Crypthecodinium cohnii* can be easily found in the market traded as a FO substitute due to its DHA content for seabream, *Sparus aurata*, microdiets (Ganuza et al., 2008). These characteristics seem to be of great importance for seabream larvae and resulted in similar performances compared to classical fisheries diets (Bec et al., 2006).

Nannochloropsis sp., *Isochrysis galbana* and *Schizochytrium mangrovei* are also widely used due to their EPA and DHA composition for rotifer production. Those microalgae were demonstrated to increase rotifer survival, productivity, the efficiency of feed assimilation, and biochemical composition (Ferreira et al., 2009; Ferreira et al., 2008).

Therefore, the potential of using microalgae in aquafeed is tremendous. Besides being a reliable and unconventional source of nutrients, microalgae biomass is rich in bioactive metabolites, which confer cultivation advantages to cultured animals.

6.5 INDUSTRIAL APPLICATIONS OF MICROALGAE IN THE FUNCTIONAL TERRESTRIAL FEED INDUSTRY

The incorporation of microalgae in non-aqua feed, while not being as common, can be seen in the literature and the market. Several products using *Spirulina* sp. as a pet feed supplement are commercialized, such as Phycon® pastes, from Phycon (The Netherlands) or Allvitae®, from Allmicroalgae (Portugal). Besides *Spirulina* sp. PUFA-rich microalgae are most commonly found in pet food – for cats and dogs, once essential to keep the animals' health intact, especially during their growth and reproduction. Together with these products, the literature supports the supplementation of *Schizochytrium* sp. into canine diets as a source of n-3 LC-PUFA DHA (Hadley et al., 2017). Souza et al., (2019) described that a 0.4% *Schizochytrium* sp. diet supplementation is pleasant to dogs' palate while increasing the metabolizable energy and nutrients digestibility. Also, it stimulates phagocytic cells and the phagocytosis of monocytes, while not affecting the animals' fecal characteristics, biochemical profile, and blood hemogram (Souza et al., 2019).

Many experiments have been applied to farm animals showing that dietary microalgae can improve their growth performance and health condition. Poultry feeding assays are reported in the literature, demonstrating the physiological and functional effects of microalgae supplementation on those animals. Waldenstedt et al., (2003) verified that *Haematococcus pluvialis* supplementation could reduce caecal colonization of *Clostridium perfringens* on female broiler chickens (Waldenstedt et al., 2003). Specific-pathogen-free chicken fed with 1–2 $g.kg^{-1}$ *Spirulina* sp. of ration exhibited optimum immune response, increased protection against heterologous virus strains, and reduced viral shedding (Abotaleb et al., 2020). The immune stimulation conferred by *Spirulina* sp. in chickens was also confirmed by Mirzaie et al. (2018). Moreover, *Spirulina* sp. supplementation decreased stress hormones and serum lipid parameters and elevated antioxidant status, while not affecting animal performance characteristics (Mirzaie et al., 2018). Kang et al., (2013) noted that 1% fresh liquid *Chlorella vulgaris* supplementation improved body weight gain, immune factors, and the production of *Lactobacillus* bacteria in the intestinal microflora of broiler chickens (Kang et al., 2013). The beneficial effects of this microalga have also been reported for ducks. Oh et al., (2015) found that the animal body weight was increased in line with the supplementation of heterotrophic *C. vulgaris,* and the meat quality was positively affected (Oh et al., 2015).

Studies on pig feeding using microalgae are scarce when compared with other animals. Furbeyre et al., (2017) demonstrated that 1% *Arthrospira platensis* and *C. vulgaris* supplementation is responsible for improving the intestine mucosal architecture and nutrient digestibility of weaned piglets. Moreover, *C. vulgaris* was demonstrated to play a role in regulating digestive disorders after weaning, avoiding diarrhea (Furbeyre et al., 2017). In growing pigs, the inclusion of 0.1% heterotrophic *C. vulgaris* improved the growth performance, nutrient digestibility, microbial shedding (decreased *Escherichia coli* and higher *Lactobacillus* sp.), and decrease the fecal noxious gas emissions (ammonia and hydrogen sulphide) (Yan et al., 2012). Coelho et al., (2020) showed that the growth performance, carcass, and meat quality traits remained stable when finishing pigs were supplemented with 5% *C. vulgaris* diets. This inclusion increased the levels of some lipid-soluble antioxidant pigments and n-3 PUFA, and decreased the n-6:n-3 fatty acid ratio, improving the nutritional value of pork fat (Coelho et al., 2020).

A limited number of ruminant feeding assays are reported in the literature. The reason is mainly attributed to the amount of microalgae necessary to perform those assays being enormous when compared to other animals (Becker, 2004), as well as the technical details necessary to conduct them. Nevertheless, one of the first microalgae feeding assays, performed by Hintz et al., (1966) demonstrated that when weaning lambs were fed with supplementation of a microalgae mixture, containing *Chlorella* spp., *Scenedesmus obliquus,* and *Scenedesmus quadricauda*, significant weight gain occurred compared to the other rations (Hintz et al., 1966). Alves et al. (2018) used sheep ruminal fluid, hypothesizing the protection of *Nannochloropsis oceanica* cell walls to EPA. These authors demonstrated that *N. oceanica* resultant EPA metabolism was remarkably reduced, demonstrating, for the first time in ruminants, the kinetics of EPA biohydrogenation class products and the formation of 20:0 fatty acids (Alves et al., 2018).

The supplementation of 10%, but not 20%, *A. platensis* to weaned lambs led to an increase in body weight and score, and body condition score when compared to the non-supplemented control group (Holman, 2012).

Kulpys et al., (2009) evaluated the effect of *A. platensis* when supplemented to lactating cows, during a 90-day trial. It was verified that a 200g daily supplementation of *Spirulina* sp. led to an increase in cow bodyweight of 8.5–11% and 21% of milk when compared to the control group. The authors even concluded that this supplementation is economically sustainable (Kulpys et al., 2009).

Although the microalgal species was not revealed, a study performed by Elzinga et al., (2019) demonstrated that a DHA-rich microalgae supplementation given to horses with equine metabolic syndrome can modulate their metabolic condition and reduce inflammation. This study is especially relevant since a big portion of the equine population is predisposed to develop this metabolic syndrome (Elzinga et al., 2019).

Several other microalgae have been experimented on in lab animals, supporting the potential of the use of this group of microorganisms in animal diets (Navarro et al., 2016). Therefore, besides minor effects being found in terms of meat quality, the inclusion of different species of microalgae in animal diets can improve their productivity by increasing growth performance parameters and stimulating their immune response. The scientific community has demonstrated the role of several microalgal strains in the most diverse terrestrial animals, increasing opportunities for the establishment of robust and scientifically supported feed products.

6.6 COMMERCIALIZED FORMULATED AQUA AND TERRESTRIAL FEED

While the potential of microalgae as a feed supplement for the most diverse animals has long been recognized, the products available on the market are still limited to a few formulations. During the 1960s, protein-rich microalgae were the main product available. The commercialization of *Dunaliella* sp. and *Haematococcus* sp. aimed at the functionality and color conferred by their pigments, β-carotene, and astaxanthin, appeared and had their boom during the 1980s. The 1990s brought the lipid-rich microalgae and EPA and DHA became a trend in feed products (Camacho et al., 2019). Nowadays, the market is populated by diverse microalgae-based products commercialized by several companies, some microalgae producers, and other feed formulators, as demonstrated in Table 6.1.

