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Potential of microalgae as a sustainable feed ingredient for aquaculture

Senthil Nagappan [a], Probir Das [a,*], Mohammad AbdulQuadir [a], Mahmoud Thaher [a], Shoyeb Khan [a], Chandan Mahata [a], Hareb Al-Jabri [a], Ann Kristin Vatland [b], Gopalakrishnan Kumar [b,*]

[a] *Algal Technology Program, Center for Sustainable Development, College of Arts and Sciences, Qatar University, Doha, Qatar*
[b] *Institute of Chemistry, Bioscience, and Environmental Engineering, Faculty of Science and Technology, University of Stavanger, PO Box 8600 Forus, 4036 Stavanger, Norway 4021 Stavanger, Norway*

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ABSTRACT

An increase in fish consumption, combined with a decrease in wild fish harvest, is driving the aquaculture industry at rapid pace. Today, farmed seafood accounts for about half of all global seafood demand for human consumption. As the aquaculture industry continues to grow, so does the market for aquafeed. Currently, some of the feed ingredients are coming from low-value forage fishes (fish meal) and terrestrial plants. The production of fish meal can't be increased as it would affect the sustainability and ecosystem of the ocean. Similarly, increasing the production of terrestrial plant-based feed leads to deforestation and increased freshwater use. Hence, alternative and environmentally sustainable sources of feed ingredients need to be developed. Microalgae biomasses represent potential feed source ingredients as the cell metabolites of these microorganisms contain a blend of essential amino acids, healthy triglycerides as fat, vitamins, and pigments. In addition to serving as bulk ingredient in aquafeed, their unique array of bioactive compounds can increase the survivability of farmed species, improve coloration and quality of fillet. Microalgae has the highest areal biomass productivities among photosynthetic organisms, including fodder crops, and thus has a high commercial potential. Also, microalgal production has a low water and arable-land footprint, making microalgal-based feed environmentally sustainable. This review paper will explore the potential of producing microalgae biomass as an ingredient of aquaculture feed.

1. Introduction

Aquaculture is the fastest-growing segment of the food industry. Aquaculture market size is estimated to be worth US$ 31.94 billion in 2019 (Marketwatch, 2020). It is expected to increase at a rate of more than 7.1% between 2020 and 2027. The aquaculture industry's growth is currently being driven by increased human consumption and market acceptance. The industry has introduced a number of new species in recent years. Aquaculture has increased the production of fish that are suitable for a plant-based diet. Fish nutrition has been fine-tuned, resulting in lower feed waste and, as an outcome, financial viability for the industry. A diet rich in functional ingredients like omega-3 fatty acids, antioxidants, and prebiotic compounds has improved the yield, survivability, and quality of farmed fish. As a result, aquaculture has gained a competitive advantage over wild fish resources.

Currently, commercial species account for 70% of all fish in the aquaculture industry (Tacon, 2020). Nearly 68% of commercial species rely on fish feed (Tacon, 2020). Fish meal, which is made from both small fish and waste products from fish, has traditionally been used as a main ingredient of fish feed. Fish meal is a highly sought-after feed ingredient for fish feed due to its following properties: (1) Excellent digestibility and palatability for fish, resulting in increased growth; the deformities are reported infrequently or not at all; (2) the well-balanced composition and concentrations of protein, minerals, essential fatty acids, and essential amino acids; (3) Low feed conversion ratio (i.e., a high percentage of feed is converted into fish biomass), resulting in less feed waste; (4) Increased immunity leading to a higher survival rate.

The demand for fish meal - a key ingredient in feed has increased by 300% in the last ten years (Indexmundi, 2021) (Fig. 1). Similarly other key ingredients like soybean meal and fish oil are witnessing price surge. Fish farming is further expected to expand in the future. Currently, the fish meal and fish oil comes from wild fish, whose harvesting is limited

* Corresponding authors.
E-mail addresses: probir.das@qu.edu.qa (P. Das), gopalakrishnan.kumar@uis.no (G. Kumar).

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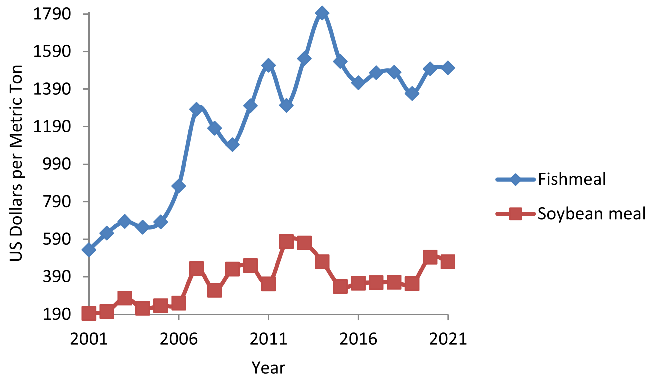

Fig. 1. Price of Fish meal and soybean meal for past 20 years.

and unpredictable. Also, pelagic fish are the source of fish feed, and their stocks are depleting as a result of the El Nino effect (Bakun and Broad, 2003) and unsustainable practice of overfishing. As a result, many industries and researchers have already begun looking for sustainable and suitable fish meal, soybean and fish oil substitutes. Before focusing on microalgae as a potential feed ingredient, this review will briefly discuss bottlenecks of alternative ingredients for fish meal.

Feed for aquatic species is rarely obtained from a single source. This is due to the fact that a single source solely fulfils the nutritional requirements, such as carbohydrate, protein, fat, minerals, and vitamin levels of an aquatic species. Common carbohydrates ingredients include corn, wheat, rice, maize starch, and potato starch (Hodar et al., 2020). Protein sources in the feed are derived from plants, insects, animals, and microbial sources. Plant protein sources typically include soybean meal, guar meal (co-product from guar gum), corn gluten, potato protein, wheat gluten, peas, co-products of cane sugar, macroalgae, canola, cassava, and wheat (Montoya-Camacho et al., 2019). Fish meal, feather meal, blood meal, animal waste, seafood waste, fish silage are major animal-based proteins in feed (Mo et al., 2018). Bacteria, yeast, and microalgae are the microbial sources of protein in fish feed (Jones et al., 2020). Fat/lipid sources include fish oil, vegetable oil, soya oil, rapeseed oil, sunflower oil, and algal oil. Other ingredients, such as fibre, vitamins, minerals, and amino acids, are minimally required for fish performance but are essential. Genetically modified crops with enhanced characteristics, such as canola and camelina with a high omega-3 fatty acid content, are also being investigated as a potential alternative ingredient in aquafeed (Jones et al., 2020).

Above-mentioned alternative feed ingredients have several advantages including nutritious, presence of bioactive compounds, and sustainable production. However, there are some disadvantages to using such alternatives. For example, one of the major disadvantages of these plant-based sources is the significant presence of ant-nutritive factors and indigestible fibers (Kokou and Fountoulaki, 2018). Examples of ant-nutritional compounds are tannins, saponins, soluble non-starch polysaccharides. This affects the growth of fish and leads to feed waste. Bacteria-based meal and Insect based proteins are of high cost. Plant-derived oils are rich in omega 6 fatty acids. However, they are poor in omega-3 fatty acids. Omega-3 fatty acids supplement helps to improve the quality of fish (Shah et al., 2018). Lack of essential amino acids and minerals in plant proteins and animal by-products (Shah et al., 2018). Even though plant-based feed has been shown to support fish growth, it lacks essential amino acids such as methionine, tryptophan, lysine, and threonine. The fish's quality may suffer as a result of the lack of essential amino acids. Plant-based protein has also been shown to be difficult to digest. Table 1 illustrates further the advantages and disadvantages of various alternate feeds in detail.

As a result of the growing demand for alternative ingredients in fish feed, several works have been published. Alternative ingredients must meet environmental sustainability and economic viability criteria. The criteria listed below can also be used to develop novel fish feed.

- Humans should not be deprived of the health benefits of eating fish that have been substituted for novel feed rather than conventional fish meal.
- Any alternative feed should have high nutritional contents such as omega-3 fatty acids and high protein content, appropriate amino acid profile, digestibility, and palatability.
- Anti-nutritional factors, non-soluble carbohydrates, fibers, and heavy metals should be in lower concentrations, as these have an adverse effect on fish growth and lead to the accumulation of unwanted waste.
- Low feed conversion ratios (input/weight gained) should be maintained.
- When scaling up a newer feed, there should be no issues with sustainability.
- One of the most important factors is the cost of feed. On the market, the new feed should be cost-effective.
- Not to be subjected to any policy restrictions, such as those imposed for GMOs.

2. Microalgae as an alternative to fish meal

Global microalgae demand is projected to reach US$3.4 billion by 2020 and is expected to grow by 4.3% over the next seven years (Globenewswire, 2021). So far, the microalgae industry has concentrated mainly on food products and cosmetic-related species such as *Spirulina* sp. and *Dunaliella salina*. Furthermore, fish hatcheries use species like Isochrysis sp., Pavlova sp., and others as live feed for larvae, but these industries are mostly small-scale. However, in recent times, microalgae are being investigated as a potential bulk-feed ingredient for fingerlings and adult fishes (Hodar et al., 2020). There are several explanations for the projection of microalgae as a promising alternative fish feed.

Microalgae's net biomass productivity is higher than any other terrestrial plant or animal (Rizwan et al., 2018). Unlike land-based plants, there is no need for microalgae to grow in fertile land; further, microalgae can be cultivated using even sea-water or waste-water (Li et al., 2019). Therefore, there is no demand on the existing patterns (or practices) of land use on agriculture and freshwater supply for large-scale production of microalgae using non-arable land or non-potable water. The nutrients requirement for microalgae is relatively simple as opposed to insects and bacteria. Microalgae could also be used for fish feed production in the biorefinery context (Arun et al., 2020; Nagappan and Nakkeeran, 2020). This concept, for example, could allow valuable metabolites like pigments to be co-produced with fish feed.

The main reason for the microalgae promise is that it has the right blend of protein, lipid, and carbohydrate – appropriate to protect the fish health. Microlagae, in particular, are a rich source of protein and lipid when compared to other alternative ingredients such as yeast and bacteria (Table 2). Microlagae also have a well-balanced amino acid profile, obviating the need for high-cost amino acid supplements in the diet (Table 3). For instance, microalgae such as *Chlorella, Chlamydomonas, Porphyridium, Isochrysis,* and *Nannochloropsis* are high in methionine, which is often lacking in plant-based ingredients (Wan et al., 2019). Type of carbohydrate is an important feed property. For instance, the content of starch - a readily digestibile carbohydrate in microalgal species ranges from 7% to 45% (Dragone et al., 2011). Species including *Tetraselmis subcordiformis*, *Chlamydomonas rheinhardtii* and *Chlorella vulgaris* comparatively have higher starch content (30–49%) than other microalgae (Dragone et al., 2011; Yao et al., 2012). The content of fiber – a complex carbohydrate in microalgae ranges from 5% to 18% (Matos et al., 2016). Unlike plants, microalgal fiber lack lignin and contain low hemicellulose suggesting better digestibility (Niccolai et al., 2019). Microalgae species including, *Spirulina* sp. and *Chlorella vulgaris* have

Table 1
Advantages and disadvantages of alternate fish feed.

Alternate Feed	Advantages	Disadvantages	Reference
Guar meal	• Soy meal could be replaced with guar meal without affecting growth efficiency In some fishes,.	• Anti-nutritional and anti-digestive compounds like Residual gum, saponin, phytate, and protease inhibitor tannin are present • Slow rate of gastrointestinal evacuation- • Poor in amino acid digestibility • The supply of guar meal in the market is influenced by the oil industry's production and the amount of guar gum consumed.	(Nidhina and Muthukumar, 2015; Ullah et al., 2016)
Macroalgae	• Apart from their nutritional value, macroalgae contain a variety of pigments, defensive compounds, and secondary metabolites that may benefit farmed fish.	• Complex polysaccharides leads to poor digestibility • Contains excess heavy metals • Presence of anti-nutritional factors like phlorotannins, lectins, and phytic acids, trypsin inhibitors and amylase inhibitors	(Garcia-Vaquero and Hayes, 2016)
Soybean meal (SBM)	• High protein content ranging from 44% to 48%	• Anti-nutritional factors like lectin and non– starch polysaccharides are present; reduced feed intake • Level of the amino acids like methionine, cystine lysine, and threonine and tyrosine are limited • Low in phosphorous	(Goda et al., 2007; Zhou et al., 2018)
Canola meal	• High protein content	• Low in phosphorous	(Wickramasuriya et al., 2015)
Corn gluten meal	• Crude protein content ranging from 60% to 73% • Corn gluten meal is now commonly used in salmon and other aquatic fish such as gilthead seabream and European seabass and aquafeeds • highly digestible	• Deficient in lysine	(Liu et al., 2020; Wickramasuriya et al., 2015)
Cottonseed meal	• protein content of 40% can be used in aquaculture diets without causing growth inhibition	• Presence of gossypol may be harmful	(Delgado et al., 2021)
Peas/lupins	• High protein digestibility	• Contain elevated amounts of non–starch polysaccharides lupins that are not metabolized; • Anti–nutrient quinolizidine alkaloids are present • Lysine and methionine are scarce	(Kokou and Fountoulaki, 2018)
Wheat	• Low in protein (<11)	• Wheat is largely an energy source due to its high starch content (typically >70%); • Lysine is a limiting amino acid.	(Draganovic et al., 2013; Sørensen et al., 2011)
Barley	• Well digested	• Low crude protein content (9–15%); • High fibre content; • Low available phosphorous; • Lysine and arginine can be limiting;	(Snow and Ghaly, 2007)
Hydrolysed feather meal	• protein content of hydrolyzed feather meal ranges from 74% to 91% crude protein, and it's high in cystine (4–5% crude protein)	• Less digestible • Low in lysine (2% of crude protein) and methionine (1% crude protein)	(Grazziotin et al., 2008; Yu et al., 2020)
Poultry by-product meal	• High protein content	• Deficient in methionine, lysine, and histidine	(Laporte et al., 2009)
Blood meal	• High protein content • Rich in lysine	• Deficient in methionine; • Heat sensitivity and drying conditions have a significant impact on protein digestibility.	(Aladetohun and Sogbesan, 2013; Hussain et al., 2011)
Fish by-products from fish processing plants	• High digestibility • Good palatability	• Potential viruses and contaminants that are toxic to both fish may be present.	(Hardy, 2000)
Insects	• Can be cultivated in food waste	• Methionine and Cysteine were the most limiting amino acids for most insect meals • Chitin is present which is an anti nutritional factor	(Bosch et al., 2014)
Bacteria	• Rapid growth rate • Least explored • Can be grown in variety of substrates	• The bacterial meal diet has a lower digestibility than the fish meal diet and can contain unidentified antinutrients.	(Skrede et al., 1998)
Yeast	• Can grow in lignocellulosic wastes • Except low methionine content, yeast protein has a favorable amino acid composition for fish • Rapid growth rate	• Production cost is high • The sulfur-containing amino acids methionine and cysteine are usually low in yeast protein.	(Blomqvist et al., 2018; Marques et al., 2004)
Microalgae and Algal oil	• Rapid growth rate • Diverse species availability with wide range of characteristics • Rich in Omega-3 fatty acids • High in antioxidants, colouring compounds and probiotic effect	• High production cost in case of formulated feed • Selected microalgae have rigid cell wall leading to difficult in digestibility	(Arun et al., 2020; Katiyar and Arora, 2020; Madeira et al., 2017)

