NUTRITION

Algae Protein: an alternative and sustainable protein source for fish diets

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Abstract

Global aquaculture as a food-producing industry will have to play an important role in covering the increased future needs of the world's human population. For aquaculture to grow sustainably, the availability of sustainable and viable compound feed and ingredients is essential. For many years fishmeal has been considered a superior ingredient and the primary protein source of choice for the aquafeed industry. Nowadays, fishmeal utilization is under drastic reduction for sustainability reasons, and is mainly replaced by alternative protein sources of plant origin. However, antinutritional factors existence in plant proteins and environmental concerns related to cropland production have led to the search for next-generation alternative protein sources. Recently, microalgae have gained increased attention from the aquafeed industry as a sustainable alternate protein source. Microalgae are well known as a rich source of nutrients and bioactive compounds. Their nutritional profile regarding the protein content and essential amino acid composition makes them a suitable alternative protein source for aquafeeds. The incorporation of microalgae as a protein source in fish diets has been investigated and reported for various fish species and life stages with promising results. Moreover, research shows that microalgae can act as a functional ingredient with extra benefits to the fish's health beyond growth. The literature review shows that microalgae, unquestionably, can be used as an alternative and sustainable source of protein. In addition they contain functional metabolites that are important for sustaining fish proper growth and health.

Background

According to the projections of the UN report, the world's human population is expected to reach 9.7 billion by 2050. The main challenge of the food sector will be the substantial increase of global food production in a sustainable manner to meet the needs of a growing population of that magnitude. In 2018, more than 50 percent of fish for human consumption originated from aquaculture (FAO, 2020). Over the last five decades, global aquaculture showed impressive growth with a recorded annual average rate of 3.1 percent from 1961 to 2017, much higher compared to the growth rate of all other animal protein food sources (2.1%) and almost double compared to the annual world population growth (FAO, 2020). Even though aquaculture, production is increasing at high rates, there are still significant constraints concerning its future sustainability (NRC, 2011). Aquaculture production heavily relies on the availability of sustainable and viable compound feeds.

For many decades, fishmeal has been considered as a superior ingredient and the primary protein source of aquafeed industry due to its desirable nutritional characteristics, such as high quality of digestible protein, amino acid, and fatty acid profile, micronutrient content, bioactive compounds, as well as lack of antinutritional factors (NRC, 2011; Larsen et al., 2012). Although the global fishmeal production has fluctuations from year to year, has been relatively stagnant for the past two decades, a potential increase beyond current average levels is not considered achievable. A considerable amount of research has been conducted over the past four decades targeting the decades targeting the replacement of fishmeal with alternative protein sources in fish feeds, mainly of plant origin (Brezas & Hardy, 2020). Even though terrestrial plant-derived ingredients such as soybean meal, corn gluten meal, pea protein, wheat gluten, sunflower meal, fava beans, etc., were contributed to a significant reduction of fishmeal in the aquafeeds, current dietary inclusion thresholds of plant proteins are determined by several factors (Ahmed et al., 2019). As global feed production is increasing considerably, intense competition for protein between feed and food has been raised, which has caused severe volatility in plant ingredients availability and prices (Sagaram, 2021).

Another important factor responsible for the limited use of plant proteins is their content in antinutritional substances, mainly responsible for causing metabolic disturbances and the deterioration of fish health, especially when carnivorous species are fed diets rich in plant proteins (Francis et al., 2001; Gatlin et al., 2007). At last, the increased use of plant proteins has raised controversies related to the adverse environmental effects that increased terrestrial crop production could have on the land use and water consumption (Naylor et al., 2021).

The current review is a short overview of the literature regarding the potential of algae biomass as an alternative and sustainable protein source substitute for fishmeal in fish diets, highlighting its effects on growth and health.

Microalgae: a novel ingredient for aquafeeds

Microalgae are unicellular microscopic organisms classified as autotrophs (utilize solar energy and carbon dioxide as a carbon source) and chemo-organotrophs or heterotrophs (organic carbon sources are used in the absence of light). Microalgae are further classified into the prokaryotic blue-green algae and the bigger eukaryotic group of green algae, golden algae, and diatoms (Parisi et al., 2020). In the general context, microalgae are considered a rich source of key nutrients like proteins, lipids, amino acids, fatty acids, micronutrients, vitamins (B complex, C, E. and A) as well as a wide range of nutraceutical and pharmaceutical compounds (Galasso et al., 2019; Parisi et al., 2020; Nagappan et al., 2021).