Allmicroalgae Natural Products, located in Portugal, and Necton, located in the same country, are two examples of companies whose core activity is microalgae production and which have a brand of aquafeed products in the market, Allvitae® and Phytobloom®, respectively.

In the aqua market, two significant categories of microalgae-derived feed products can be found: microalgae cultures, usually concentrated, mostly used for larval fish, shrimp, and bivalves, and formulated rations, supplemented with microalgae. Algagen LLC, located in Florida (USA), supplying concentrated microalgae cultures for the most diverse aqua cultivation. However, in Belgium Proviron commercialized their microalgae for feed application in powder form. Others, like Sera in Germany formulated products on the market, instead of pure microalgae.

Most of the commercialized microalgae for feed applications are being used fresh, either as the only component or as an additive. Some suppliers produce microalgae seed cultures so that the aquaculture farmers can have an on-site microalgae cultivation for further utilization. Algagen LLC is one company with a diverse offer of seed microalgae species. There is also a large number of options in terms of species. As an example, GreenSea, located in France, has more than ten species available as a product.

The market of microalgae-based feed for terrestrial animals is not as developed as that of aquatic animals. The products only started to appear on the market late in the 20th century, and the majority are nutritional supplements. *Chlorella* sp. and *Spirulina* sp. are the predominant microalgae in these products, such as pâtés and biscuits for cats and dogs commercialized by Yarrah, in the Netherlands, or Equialgae, the ration sold by NeoAlgae for horses. However, other species appear supplemented in the animal feed, such as *Tetraselmis chui*, *Nannochloropsis oceanica* and *Scenedesmus obliquus* used in Allvitae® by Allmicroalgae Natural Products, as is demonstrated in Table 6.2.

TABLE 6.1
Description of Some Aqua-feed Microalgae-based Products Available in the Market

Product	Composition	Microalgae Species	Company	Price	Applications
Instant Algae® Nanno 3600	Microalgae concentrate	*Nannochloropsis* sp.	Reed Mariculture Inc. (California, USA)	62.50€ (1L)	Finfish and shellfish hatchery
Instant Algae® Tetraselmis 3600		*Tetraselmis* sp.		70.83€ (1L)	Finfish, shellfish and shrimp hatchery
Instant Algae® Iso 1800		*T- Isochrysis*		45.81€ (1L)	Finfish, shellfish and shrimp hatchery
Instant Algae® Pavlova 1800		*Pavlova* sp.		45.81€ (1L)	Finfish, shellfish and shrimp hatchery
Instant Algae® TW 1800		*Thalassiosira weissflogii* and *T. pseudonana*		30.83€ (1L)	Finfish, shellfish and shrimp hatchery
Rotifer Diet®		*Nannochloropsis* sp. and *Tetraselmis* sp.		62.50€ (1L)	Finfish hatchery
RotiGrow® OneStep	Liquid	High-yield microalgal blend		59.38€ (1L)	Finfish hatchery
RotiGrow® Plus		Omega algal blend		59.38€ (1L)	Finfish and shellfish hatchery
Rotigrow® Nanno		*Nannochloropsis* sp.		59.38€ (1L)	Finfish and shellfish hatchery
Chlorella V12	Live microalgae	*Chlorella* sp.		n.d	Finfish hatchery
N-Rich® High Pro	Liquid	Concentrated blend of microalgae		45.81€ (1L)	Finfish hatchery
N-Rich® PL Plus					
N-Rich® Ultra PL					
RotiGreen® Nanno		*Nannochloropsis* sp.		62.50€ (1L)	Finfish hatchery
RotiGreen® Iso		*T- Isochrysis*		45.81€ (1L)	
Rotigreen® Omega		Blend of *Nannochloropsis* sp. and other DHA-producing microalgae (n.d.)		45.81€ (1L)	
Shellfish Diet® 1800	Microalgae concentrate	Blend of *Isochrysis* sp., *Pavlova* sp., *Tetraselmis* sp., *Thalassiosira weissflogii* and *T. pseudonana*		44.05€ (1L)	Shellfish hatchery

(*Continued*)

TABLE 6.1 (Continued)
Description of Some Aqua-feed Microalgae-based Products Available in the Market

Product	Composition	Microalgae Species	Company	Price	Applications
LPB Frozen Shellfish Diet®		Blend of *Tetraselmis* sp., *Thalassiosira weissflogii* and T. *pseudonana*			Shellfish hatchery
TDO Chroma BOOST™		*Haematococcus* sp.		22.92 to 31.25€ (1Kg)	Finfish hatchery
Phytobloom Green Formula	Microalgae concentrate	*Nannochloropsis* sp.	Necton, S.A. (Algarve, Portugal)	n.d	Finfish, shellfish and shrimp hatchery
		Isochrysis sp.		n.d	Shellfish hatchery
		Tetraselmis sp.		n.d	n.d.
Phytobloom Green Ice	Frozen paste	*Nannochloropsis* sp.		n.d	Finfish, shellfish and shrimp hatchery
		Isochrysis sp.		n.d	Finfish and shrimp hatchery
		Phaeodactylum sp.		n.d	Shellfish hatchery
		Tetraselmis sp.		n.d	Shrimp hatchery
Phytobloom Green Prof	Microalgae powder	*Nannochloropsis* sp.		n.d	Finfish, shellfish and shrimp hatchery
		Isochrysis sp.		n.d	Finfish, shellfish and shrimp hatchery
		Phaeodactylum sp.		n.d	Shellfish hatchery
		Tetraselmis sp.		n.d	Shellfish and shrimp hatchery
AlgaGenPods™ *Tisbe*	Liquid	Live *Tisbe biminiensis* and microalgae (n.d.)	Algagen LLC (Florida, USA)	85€ (1Kg)	Seahorse, mandarins, wrasses, invert larvae
AlgaGenPods™ *Apocylops*		*Apocylops panamensis* and microalgae (n.d.)		85€ (1Kg)	Corals and marine-fish-larvae
AlgaGenPods™ *Pseudodiaptomus*		*Pseudodiaptomus pelagicus* and microalgae (n.d.)		15€ (1Kg)	Leafy seadragon, fish, reidi seahorse, clown and mandarin larval fish, seahorses, sessile invertebrates, and small reef fish

(*Continued*)

TABLE 6.1 (Continued)
Description of Some Aqua-feed Microalgae-based Products Available in the Market

Product	Composition	Microalgae Species	Company	Price	Applications
AlgaGenPods™ Parvocalanus		*Parvocalanus crassirostris* and microalgae (n.d.)		103€ (1Kg)	Sea tang, sergeant major, anthias, several larval fish, sessile invertebrates, corals and small reef fish
AlgaGenPods™ Acartia		*Acartia tonsa* and microalgae (n.d.)		85€ (1Kg)	Aquatic toxicology, ideal for fish breeding efforts
Phyto-Plasm™ Brown		Three types of brown phytoplankton/microalgae and zooxanthellae		45.83€ (1L)	Most pods, clams, flame scallops, shrimp and corals
Phyto-Plasm™ Green		Two types of green phytoplankton/ microalgae and zooxanthellae			Amphipods, copepods, shrimp larvae, sponges, feather-duster, gorgonians and soft corals.
Phyto-Plasm™ Zooxanthellae		Blend of two species of symbiotic microalgae			Corals
Phyto-Plasm™ Gallon		Blend of microalgae algae either green or brown and zooxanthellae			Corals
PhycoPure™ Reefblend		Blend of microalgae/phytoplankton		55€ (1Kg)	Packed and diverse reef tanks, including zooxanthellae
PhycoPure™ Copepod Blend				n.d	Clams and corals
PhycoPure™ Greenwater				n.d	Rotifers, feather dusters and leathers
PhycoPure™ Zooxanthellae				132€ (1Kg)	Clams and corals