low fiber content of 8.5% and 5.6% respectively while species of genus *Nannochloropis*, *Tetraselmis*, *Tisochrysis* and *Phaeodactylum* have higher fiber content (Niccolai et al., 2019). Moreover, nutrient limitation have been shown to further increase the carbohydrate and lipid content in microalgae (Chen et al., 2017; Nagappan et al., 2019a, 2019b).

Microalgae also have a variety of pigments with antioxidant properties, and some microalgae produce abundant vitamins and immunostimulants in their cells, which can contribute to the health of aquatic species (Prabha et al., 2020; Zhou et al., 2019). Microalgal pigments, like astaxanthin, also could give attractive colour to the fish -increasing their marketability (Posten and Schaub, 2009). Microalgae contain an abundance of organic minerals (Mustafa, 1995). This is because microalgae have structural features that allow them to bind metal with high affinity and also due to a large surface-to-volume ratio. With minerals-rich microalgal biomass, the disadvantage of mineral leaching before fish ingestion could be avoided. Certain microalgae are high in omega-3 fatty acids like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA); these fatty acids are health-beneficial not only to fish but also to humans (Ryckebosch et al., 2012). Naturally, omega-3 fatty acids can be found in high concentrations in selected marine fishes derived from algae feeding. In a study involving various alternative feeds, omega-3 fatty acid-rich microalgae were found to be a suitable substitute for lipids in feed, as well as fish oil (Cottrell et al., 2020).

3. Effect of microalgae on growth and weight gain of fish

When aquatic species were fed a diet containing low and moderate concentrations (2–10%) of microalgae, the weight gain was similar to or even better than that of control (Table 4). In one study, a 31% higher

Table 2
Nutritional content of Alternate feed.

Feed ingredient	Protein (%)	Lipid (%)	Carbohydrate (%)	References
Anabaena cylindrica	43–56	4–7	25–30	(Becker, 2007)
Botryococcus braunii	39.9	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)
Nannochloropsis granulata	33.5	23.6	36.2	(Tibbetts et al., 2017)
Pavlova sp.	24–29	9–14	6–9	(Madeira et al., 2017)
Phaeodactylum tricornutum	39.6	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	17.72	14.46	(Madeira et al., 2017)
Hydrolyzed feather meal	84.2	10.4	_	(Yu et al., 2020)
Hermetia illucens	43.6	33.1	_	(Varelas, 2019)
Saccharomyces cerevisiae	50.1	1.8	4.6	(Blomqvist et al., 2018)
Fish meal	63	11	–	(Hodar et al., 2020)
Corn-gluten meal	62	5	18.5	(Liu et al., 2020)
Soybean meal	44	2.2	39	(El-Sayed, 1994)
Wheat meal	12.2	2.9	69	(Sørensen et al., 2011)
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)

Table 3
Amino acid composition of alternate fish feed (values represented as g 100 g protein).

	Spirulina	*Phaeodactylum*	*Nannochloropsis granulata*	*Botryococcus braunii*	*Porphyridium aerugineum*	*Tetraselmis chuii*	*Chlorella*	Fish Meal	Black Soldier Fly Meal	*Saccharomyces cerevisiae*	*Gracilaria sp.*	Soybean concentrate
Essential amino acids												
Threonine	2.7	2.7	5.4	3.7	5.8	4.0	4.0	3.5	3.6	8.2	3.8	2.9
Valine	3.0	4.6	7.1	4.4	7.3	4.8	5.3	4.0	5.5	9.6	7.2	2.5
Methionine	1.2	7.0	3.5	2.5	3.7	2.4	2.2	2.5	1.8	3.4	0.7	0.2
Isoleucine	2.9	4.8	5.6	3.4	7.1	3.4	3.8	3.7	3.9	8.1	4.7	2.5
Leucine	4.8	1.5	11.0	7.1	11.9	7.3	7.8	6.2	6.1	12.2	8.3	5.3
Phenylalanine	2.5	6.4	6.2	4.4	6.3	4.7	4.7	3.3	3.6	7.3	4.2	3.4
Lysine	2.5	2.6	8.5	4.7	8.0	5.6	5.2	3.7	6.2	12.0	6.2	4.3
Histidine	0.8	5.7	2.3	1.5	1.9	1.6	1.8	-	3.0	3.7	1.0	1.8
Arginine	3.4	0.9	7.4	20.5	8.6	9.4	5.5	6.8	4.7	8.1	10.3	5.1
Non essential Amino acids												
Asparagic	5.1	18.8	11.4	8.7	15.0	14.1	7.8	-	8.5	17.1	12.8	8.4
Serine	2.6	7.1	5.6	3.5	7.0	4.2	3.3	-	3.9	7.9	5.4	4.0
Glutamic	7.8	5.5	14.1	12.7	15.6	12.0	9.7	-	10.2	23.4	13.0	15.4
Glycine	2.6	1.5	7.5	4.9	7.0	6.5	5.2	1.7	5.4	7.9	6.0	3.1
Alanine	3.9	3.4	7.1	6.4	8.4	6.0	7.2	-	5.6	11.1	10.0	3.3
Cysteine		1.5	1.6	1.4	2.2	2.8	–	0.4	0.8	2.4	0.1	1.1
Tyrosine	2.1	3.4	4.2	2.8	5.8	3.0	–	-	0.0	6.6	1.5	2.1
Proline	1.7	7.3	11.2	4.6	5.0	3.6	4.2	-	5.2	7.1	4.8	3.6

Note: The values are average values referred from following: (El-Sayed, 1994; Hodar et al., 2020; Liu et al., 2020; Samuelsen et al., 2018; Sørensen et al., 2016, 2011; Tavakoli et al., 2021; Tibbetts et al., 2017; Becker, 2007; Blomqvist et al., 2018; Madeira et al., 2017; Tulli et al., 2012; Varelas, 2019; Wan et al., 2019; Yu et al., 2020) weight gain was achieved in Atlantic salmon (*Salmo salar L.*) when fed a diet containing 5% *Schizochytrium* sp. oil than with a diet lacking it (Wei et al., 2021). Similarly, a 30% higher weight gain in post-larvae Pacific white shrimp (*Litopenaeus vannamei*) was achieved with a diet containing 0.75% *Tetraselmis suecica* compared to a diet lacking it (Sharawy et al., 2020a). Compared to other fish species, microalgae-based diets yielded more promising results for Tilapia. A 69%, 58%, and 46% higher weight gains were achieved in Nile tilapia (*Oreochromis niloticus*) when it was fed a diet containing 15% *Chlorella* sp., 14% Defatted *Nannochloropis oculata* and *Schizochytrium* sp., and 10% *N. oculata,* respectively compared to the control diets (Table 4). Also, weight gains for Senegalese sole juveniles was significantly higher (i.e., 84–97%) with a microalgae-based diet than with the control diet in (Vizcaíno et al., 2018). Although various factors could have contributed to wide differences in the weight gain values in different species, the positive effect of microalgae's low and moderate inclusion in the diet is evident, as shown by many studies.

A high microalgae concentrations in fish feed (generally greater than 15%) could reduce the growth rate and the fish weight. Studies have shown that the inclusion of *Arthospira* biomass in the fish meal at a level higher than 30% decreased the growth of silver seabream (*Rhabdosargus sarba*) (El-Sayed, 1994). Similarly, when a mixed culture with green microalgae and cyanobacteria replaced 15–20% of fish meal, rainbow trouts showed a decrease in growth (Dallaire et al., 2007). Recalcitrant cell wall and digestive enzyme inhibitors may be the cause of the negative growth effect of microalgal diet at high concentrations. The exact level of microalgae feed substitution varies depending on microalgal species, aquatic species, and pellet processing conditions. Therefore, for each combination of selected microalgae and fish, individual studies must be carried out Since many microalgae are yet to be tested on fishes more studies have to be conducted in future.

4. Effect of microalgae on the feed conversion ratio and feed intake

The feed conversion ratio (FCR) is calculated by the feed amount converted into the desired product. Typically, fish require energy and nutrient-dense feed as compared to animals (Cottrell et al., 2020). Therefore, the feed conversion ratio of fish compared to animals is low. However, the feed conversion ratio varies depending on the ingredient of fish feed. Table 5 shows that the feed conversion ratio in aquatic species was not affected or even considerably reduced when fed with a diet containing a low and moderate amount of microalgae (2–10%). Nearly 24% reduction in FCR was achieved with a diet containing 0.5% *Tetraselmis suecica* compared to the control diet in post-larvae Pacific white shrimp (*Litopenaeus vannamei*) (Sharawy et al., 2020a). A 30% reduction in FCR was achieved with a diet containing 15% *Chlorella* sp. compared to the control diet in Nile tilapia (*Oreochromis niloticus*) (Fadl et al., 2017a). Feed intake was proportional to the amount of microalgae in the feed (Abdelghany et al., 2020; Gong et al., 2020; Yu et al., 2020). In general, the diets containing up to 15% microalgae resulted in comparable feed intake to control (Abdelghany et al., 2020; Gong et al., 2020; Walker and Berlinsky, 2011; Yu et al., 2020). In the above cases, the feed intake improved as the study progressed. However higher algal content (greater than 25%) suggested starvation in species like Atlantic cod, *Gadus morhua* (Walker and Berlinsky, 2011). In summary, the type and concentration of microalgae have to be optimized in the fish feed so that selected fish species will have a low feed conversion ratio and better feed intake.

5. Effect of microalgae on digestibility

Quantitative digestibility tests to identify highly digestible microalgae could lower feed prices, reduce feed conversion ratios, and decrease the adverse environmental impacts such as eutrophication (Moheimani et al., 2018; Niccolai et al., 2019). Non-starch polysaccharides (fiber; e.g., pectin, algenan, cellulose, etc.,) found in microalgae are generally indigestible (Gong et al., 2018). The recalcitrant cell wall of microalgae is another major factor that can impair nutritional digestibility in monogastric animals like fish and shrimp (Niccolai et al., 2019). The morphology of microalgal cell walls differs from one species to the other (Table 6). For example, microalgae including *Nannochloropsis gaditana* and *Desmodesmus* contain cell wall rich in non-starch polysaccharides algenan and pectin, repectively (Becker, 2007; Kaur et al., 2012; Scholz et al., 2014). However, the cell wall of *Spirulina* sp. consists of mucopeptides, and hence it could be easily consumed by fish (Becker, 2007; Bleakley and Hayes, 2017). The protein digestibility is important for microalgae-based feed formulation. The protein digestibility of thick cellulosic cell-walled microalgal species including *Chlorella* sp., *Desmodesmus* sp., *Nannochloropsis* sp., and *Tetraselmis* sp. in aquatic species ranged 80–89%, 54–67%, 69–81%, and 70–73%, respectively (Gong et al., 2018; Moheimani et al., 2018; Niccolai et al., 2019; Skrede et al., 2011). Other hand non-cellulosic species like *Isochrysis* sp. and *Spirulina* sp. had similar higher protein digestibility of 86% (Niccolai et al., 2019; Skrede et al., 2011). The dry matter digestibility also reflected the same trend of superior values for non-cellulosic microalgal species compared to thick-walled cellulosic species. Recent studies with farmed marine aquaculture species have shown that dietary supplementation with various microalgae up to 25% is acceptable based on intestinal histological parameters, organ weights, sensory evaluation, and digestive enzyme activities (Patterson and Gatlin III, 2013; Tibaldi et al., 2015; Vizcaíno et al., 2018).