Furthermore, microalgal biomass productivity is considered reasonably efficient and superior compared to any other terrestrial plant and animal organism, they do not rely on production inputs which compete with the needs of other food production systems, while their nutritional value could be modulated considerably (through the manipulation of their culture conditions) due to their remarkable metabolic plasticity (Glencross et al., 2020; Parisi et al., 2020; Nagappan et al., 2021). For the above-mentioned reasons, microalgae have attracted the interest of many different industries and sectors for a diverse array of applications like nutraceuticals, pharmaceuticals, biofuels, feed and human food (Azari et al., 2018) (Figure 1). Microalgae can be produced in huge quantities in super-intensive mass-culture systems using nonconventional photo-bioreactors or through heterotrophic methods, minimizing the environmental impact as their large-scale cultivation does not antagonize for precious natural resources (freshwater use and arable land) (Figure 2) (Guedes & Malcata, 2012; Parisi et al., 2020).

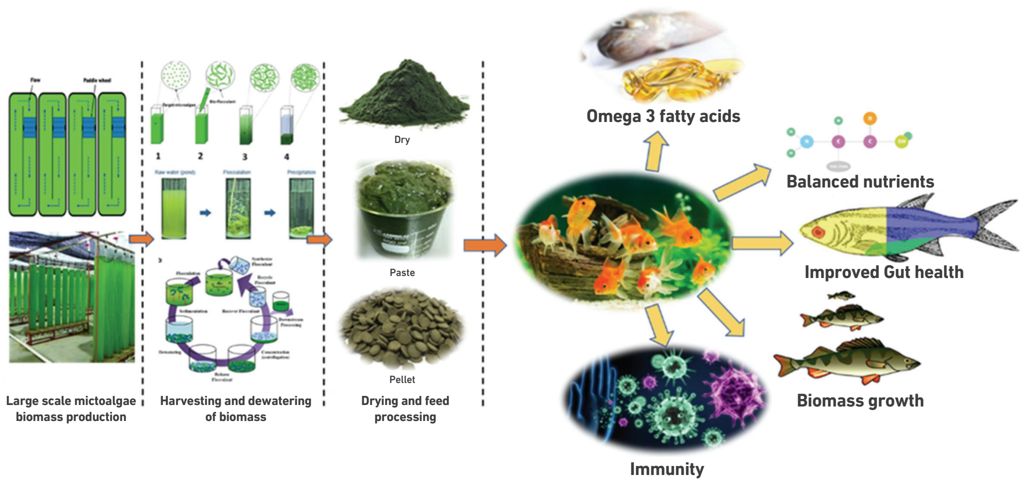

Figure 1. **Technology process lineup for the production of microalgae and their dietary benefits in farmed fish.**
Adapted from Ahmad et al., 2022

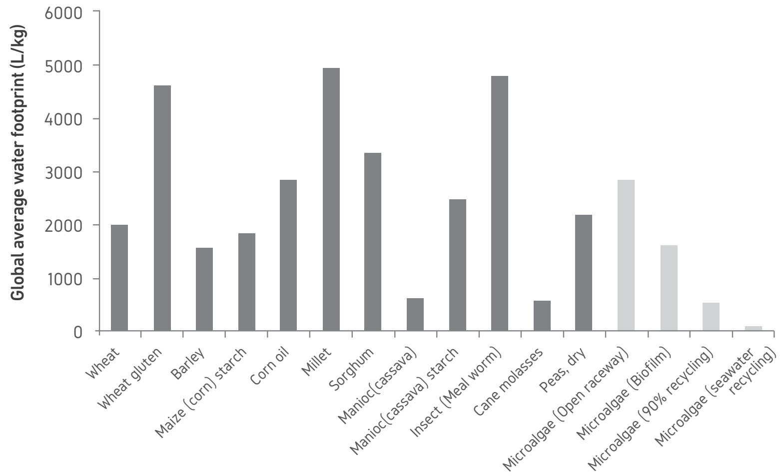

Figure 2. **Global average water footprint of various feed ingredients.**
Adapted from Nagappan et al., 2021

One of the main reasons that microalgae are gaining momentum as next-generation feed ingredients sources is the current significant technical as well as biotechnological achievements towards more efficient production and downstream processing, targeting products of high quality and of substantially increased availability (Shah et al., 2018; Sagaram et al., 2021).