(*Continued*)

TABLE 6.1 (Continued)
Description of Some Aqua-feed Microalgae-based Products Available in the Market

Product	Composition	Microalgae Species	Company	Price	Applications
Proviron ChaetoPrime	Microalgae powder	*Chaetoceros muelleri*	Proviron (Hemiksem, Belgium)	270€ (1Kg)	Bivalves and shrimp or prawn larviculture
Proviron NannoPrime		*Nannochloropis* sp.		240€ (1Kg)	Finfish and shrimp larviculture
Proviron IsoPrime		*Isochrysis aff. galbana* T-ISO		270€ (1Kg)	Finfish, shrimp and prawn larviculture
Proviron TetraPrime S		*Tetraselmis suecica*		240€ (1Kg)	Bivalve, shrimp and prawn larviculture
Proviron TetraPrime C		*Tetraselmis chui*		240€ (1Kg)	Bivalve, shrimp and prawn larviculture
Proviron ThalaPrime W		*Thalassiosira weissflogii*		270€ (1Kg)	Bivalve and shrimp larviculture
Phycom® Algae flakes	Flakes of different sizes	*Chlorella vulgaris*	Phycom® (Veenendaal, Netherlands)	n.d	Freshwater fish
Phycom® Algae flakes		*Chlorella sorokiniana*		n.d	Freshwater fish
Phycom® Algae powder	Microalgae powder	*Chlorella vulgaris*		n.d	Freshwater fish
Phycom® Algae powder		*Chlorella sorokiniana*		n.d	Freshwater fish
Phycom® Algae paste		*Chlorella vulgaris*		n.d	Freshwater fish
Phycom® Algae paste	Microalgae paste	*Chlorella sorokiniana*		n.d	Freshwater fish
Phycom® Algae pellets (IQF)	Soluble pellets	*Chlorella vulgaris*		n.d	Freshwater fish
Phycom® Algae pellets (IQF)		*Chlorella sorokiniana*		n.d	Freshwater fish
Sera Crabs Nature	Sinking loops	Blend of *Spirulina* sp. and *Haematococcus* sp.	Sera (Heinsberg, Germany)	n.d	Crustaceans
Sera Micron Nature	Powder	Zooplankton, *Spirulina* sp. (51%) and *Haematococcus* sp.		n.d	Fish, amphibians and *Artemia nauplii*

(*Continued*)

TABLE 6.1 (Continued)
Description of Some Aqua-feed Microalgae-based Products Available in the Market

Product	Composition	Microalgae Species	Company	Price	Applications
Sera Plankton Tabs Nature	Sinking tablets	Zooplankton, *Spirulina* sp. (24%) and *Haematococcus* sp.		n.d	Fish that eat at the bottom and invertebrates
Sera Shrimps Nature	Granules	*Spirulina* sp. (5%) and *Haematococcus* sp.		n.d	Shrimp
Sera Catfish Chips Nature	Sinking tablets	*Spirulina* sp. (4%) and *Haematococcus* sp.		n.d	Rasping and suckermouth bottom fish
Sera Herbs'n'Loops Nature	Powder loops	*Spirulina* sp. and *Haematococcus* sp.		n.d	Tortoises and other herbivorous reptiles
Sera Guppy Gran Nature	Soft granules	*Spirulina* sp. (4%) and *Haematococcus* sp.		n.d	Herbivorous fish that mainly eat in the middle water layers, such as guppies
Sera KOI Professional Spirulina Color Food	Granules	*Spirulina* sp. (6.3%) and *Haematococcus* sp.		n.d	n.d.
Sera Spirulina Tabs Nature	Attaching tablets	*Spirulina* sp. (24%) and *Haematococcus* sp.		n.d	Herbivorous fish and invertebrates
Sera Cichlid Green XL Nature	Floating granules	*Spirulina* sp. (10%) and *Haematococcus* sp.		n.d	Herbivorous fish
Sera Discus Color Nature	Soft granules	*Spirulina* sp. and *Haematococcus* sp.		n.d	Discus fish
Sera Flora Nature	Flakes	*Spirulina* sp. (7%) and *Haematococcus* sp.		n.d	Herbivorous ornamental fish
Hagen Nutrafin MAX Spirulina Flakes	Flakes	*Spirulina* sp.	Hagen (Quebec, Canada)	n.d	Ornamental fish

(*Continued*)

TABLE 6.1 (Continued)
Description of Some Aqua-feed Microalgae-based Products Available in the Market

Product	Composition	Microalgae Species	Company	Price	Applications
Allvitae® Aqua	Microalgae paste	*Nannocloropsis* sp., *Chlorella* sp. *Phaeodactylum tricornutum*., *Tetraselmis* sp.	Allmicroalgae Natural Products S.A. (Pataias, Portugal)	n.d.	Finfish, shellfish and shrimp hatchery
	Powder	*Nannocloropsis* sp., *Chlorella* sp. *Phaeodactylum tricornutum*., *Tetraselmis* sp.			
Algamune™	Powder	*Euglena gracilis*	Algal Scientific Corporation (Michigan, USA)	n.d.	Shrimp
Green feed Nanno CLA101	Microalgae concentrate	*Nannochloropsis oculata*	GreenSea (Mèze, France)	n.d.	Finfish hatchery
Green Feed Chaeto CLA110-G		*Chaetoceros gracilis*		n.d.	Shellfish and shrimp hatchery
Green Feed Chaeto CLA110-C		*Chaetoceros calcitrans*		n.d.	Shellfish and shrimp hatchery
Green Feed Tetra CLA106-S		*Tetraselmis suecica*		n.d.	n.d.
Green Feed Tetra CLA106-C		*Tetraselmis chui*		n.d.	Shellfish and shrimp hatchery
Green Feed Iso CLA112		*Tisochrysis lutea*		n.d.	Finfish hatchery
Green Feed Phaeo CLA102		*Phaeodactylum tricornutum*		n.d.	Shellfish and shrimp hatchery
Green Feed Duna CA114	Live culture	*Dunaliella tertiolecta*		n.d.	n.d.
Green Feed Porphy CLA103	Microalgae concentrate	*Porphyridium cruentum*		n.d.	n.d.