Cell disruption can increase the nutrient digestibility of microalgae. The degree of cell disruption affects nutrient digestibility (Agboola et al., 2019). Adopting the most appropriate approach to disrupt the cell wall makes it possible to improve microalgae's nutritional accessibility and digestibility by fish (Batista et al., 2020b). In a study, pasteurization, melting, freeze-drying, cold pasteurization, and bead milling were used to break the cell wall of *Nannochloropsis gaditana* biomass, and then the treated biomass was used as a feed ingredient for African catfish and Nile tilapia (Agboola et al., 2019); the fish weight gain and feed conversion ratio for cold pasteurization and bead milling diets improved by 13% and 11%, respectively, as compared to the control diet. Diets containing enzymatically processed *Nannochloropsis oceanica* and physically processed *Chlorella vulgaris* and *Tetraselmis* sp. had higher protein and energy digestibility compared to non-treated control (Batista et al., 2020b). While the enzyme Bromelain improved the digestibility of *Spirulina*-based fish feed in Mozambique tilapia fingerlings (*Oreochromis mossambicus*), other two enzymes (i.e., papain and trypsin) were found to be less effective (Sharma et al., 2021). The extrusion process can disrupt algae cell walls, allowing fishes to consume more nutrients (Maehre et al., 2016; Shi et al., 2016; Venou et al., 2009). In a study involving the feeding of *Nannochloropsis* sp. biomass to Atlantic salmon, *S. salar*, extrusion processed feed was shown to be more digestible than non-extrusion processed feed in terms of ash and dry matter (Gong et al., 2018). In another study involving gibel carp, the extruded feeds displayed higher protein and dry matter digestibility than the pelleted feeds (Shi et al., 2016).

Due to variations in eating patterns and digestive physiologies among various fish species, the nutritional benefits of a microalga in one target animal species do not generally guarantee the same in others. For example, shrimp do not have an acidic stomach, and trout have a longer gut transit period which might lead to poor digestibility of complex forms of algal nutrients (Tibbetts et al., 2017). As a result, a thorough microalgae digestibility analysis is needed. If physical/mechanical and enzymatic cell disruption of microalgae are used to increase microalgal digestibility, it should also be industrially scalable.

6. Microalgae-based diet and fish health

6.1. Effect of microalgae-based diet on survivability

Several studies have shown that the survivability of fish could be

Table 4
Effect of microalgae based feed on growth performance.

Fish	Microalgae	Inclusion level	Replacing	Pellet	Weight gain (%) of algae based feed	Weight gain (%) of Reference	% difference between weight gain of algal and that of reference diet	Specific growth of algae based feed	Specific growth rate of reference diet	% difference between Specific growth rate of algal and that of reference diet	Ref.
Salmonids											
Atlantic salmon (Salmo salar L.)	Scenedesmus sp.	10	Fish Meal	Extrusion	95	107	-11	0.8	0.82	-2	(Gong et al., 2019)
Atlantic salmon (Salmo salar L.)	Defatted N. oceanica	10	Fish Meal	Extrusion	96	100	-4	1	1.06	-6	(Sørensen et al., 2017)
Atlantic salmon (Salmo salar L.)	Defatted N. oceanica	10	Fish Meal	Extrusion	82	85	-4	1.03	1.12	-8	(Gong et al., 2020)
Atlantic salmon (Salmo salar L.)	N. oceanica + Digestarom® PEP MGE150 0.06% feed	10	Fish Meal	Extrusion	86	85	1	0.87	0.91	-4	(Gong et al., 2020)
Atlantic salmon (Salmo salar L.)	N. oceanica + ZEOFeed 1% feed	10	Fish Meal	Extrusion	85	85	0	0.9	0.91	-1	(Gong et al., 2020)
Atlantic salmon (Salmo salar L.)	Defatted Nannochloropsis sp.	5	Fish Meal	Extrusion	137	147	-7	0.91	0.91	0	(Valente et al., 2019)
Atlantic salmon (Salmo salar L.)	Schizochytrium sp. oil	5	Fish Oil	_	426	326	31	1.5	1.3	15	(Wei et al., 2021)
Juvenile rainbow trout (Oncorhynchus mykiss)	Nannochloropsis sp. & Isochrysis sp.	7& 2.4	Fish Meal	_	319	395	-19	1.77	1.95	-9	(Sarker et al., 2020b)
Juvenile rainbow trout (Oncorhynchus mykiss)	Nannochloropsis sp. & Schizochytrium sp.	7& 2.5	Fish Meal Fish Oil	_	349	395	-12	1.59	1.63	-2	(Sarker et al., 2020b)
Juvenile rainbow trout (Oncorhynchus mykiss)	Nannochloropsis sp., Isochrysis sp. & Schizochytrium sp.	7& 2.4& 3.2	Fish Meal Fish Oil	_	343	395	-13	1.7	1.9	-11	(Sarker et al., 2020b)
Rainbow trout (Oncorhynchus mykiss)	Scenedesmus almeriensis	10	Fish Meal	Extrusion	325	389	-16	1.8	1.9	-5	(Tomás-Almenar et al., 2018)
Rainbow trout (Oncorhynchus mykiss)	Scenedesmus sp.	5	Fish Meal Fish Oil	_	104	108	-3	1.8	1.9	-5	(Skalli et al., 2020)
Shrimp											
Post larvae Pacific white shrimp (Litopenaeus vannamei)	Schizochytrium sp.	7.5	Fish Oil	Extrusion	637	562	13	2.36	2.24	5	(Allen et al., 2019)
Post larvae Pacific white shrimp (Litopenaeus vannamei)	Tetraselmis suecica	0.75	_	Cold pelletized	16,255	12,529	30	5.56	5.38	3	(Sharawy et al., 2020b)
Pacific white shrimp (Litopenaeus vannamei) larvae	Schizochytrium sp. meal	6	Fish Oil	_	2988	2869	4	5.63	5.38	5	(Wang et al., 2017b)
Juvenile Pacific white shrimp (Litopenaeus vannamei)	Aurantiochytrium sp. meal	8	Fish Oil	Cold pelletized	293	297	-1	5.66	5.38	5	(Guimarães et al., 2019)
Black tiger shrimp (Penaeus monodon)	Aurantiochytrium sp.	2	Wheat	Extrusion	1094	971	13	8.85	8.47	4	(Jaseera et al., 2021)
Black tiger shrimp (Penaeus monodon)	Aurantiochytrium sp.	2	Wheat	Extrusion	1217	971	25	9.21	8.81	5	(Jaseera et al., 2021)
Sea bass											
	TIsochrysis lutea & Tetraselmis suecica	12 & 6	Fish Meal Fish Oil	Cold pelletized	105	101	4	0.68	0.66	3	(Cardinaletti et al., 2018)

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Table 4 (*continued*)

Fish	Microalgae	Inclusion level	Replacing	Pellet	Weight gain (%) of algae based feed	Weight gain (%) of Reference	% difference between weight gain of algal and that of reference diet	Specific growth of algae based feed	Specific growth rate of reference diet	% difference between Specific growth rate of algal and that of reference diet	Ref.
European sea bass (Dicentrarchus labrax)											
European sea bass (Dicentrarchus labrax)	Microalgal consortium	20	Fish Oil& Wheat	Cold pelletized	243	239	2	1.66	1.65	1	(Pascon et al., 2021)
European sea bass (Dicentrarchus labrax)	Nannochloropsis sp.	10	Fish Oil& Wheat	Cold pelletized	246	239	3	1.68	1.65	2	(Pascon et al., 2021)
European sea bass (Dicentrarchus labrax)	Nannochloropsis sp.	8.3	Fish Meal& Wheat	Extrusion	175	172	2	1.2	1.2	0	(Batista et al., 2020a)
Sea bream											
Juvenile Gilthead Seabream (Sparus aurata)	Defatted Tetraselmis sp.	10	Soybean	Extrusion	245	248	-1	2.03	2.04	0	(Pereira et al., 2020)
Gilthead seabream (Sparus aurata)	Phaeodactylum tricornutum + 16 mg g−1 fucoxanthin	2.5	Wheat	Extrusion	79	78	1	0.69	0.69	0	(Ribeiro et al., 2017)
Juvenile red seabream (Pagrus major)	Schizochytrium sp.	11	Fish Meal & Fish Oil	_	382	420	-9	1.86	1.96	-5	(Seong et al., 2019)
Juvenile Gilthead Seabream (Sparus aurata)	Nannochloropsis gaditana	5	Soybean	_	105	98	8	2.02	1.9	6	(Ayala et al., 2020)
Juvenile Gilthead Seabream (Sparus aurata)	Cellulose hydrolysed Nannochloropsis gaditana	5	Soybean	_	102	98	4	1.96	1.9	3	(Ayala et al., 2020)
Tilapia											
Nile tilapia (Oreochromis niloticus)	Anabaena sp.	1.5	Fish Meal	_	88	73	20	6.34	6.03	5	(Fadl et al., 2020)
Nile tilapia (Oreochromis niloticus) Fry	Nannochloropsis salina	82	Fish Meal Fish Oil	_	215	232	-7	3.19	3.34	-4	(Gbadamosi and Lupatsch, 2018)
Nile tilapia (Oreochromis niloticus) juveniles	Nannochloropsis oculata	10	starch	_	212	145	46	1.89	1.49	27	(Abdelghany et al., 2020)
Juvenile Nile tilapia (Oreochromis niloticus)	Autotrophic biofloc technology system - Chlorella vulgaris and Scenedesmus obliquus	_	_	_	106	106	1	1.3	1.3	0	(Jung et al., 2017)
Juvenile Nile tilapia (Oreochromis niloticus)	Nannochloropsis gaditana	30	Whole	Extrusion	212	212	0	2.76	2.71	2	(Teuling et al., 2019)
Nile tilapia (Oreochromis niloticus)	Spirulina sp.	15	_	_	18	22	-18	0.27	0.32	-16	(Fadl et al., 2017b)
Nile tilapia (Oreochromis niloticus)	Chlorella sp.	15	_	_	38	22	69	0.51	0.32	59	(Fadl et al., 2017b)
	Spirulina sp. & Chlorella sp.	15 and 15	_	_	31	22	39	0.43	0.32	34	(Fadl et al., 2017b)

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Table 4 (*continued*)

Fish	Microalgae	Inclusion level	Replacing	Pellet	Weight gain (%) of algae based feed	Weight gain (%) of Reference	% difference between weight gain of algal and that of reference diet	Specific growth of algae based feed	Specific growth rate of reference diet	% difference between Specific growth rate of algal and that of reference diet	Ref.
Nile tilapia (Oreochromis niloticus)											
Nile tilapia (Oreochromis niloticus)	Defatted N.oculata and Schizochytrium sp.	14.2	Fish Meal & Fish Oil	Extrusion	504	319	58	0.87	0.62	40	(Sarker et al., 2020a)
Juvenile Nile tilapia (Oreochromis niloticus)	Defatted N.oculata and Schizochytrium sp.	12&3	Fish Meal	Extrusion	352	235	50	3.02	2.29	32	(Ju et al., 2017)
Outdoor juvenile tilapia (Oreochromis mossambicus)	Defatted N.oculata and Schizochytrium sp.	12&3	Fish Meal	Extrusion	128	143	-11	3.2	3.4	-6	(Ju et al., 2017)
Others											
Hybrid striped bass (Morone sp.)	Defatted Chlorella sp.	11.6	Fish Meal	Extrusion	352	386	-9	3.08	3.23	-5	(de Cruz et al., 2018)
Hybrid striped bass (Morone sp.)	Chlorella sp.	9.5	Fish Meal	Extrusion	353	386	-9	3.08	3.23	-5	(de Cruz et al., 2018)
Hybrid striped bass (Morone sp.)	Phaeodactylum tricornutum	10.1	Fish Meal	Extrusion	349	386	-10	3.07	3.23	-5	(de Cruz et al., 2018)
Hybrid striped bass (Morone sp.)	Phaeodactylum tricornutum & Nanochloropsis salina	13.2	Fish Meal	Extrusion	331	386	-14	2.98	3.23	-8	(de Cruz et al., 2018)
Hybrid striped bass (Morone sp.)	Nanochloropsis salina	6.4	Fish Meal	Extrusion	354	386	-8	3.09	3.23	-4	(de Cruz et al., 2018)
Hybrid striped bass (Morone sp.)	Nanochloropsis salina & Amphora sp	11.9	Fish Meal	Extrusion	156	168	-7	1.92	2.01	-4	(de Cruz et al., 2018)
Hybrid striped bass (Morone sp.)	Nanochloropsis salina & Cylindro sp	11.4	Fish Meal	Extrusion	158	168	-6	1.93	2.01	-4	(de Cruz et al., 2018)
Hybrid striped bass (Morone sp.)	Phaeodactylum tricornutum & Nanochloropsis salina	9.9	Fish Meal	Extrusion	176	168	5	2.07	2.01	3	(de Cruz et al., 2018)
Hybrid striped bass (Morone sp.)	Bluegreen algae biomass	9.3	Fish Meal	Extrusion	168	168	0	2.01	2.01	0	(de Cruz et al., 2018)
Juvenile turbot (Scophthalmus maximus L.)	Nannochloropsis sp.	10	Fish Meal	Extrusion	158	150	6	1.36	1.3	5	(Qiao et al., 2019)
Trachinotus ovatus larvae	Isochrysis galbana	4.8	Fish Oil	Cold pelletized	364	336	8	1.92	1.84	4	(He et al., 2018)
Juvenile yellow perch (Perca flavescens)	Defatted Haematococcus pluvialis	5	Fish Meal	Extrusion	177	192	-7	1.82	1.9	-4	(Jiang et al., 2019)
Gibel carp (Carassius auratus gibelio)	Oedocladium sp.	4	Soybean oil and cellulose	Cold pelletized	40	43	-8	0.85	0.92	-8	(Chen et al., 2019)
Gibel carp (Carassius auratus gibelio)	Tribonema sp.	5	Soybean oil and cellulose	Cold pelletized	40	43	-8	0.85	0.92	-8	(Chen et al., 2019)
	Scenedesmus obliquus	12	Fish Meal	Extrusion	77	88	-12	0.66	0.72	-8	(Knutsen et al., 2019)