Microalgae have been introduced to the aquaculture industry for the past 40 years, as different microalgae cultures have been used as live feeds for zooplankton and fish larvae in the hatchery production of many marine fish species (Shields & Lupatsch, 2013; Villar-Navarro et al., 2021). However, due to the continuously increased demand for novel feed ingredients, microalgae biomass has gained vast attention in the aquafeed industry as a viable and feasible feed alternative ingredient. Nowadays, microalgae derived products are regularly used in aquafeeds in a refined form as added-value products alternatives, such as astaxanthin, omega-3 long chain polyunsaturated fatty acids, etc., (Bleakley & Hayes, 2017; Sagaram et al., 2021).

Microalgae protein as an alternative protein source in the diets of different fish species

Generally, the nutritional composition of microalgae is well known (Table 1) and is considered a rich source of macronutrients such as proteins and lipids. However, their protein concentration can be varied from species to species and method of culture, ranging from 40 to 70% (in dry weight), having an adequate profile of essential amino acids, which makes them a suitable alternative protein source for aquafeeds (Becker, 2007; Kovac et al., 2013). Brown (1991) studied and determined the biochemical composition (sugar and amino acids) of 16 microalgal species used in aquaculture, grew under similar experimental conditions and harvested at the same growth phase. The results showed only minor differences in relation to the amino acid profile between most of the microalgal species under investigation. Remarkably, microalgae protein digestibility presents noteworthy differences among the fish species. In a detailed review by Annamalai et al. (2021), commonly used microalgae species like *Nannohloropsis, Chlorella, Spyrulina, Schizochitrium* and *Isochrysis* showed to have high protein digestibility over various fish species like Atlantic salmon, European sea bass, rainbow trout, Nile tilapia and African catfish among others.

The incorporation of algae biomass as a protein source in fish diets has been evaluated in different fish species and life stages. Different microalgae species were investigated as potential alternatives to fishmeal in Atlantic salmon and rainbow trout in different life stages. Two marine microalgae, *Nanofrustulum* and *Tetraselmis* were examined each one at two levels of 5 and 10%, as fishmeal protein replacements in the diets of Atlantic salmon post-smolts of the initial average weight of 173.1 g (Kiron et al., 2012). These authors reported that no significant differences were detected either in growth and feed utilization or in whole-body proximate composition after a 12-week feeding period (Kiron et al., 2012). Norambuena et al., (2015) found that the dietary inclusion of *Entomoneis spp* biomass at 2.5 and 5.0% in low fish meal diets did not negatively affect the growth and feed efficiency of Atlantic Salmon (33.7g initial weight). An additional finding of this study was the increase in the content of the omega-3 polyunsaturated fatty acids in the whole body of the salmon fed with a higher level of microalgae. Another 70-day feeding trial with Atlantic salmon (167 g) showed the marine microalga, *Desmodesmus sp* can successfully replace fishmeal both at 10 and 20% levels without compromise the growth indices such as condition factor and specific growth rate as well as the intestinal health and immune system (Kiron et al., 2016). Sorensen et al. (2016) showed that the use of the microalgae *Phaeodactylum tricornutum* at 3 and 6% fishmeal replacement did not impact the growth, nutrient retention and health of Atlantic salmon (325 g) after 82 days feeding period.

On the contrary, the 20% dietary incorporation of defatted *Nannochloropsis oceania* negatively affected the growth performance and feed efficiency of Atlantic salmon (215 g) in an 84-day feeding trial, while the 10% inclusion rate did not exhibit any negative effect (Sorensen et al., 2017). In line with the previous study, Gong et al., (2019) demonstrated that up to 10% dietary incorporation of the microalga *Scenedesmus sp* did not impact growth parameters and health of Atlantic salmon (229 g) for a period of 65 days, while the total omega-3 whole body content found to be improved.