(*Continued*)

TABLE 6.1 (Continued)
Description of Some Aqua-feed Microalgae-based Products Available in the Market

Product	Composition	Microalgae Species	Company	Price	Applications
Nutritional mix CLA006		*Tetraselmis* sp., *Porphyridium* sp., *Nannochloropsis* sp., *Phaeodactylum* sp. blend		n.d.	Shellfish hatchery, invertebrates and corals
Nutritional mix CLA007		*Tetraselmis* sp., *Porphyridium* sp., *Nannochloropsis* sp., *Phaeodactylum* sp. blend		n.d.	Shellfish hatchery, invertebrates and corals
Green feed Nanno CLA1011		*Nannochloropsis oculata*		n.d.	Finfish hatchery
BIOMIN PA101	Powder	*Tetraselmis chui*		n.d.	Shellfish and shrimp hatchery
BIOMIN PA102		*Phaeodactylum tricornutum*		n.d.	Shellfish and shrimp hatchery
BIOMIN PA103		*Dunaliella* spp.		n.d.	Fish feed formulation
BIOMIN PA106		*Spirulina* sp.		n.d.	Fish feed formulation
BIOMIN PA109		*Chlorella* sp.		n.d.	Fish feed formulation
BIOMIN PA118		*Haematococcus* sp.		n.d.	Shrimp hatchery and fish feed formulation
Nanno CLA101F20	Frozen paste	*Nannochloropsis* sp.		n.d.	Finfish hatchery
CA104	Microalgae concentrate	*Chlorella vulgaris*		n.d.	Fresh water organisms
CA105		*Selenastrum capricornutum*		n.d.	Fresh water organisms
CA111		*Rhodomonas salina*		n.d.	Copepods
CA117		*Scenedesmus subspicatus*		n.d.	Fresh water organisms

n.d. – not described

TABLE 6.2
Description of Some Terrestrial Feed Microalgae-based Products Available in the Market

Product	Composition	Microalgae Species	Company	Price	Applications
Phycom® Algae flakes	Brittle flakes	*Chlorella vulgaris*	Phycom (Veenendaal, Netherlands)	n.d.	Poultry and piglets, horses, dogs and cats, freshwater fish
Phycom® Algae flakes		*Chlorella sorokiniana*		n.d.	
Phycom® Algae powder	Microalgae powder	*Chlorella vulgaris*		n.d.	
Phycom® Algae powder		*Chlorella sorokiniana*		n.d.	
Phycom® Algae paste	Microalgae paste	*Chlorella vulgaris*		n.d.	
Phycom® Algae paste		*Chlorella sorokiniana*		n.d.	
Phycom® Algae pellets (IQF)	Pellets	*Chlorella vulgaris*		n.d.	
Phycom® Algae pellets (IQF)		*Chlorella sorokiniana*		n.d.	
ALLVITAE Mature Pets (7+)	Enriched with microalgae powder	*Chlorella vulgaris*, *Tetraselmis chui*, *Nannochloropsis oceanica* and/or *Scenedesmus obliquus*	Allmicroalgae Natural Products S.A. (Pataias, Portugal)	n.d.	Cats and dogs
ALLVITAE Junior Pets		*Chlorella vulgaris*, *Tetraselmis chui*, *Nannochloropsis oceanica* and/or *Scenedesmus obliquus*			Cats and dogs
ALLVITAE Piglets		*Chlorella vulgaris*, *Tetraselmis chui*, *Nannochloropsis oceanica* and/or *Scenedesmus obliquus*			Swine

(Continued)

TABLE 6.2 (Continued)
Description of Some Terrestrial Feed Microalgae-based Products Available in the Market

Product	Composition	Microalgae Species	Company	Price	Applications
ALLVITAE Performance		*Chlorella vulgaris*, *Tetraselmis chui*, *Nannochloropsis oceanica* and/or *Scenedesmus obliquus*			Swine
ALLVITAE Organic Eggs		*Chlorella vulgaris*, *Tetraselmis chui*, *Nannochloropsis oceanica* and/or *Scenedesmus obliquus*			Aviary, game birds
ALLVITAE Game Birds		*Chlorella vulgaris*, *Tetraselmis chui*, *Nannochloropsis oceanica* and/or *Scenedesmus obliquus*			Aviary, game birds
Yarrah Organic dog food pâté with beef and chicken	Microalgae-enriched pâté	*Spirulina* sp.	Yarrah (Harderwijk, Netherlands)	9.70€ (1Kg)	Dogs
Yarrah Organic Sensitive dry dog food	Microalgae-enriched pâté	*Spirulina* sp.		10€ (1Kg)	Dogs with stomach or digestion problems
Yarrah Organic dog food pâté with chicken	Microalgae-enriched pâté	*Spirulina* sp.		6.70€ (1Kg)	Dogs
Yarrah Organic cat food chunks with fish	Microalgae-enriched chunks	*Spirulina* sp.		11.50€ (1Kg)	Cats
Yarrah Organic cat food pâté with fish	Microalgae-enriched pâté	*Spirulina* sp.		6.70€ - (1Kg)	Cats

(*Continued*)

TABLE 6.2 (Continued)
Description of Some Terrestrial Feed Microalgae-based Products Available in the Market

Product	Composition	Microalgae Species	Company	Price	Applications
Yarrah Organic vegan dog biscuits for smaller dogs	Microalgae-enriched biscuits	*Spirulina* sp.		14.80€ (1Kg)	Dogs
Dr. Mercola SpiruGreen	Tablets (supplement)	Blend of *Arthrospira platensis* (97%) and *Haematococcus pluvialis* (3%)	Cape Dr. Mercola (Cape Coral, USA)	n.d.	Cats and dogs
Algae-to-Omega – Horse Omega-3 Supplement	Enriched with microalgae powder	n.d.	Equi-Force Equine Products, LLC (Kentucky, USA)	64.88€ (1Kg)	Horses
Equialgae – Horses	Enriched with microalgae powder	Blend of *Spirulina* sp. and *Chlorella* sp.	NeoAlgae (Gijón, Espanha)	27.30€ (1Kg)	Horses
Arenus Animal Health-Aleira	Enriched with microalgae powder	Algae-derived DHA	Arenus Animal Health (Fort Collins, USA)	106.62€ (1Kg)	Horses
Arenus Animal Health-Releira	Enriched with microalgae powder	Algae-derived DHA		55.19€ (1Kg)	
AlgenPower CHLORELLA – Premium Vitties	Microalgae-enriched biscuits	Enriched with 2% *Chlorella* sp.	AlgenPower (Hausmannstätten, Austria)	49.76€ (1Kg)	Dogs
AlgenPower CHLORELLA – Premium Vitties	Microalgae-enriched biscuits	Enriched with 2% *Chlorella* sp.		49.76€ (1Kg)	Horses
AlgEnerg Pvt Ltd - Animal Feed Supplement	Microalgae powder	Enriched with *Spirulina* sp.	AlgEnerg Pvt Ltd (New Delhi, India)	4.99€ (1Kg)	Cows, buffalo, calves, sheeps, goats, horses and poultry

(*Continued*)

TABLE 6.2 (Continued)
Description of Some Terrestrial Feed Microalgae-based Products Available in the Market

Product	Composition	Microalgae Species	Company	Price	Applications
SmartPak Equine - Smart & Simple™ Spirulina Pellets	Pellets	Enriched with *Spirulina* sp.	SmartPak Equine (Plymouth, USA)	26.20€ (1Kg)	Horses
SmartPak Equine - SmartBreathe® Ultra Pellets	Pellets			21.20€ (1Kg)	
SmartPak Equine - SmartItch-Ease™	n.d.			54.90€ (1Kg)	
Crypto Lina	Microalgae powder	Enriched with *Spirulina* sp.	Crypto Aero Wholefood Horse Feed (Wellington, Florida)	22.75€ (1Kg)	Horses
Crypto Aero Metabolism				83.44€ (1Kg)	
Crypto Aero Plus				100.82€ (1Kg)	
Air-Way EQ (Pellets)	Microalgae powder	Enriched with *Spirulina* sp.	Med-Vet Pharmaceuticals (Eden Prairie, USA)	928.81€ (1Kg)	Horses
Wholistic Spirulina	Microalgae powder	Enriched with *Spirulina* sp.	Wholistic Pet Organics (Bedford, USA)	148.07€ (1Kg)	Dogs
Algamune™	Powder	*Euglena gracilis*	Algal Scientific Corporation (Michigan, USA	n.d.	Pigs and poultry

n.d. – not described

The market perspective for microalgae-based feed products is positive for the coming years. Besides representing an excellent alternative to the classical ingredients used for aquafeed, research and development support the evidence of the health-promoting effects induced by this group of organisms. There is also a growing need to diminish the number of antibiotics used during fish production. It is then expected that the offer on products supplemented with microalgae shall increase significantly in the next few years.