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Table 4 (*continued*)

Fish	Microalgae	Inclusion level	Replacing	Pellet	Weight gain (%) of algae based feed	Weight gain (%) of Reference	% difference between weight gain of algal and that of reference diet	Specific growth of algae based feed	Specific growth rate of reference diet	% difference between Specific growth rate of algal and that of reference diet	Ref.
Spotted wolffish juveniles (Anarhichas minor)											
Juveniles African catfish (Clarias gariepinus, Fleuren en Nooijen strain)	Nannochloropsis gaditana	30	Whole	Extrusion	181	180	1	3.04	2.99	2	(Agboola et al., 2019)
Senegalese sole Juveniles	TIsochrysis lutea	15	Fish Meal & Fish Oil	Extrusion	318	161	97	1.68	1.69	-1	(Vizcaíno et al., 2018)
Senegalese sole Juveniles	Nannochloropsis gaditana	15	Fish Meal & Fish Oil	Extrusion	296	161	84	1.63	1.69	-4	(Vizcaíno et al., 2018)
Senegalese sole Juveniles	Scenedesmus almeriensis	15	Fish Meal & Fish Oil	Extrusion	301	161	86	1.63	1.69	-4	(Vizcaíno et al., 2018)

improved by feeding microalgae-containing diet. The inclusion of microalgae, including *Pavlova* sp., *Chaetoceros* sp., *Nannochloropsis oculata*, and *Isochrysis* sp., in feed, the seahorses (*Hippocampus reidi*) and oysters (*Pinctada margaritifera*), increased their survivability (Martínez-Fernández and Southgate, 2007; Mélo et al., 2016). The survival rate of *L. vannamei* shrimp increased when 1–2% of its feed was supplemented with *Dunaliella salina* (Medina-Félix et al., 2014). The enhancement of fish survivability by the microalgae-based feeds could also be linked with their functional characteristics, including the effects of probiotics, prebiotics, immunostimulants, antiviral, antibacterial, etc.

6.2. Microalgae as probiotic

Probiotics are either micro-organisms or components of micro-organisms that help improve intestinal health after consumption. Microalgae are known to have probiotic effects on fishes. When a fish consumes algal cells, the microbiome in the intestine digests the algal cell, releasing probiotic materials that inhibit pathogens (Austin, 2006; Ghanbari et al., 2015; Nayak, 2010). In a study, the *Tetraselmis suecica* live cells were fed to white shrimp (*Fenneropenaeus indicus*); the load of pathogenic bacteria in its gut was reduced when compared to control (Regunathan and Wesley, 2004). The addition of a 1.2% *Schizochytrium* sp. meal to the diet influenced the gut microbiota, resulting in improved Nile tilapia health (Souza et al., 2020). The inclusion of freeze-dried microencapsulated *Chaetoceros* sp. directly in the water supported the growth of beneficial bacteria in the gut of Pacific white shrimp (*Litopenaeus vannamei*); study also reported that the survival of *L. vannamei* at larval and stages beyond larvae increased (Nimrat et al., 2011).

6.3. Microalgae as prebiotic

Similar to probiotics, prebiotics are beneficial to fish health as these too improve gut health. Prebiotics differ from probiotics in the sense that these particularly refer to the indigestible cell wall polysaccharides/fibres that can provoke the growth of beneficial bacteria like *Bifidobacteria*, *Lactobacilli*, etc. in the intestine (Dawood et al., 2018; Wang et al., 2017a). Despite the fact that fiber, polysaccharide, and oligosaccharide from microalgae, such as beta-glucan from *C. vulgaris* and homogalactan from *Gyrodinium* sp., are frequently reported, their prebiotic effect in fishes has not been thoroughly investigated (Hemaiswarya et al., 2011). On the other hand, the whole biomass of *Spirulina platensis* and *Isochrysis galbana* have been shown to promote the growth of beneficial bacteria in vitro and in vivo studies (Dineshbabu et al., 2019; Hemaiswarya et al., 2011). Currently, algae-based companies like Algatech are commercially selling beta-glucan – a prebiotic compound from microalgae, *Euglena* sp. After the ban of antibiotics in animal feed in several countries, prebiotics, in addition to probiotics, have witnessed steady growth in the market (Defoirdt et al., 2007). In this scenario, the probiotic effects can be added as a selling point for macroalgae-based fish feed.

6.4. Immunostimulants

Feeding of cell wall compounds like glucans, peptidoglycans, lipopolysaccharides, fucoidan, chitin, and whole algae can enhance the immune system in aquatic species (Dawood et al., 2018). The cell-wall polysaccharides have been shown to increase immune response by increasing cytokine, phagocytosis, and proliferation of immune cells, including neutrophils and monocyte-macrophages in aquatic species (Dawood et al., 2018). Paramylon, a β-1,3 polymer of glucose (beta-glucan) found in the cell wall of *Euglena* sp., has been shown to act as immunostimulant in species such as Atlantic salmon, mussels, red drum, and matrinxã (Bianchi et al., 2015; Kiron et al., 2016; Montoya et al., 2017; Yamamoto et al., 2018).

When 6–8% of fish meal was replaced with *Chlorella vulgaris*, post-larvae of *Macrobrachium rosenbergii* displayed improved immune response (prophenol oxidase activity and total haemocyte count), and

Table 5
Effect of microalgae based diet on Feed conversion ratio.

Fish	Algae	Inclusion level in Diet	Targeting ingredient for Replacement	Pellet	Feed conversion ratio of microalgae based diet	Feed conversion ratio of Reference diet	% Difffernce between FCR of algae and that of reference dier	Reference
Salmonids								
Atlantic salmon (*Salmo salar* L.)	*N. oceanica* + Digestarom® PEP MGE150 0.06% feed	10	Fish Meal	Extrusion	0.89	0.90	-1	(Gong et al., 2020)
Atlantic salmon (*Salmo salar* L.)	*N. oceanica* + ZEOFeed 1% feed	10	Fish Meal	Extrusion	0.89	0.90	-1	(Gong et al., 2020)
Rainbow trout (*Oncorhynchus mykiss*)	*Scenedesmus* sp.	5	Fish Meal Fish Oil	_	1.14	1.15	-1	(Skalli et al., 2020)
Atlantic salmon (*Salmo salar* L.)	*Schizochytrium* sp. oil	10	Fish Oil	_	0.90	0.90	0	(Wei et al., 2021)
Atlantic salmon (*Salmo salar* L.)	Defatted *Nannochloropsis* sp.	5	Fish Meal	Extrusion	1.52	1.48	3	(Valente et al., 2019)
Atlantic salmon (*Salmo salar* L.)	*N. oceanica*	10	Fish Meal	Extrusion	0.95	0.90	6	(Gong et al., 2020)
Rainbow trout (*Oncorhynchus mykiss*)	*Scenedesmus almeriensis*	2.5	Fish Meal	Extrusion	0.89	0.84	6	(Tomás-Almenar et al., 2018)
Atlantic salmon (*Salmo salar* L.)	Defatted *N. oceanica*	10	Fish Meal	Extrusion	0.86	0.81	6	(Sørensen et al., 2017)
Atlantic salmon (*Salmo salar* L.)	*Scenedesmus* sp.	10	Fish Meal	Extrusion	0.88	0.76	16	(Gong et al., 2019)
Shrimp								
Post larvae Pacific white shrimp (*Litopenaeus vannamei*)	*Tetraselmis suecica*	0.5	_	Cold pelletized	1.04	1.36	-24	(Sharawy et al., 2020b)
Black tiger shrimp (*Penaeus monodon*)	*Aurantiochytrium* sp. meal	2	Wheat	Extrusion	1.12	1.28	-13	(Jaseera et al., 2021)
Post larvae of Pacific white shrimp (*Litopenaeus vannamei*)	*Schizochytrium* sp.	7.5	Fish Oil	Extrusion	2.07	2.16	-4	(Allen et al., 2019)
Juvenile Pacific white shrimp (*Litopenaeus vannamei*)	*Aurantiochytrium* sp. meal	8	Fish Oil	Cold pelletized	2.10	2.08	1	(Guimarães et al., 2019)
European sea bass								
European sea bass (*Dicentrarchus labrax*)	*TIsochrysis lutea*, *Tetraselmis suecica*	12 & 6	Fish Meal Fish Oil	Cold pelletized	1.69	1.75	-3	(Cardinaletti et al., 2018)
European sea bass (*Dicentrarchus labrax*)	*Nannochloropsis* sp	8.3	Fish Meal, wheat	Extrusion	1.60	1.60	0	(Batista et al., 2020a)
European sea bass (*Dicentrarchus labrax*) juveniles	Microalgal consortium	10	Fish Oil, Wheat	Cold pelletized	1.16	1.15	1	(Pascon et al., 2021)
European sea bass (*Dicentrarchus labrax*) juveniles	*Nannochloropsis* sp.	10	Fish Oil, Wheat	Cold pelletized	1.16	1.15	1	(Pascon et al., 2021)
Sea bream								
Juvenile Gilthead Seabream (*Sparus aurata*)	*Nannochloropsis gaditana*	5	Soybean	_	1.09	1.16	-6	(Ayala et al., 2020)
Juvenile gilthead seabream (*Sparus aurata*)	Defatted *Tetraselmis* sp.	10	Soybean	Extrusion	1.40	1.42	-1	(Pereira et al., 2020)
Gilthead seabream (*Sparus aurata*)	*Phaeodactylum tricornutum* + 12 mg g− 1 fucoxanthin	2.5	Wheat	Extrusion	1.74	1.69	3	(Ribeiro et al., 2017)
Juvenile red seabream (*Pagrus major*)	*Schizochytrium* sp.	11	Fish Meal & Fish Oil	_	1.15	1.01	14	(Seong et al., 2019)
Tilapia								
Nile tilapia (*Oreochromis niloticus*)	*Chlorella* sp.	15	_	_	1.24	1.78	-30	(Fadl et al., 2017b)
Nile tilapia (*Oreochromis niloticus*)	*Spirulina* sp. & *Chlorella* sp.	15 & 15	_	_	1.49	1.78	-16	(Fadl et al., 2017b)
		14.2		Extrusion	1.40	1.61	-13	

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Table 5 (*continued*)

Fish	Algae	Inclusion level in Diet	Targeting ingredient for Replacement	Pellet	Feed conversion ratio of microalgae based diet	Feed conversion ratio of Reference diet	% Difffernce between FCR of algae and that of reference dier	Reference
Nile tilapia (*Oreochromis niloticus*)	Defatted *N.oculata* and *Schizochytrium* sp		Fish Meal & Fish Oil					(Sarker et al., 2020a)
Juvenile Nile tilapia (*Oreochromis niloticus*)	*Nannochloropsis gaditana*	30	Whole	Extrusion	0.90	0.96	-6	(Teuling et al., 2019)
Nile tilapia (*Oreochromis niloticus*) juveniles	*Nannochloropsis oculata*	15	Starch	_	1.89	1.91	-1	(Abdelghany et al., 2020)
Juvenile Nile tilapia (*Oreochromis niloticus*)	Defatted *N.oculata* and *Schizochytrium* sp	12 & 3	Fish Meal	Extrusion	1.72	1.72	0	(Ju et al., 2017)
Nile tilapia (*Oreochromis niloticus*) fry	*Nannochloropsis* salina	82	Fish Meal Fish Oil	_	1.28	1.20	7	(Gbadamosi and Lupatsch, 2018)
Juvenile Nile tilapia (*Oreochromis niloticus*)	*Nannochloropsis gaditana*	30	Whole	Extrusion	1.04	0.96	8	(Teuling et al., 2019)
Juvenile Nile tilapia (*Oreochromis niloticus*)	Defatted *N.oculata* and *Schizochytrium* sp	12 & 3	Fish Meal	Extrusion	1.88	1.72	9	(Ju et al., 2017)
Other species								
Juvenile turbot (*Scophthalmus maximus L.*)	*Nannochloropsis* sp.	10	Fish Meal	Extrusion	0.81	0.86	-6	(Qiao et al., 2019)
Juveniles African catfish (*Clarias gariepinus, Fleuren en Nooijen* strain)	*Nannochloropsis gaditana*	30	Whole	Extrusion	0.92	0.93	-1	(Agboola et al., 2019)
Senegalese sole Juveniles	*Scenedesmus almeriensis*	15	Fish Meal & Fish Oil	Extrusion	1.26	0.93	2	(Vizcaíno et al., 2018)
Juvenile yellow perch (*Perca flavescens*)	Defatted *Haematococcus pluvialis*	5	Fish Meal	Extrusion	1.19	1.17	2	(Jiang et al., 2019)
Trachinotus ovatus larvae	*Isochrysis galbana*	4.8	Fish Oil	Cold pelletized	1.83	1.78	3	(He et al., 2018)
Senegalese sole Juveniles	*TIsochrysis lutea*	15	Fish Meal & Fish Oil	Extrusion	1.28	0.93	3	(Vizcaíno et al., 2018)
Senegalese sole Juveniles	*Nannochloropsis gaditana*	15	Fish Meal & Fish Oil	Extrusion	1.30	0.93	5	(Vizcaíno et al., 2018)

survivability against *Aeromonas hydrophila* infection (Maliwat et al., 2017). In another study, administration of (*Tetraselmis chuii*, *Nannochloropsis gaditana,* and *P. tricornutum*) orally enhanced the defence activity of gilthead seabream (*S. aurata*) (Cerezuela et al., 2012). When *Euglena viridis* biomass was fed to Rohu fish (*Labeo rohita*), it had immunostimulatory effects such as increased serum bactericidal activity, pathogen lysozyme, and superoxide anion production against *A. Hydrophila* (Das et al., 2009). *Dunaliella salina* increased the survival

Table 6
Cell wall morphology of different microalgae.