In a study with the rainbow trout fry (initial weight of 900 mg), a 12.5% dietary incorporation of a mixture of microalgae biomass composed of *Scenedesmus sp.* and *Chlamydomonas sp.* did not affect survival, growth and carcass quality of the fish for 56 days (Dallaire et al., 2007). On the contrary, the higher inclusion levels (25% and 50%) impacted negatively the growth parameters of rainbow trout fry.
Tomas-Almenar et al. (2018), evaluated the *Scenedesmus almeriensis* biomass as a replacer of fishmeal at 5%, 10%, 20% and 40% levels in the diets of rainbow trout (initial weight of 75 g) for 82 days. Although a significant reduction in growth was recorded for all treatments, which had included microalgae biomass compared to the control diet, those values were typical for the trout in that growing period. Moreover, no negative effects on the health and final quality of the fish were observed.

In another study, the replacement of soybean meal with Spirulina at 20, 40, 60 and 80% inclusion levels in the diets of rainbow trout (30g initial weight) showed positive effects on body length, carcass mean weight and proximate composition, with the 60% found to be the most beneficial among the treatments (Ahmadzadenia et al., 2011).
Spirulina maxima meal was used to replace fishmeal in the diets of red tilapia fingerlings (*Oreochromis sp.*) (initial weight, 2.5 g) at three levels of substitution (10, 20, and 30%) in 90 days feeding trial (Rincon et al., 2012). The results of this study showed no differences among the treatments in terms of survival, growth performance and protein retention efficiency, thus, indicating that the replacement of 30% fishmeal by Spirulina is feasible for red tilapia fingerlings.

Gbadamosi and Lupatsch, (2018) studied the effects of *Nannochloropsis salina* as the solely source of protein in the diets of Nile tilapia (initial weight 12.70 g) against fishmeal and soybean meal. They reported that fish fed the Nannochloropsis salina based diet showed similar weight gain compared to the soybean diet but lower than the fishmeal diet, while it presented a significantly lower feed conversion ratio compared to fish fed the soybean diet but higher than fishmeal diet.

On the other hand, fish fed the *Nannochloropsis salina* based diet exhibited the highest protein retention efficiency among the dietary groups.
Notably, total fishmeal and fish oil replacement were achieved in the diets of *Oreochromis niloticus* (initial weight, 34.5g) by a combination of a mixture of defatted *Nannochloropsis oculata* and *Schizochytrium sp.* biomass (Sarker et al., 2020). After 184 days of the trial duration, significantly higher values were found in final weight and SGR for the fishmeal free diet, which contained a mixture of microalgae compared to the control fishmeal diet.

Recently, Valente et al., (2019) investigated the partial substitution of dietary fishmeal in European sea bass (initial weight, 21.76 g) with the defatted biomass of the microalgae *Nannochloropsis sp.* originated from biorefinery, for 93 days. These authors concluded that the dietary incorporation of 15% *Nannochloropsis sp.* biomass replacing 9.5% fishmeal resulted in similar growth performance and whole-body composition compared to the fishmeal control diet.

The microalgae *Scenedesmus almeriensis* was tested as an ingredient at different inclusion levels (12%, 20%, 25%, and 39%) in the feed of gilthead sea bream (Sparus aurata) juveniles (initial weight, 8g) in a 45-day trial (Vizcaino et al., 2014). They reported that no negative effects were found on growth performance or feed efficiency and nutrient utilization of fish compared to control fishmeal-based diets.

Table 1. Nutritional composition of major microalgae species
Adapted freom Nagappan et al., 2021