6.7 CHALLENGES AND FUTURE PERSPECTIVES

The recent rapid evolution in the microalgae biotechnology field led to an increase in the algal bioeconomy applied to the feed industry. Microalgae researchers have been focusing on increasing biomass productivity while reducing production costs. Significant achievements have been reported for bioreactor design, harvesting techniques, strain development, adaptation, and genetic and metabolic engineering, allowing improvements in biomass and added-value compounds productivity. Culturing conditions have also been manipulated to increase the biomass content in carbohydrates, proteins, lipids, pigments, and other metabolites of interest. All those improvements allowed for the achievement of a cell factory undergoing continuous improvement and becoming more effective in carbon capture and more suitable for market applications, such as feed. In spite of all these improvements, the price of microalgal feed is still higher when compared to the traditional ingredients, while conferring to the product functionalities that are not seen in the other crop ingredients. Another constraint in microalgae marketing relates to aquaculture demand for live biomass instead of dried powder. The sourcing and transport of concentrated and frozen biomass inevitably increase the product's final cost.

The balanced nutritional profile and functionality of the biomass are critical factors for the use of these microorganisms in animal feed. Further studies are needed, especially for product formulation, but the future seems promising for this market.

6.8 SUMMARY

Scientifically validated evidence showing that microalgal metabolites develop functional roles when incorporated in feed has been increasing in recent times. Biotechnological research in this field is promising. New bioactive metabolites relevant in the most diverse areas, from human to animal health, are likely to be found in the near future. The microalgae market is also a likely to become a growing sector once consumers become more aware of its nutritional and functional properties and are consequently willing to pay to benefit from that. The possible use of microalgae is then destined to become a prominent reality, intending to promote the growth and health of the fed animal. Beyond microalgae functionality being well documented, further studies are needed in the field of product formulation.

REFERENCES

Abotaleb, M.M., Mourad, A., Abousenna, M.A., Helal, A.M., Nassif, S.A., Elsafty, M.M., 2020. The effect of spirulina algae on the immune response of spf chickens to commercial inactivated Newcastle vaccine in poultry. *VacciMonitor*. 29, 2.

Alves, S.P., Mendonça, S.H., Silva, J.L., Bessa, R.J.B., 2018. *Nannochloropsis oceanica*, a novel natural source of rumen-protected eicosapentaenoic acid (EPA) for ruminants. *Sci. Reports*. 8, 1.

Barkia, I., Saari, N., Manning, S.R., 2019. Microalgae for high-value products towards human health and nutrition. *Mar. Drugs* 24, 304.

Barros, A., Pereira, H., Campos, J., Marques, A., Varela, J., Silva. J., 2019. Heterotrophy as a tool to overcome the long and costly autotrophic scale-up process for large scale production of microalgae. *Sci. Reports* 9, 13935.

Barsanti, L., Vismara, R., Passarelli, V., Gualtieri, P., 2001. Paramylon (β-1,3-Glucan) content in wild type and WZSL mutant of *Euglena gracilis*. Effects of growth conditions. *J. Appl. Phycol.* 13, 59–65.

Bec, A., Martin-Creuzburg, D., Von Elert., E., 2006. Trophic upgrading of autotrophic picoplankton by the heterotrophic nanoflagellate *Paraphysomonas* sp. *Limnol. Oceanog*. 51, 1699–1797.

Becker, W., 2004. Microalgae in human and animal nutrition, in: Richmond, A. (Ed.), *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, p. 18. Blackwell Science, London.

Berthon, J.Y., Nachat-Kappes, R., Bey, M. Cadoret, J.P., Renimel, I., Filaire, E. 2017. Marine algae as attractive source to skin care. *Free Radical Res*. 510, 555–567.

Bingula, R., Dupuis, C., Pichon, C., Berthon, J.Y., Filaire, M., Pigeon, L., Filaire, E., 2016. Study of the effects of betaine and/or C-phycocyanin on the growth of lung cancer A549 cells in vitro and in vivo. *J. Oncol.* 2016, 8162952.

Borowitzka, M.A., 1999. Commercial production of microalgae: Ponds, tanks, tubes and fermenters. *Prog. Ind. Microbiol.* 35, 313–321.

Borowitzka, M.A., 2018. Biology of microalgae, in: *Microalgae in Health and Disease Prevention*, pp. 23–72.

Calder, P.C., 2006. N-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am. J. Clin. Nutr.* 83, 1505S–1519S.

Camacho, F. Macedo, A., Malcata, F., 2019. Potential industrial applications and commercialization of microalgae in the functional food and feed industries: A short review. *Mar. Drugs.* 17, 312.

Canales-Gómez, E., Correa, G., Teresa Viana, M., 2010. Effect of commercial carotene pigments (Astaxanthin, Cantaxanthin and β-Carotene) in Juvenile abalone *Haliotis rufescens* diets on the color of the shell or nacre. *Vet. Mex*. 41, 3.

Carneiro, W.F., Dias Castro, T.F., Orlando, T.M., Meurer, F., Paula, D.A.J., Virote, B.C.R., Vianna, A.R.C.B., Murgas, L.D.S., 2020. Replacing fish meal by *Chlorella* sp. meal: Effects on zebrafish growth, reproductive performance, biochemical parameters and digestive enzymes. *Aquaculture* 528, 735612.

Casadevall, E., Dif, D., Largeau, C., Gudin, D., Chaumont, D., Desanti, O., 1985. Studies on batch and continuous cultures of *Botryococcus braunii*: Hydrocarbon production in relation to physiological state, cell ultrastructure, and phosphate nutrition. *Biotechnol. Bioeng*. 27, 286–295.

Chen, F., 1996. High cell density culture of microalgae in heterotrophic growth. *Trends Biotech*. 14, 21–426.

Choopani, A., Poorsoltan, M., Fazilati, M., Mohammad Latifi, A., Salavati, H., 2016. Spirulina: A source of gamma-linoleic acid and its applications. *J. Appl. Biotech. Rep.* 3, 483–488.

Coelho, D., Pestana J., Almeida, J.M., Alfaia, C.M., Fontes, C.M.G.A., Moreira, O., Prates, J.A.M., 2020. A high dietary incorporation level of *Chlorella vulgaris* improves the nutritional value of pork fat without impairing the performance of finishing pigs. *Animals* 10, 12.

D'Alessandro, E.B., Filho, N.R.A., 2016. Concepts and studies on lipid and pigments of microalgae: A review. *Renew. Sustain. Energy Rev.* 58, 832–841.