Division	Species	Cell wall
Cyanophyta	*Spirulina platensis, Aphanizomenon flos-aquae*	Has four layers. Outer fibrillar layer; then a peptidoglycan layer (known as murein) gives rigidity; third layer is again a fibrillar layer and an outer membrane covered with acidic polysaccharides; Lipopolysaccharide also present
Chlorophyta	*Chlorella sorokiniana, Chlorella zofingiensis, C. homosphaera*	Two layers; Outer layer is a trilaminar structure and has algaenan – a nonhydrolyzable polymer; Inner is a fibrillar rigid layer made up of polysaccharides (cellulose/hemicellulose/pectin), glucosamine (Chitin)
Chlorophyta	*Chlorella vulgaris*	Two layers; Outer layer is homogenous lacking trilaminar and algaenan. Inner is a rigid fibrillar structure and has cellulose rich matrix
Chlorophyta	*Tetraselmis suecica*	Scales
Chlorophyta	*Scenedesmus* sp.	Rigid cell wall; Has three layers: outer pectic layer; a thin algaenan middle layer; an inner fibrillar layer made up of cellulose; Cell wall contains mannose, glucose, and galactose
Dinophyta	*Dunaliella salina, Dunaliella tertiolecta*	Lack cell wall or contain few cellulose
Cryptophyta	*Cryptomonas rufescens*	Periplast covering
Euglenophyta	*Euglena gracilis*	Lack cell wall
Rhodophyta	*Porphyridium* sp.	Polysaccharide capsule containing Glucose, xylose, glucuronic acid, galactose, and methyl-glucuronic acid
Heterokontophyta	*Phaeodactylum tricornutum*	Naked or covered by scales or with large quantities of silica
Bacillariophyta	*Chaetoceros calcitrans, Chaetoceros gracilis*	Frustules with hydrated silica
Eutomatophyceae	*Nannochloropsis oculata*	Thick cell wall; Has three layers: Outer is a thin Algaenan layer. Middle is a cellulose based layer and a porous inner layer. Cell wall contains amino acids and other sugars (ribose,fucose, xylose, rhamnose, mannose,and galactose) present in small amount

Note: Reference from (Blomqvist et al., 2018; El-Sayed, 1994; Hodar et al., 2020; Liu et al., 2020; Madeira et al., 2017; Samuelsen et al., 2018; Sørensen et al., 2016, 2011; Tavakoli et al., 2021; Tibbetts et al., 2017; Tulli et al., 2012; Varelas, 2019; Wan et al., 2019; Yu et al., 2020)

Table 7
Fatty acid composition of microalgae including other alternative ingredients.

Fattyacid	*Chroococcus*	*Synechococcus*	*Isochrysisgalbana*	*Pavlova*	*Phaeodactylumtricornutum*	*Porphyridiumcruentum*	*Rhodomonasbaltica*	*Oocystis*	*Pseudokirchneriellasubcapitata*	*Tetraselmis*	*Tribonema*	*Nannochloropsisoceanica*	*Scizhochytriumsp.*	***SoybeanOil***	***Blackflysoldier***	***Yeast****S.cerivisiae*	*Gracilariasp*	*Sargassumsp*	*Ulvasp*
Saturated																			
C10:0	_	_	_	_	_	_	_	_	_	_	_	_	_	_	1.1	_	_	_	_
C12:0	2.1	0.7	_	_	_	_	2	_	_	_	_	1.2	0.2	_	24.6	0.1	0.1	_	_
C14:0	0.1	5.6	8.9	7.5	8.8	_	4.1	0.2	0.1	0.5	1.1	16.9	9.0	_	3.3	0.1	1.7	1.6	1.1
c15:0	_	_	_	_	_	_	_	_	_	_	_	_	2.2	_	_	_	0.7	0.3	0.4
C16:0	21.3	3.4	11.5	13.4	16.6	5.9	6	3.8	16.2	6.3	2.5	17.2	38.0	14.7	18.4	15.7	65.0	43.1	61.1
C 17:0	_	_	_	_	_	_	_	_	_	_	_	_	0.9	_	_	_	_	0.2	0.2
C 18:0	0.3	_	_	0.4	0.6	0.2	0.8	_	1.3	1.2	0.1	1.8	1.5	5.4	8.0	1.3	1.7	1.6	1.5
C 20:0	0.2	_	_	_	_	_	0.1	_	0.2	_	_	_	0.5	0.4	_	_	_	1.1	0.3
C 21:0	_	_	_	_	_	_	_	_	_	_	_	_	0.3	_	_	_	_	_	_
C 22:0	_	_	_	_	_	_	_	_	_	_	_	_	0.4	0.3	_	_	_	0.8	2.3
C 24:0	_	_	_	_	1.6	_	4	0.1	0.7	_	_	_	_	_	_	_	_	_	0.1
Sum	24	9.7	20.4	21.3	27.6	6.1	17	4.1	18.5	8.0	3.7	37.1	52.9	20.8	55.4	17.2	69.2	48.7	67.0
Monosaturated																			
C14:1	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0.2	_	_	0.2
C 16:1	1.1	10.8	3.3	12.8	26	_	0.4	1.5	1.0	1.3	5.1	18.2	_	_	0.8	43.2	0.0	2.7	0.1
C 17:1	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0.0	0.2	0.3
C18:1[c]	0.4	_	13.1	2.9	1.8	0.1	3.4	3.9	31.1	10.7	0.2	4.1	_	26.8	24.5	39.3	17.3	19.7	16.6
C 20:1[c]	_	_	_	_	_	_	0.1	_	0.9	0.9	_	0.5	_	_	_	_	_	0.4	0.0
C 22:1[c]	0.1	_	0.6	0.8	0.3	_	0.1	_	0.8	_	_	_	_	_	_	_	_	_	_
Sum	1.6	10.8	17	16.5	28.1	0.1	4	5.4	33.8	12.9	5.3	22.8	0.0	26.8	25.3	82.7	17.3	23.0	17.1
Polyunsaturated																			
C 18:2[b]	10.7	_	7	2.1	1.5	2.1	11.7	6.4	5.1	2.5	0.2	9.7	0.2	44.4	19.3	_	10.3	9.6	8.2
C 18:3[a]	1	_	3.8	1.8	0.3	_	12	8.1	11.4	6.4	_	0.5	0.8	8.0	_	_	0.5	2.6	1.4
C 18:4[a]	_	_	12.5	4.3	3.3	_	5.1	0.7	3.0	4.1	0.1	_	_	_	_	_	_	_	_
C 20:2[b]	0.1	_		_	_	0.3	0.1	_	_	_	_	0.5	_	_	_	_	_	0.7	_
C 20:3	_	_	_	_	_	_	_	_	_	_	_	_	0.3	_	_	_	_	0.7	_
C 20:4[b]	0.1	_		0.4	2.2	6	0.2	0.5	_	0.6	_	3.7	0.5	_	_	_	_	8.9	_
C 20:5[a]	_	_	0.8	18	28.4	6.1	4.4	1.1	_	4.8	3.2	23.4	0.7	_	_	_	_	0.6	_
C22:5[b]	_	_		_	1.3	_	0.2	_	2.1	_	_	_	6.7	_	_	_	0.7	0.3	0.8
C22:6[a]	_	_	15.8	13.2	0.2	_	_	_	0.1	0.2	_	_	37.6	_	_	_	0.1	0.5	0.2
Sum	11.9	0	39.9	39.8	37.2	14.5	33.7	16.8	21.7	18.6	3.5	37.8	47.0	52.4	19.3	0.0	11.5	23.8	10.6

rate of *P. monodon* infected by white spot syndrome by increasing the antioxidant factors like superoxide dismutase and catalase in shrimp (Madhumathi and Rengasamy, 2011). A diet containing a mixture of *Lactobacillus sakei* and *Navicula* sp. improved the humoral immune parameters of pacific red snapper (*Lutjanus peru*) (Reyes-Becerril et al., 2013). Feed containing microalgae *P. incisa* increased the survival rate by increasing lysozyme levels in Guppy fish (*Poecilia reticulata*) (Nath et al., 2012). *Spirulina* has been shown to invoke non-specific immune responses against pathogens in many species (Cao et al., 2018; Sheikhzadeh et al., 2019). The white blood count, red blood count, haemoglobin, albumin levels total protein increased when a 10% *A. Platensis* was fed to rainbow trout (*O. mykiss*) (Yeganeh et al., 2015). Pathogens that affect fish survival and growth could be inhibited by microalgal immunostimulant properties, giving algae-based feed more value.

7. Effect of microalgae on quality of fish

7.1. Firmness and taste

Gaping occurs as connective tissue between muscle layers tears, resulting in slits gaps in the fillet and loss of firmness. Fillet firmness is an important trait for consumer acceptance of farmed fish, and soft fillets are devalued by the food industry. Microalgae have been shown to reduce gaping in the fillet. In Atlantic salmon, a 5% *Schizochytrium* sp. diet improved fillet quality by reducing gaping when compared to a control diet (Kousoulaki et al., 2016). In another study, the fish feed containing 5% *Spirulina* improved the texture and taste of Striped jack (*Pseudocaranx dentex*) (Watanabe, 1990). Fish feed containing 2% *Spirulina* increased the increased firmness, muscle quality, and fibrousness of Nile Tilapia *(Oreochromis niloticus)* (Mustafa, 1995). Salmon filets produced from *Schizochytrium limacinum* were found to have the identical taste and odour as filets produced from conventional fish oil (Katerina et al., 2020).

Organic minerals such as selenium, glutamate (a functional amino acid), vitamin E, and PUFA levels have been shown to greatly minimize gaping (Tavakoli et al., 2021). Microalgae can accumulate high levels of various vitamins, including type E (Madeira et al., 2017). Also, microalgae have a mineral composition ranging from 2.2% to 4.8% of total dry weight (Guedes et al., 2015). Calcium, potassium, iron, copper, sodium, sulfur, zinc, phosphorous, and magnesium are all abundant in microalgae (Dineshbabu et al., 2019). According to a study, adding mineral-rich microalgae to salmon diets increases the fish's texture and flavour (Guedes et al., 2015). Even though an important parameter in the saleability of fish, there is not many studies on the effect of algae and algal substances on filet quality like gaping, texture, etc.

7.2. Healthy fat

The level of lipid in microalgae is generally high compared to other species (20–60%) (Ferreira et al., 2019; Madeira et al., 2017; Nagappan et al., 2020). One of the lipid classes in macroalgae with a high nutritional value is polyunsaturated fatty acids (PUFAs). The beneficial effects of PUFAs on human well-being are well known (Katiyar and Arora, 2020). The docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are nutritionally important PUFAs, which could be found at high levels in several microalgae (Table 7). Since long-chain PUFAs are difficult to be synthesized artificially, the microalgae and fish oils that contain these lipids are highly commercialized. In general, vegetable oils from canola, palm, and soybean seeds lack PUFAs or may contain these in very low levels (Hashempour-Baltork et al., 2016). PUFA rich microalgae include *Schizochytrium* and *Crypthecodinium*, which produces DHA, *Phaeodactylum*, *Nannochloropsis*, *Isochrysis*, *Nitzschia*, *Diacronema,* which produce EPA, *Porphyridium*, which produce arachidonic acid and *Desmodesmus* sp. which produce alpha-linolenic acid (Lu et al., 2021; Nagappan and Verma, 2018). Also, species like *Monodus* sp.*, Aurantiochytrium* sp., *Thraustochytrium* sp., *Thalassiosira* sp.*, Isochrysis* sp., *Phaeodactylum* sp., and *Pavlova* sp. contain a significant amount of PUFA (Lu et al., 2021). PUFA in these microalgal species ranges from as low as 2.2% to high as 37% (Ferreira et al., 2019). One of the rich sources of PUFAs, especially omega-3 fatty acids, are thraustochytrids (Leyland et al., 2017). Commercially salmon is fed with thraustochytrid *Schizochytrium* sp. solely for PUFA (Ren et al., 2010). Also, thraustochytrids were used to enrich zooplankton and fed to finfish larvae (Barclay and Zeller, 1996).

7.3. Microalgae as a colouring agent

Customers use colour as one of their first cues when selecting seafood. Fish pigmentation is known to be influenced by microalgal biomasses. The most popular microalgae used specifically for colour enhancement is *Haematococcus pluvialis* which is rich in astaxanthin. Both whole cell *H. pluvialis* and extracted astaxanthin are used as feed additives (1.5% in range) in the aquaculture industry (Chen et al., 2017). Lutein pigment is also used as a feed additive mainly for yellow/red colour (Dawood et al., 2018). Microalgae that contain a high amount of lutein are *Chlorella* and *Scenedesmus* species (Dawood et al., 2018). The coloration of several species such as Koi, Red tilapia, Striped jack, yellow catfish, and Black tiger prawn was enhanced by *Spirulina* sp. (Ansarifard et al., 2018; Dineshbabu et al., 2019; Liu et al., 2021; Nur-E-Borhan, 1993). In another study, the Yellow catfish (*Pelteobagrus fulvidraco*) fed with 4% defatted-*Spirulina* and 0.4% *Spirulina*-lipid-extract had significantly better skin colour than those fed with a control diet (Liu et al., 2021). In another study, the pigmentation of Showa koi was found to be modified by supplementing the diet with 7.5% *Spirulina platensis* (Sun et al., 2012). A diet containing 2.5% *Phaeodactylum tricornutum*, a diatom having high fucoxanthin content, has been shown to give gilthead seabream a bright yellow pigmentation (Ribeiro et al., 2017). Even though microalgae can improve colour, synthetic feed additives are favoured in the fish industry due to their low cost. However, factors such as customer preference for natural products can enable algal pigments to penetrate the market.