Feed ingredient	Protein (%)	Lipid (%)	Carbohydrate (%)	References
Anabaena cylindrical	43 - 56	4-7	25 - 30	(Becker, 2007)
Botryococcus braunii	40	34.4	18.5	(Tavakoli et al., 2021)
Chlamydomonas rheinhardii	43 - 56	14 - 22	2.9 - 17	(Becker, 2007)
Chlorella pyrenoidosa	57	2	26	(Becker, 2007)
Chlorella vulgaris	51 - 58	14 - 22	12 -17	(Becker, 2007)
Dunaliella salina	49 - 57	6 - 8	4 - 32	(Becker, 2007)
Euglena gracilis	39 - 61	14 - 20	14 - 18	(Becker, 2007)
granulata	34	23.6	36.2	(Tibbetts et al., 2017)
Pavlova sp.	24 - 29	9 - 14	6 - 9	(Madeira et al., 2017)
Phaeodactylumtricornutum	40	18.2	25.2	(Sørensen et al., 2016)
Porphyridium aerugineum	31.6	13.7	45.8	(Madeira et al., 2017)
Scenedesmus obliqus	50 - 56	12 - 14	10 - 52	(Becker, 2007)
Schizochytrium	12.5	40.2	38.9	(Samuelsen et al., 2018)
Spirulina platensis	55.8	14.2	22.2	(Madeira et al., 2017)
Spirulina maxima	60 -71	6 -7	13 -16	(Madeira et al., 2017)
Spirogyra sp.	6 - 20	11 - 21	33 - 64	(Becker, 2007)
Synechococcus sp.	63	11	15	(Becker, 2007)
Tetraselmis sp.	27.2	14	45.4	(Tulli et al., 2012)
Tetraselmis chuii (PLY-429)	46.5	12.3	25	(Makridis et al., 2006)
Dunaliella sp.	40.46	15.51	20.44	(Madeira et al., 2017)
Haematococcus	30.87	23.07	37.93	(Madeira et al., 2017)
Isochrysis	41.00	17.72	14.46	(Madeira et al., 2017)
Brown macroalgae	2.4 - 16.8	0.3 - 9.6	38 - 61	(Wan et al., 2019)
Green macroalgae	3.2 - 35.2	0.3 - 2.8	15 - 65	(Wan et al., 2019)
Red macroalgae	6.4 - 37.6	0.2 - 12.9	36 - 66	(Wan et al., 2019)

Fish health benefits from dietary microalgae

Recently, functional ingredients have been gaining much attention for use in aquafeeds. A functional ingredient confers extra benefits to the fish's growth and health, such as immunostimulants, probiotics, antioxidants, antibacterial, etc., (Martin and Król, 2017). Among the microalgae, several species like Chlorella, Spirulina, Tetraselmis and *Schizochytrium* among others, have shown to contain biomolecules with health protective and promoting attributes (Li et al., 2015; Xiao & Zheng 2016; Shah et al., 2018; Yarnold et al., 2019; Ahmad et al., 2022).

The incorporation of 10% of Spirulina platensis in the diets of rainbow trout (*Oncorhynchus mykiss*) (initial weight, 101g) was found to improve the health physiological status of the fish by decreasing stress plasma indices like cortisol and glucose (Yeganeh et al., 2015). Cerezeula et al., (2012), reported that the dietary microalgae (*Nannochloropsis gaditana, Tetraselmis chuii* and *Phaeodactylum tricornutum*) boosted gilthead sea bream (*Sparus aurata L.*) immune defense system by triggering the natural haemolytic complement activity, phagocytic capacity and the expression of antimicrobial peptides genes.

The innate and adaptive immune status of gibel carp (*Carassius auratus gibelio*) (initial weight, 29.91g) was improved by the supplementation of its feed with 0.4% Chlorella through regulation of certain immunoglobulins (M and D), interleukin-22 and chemokine (C-C motif) ligand 5 in blood, liver and kidney tissues (Zhang et al., 2014). Ibrahem et al. (2013) fed tilapia (*O. niloticus*) fingerlings (initial weight, 4g) with graded inclusion levels of dried S. platensis (5, 7.5, 10, 15 and 20g/kg) for 3 months and at the end of the feeding trial they challenged the fish with *Pseudomonas fluorescens.* They observed a stimulated response of the nonspecific immune system, since the nitroblue tetrazolium, neutrophil adherence and lysozyme activity were significantly increased in most of the supplemented groups. All Spirulina supplemented diets increased the resistance to bacterial challenge infections compared with the Spirulina free control diet. In contrast, the diet with the 10g/kg supplementation showed the best results.

Lastly, Rahimnejad and Lee (2017) reported a higher antioxidant catalase activity in olive flounder (*Paralichthys olivaceus*) (initial weight, 104g) when fish fed a diet containing 15% defatted Chlorella vulgaris.

Conclusions

Microalgae have been proven as valuable natural sources which include highly beneficial nutrients such as essential amino acids and fatty acids, micronutrients, vitamins and bioactive compounds for use in fish diets. Microalgae biomass unquestionably can be used as an alternative source of protein and functional metabolites that are important for sustaining the proper growth and health of various cultured fish species. In addition, it is considered a sustainable and viable novel ingredient for the aquafeed industry.

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