Das, B.K., Pradhan, J., Sahu, S., 2009. The effect of *Euglena viridis* on immune response of Rohu, Labeo Rohita (Ham.). *Fish Shellfish Immunol.* 26, 871–876.

Delattre, C., Guillaume P., Laroche, C., Michaud, P., 2016. Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides. *Biotech. Adv.* 15, 1159–1179.

Del Mondo, A., Smerilli, A., Sané, E., Sansone, C., Brunet, C., 2020. Challenging microalgal vitamins for human health. *Microbial Cell Factories*. 201.

De Morais, M.G., Vaz, B.D.S., De Morais, E.G., Costa, J.A.V., 2015. Biologically active metabolites synthesized by microalgae. *BioMed. Res. Int.* 215.

Dubey, K.K., Kumar, S., Dixit, D., Kumar, P., Kumar, D., Jawed, A., Haque, S., 2015. Implication of industrial waste for biomass and lipid production in *Chlorella minutissima* under autotrophic, heterotrophic, and mixotrophic grown conditions. *Appl. Biochem. Biotech.* 176, 1581–1595.

El-Sayed, A.F.M., 1994. Evaluation of soybean meal, spirulina meal and chicken offal meal as protein sources for silver seabream (*Rhabdosargus sarba*) fingerlings. *Aquaculture*. 127, 169–176.

Elzinga, S.E., Betancourt, A., Stewart, J.C., Altman, M.H., Barker, V.D., Muholland, M., Bailey, S., Brennan, K.M., Adams, A.A., 2019. Effects of Docosahexaenoic acid–rich microalgae supplementation on metabolic and inflammatory parameters in horses with equine metabolic syndrome. *J. Equine Veterinary Sci.* 83, 102811.

FAO, 2018. *The State of Fisheries and Aquaculture in the World 2018 – Meeting the sustainable development goals*. Rome. Licence: CC BY-NC-SA 3.0 IGO.

Ferreira, M., Coutinho P., Seixas, P., Fábregas, J., Otero, A., 2009. Enriching rotifers with 'premium' microalgae. *Nannochloropsis gaditana*. *Mar. Biotechol.* 22, 3.

Ferreira, M., Maseda, A., Fábregas, J., Otero, A., 2008. Enriching rotifers with "premium" microalgae. Isochrysis aff. galbana clone T-ISO. *Aquaculture* 279(2008), 126–130.

Furbeyre, H.J., Van Milgen, T., Mener Gloaguen, M., Labussière, E., 2017. Effects of dietary supplementation with freshwater microalgae on growth performance, nutrient digestibility and gut health in weaned piglets. *Animal*. 11, 183–192.

Ganuza, E., T. Benítez-Santana, E. Atalah, O. Vega-Orellana, R. Ganga, and M. S. Izquierdo., 2008. *Crypthecodinium cohnii* and *Schizochytrium* sp. as Potential substitutes to fisheries-derived oils from seabream *(Sparus aurata*) microdiets. *Aquaculture.* 277, 109–116.

García, José L., de Vicente, M., Galán., B., 2017. Microalgae, old sustainable food and fashion nutraceuticals. *Microbio. Biotech.* 10, 1017–1024.

Ghidossi, T., Marison, I., Devery, R., Gaffney, D., Forde. C., 2017. Characterization and optimization of a fermentation process for the production of high cell densities and lipids using heterotrophic cultivation of *Chlorella protothecoides*. *Industr. Biotech.* 13.

Guedes, A.C., Malcata, F.X., 2012. Nutritional Value and Uses of Microalgae in Aquaculture. In Muchlisin, Z. (Ed.), *Aquaculture*. In Tech, Rijeke, Croatia.

Hadley, K.B., Bauer, J., Milgram, N.W., 2017. The oil-rich alga *Schizochytrium* sp. as a dietary source of docosahexaenoic acid improves shape discrimination learning associated with visual processing in a canine model of senescence. *Prostaglandins Leukot. Essent. Fatty Acids*. 118, 10–18.

Hajiahmadian, M., Vajargah, M.F., Farsani, H.G., Mohammad., M., 2012. Effect of *Spirulina platensis* meal as feed additive on growth performance and survival rate in golden barb fish, *Puntius gelius* (Hamilton, 1822). *J. Fisheries Int*. 7, 61–64.

Hernández-Carmona, G., Carrillo-Domínguez, S., Arvizu-Higuera, D.L., Rodríguez-Montesinos, Y.E., Murillo-Álvarez, J.I., Muñoz-Ochoa, M., María Castillo-Domínguez, R., 2009. Monthly variation in the chemical composition of *Eisenia arborea* J.E. Areschoug. *J. Appl. Phycol*. 21, 607–616.

Hintz, H. F., Heitman, Weir, H.W.C., Torell, D.T., Meyer., J.H., 1966. Nutritive Value of Algae Grown on Sewage2. *J. Anim. Sci.* 25.

Ho, S.H., Chen, C.Y., Chang, J.S., 2012. Effect of Light Intensity and Nitrogen Starvation on CO_2 Fixation and Lipid/Carbohydrate Production of an Indigenous Microalga *Scenedesmus obliquus* CNW-N. Biores. *Technol*. 113, 244–252.

Holman, B., 2012. Growth and Body Conformation Responses of Genetically Divergent Australian Sheep to Spirulina (*Arthrospira platensis*) Supplementation. *Am. J. Exper. Agriculture*. 2, 2.

Hu, J., Nagarajan, D., Zhang, Q., Chang, J.S., Lee, D.J., 2018. Heterotrophic Cultivation of Microalgae for Pigment Production: A Review. *Biotectnol. Adv*. 36, 54–67.

Hussein, E.E.S., Dabrowski, K., El-Saidy, M.S.D., Lee, B.J., 2013. Enhancing the Growth of Nile Tilapia Larvae/Juveniles by Replacing Plant (Gluten) Protein with Algae Protein. *Aquaculture Res*. 44, 1365–2109.

Illman, A. M., Scragg, A.H., Shales, S.W., 2000. Increase in Chlorella Strains Calorific Values When Grown in Low Nitrogen Medium. *Enzyme Microb.Technol.* 27, 631–635.

Jacob-Lopes, E., Maroneze, M.M., Deprá, M.C., Sartori, R.B., Dias, R.R., Zepka, L.Q., 2019. Bioactive Food Compounds from Microalgae: An Innovative Framework on Industrial Biorefineries. Curr. Opinion *Food Sci*. 25, 1–7.

Jaseera, K.V., Ebeneezar, S., Sayooj P., Nair, A.V., Kaladharan, P., 2021. Dietary Supplementation of Microalgae, *Aurantiochytrium* sp. and Co-Feeding with Artemia Enhances the Growth, Stress Tolerance and Survival in *Penaeus monodon* (Fabricius, 1798) Post Larvae. *Aquaculture*. 533, 1–12.

Kang, H. K., Salim, H.M., Akter Kim, N.D.W., Kim, J.H., Bang, H.T., Kim, M.J., Na, J.C., Choi, H.C., Suh, O.S., 2013. Effect of various forms of dietary Chlorella supplementation on growth performance, immune characteristics, and intestinal microflora population of broiler chickens. *J. Appl. Poultry Res*. 22, 100–108.