8. Current methodologies for fish feed preparation

8.1. Off-the-shelf feeds: microalgal paste

Microalgal paste in dry, flocculated, microencapsulated, or cryopreserved forms is an alternative to a fresh or live feed (Raja et al., 2018). Algal pastes are being used as a protein substitute in aquaculture to feed shrimps, larval fishes, zooplankton, and bivalves (Hemaiswarya et al., 2011). Microalgal paste, in particular, is beneficial to small hatchery systems because it eliminates operation of algal reactor along hatchery. The most widely used technique for producing microalgal paste is centrifugation (McCausland et al., 1999). Another popular technique is the chemical flocculation of microalgae by modifying the pH (Pugazhendhi et al., 2019). However, chemical flocculation is unsuitable for microalgal feed because of the presence of chemical flocculants and co-precipitation of undesirable compounds along with microalgae (Pugazhendhi et al., 2019). Bio-flocculation with chitosan, microbes, and exo-polysaccharides is an alternative to chemical flocculation that avoids contamination but is more difficult to set up and more expensive. Selection of self-settling, auto flocculating, and filamentous microalgae can be an economical option to produce an algal paste.

To prolong the shelf life of microalgae paste, investigators have used low-temperature preservation, freezing, vacuum packaging, and lyophilisation of biomass, as well as antioxidants, food acids, and vitamins (Amouzad Khalili et al., 2019). One issue that has to be resolved in paste preservation is that with a longer time of storage, the reactivation of cells will be delayed (McCausland et al., 1999; Raja et al., 2018). The longer the shelf life of a concentrate, the more likely it is to be marketed and used in aquaculture (Sales and de Souza-Santos, 2020). The minimum requirement of off-the-shelf algal paste is that there should be enough time to manufacture, transport, and use the items. Dhert et al. (2001) recommended a 2-week minimum shelf life, while Robert and Trintignac (1997) proposed 2-month minimum shelf life. When the algal paste was stored in a refrigerator (5–10 °C) or in a biofreezer (20–22 °C) without cryoprotectant but with NaOH added, it was able to revive even after 18 months (Jereos-Aujero and Millamena, 1981). Commercial concentrates, such as Reed Mariculture's Nanno 3600, have a shelf life of 12–14 weeks in the refrigerator; however, pasteurization was used to inactivate the cells for this product. Nauplii (Brazil) offers another concentrate – LiveNanno that includes live *Nannochloropsis* sp. cells and has a three-month shelf life. The acceptability of microalgal paste as larval food in the hatchery determines its usefulness. To maintain the nutritional value and cell viability of concentrated paste, its quality must be maintained. When compared to live microalgal diets, there was no nutrient loss during the concentrating, transportation, and preservation of the *Skeletonema costatum* and *Chaetoceros calcitrans* algal pastes (McCausland et al., 1999).

8.2. *Off-the-shelf feeds: pellet based formulated feeds*

Dry feed is expected to account for around 36% of total aquafeed demand in 2019 (Allaboutfeed, 2021). The dry feed can be manufactured with a variety of pellet sizes. Pelletizing can be done using a cold pelleting or an extruder, with the latter being the more popular method. Extrusion is a process that involves high pressure (20–30 bar), high temperature (120–130 °C), and shear forces (Dalbhagat et al., 2019). The biochemical properties of microalgae can affect the extrusion process (Gong et al., 2020). For instance, lipid-rich microalgae can act as a lubricant in the extruder barrel, reducing viscous heat dissipation and lowering pellet quality (Samuelsen et al., 2018). Therefore, the recommended ipid level for making fish feed pellets, obtained via an extruder, is 120 g/kg (Rokey, 1994). If a high lipid feed (>30%) is needed, as in the case of salmon feed, then oil has to be coated on the dried pellet using a vacuum coating process (Samuelsen et al., 2018). Extrusion can break the microalgae's recalcitrant cell wall, improving nutrient availability and digestibility (Gong et al., 2018). However, high pressure and temperature in the extrusion process can degrade the functional compounds of microalgae, so cold pelletization techniques may be preferable in such cases.

8.3. *Green water and poly-aquaculture*

The drawbacks of off-the-shelf feeds include cell settling, bacterial degradation, nutrient leaching, aggregation, loss of nutritive and functional value, and disintegration for dried algae (Hemaiswarya et al., 2011; Wan et al., 2019). Also, harvesting is a very expensive step in the production of off-the-shelf feed. Green water and poly-aquaculture techniques can help solve the problems associated with off-the-shelf feeds. Here, the energy-intensive harvesting step is eliminated by growing microalgae directly in the ponds where the fish are raised. The growth of microalgae in ponds can be aided by the use of fertilizers, waste, and other materials (Neori, 2011). Aside from microalgae, green water ponds also support the growth of bacteria, macrophytes, zooplankton, and other microbes which are consumed by fish. The overall goal will be to maintain an optimal oxygen balance in the water (Pekàr and Olah, 1997). Water exchange, periphyton substrate, and fertilization timings can all influence the growth of organisms in green water. Tilapia, Silver carp, Catla, grass carp, and Rohu carp were found suitable to be grown in green water (Muller-Feuga, 2000). Poly-aquaculture was demonstrated in a pilot study in Australia where phytoplankton was grown in nutrient-rich wastewater and fed to invertebrates of various trophic levels, such as sponges, annelid worms and bivalves, which were in turn used as animal feeds for shrimp (Palmer, 2010).

Green water/poly-aquaculture techniques are generally low-cost and require little maintenance (Muller-Feuga, 2000). According to a study, shrimp grown in "green water" costs US$ 1–3 per kg, while shrimp grown in a conventional feed supplemented system costs US$ 4–8 per kg (Biao and Kaijin, 2007; Neori, 2011). Pathogen inhibition is higher in green water ponds than in clear water ponds (Palmer et al., 2007). Species growth was higher in green water than clear water (fed with fish meal), as seen in Asian tiger shrimp (Glencross et al., 2014). Ammonia excreted by fish could act as a nitrogen source for microalgae; thus, green water/poly-aquaculture techniques reduce eutrophication potential.

9. The production cost of microalgae biomass and microalgae-based fish feed

The cost of producing microalgal biomass is determined by the

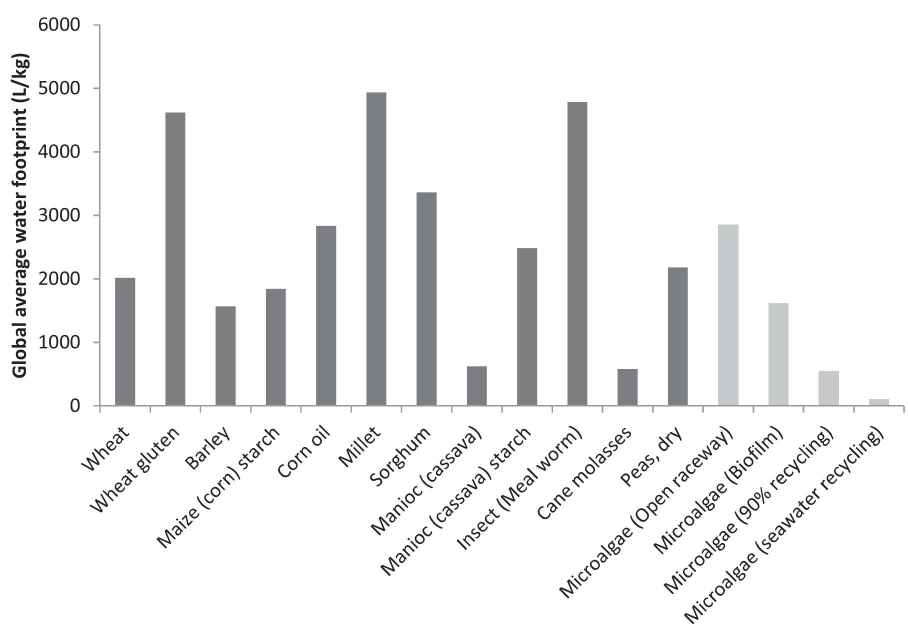

Fig. 2. Global average water footprint of various feed ingredient.
Note: Reference from (Mekonnen and Hoekstra, 2011; Miglietta et al., 2015; Pugazhendhi et al., 2020).

reactor type, reactor size, biomass productivity, waste stream utilization, value-added co-products, and automation. A small-scale open raceway pond of 1 ha containing microalgae was estimated to have a production cost of 12.9 $/kg (Sui et al., 2020). However, by increasing the raceway reactor size to 5 ha, the cost of producing microalgae biomass can be reduced to 5.3 $/kg (Fernández et al., 2019). The same study showed that using a thin layer cascade instead of an open raceway pond resulted in a 50% lower production cost for a similar plant size of 5 ha. When wastewater is used to produce microalgal biomass, labor costs are reduced through automation, and revenue from wastewater treatment are factored in, the cost of producing microalgae is reduced by 74%. So, for a 5 ha thin-layer cascade, the final cost was estimated to be 0.71 $/kg of microalgal biomass (Fernández et al., 2019). In case of photobioreactor (size of 25 m^2), tubular reactors were 54–68% cheaper than bubble columns for microalgal biomass production (Oostlander et al., 2020). Same study reported that in a photobioreactor, using artificial light rather than natural light (Dutch climate) can reduce costs slightly by 2–13%. The most significant reduction was achieved by increasing the size of the photobioreactor by 3.75 times (from 25 to 1500 m^2), which resulted in a nearly 92% reduction in the cost of microalgae production (Oostlander et al., 2020). Microalgae production using tubular photobioreactors, installed in a 1500 m^2 greenhouse, was estimated to cost 50.5 $/kg (Oostlander et al., 2020).

In recent years, more large-scale cultivation facilities (over 200 ha) have been built. According to several estimates, large-scale facilities can produce microalgal biomass at even lower costs. Microalgae biomass production can be as low as 0.65 $/kg, according to a study conducted on a 405 ha open raceway pond (Hoffman et al., 2017). These figures are based on waste water usage, but revenue from waste water treatment was not taken into account. The study assumed 20 gm^{-2} day^{-1} algal biomass productivity. With higher biomass productivities, production costs can be reduced even more. Microalgal biomass harvesting is energy-intensive and accounts for nearly 20–30% of total production costs (Barros et al., 2015). Auto-flocculating and naturally sedimenting microalgae could solve the harvesting of microalgae biomass, which otherwise could be very energy-intensive. Also, novel reactors like algal turf systems have shown to lower harvesting costs by producing high-density biomass that is easy to harvest. The biomass production cost of a 405 ha algal turf system was estimated to be only 0.49 $/kg (Hoffman et al., 2017). Profits from waste-stream treatment and automation technologies were not included in the study, which could have resulted in lower production costs.

Apart from microalgae, the animal and plant-based ingredients determine the cost of microalgae-based feed. According to one study, the cost of microalgae-based feed was 0.68 $/kg (Sarker et al., 2020a). This was calculated for Tilapia feed, in which fishmeal was completely replaced by *Nannochloropsis* sp, and fish oil was replaced by *Schizochytrium* oil. In comparison, microalgae-based feed costs lesser than the fish meal (1.5$/kg) and insect-based feed (3–5.9$/kg) but higher than the plant-based feed (0.64$/kg) (Arru et al., 2019; Indexmundi, 2021; Sarker et al., 2020a). However, as large-scale microalgae-based industries emerge at a rapid pace, the cost of microalgae-based feed is expected to fall even further in the future, making it cost-competitive with plant-based feed.

10. Environmental impact of microalgae feed

10.1. Water footprint of microalgae biomass production for fish feed

A water footprint indicator can represent water use for fish feed production. The water footprint is the freshwater amount that a process or product consumes. The water footprint of fish meal is comparatively zero since there is no water required. However, as mentioned earlier, fish meals are not sustainable in the long term. The water footprints of microalgae biomass production are 2857 L kg^{-1}, and 1618 L kg^{-1} for open raceway pond, and biofilm photobioreactor, respectively (Ozkan et al., 2012). However this study was based on the use of freshwater. The use of wastewater, seawater, and water recycling can reduce water depletion or footprint. For example, when the growth media is recycled, the water footprint of microalgae can be reduced by 90% (Pugazhendhi et al., 2020). Fig. 2 shows that the water footprint of microalgae production is lower than that of plant and insect production. In open cultivation system, the water loss due to evaporation is a major contributor to the water footprint of microalgal biomass production. The evaporation water loss could be as high as 2.0 cm day^{-1} from a 20 cm deep raceway pond (Das et al., 2016). For the cultivation of most of the marine microalgae, maintaining salinity similar to seawater is essential; freshwater must be added to compensate for evaporation loss. However, several strains (e.g., *Tetraselmis* sp., *Picochlorum* sp., *Dunaliella* sp.) could adapt to incremental salinity and maintain their biomass productivities over a wide salinity range (Das et al., 2019b; Pick, 2002). Such halo-tolerant strains could significantly reduce the water footprint of microalgae production in open cultivation system.

10.2. Global warming potential and other environmental parameters

A number of unit processes are involved in the production of microalgae biomass; depending on the strain and the combination of these unit operations, there could be a net positive greenhouse gas emission (Kim et al., 2019). A net positive emission means more carbon dioxide is emitted than is absorbed by a process. Sourcing water and CO_2, harvesting, extraction, and drying are all energy-intensive steps in microalgal biomass or metabolite production (Fasaei et al., 2018). In addition, the loss of CO_2 to the atmosphere and parasitic energy consumption are major issues, especially in raceway ponds; in the case of a fermentor, the impact is due to the carbon source (Kroumov et al., 2016). As a result, some studies have found that microalgae feed could have a much higher global warming potential than other alternative feeds and fish meal.

Microalgal feed's global warming potential could be reduced in a number of ways. Carbon dioxide loss can be reduced by carefully controlling process parameters. The use of advanced control techniques such as model predictive control, for example, has been shown to significantly reduce carbon dioxide loss in the atmosphere (García Sánchez et al., 2003). Another option is to use sodium bicarbonate salt as a carbon source for microalgae cultivation rather than carbon dioxide (Kim et al., 2019). Another option is to implement large-scale cultivation. According to one study, microalgae-based fish feed produced on a large scale (2.5 ha) had 20 times lower carbon footprint than fish feed produced on a pilot scale (0.024 ha) (Taelman et al., 2013).

Improvements in biomass yield, better reactor design, reactor installation in suitable climatic conditions, energy-efficient harvesting methods, use of renewable energy such as solar energy, use of flue gas, food wastes, and other wastes as carbon sources are some other options for reducing global warming potential/carbon footprint (Jeno et al., 2021; Nagappan et al., 2019b; Taelman et al., 2013). The environmental sustainability of microalgae can be improved by utilizing nutrient-rich waste streams (Van Den Hende et al., 2014). For instance, the potential utilization of waste nitrogen fertilizer from a fertilizer industry using marine microalgae can be a viable option (Al-Jabri et al., 2021). The end use of microalgae biomass also influences the environmental sustainability parameters. According to a study by (Sfez et al., 2015), the feeding of microalgal biomass produced from high rate algal ponds to shrimp had less marine eutrophication potential than algae used for biogas production; however, freshwater eutrophication potential was similar for both scenarios. Implementing Integrated Multitrophic Aquaculture Systems is another exciting option for using algae in an environmentally friendly manner. Microalgae-based polyculture, for example, could be integrated with other systems such as aquaponics and conventional agriculture (Yarnold et al., 2019). Microalgal fish feed could not only be environmentally sustainable but also economically viable if appropriate policies are implemented (e.g., carbon taxation).

11. Safety aspects of microalgae

Several microalgae have been deemed safe by the US-FDA (US Food drug administration) and the European Food Safety Authority. The US-FDA already approved *Haematococcus pluvialis* for use as a colour enhancer in salmonids and shrimp feed (Han et al., 2013). Moreover, FDA-USA has classified oil extracts from *Crypthecodinium cohnii*, *Schizochytrium* sp., and *Ulkenia* sp. and dried *Spirulina platensis, Chlorella protothecoides*, and *Dunaliella bardawil* as generally recognized as safe (GRAS) (Jha et al., 2017). The European Union ratified algal oil and meals as salmon feed for commercial purposes in 2017 (Lähteenmäki-Uutela et al., 2021). The European Food Safety Authority approved carotenoids from Dunaliella salina and DHA from *C. cohnii* (Enzing et al., 2014). The European Union has approved *Aphanizomenon* flos-aquae, *Chlorella luteoviridis*, *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Odontella aurita*, and *Tetraselmis chuii* as foods or food ingredients under the Novel Food Catalogue (Lähteenmäki-Uutela et al., 2021). *Chaetoceros gracilis*, *Isochrysis* sp., *Tetraselmis suecica*, *Skeletonema costatum*, *Pavlova lutheri*, *Dunaliella tertiolecta*, *Phaeodactylum tricornutum*, *Nannochloropsis* sp., and *Chlorella* sp. have so far not been reported to contain any toxins (Enzing et al., 2014). *Spirulina* and *Chlorella*, in addition to the aforementioned strains, are sold as supplements in many parts of the world. Several toxicological studies have shown that various microalgae are safe to use as feed supplements (Dineshbabu et al., 2019).

12. Challenges and future direction of algae in aquafeed

For microalgae to become a successful alternative to fish meal, it has to overcome a number of obstacles. Similar to many alternate feeds, the microalgae-based fish feed has a low palatability, but this can be improved by changing the texture of the feed and adding attractants/stimulants that fish's chemosensory systems accept. Microalgae like *Chlorella* sp., *Nannochloropsis* sp., etc., have low digestibility due to the presence of non-starch substances and rigid cell walls (Skrede et al., 2011). Starch that could be digested well by fish could be accumulated in microalgae grown under nutritional stress or by two-stage cultivation (Nagappan and Kumar, 2021). In the case of rigid cell walls, mechanical methods can be used to break them down, increasing digestibility and exposing nutrients to the fish. However, any pretreatment techniques for the cell rupture would incur additional costs for the fish feed production. Selected microalgal components can have an impact on fish health; for example, extracellular polysaccharides from *D. tertiolecta* have been shown to reduce nutrient absorption (Mohebbi et al., 2016). In some cases, microalgae have lower protein content and higher carbohydrate content than conventional feed -reducing feed suitability (Skrede et al., 2011). All of this suggests that careful species selection and evaluation of growth in various environments are required to reduce the production cost.

In many cases, the harvested microalgal biomass is unsuitable for direct use as fish feed. This is especially true for marine microalgae, which have a high salt content that must be removed before feeding. In many cases, the microalgae may have accumulated trace elements that must be removed as well. Some microalgae have been found to contain toxins that are harmful not only to fish but also to humans (Caruana and Amzil, 2018). In general, microalgae production for fish feed will take place in open ponds, which may contain other toxic microalgae. An in-house testing of harvested biomass for toxic compounds and toxic microalgae can overcome above problem. Pigments like carotenoids, which give microalgal functional properties like antioxidant activity to fishes, are easily degraded (Chen et al., 2017). Adding of appropriate preservatives during biomass processing, use of appropriate drying methods and use of cold pelletization can prevent degradation. But any additional steps in the preparation of microalgal biomass for fish feed, such as those mentioned above, will raise the cost of production even more. As a result, low-cost processing methods for microalgae for fish feed preparation must be investigated.

One of the most significant obstacles to using microalgae as fish feed is the high cost of production (Fasaei et al., 2018). The low productivity in large-scale open ponds, energy-intensive harvesting techniques, and more expensive downstream processing techniques all contribute to the high production costs. Seasonal variations in light and temperature may also have an impact on biomass productivity. But the selection of robust species can overcome the above problem, as shown in our group's recent study involving long-term semi-continuous cultivation of a halo-tolerant *Tetraselmis* sp. using recycled growth media (Das et al., 2019a). Furthermore, pests, grazers, and pathogens pose constant contamination problems in large-scale production. (Hannon et al., 2010). Large cold rooms or freezers are required to preserve the nutritional and functional properties of feed. This raises the production cost even more. There are several approaches to lowering production costs, and active research is required to address these issues. Biorefinery techniques, the use of waste flue gas from industries, and heterotrophic cultivation methods based primarily on organic carbon sources are some of the approaches. A reliable strain should be found and optimized for year-round cultivation. Economic and environmental sustainability issues can be addressed by expanding the use of low-cost harvesting techniques such as natural sedimentation and cross-flow filtration.

Microalgae feed is also recommended because it improves the gut health and survival of fishes. However, many studies that have suggested a pro/prebiotic effect are based on a consortium of algae, bacteria, and other organisms rather than just microalgae (Shah et al., 2018; Tacon, 2020). As a result, more research is needed to prove that microalgae can have probiotic, prebiotic, and increase fish survivability by reducing pathogenic bacteria load. The exact composition and structure-activity relationship of functional compounds in algae must be characterized urgently as claims of algae having a pro/prebiotic effect increase. This will allow better pro/prebiotic activity screening in the future. Also, one of the major advantages of microalgae feed is that it contains higher levels of omega-3 fatty acids than other fish feeds. However, genetically modified plants with a higher omega-3 fatty acid content, such as camelina and canola, have recently entered the market. As a result, microalgae should clearly establish additional selling points in the fish feed sector, such as superior probiotics, immunostimulants, and so on.

There is a vast collection of microalgae that has yet to be explored for fish feed. Nonetheless, current research on algae of various genera has revealed a wide range of feed conversion ratios, digestibility, nutritional, and functional values. As a result, more screening studies of new microalgae on fish feed selection can reveal its true potentiality. In many cases, when compared to fish meal, the microalgal feed resulted in lower fish intake. However, if ingredients like taurine are added to microalgal feed, fish will have better intake, resulting in superior growth performance (Takagi et al., 2008). Production systems, harvesting, and processing technologies are yet to be optimized at large scales. In the future, innovative manufacturing in feed combined with novel upstream and downstream processing technologies for microalgae biomass production can effectively replace fish meal and provide a sustainable solution.

13. Conclusion

Aquaculture products and aquafeed are in higher demand around the world. Traditional fish feed comprising fish meal and soybean meal as bulk ingredient, however, does not meet demand and unsustainable. Fish feed made from microalgae has a lot of potentials to replace fish meal and soybean meal. It has characteristics such as a faster growth rate and the ability to grow and produce high-value products without the use of arable land and freshwater. Apart from serving as a source of protein, lipids, and carbohydrates, microalgae contain a number of functional compounds. This includes activities such as prebiotics, probiotics, and disease resistance. Microalgae can be used in aquaculture in various ways, including algal paste, extruded pellets, and polyaquaculture. The high cost of production is one of the most significant problems with microalgae. The selection of microalgal strains with desired cellular composition and lower production cost as feed could meet a significant demand for fish feed in the coming future.

CRediT authorship contribution statement

Probir Das, Gopalakrishnan Kumar: Conceptualization, Writing – review & editing. **Senthil Nagappan**, **Ann Kristin Vatland**: Data curation, Writing – original draft. **Mohammad AbdulQuadir, Mahmoud Thaher, Shoyeb Khan, Chandan Mahata:**, Data curation, Investigation, Validation. **Hareb Al-Jabri**: Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abdelghany, M.F., El-Sawy, H.B., Abd El-Hameed, S.A., Khames, M.K., Abdel-Latif, H.M., Naiel, M.A., 2020. Effects of dietary Nannochloropsis oculata on growth performance, serum biochemical parameters, immune responses, and resistance against Aeromonas veronii challenge in Nile tilapia (Oreochromis niloticus). Fish. Shellfish Immunol. 107, 277–288.

Agboola, J.O., Teuling, E., Wierenga, P.A., Gruppen, H., Schrama, J.W., 2019. Cell wall disruption: an effective strategy to improve the nutritive quality of microalgae in African catfish (Clarias gariepinus). Aquac. Nutr. 25, 783–797.

Aladetohun, N., Sogbesan, O., 2013. Utilization of blood meal as a protein ingredient from animal waste product in the diet of Oreochromis niloticus. Int. J. Fish. Aquat. 5, 234–237.

Al-Jabri, H., Das, P., Thaher, M., Khan, S., AbdulQuadir, M., 2021. Potential utilization of waste nitrogen fertilizer from a fertilizer industry using marine microalgae. Sci. Total Environ. 755, 142532.

Allaboutfeed, 2021. The aquafeed market: Growth, trends and future outlook. https://www.allaboutfeed.net/, (accesed 27 August 2021).

Allen, K.M., Habte-Tsion, H.-M., Thompson, K.R., Filer, K., Tidwell, J.H., Kumar, V., 2019. Freshwater microalgae (Schizochytrium sp.) as a substitute to fish oil for shrimp feed. Sci. Rep. 9, 1–10.

Amouzad Khalili, M., Abedian Kenari, A., Rezaei, M., Mirzakhani, M.K., 2019. Antioxidant and antibacterial effects of vitamins c and e alone or a combination on microalgae (*Nannochloropsis oculata*) paste quality during cold storage. J. Aquat. Food Prod. Technol. 28, 1051–1062.

Ansarifard, F., Rajabi Islami, H., Shamsaie Mehrjan, M., Soltani, M., 2018. Effects of Arthrospira platensis on growth, skin color and digestive enzymes of Koi, Cyprinus carpio. Iran. J. Fish. Sci. 17, 381–393.

Arru, B., Furesi, R., Gasco, L., Madau, F.A., Pulina, P., 2019. The introduction of insect meal into fish diet: The first economic analysis on European sea bass farming. Sustainability 11, 1697.

Arun, J., Gopinath, K.P., SundarRajan, P., Felix, V., JoselynMonica, M., Malolan, R., 2020. A conceptual review on microalgae biorefinery through thermochemical and biological pathways: bio-circular approach on carbon capture and wastewater treatment. Bioresour. Technol. Rep. 11, 100477.

Austin, B., 2006. The bacterial microflora of fish, revised. Sci. World J. 6, 931–945.

Ayala, M.D., Galián, C., Fernández, V., Chaves-Pozo, E., García de la Serrana, D., Sáez, M. I., Galafaz Díaz, A., Alarcón, F.J., Martínez, T.F., Arizcun, M., 2020. Influence of low dietary inclusion of the microalga nannochloropsis gaditana (Lubián 1982) on performance, fish morphology, and muscle growth in juvenile gilthead seabream (sparus aurata). Animals 10, 2270.

Bakun, A., Broad, K., 2003. Environmental 'loopholes' and fish population dynamics: comparative pattern recognition with focus on El Niño effects in the Pacific. Fish. Oceanogr. 12, 458–473.

Barclay, W., Zeller, S., 1996. Nutritional enhancement of n-3 and n-6 fatty acids in rotifers and artemia nauplii by feeding spray-dried Schizochytrium sp. J. World Aquac. Soc. 27, 314–322.