Kim, S.S., Rahimnejad, S., Kim, S.K.W., Lee, K.J., 2013. Partial replacement of fish meal with *Spirulina pacifica* in diets for parrot fish (Oplegnathus Fasciatus). *Turk. J. Fisheries Aquatic Sci*. 13, 197–204.

Kiron, V., Kulkarni, A., Dahle, D., Vasanth, G., Lokesh, J., Elvebo, O., 2016. Recognition of purified beta 1,3/1,6 glucan and molecular signalling in the intestine of Atlantic Salmon. *Dev. Comp. Immunol*. 56, 57–65.

Kiron, V., Sørensen, M., Huntley, M., Vasanth, G.K., Gong, Y., Dahle, D., Palihawadana, A.M., 2016. Defatted biomass of the Microalga, *Desmodesmus* sp., can replace fishmeal in the feeds for Atlantic Salmon. *Front. Mar. Sci*. 17 (May). 10.3389/fmars.2016.00067

Krzemińska, I., Piasecka, Nosalewicz, A.A., Simionato, D., Wawrzykowski, J., 2015. Alterations of the lipid content and fatty acid profile of *Chlorella protothecoides* under different light intensities. *Bioresour. Technol*. 196, 72–77.

Kulpys, J., Paulauskas, E., Pilipavicius, V., Stankevicius, R., 2009. Influence of Cyanobacteria *Arthrospira* (Spirulina) *platensis* biomass additives towards the body condition of lactation cows and biochemical milk indexes. *Agronomy Res*. 7, 823–835.

Lafarga, T., 2020. Cultured microalgae and compounds derived thereof for food applications: strain selection and cultivation, drying, and processing strategies. *Food Rev. Int.* 36, 559–583.

Levasseur, W., Perré, P., Pozzobon, V., 2020. A review of high value-added molecules production by microalgae in light of the classification. *Biotech Adv*. 41, 107547.

Li, P., Mai, K., Trushenski, J., Wu, G., 2009. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids*. 37, 43–53.

Lim, K.C., Yusoff, F.M., Shariff, M., Kamarudin, M.S., 2018. Astaxanthin as feed supplement in aquatic animals. *Rev. Aquac.* 10(3), 738–773.

Maliwat, Gian Carlo, Stephanie Velasquez, Jan Lorie Robil, Merab Chan, Rex Ferdinand Tayamen, T.M., Ragaza, J.A., 2017. Growth and immune response of giant freshwater prawn *Macrobrachium rosenbergii* (De Man) postlarvae fed diets containing Chlorella vulgaris (Beijerinck). *Aquaculture Res*. 48, 1666–1676.

Martínez-Francés, E., Escudero-Oñate, C., 2018. Cyanobacteria and microalgae in the production of valuable bioactive compounds, in: Martínez-Francés, E. and Escudero-Oñate, C. (Eds.), *Microalgal Biotechnol.* 6, 104–128.

Matos, J., Cardoso, C., Bandarra, N.M., Afonso, C., 2017. Microalgae as healthy ingredients for functional food: A review. *Food Funct*. 1, 2672–2685.

Mirzaie, S., Zirak-Khattab, F., Hosseini, A.H., Donyaei-Darian, H., 2018. Effects of dietary spirulina on antioxidant status, lipid profile, immune response and performance characteristics of broiler chickens reared under high ambient temperature *Asian-Australasian J. Anim. Sci.* 31, 556–563.

Mishra, A., Jha, B., 2009. Isolation and characterization of extracellular polymeric substances from micro-algae *Dunaliella salina* under Salt Stress. *Bioresource Technol*. 100, 3382–3386.

Napier, J.A., Olsen, R.E., Tocher, D.R., 2019. Update on GM canola crops as novel sources of omega-3 fish oils. *Plant Biotechnol. J*. 17, 703–705.

Navarro, F., Forján, E., Vázquez, M., Montero, Z., Bermejo, E., Castaño, M.A., Alberto Toimil, A., et al., 2016. Microalgae as a safe food source for animals: Nutritional characteristics of the acidophilic microalga *Coccomyxa onubensis*. *Food Nutr. Res*. 60, 30472.

Oh, S. T., Zheng, L., Kwon, H.J., Choo, Y.K., Lee, K.W., C. W. Kang, An, B.K., 2015. Effects of dietary fermented *Chlorella vulgaris* (CBT®) on growth performance, relative organ weights, cecal microflora, tibia bone characteristics, and meat qualities in pekin ducks. *Asian-Australasian J. Anim. Sci*. 28, 95–101.

Oliver, L., Dietrich, T., Marañón, I., Villarán, M.C., Barrio, R.J., 2020. Producing omega-3 polyunsaturated fatty acids: A review of sustainable sources and future trends for the EPA and DHA market. *Resources*, 9, 148.

Packer, M.A., Harris, G.C., Adams, S.L., 2016. Food and feed applications of algae, in: *Algae Biotechnology, Green Energy and Technology*. Springer, Cham.

Patras, D., Moradu, C.V., Socaciu, C., 2019. Bioactive ingredients from microalgae: food and feed applications. *Bull. Univ. Agr. Sci. Vet. Med. Cluj-Napoca Food Sci. Techno.* 76, 1.

Perez-Garcia, O., Bashan, Y., Bashan, Y., Bashan, Y., 2015. Microalgal heterotrophic and mixotrophic culturing for bio-refining: From metabolic routes to techno-economics, in: *Algal Biorefineries: Vol. 2: Products and Refinery Design*. Springer International Publishing, Switzerland.

Pires, J.C.M., 2015. Mass production of microalgae, in: *Handbook of Marine Microalgae: Biotechnology Advances*, pp. 55–68, Elsevier, Amsterdam.

Quico, A.C., Astocondor, M.M., Ortega, R.A., 2021. Dietary supplementation with *Chlorella peruviana* improve the growth and innate immune response of rainbow trout *Oncorhynchus mykiss* fingerlings. *Aquaculture*. 533, 736117.

Rammuni, M.N., Ariyadasa, T.U.A., Nimarshana, P.H.V., Attalage, R.A.A., 2019. Comparative assessment on the extraction of carotenoids from microalgal sources: Astaxanthin from H. pluvialis and β-carotene from D. salina. *Food Chem*. 30, 128–134.

Ramus, J., 1972. The production of extracellular polysaccharide by the unicellular red alga Porphyridium aerugineum. *J. Phycol.* 8, 97–111.

Rehberg-Haas, S., Meyer, S., Tielmann, M., Lippemeier, S., Vadstein, O., Bakke, I., Kjørsvik, E., Evjemo, J.O., Schulz, C., 2015. Use of the microalga Pavlova viridis as enrichment product for the feeding of Atlantic cod larvae (*Gadus morhua*). *Aquaculture.* 438(141), 150.

Remize, M., Brunel, Y., Silva, J.L.S., Berthon, J.Y., Filaire, E., 2021. Microalgae n-3 PUFAs production and use in food and feed industries. *Mar. Drugs*. 19, 113.

Rincón, D., Velásquez, D.H.A., Dávila, M.J., Semprun, A.M., Morales, E.D., Hernández, J.L., 2012. Substitution levels of fish meal by *Arthrospira* (=Spirulina) *maxima* meal in experimental diets for red tilapia fingerlings (*Oreochromis* sp.). *Revista Colombiana de Ciencias Pecuarias*. 25, 3.

Rodrigues, D.B., Menezes, C.R., Mercadante, A.Z., Jacob-Lopes, E., Zepka, L.Q., 2015. Bioactive pigments from microalgae *Phormidium autumnale*. *Food Res. Int.* 77, 273–279.

Rosenberg, J.N., Kobayashi, N., Barnes, A., Noel, E.A., Betenbaugh, M.J., Oyler., G.A., 2014. Comparative analyses of three chlorella species in response to light and sugar reveal distinctive lipid accumulation patterns in the microalga *C. sorokiniana*, in: Duhalt, R.V. (Ed.), *PLoS One*. 9, e92460.

Rumin, J., Nicolau, E., Gonçalves de Oliveira, R., Fuentes-Grünewald, C., Picot, L., 2020. Analysis of scientific research driving microalgae market opportunities in Europe. *Marine Drugs*. 18, 264.

Sahu, A., Pancha, I., Jain, D., Paliwal, C., Ghosh, T., Patidar, S., Bhattacharya, S., Mishra, S., 2013. Fatty acids as biomarkers of microalgae. *Phytochem*. 89, 53–58.

Santiago-Santos, MaC, Ponce-Noyola, T., Olvera-Ramirez, R., Ortega-Lopez, J., Ca~nizares-Villanueva, R. O. 2004. Extraction and purification of phyco-cyanin from Calothrix sp. *Process. Biochem.* 39, 2047–2052.

Sarker, C., Kapuscinski, P.K., McKuin, A.R., Fitzgerald, B., Nash, D.S.F., Greenwood, H.M., 2020. Microalgae-blend tilapia feed eliminates fishmeal and fish oil, improves growth, and is cost viable. *Sci. Rep.* 10, 19326.

Sathasivam, R., Radhakrishnan, R., Hashem, A., Allah, E.F.A., 2019. Microalgae metabolites: A rich source for food and medicine *Saudi J. Biol. Sci*, 26, 709–722.

Shah, M.R., Lutzu, G.A., Alam, A., Sarker, P., Chowdhury, M.A.K., Parsaeimehr, A., Liang, Y., Daroch., M., 2018. Microalgae in aquafeeds for a sustainable aquaculture industry. *J. Appl. Phycol.* 30, 197–213.

Sheikhzadeh, N., Tayefi-Nasrabadi, H., Oushani, A.K., Enferadi, M.H.N., 2012. Effects of *Haematococcus pluvialis* supplementation on antioxidant system and metabolism in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.* 38, 413–419

Sørensen, M., Berge, G.M., Reitan, K.I., Ruyter, B., 2016. Microalga *Phaeodactylum tricornutum* in feed for Atlantic Salmon (*Salmo salar*) – Effect on nutrient digestibility, growth and utilization of feed. *Aquaculture*. 460, 116–123.

Souza, C.M.M., De Lima, D.C., Bastos, D.S., de Oliveira, S.G., Beirão, B.C.B., Félix, A.P., 2019. Microalgae *Schizochytrium* sp. as a source of docosahexaenoic acid (DHA): Effects on diet digestibility, oxidation and palatability and on immunity and inflammatory indices in dogs. *Anim. Sci. J.* 90, 1567–1574.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. *J.Biosc. Bioeng*. 101, 87–96.

Stengel, D. B., Connnan, S., Popper, Z. A. 2011. Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application. *Biotechnol. Adv.* 29, 483–501.

Tanaka, T., Shnimizu, M., Moriwaki, I., 2012. Cancer chemoprevention by carotenoids. *Molecules*, 14, 3202–3240.

Trabelsi, L., Ouada, H.B., Zili, F., Mazhoud, N., Ammar, J., 2013. Evaluation of *Arthrospira platensis* extracellular polymeric substances production in photoautotrophic, heterotrophic and mixotrophic conditions. *Folia Microbiol*. 58, 39–45.

Tulli, F., Zittelli, G.C., Giorgi, G. Poli, B.M., Tibaldi, E., Tredici, M.R., 2012. Effect of the inclusion of dried *Tetraselmis suecica* on growth, feed utilization, and fillet composition of European sea bass Juveniles fed organic diets. *J. Aquatic Food Product Technol*. 21, 188.

Velasquez, S.F., Chan, M.A., Abisado, R.G., Traifalgar, R.F.M., Tayamen, M.M., Maliwat, G.C.F., Ragaza, J.A., 2016. Dietary spirulina (*Arthrospira platensis*) replacement enhances performance of juvenile Nile Tilapia (*Oreochromis niloticus*). *J. Appl. Phycol.* 28, 1023–1030.

Venkataraman, L.V., 1997. *Spirulina platensis* (Arthrospira): Physiology, cell biology and biotechnology, in: Vonshak, A. (Ed.), *J. Appl. Phycol*. 9, 295–296.

Vetvicka, V., Teplyakova, T.V., Shintyapina, A.B., Korolenko, A., 2021. Effects of medicinal fungi-derived β-Glucan on tumor progression. *J. Fungi*. 7, 250.

Vieira, M.V., Pastrana, L.M., Fuciños, P., 2020. Microalgae encapsulation systems for food, pharmaceutical and cosmetics applications. *Mar. Drugs* 18, 644.

Waldenstedt, L., Inborr, J., Hansson, I., Elwinger., K., 2003. Effects of astaxanthin-rich algal meal (*Haematococcus pluvalis*) on growth performance, caecal campylobacter and clostridial counts and tissue astaxanthin concentration of broiler chickens. *Anim. Feed Sci. Technol*. 108, 119–132.

Walker, A.B., Berlinsky, D.L., 2011. Effects of partial replacement of fish meal protein by microalgae on growth, feed intake, and body composition of Atlantic Cod. *North Am. J. Aquaculture.* 73, 76–83.

Wang, Xi, Fosse, H.K., Li, K., Chauton, M.S., Vadstein, O., Reitan, K.I., 2019. Influence of nitrogen limitation on lipid accumulation and EPA and DHA content in four marine microalgae for possible use in aquafeed. *Front. Mar. Sci*. 6, 95. doi: 10.3389/fmars.2019.00095

Watanuki, H., Ota, K., Tassakka, A.C.M.A.R., Kato, T., Sakai, M., 2006. Immunostimulant Effects of Dietary *Spirulina platensis* on Carp, *Cyprinus carpio*. *Aquaculture* 258, 1–4.

Wu, Z., Dejtisakdi, W., Kermanee, P., Ma, C., Arirob, W., Sathasivam, R., Juntawong, N., 2017. Outdoor cultivation of *Dunaliella salina* KU 11 using brine and saline lake water with raceway ponds in Northeastern Thailand. *Biotechnol. Appl. Biochem.* 64, 938–943.

Xu, H., Miao, X., Wu, Q., 2006. High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. *J. Biotech*. 126, 499–507.

Yan, L., Lim, S.U., H. Kim, I.H., 2012. Effect of fermented Chlorella supplementation on growth performance, nutrient digestibility, blood characteristics, fecal microbial and fecal noxious gas content in growing pigs. *Asian-Australas J. Anim. Sci*. 25, 1742–1747.

Yuan, J.P., Peng, J., Yin, K., Wang., J.H., 2011. Potential health-promoting effects of Astaxanthin: A high-value carotenoid mostly from microalgae. *Mol. Nutr. Food Res*. 55, 150–165.