Barros, A.I., Gonçalves, A.L., Simões, M., Pires, J.C., 2015. Harvesting techniques applied to microalgae: a review. Renew. Sustain. Energy Rev. 41, 1489–1500.

Batista, S., Pereira, R., Oliveira, B., Baião, L.F., Jessen, F., Tulli, F., Messina, M., Silva, J. L., Abreu, H., Valente, L.M., 2020a. Exploring the potential of seaweed Gracilaria gracilis and microalga Nannochloropsis oceanica, single or blended, as natural dietary ingredients for European seabass Dicentrarchus labrax. J. Appl. Phycol. 32, 2041–2059.

Batista, S., Pintado, M., Marques, A., Abreu, H., Silva, J.L., Jessen, F., Tulli, F., Valente, L. M., 2020b. Use of technological processing of seaweed and microalgae as strategy to improve their apparent digestibility coefficients in European seabass (Dicentrarchus labrax) juveniles. J. Appl. Phycol. 32, 3429–3446.

Becker, E.W., 2007. Micro-algae as a source of protein. Biotechnol. Adv. 25, 207–210.

Bianchi, V.A., Castro, J.M., Rocchetta, I., Nahabedian, D.E., Conforti, V., Luquet, C.M., 2015. Long-term feeding with Euglena gracilis cells modulates immune responses, oxidative balance and metabolic condition in Diplodon chilensis (Mollusca, Bivalvia, Hyriidae) exposed to living Escherichia coli. Fish. Shellfish Immunol. 42, 367–378.

Biao, X., Kaijin, Y., 2007. Shrimp farming in China: operating characteristics, environmental impact and perspectives. Ocean Coast. Manag 50, 538–550.

Bleakley, S., Hayes, M., 2017. Algal proteins: extraction, application, and challenges concerning production. Foods 6, 33.

Blomqvist, J., Pickova, J., Tilami, S.K., Sampels, S., Mikkelsen, N., Brandenburg, J., Sandgren, M., Passoth, V., 2018. Oleaginous yeast as a component in fish feed. Sci. Rep. 8, 1–8.

Bosch, G., Zhang, S., Oonincx, D.G., Hendriks, W.H., 2014. Protein quality of insects as potential ingredients for dog and cat foods. J. Nutr. Sci. 3, 29.

Cao, S., Zhang, P., Zou, T., Fei, S., Han, D., Jin, J., Liu, H., Yang, Y., Zhu, X., Xie, S., 2018. Replacement of fishmeal by spirulina Arthrospira platensis affects growth, immune related-gene expression in gibel carp (Carassius auratus gibelio var. CAS III), and its challenge against Aeromonas hydrophila infection. Fish. Shellfish Immunol. 79, 265–273.

Cardinaletti, G., Messina, M., Bruno, M., Tulli, F., Poli, B., Giorgi, G., Chini-Zittelli, G., Tredici, M., Tibaldi, E., 2018. Effects of graded levels of a blend of Tisochrysis lutea and Tetraselmis suecica dried biomass on growth and muscle tissue composition of European sea bass (Dicentrarchus labrax) fed diets low in fish meal and oil. Aquaculture 485, 173–182.

Caruana, A.M., Amzil, Z., 2018. Microalgae and toxins. Microalgae in Health and Disease Prevention. Elsevier, pp. 263–305.

Cerezuela, R., Guardiola, F.A., Meseguer, J., Esteban, M.A., 2012. Enrichment of gilthead seabream (Sparus aurata L.) diet with microalgae: effects on the immune system. Fish. Physiol. Biochem. 38, 1729–1739.

Chen, B., Wan, C., Mehmood, M.A., Chang, J.-S., Bai, F., Zhao, X., 2017. Manipulating environmental stresses and stress tolerance of microalgae for enhanced production of lipids and value-added products–a review. Bioresour. Technol. 244, 1198–1206.

Chen, W., Wang, Y., Han, D., Zhu, X., Xie, S., Han, D., Hu, Q., 2019. Two filamentous microalgae as feed ingredients improved flesh quality and enhanced antioxidant capacity and immunity of the gibel carp (Carassius auratus gibelio). Aquac. Nutr. 25, 1145–1155.

Cottrell, R.S., Blanchard, J.L., Halpern, B.S., Metian, M., Froehlich, H.E., 2020. Global adoption of novel aquaculture feeds could substantially reduce forage fish demand by 2030. Nat. Food 1, 301–308.

de Cruz, C.R., Lubrano, A., Gatlin III, D.M., 2018. Evaluation of microalgae concentrates as partial fishmeal replacements for hybrid striped bass Morone sp. Aquaculture 493, 130–136.

Dalbhagat, C.G., Mahato, D.K., Mishra, H.N., 2019. Effect of extrusion processing on physicochemical, functional and nutritional characteristics of rice and rice-based products: a review. Trends Food Sci. Technol. 85, 226–240.

Dallaire, V., Lessard, P., Vandenberg, G., De La Noüe, J., 2007. Effect of algal incorporation on growth, survival and carcass composition of rainbow trout (Oncorhynchus mykiss) fry. Bioresour. Technol. 98, 1433–1439.

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.

Das, P., Thaher, M.I., Hakim, M.A.Q.M.A., Al-Jabri, H.M.S., Alghasal, G.S.H., 2016. A comparative study of the growth of Tetraselmis sp. in large scale fixed depth and decreasing depth raceway ponds. Bioresour. Technol. 216, 114–120.

Das, P., Thaher, M., AbdulQuadir, M., Khan, S., Chaudhary, A., Al-Jabri, H., 2019a. Long-term semi-continuous cultivation of a halo-tolerant Tetraselmis sp. using recycled growth media. Bioresour. Technol. 276, 35–41.

Das, P., Thaher, M., Khan, S., AbdulQuadir, M., Al-Jabri, H., 2019b. The effect of culture salinity on the harvesting of microalgae biomass using pilot-scale tangential-flow-filter membrane. Bioresour. Technol. 293, 122057.

Dawood, M.A., Koshio, S., Esteban, M.Á., 2018. Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev. Aquac. 10, 950–974.

Defoirdt, T., Boon, N., Sorgeloos, P., Verstraete, W., Bossier, P., 2007. Alternatives to antibiotics to control bacterial infections: luminescent vibriosis in aquaculture as an example. Trends Biotechnol. 25, 472–479.

Delgado, E., Valles-Rosales, D.J., Flores, N.C., Reyes-Jáquez, D., 2021. Evaluation of fish oil content and cottonseed meal with ultralow gossypol content on the functional properties of an extruded shrimp feed. Aquac. Rep. 19, 100588.

Dhert, P., Rombaut, G., Suantika, G., Sorgeloos, P., 2001. Advancement of rotifer culture and manipulation techniques in Europe. Aquaculture 200, 129–146.

Dineshbabu, G., Goswami, G., Kumar, R., Sinha, A., Das, D., 2019. Microalgae–nutritious, sustainable aqua-and animal feed source. J. Funct. Foods 62, 103545.

Draganovic, V., der Goot, Van, Boom, A., Jonkers, J, R., 2013. Wheat gluten in extruded fish feed: effects on morphology and on physical and functional properties. Aquac. Nutr. 19, 845–859.

Dragone, G., Fernandes, B.D., Abreu, A.P., Vicente, A.A., Teixeira, J.A., 2011. Nutrient limitation as a strategy for increasing starch accumulation in microalgae. Appl. Energy 88, 3331–3335.

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.

Enzing, C., Ploeg, M., Barbosa, M., Sijtsma, L., 2014. Microalgae-based products for the food and feed sector: an outlook for Europe. JRC Sci. Policy Rep. 19–37.
Fadl, S.E., ElGohary, M., Elsadany, A.Y., Gad, D.M., Hanaa, F.F., El-Habashi, N.M., 2017a. Contribution of microalgae-enriched fodder for the Nile tilapia to growth and resistance to infection with Aeromonas hydrophila. Algal Res. 27, 82–88.
Fadl, S.E., ElGohary, M., Elsadany, A.Y., Gad, D.M., Hanaa, F.F., El-Habashi, N.M., 2017b. Contribution of microalgae-enriched fodder for the Nile tilapia to growth and resistance to infection with Aeromonas hydrophila. Algal Res. 27, 82–88.
Fadl, S.E., Elsadany, A.Y., El-Shenawy, A.M., Sakr, O.A., El Gammal, G.A., Gad, D.M., Norag, M.A.A., Eissa, I., 2020. Efficacy of cyanobacterium Anabaena sp. as a feed supplement on productive performance and immune status in cultured Nile tilapia. Aquac. Rep. 17, 100406.
Fasaei, F., Bitter, J., Slegers, P., Van Boxtel, A., 2018. Techno-economic evaluation of microalgae harvesting and dewatering systems. Algal Res. 31, 347–362.
Fernández, F.A., Sevilla, J.M.F., Grima, E.M., 2019. Costs Analysis of Microalgae Production. Biofuels From Algae. Elsevier, pp. 551–566.
Ferreira, G., Pinto, L.R., Maciel Filho, R., Fregolente, L., 2019. A review on lipid production from microalgae: Association between cultivation using waste streams and fatty acid profiles. Renew. Sustain. Energy Rev. 109, 448–466.
García Sánchez, J., Berenguel, M., Rodríguez, F., Fernández Sevilla, J., Brindley Alias, C., Acién Fernández, F., 2003. Minimization of carbon losses in pilot-scale outdoor photobioreactors by model-based predictive control. Biotechnol. Bioeng. 84, 533–543.
Garcia-Vaquero, M., Hayes, M., 2016. Red and green macroalgae for fish and animal feed and human functional food development. Food Rev. Int. 32, 15–45.
Gbadamosi, O.K., Lupatsch, I., 2018. Effects of dietary Nannochloropsis salina on the nutritional performance and fatty acid profile of Nile tilapia, Oreochromis niloticus. Algal Res. 33, 48–54.
Ghanbari, M., Kneifel, W., Domig, K.J., 2015. A new view of the fish gut microbiome: advances from next-generation sequencing. Aquaculture 448, 464–475.
Glencross, B., Irvin, S., Arnold, S., Blyth, D., Bourne, N., Preston, N., 2014. Effective use of microbial biomass products to facilitate the complete replacement of fishery resources in diets for the black tiger shrimp, Penaeus monodon. Aquaculture 431, 12–19.
Globenewswire, 2021. Microalgae-Based Products Market Forecast to 2028. https://www.globenewswire.com/ (accesed 27 August 2021).
Goda, A.M.A.S., Wafa, M., El-Haroun, E., Kabir Chowdhury, M., 2007. Growth performance and feed utilization of Nile tilapia Oreochromis niloticus (Linnaeus, 1758) and tilapia galilae Sarotherodon galilaeus (Linnaeus, 1758) fingerlings fed plant protein-based diets. Aquac. Res. 38, 827–837.
Gong, Y., Guterres, H., Huntley, M., Sørensen, M., Kiron, V., 2018. Digestibility of the defatted microalgae N annochloropsis sp. and D esmodesmus sp. when fed to Atlantic salmon, S almo salar. Aquac. Nutr. 24, 56–64.
Gong, Y., Bandara, T., Huntley, M., Johnson, Z.I., Dias, J., Dahle, D., Sørensen, M., Kiron, V., 2019. Microalgae Scenedesmus sp. as a potential ingredient in low fishmeal diets for Atlantic salmon (Salmo salar L.). Aquaculture 501, 455–464.
Gong, Y., Sørensen, S.L., Dahle, D., Nadanasabesan, N., Dias, J., Valente, L.M., Sørensen, M., Kiron, V., 2020. Approaches to improve utilization of Nannochloropsis oceanica in plant-based feeds for Atlantic salmon. Aquaculture 522, 735122.
Grazziotin, A., Pimentel, F.A., De Jong, E.V., Brandelli, A., 2008. Poultry feather hydrolysate as a protein source for growing rats. Braz. J. Vet. Res. Anim. Sci. 45, 61–67.
Guedes, A.C., Sousa-Pinto, I., Malcata, F.X., 2015. Application of Microalgae Protein to Aquafeed. Handbook of Marine Microalgae. Elsevier, pp. 93–125.
Guimarães, A.M., Dias Schleder, D., Nagata, M., Nóbrega, R.O., Fracalossi, D.M., Quadros Seiffert, W., do Nascimento Vieira, F., 2019. Aurantiochytrium sp. meal can replace fish oil in practical diets for the juvenile Pacific white shrimp. Aquac. Nutr. 25, 798–807.
Han, D., Li, Y., Hu, Q., 2013. Biology and commercial aspects of Haematococcus pluvialis. Handb. Micro Cult. Appl. Phycol. Biotechnol. 2, 388–405.
Hannon, M., Gimpel, J., Tran, M., Rasala, B., Mayfield, S., 2010. Biofuels from algae: challenges and potential. Biofuels 1, 763–784.
Hardy, R.W., 2000. New developments in aquatic feed ingredients, and potential of enzyme supplements. Av. En. Nutr. Acuicola.
Hashempour-Baltork, F., Torbati, M., Azadmard-Damirchi, S., Savage, G.P., 2016. Vegetable oil blending: a review of physicochemical, nutritional and health effects. Trends Food Sci. Technol. 57, 52–58.
He, Y., Lin, G., Rao, X., Chen, L., Jian, H., Wang, M., Guo, Z., Chen, B., 2018. Microalga Isochrysis galbana in feed for Trachinotus ovatus: effect on growth performance and fatty acid composition of fish fillet and liver. Aquac. Int. 26, 1261–1280.
Hemaiswarya, S., Raja, R., Kumar, R.R., Ganesan, V., Anbazhagan, C., 2011. Microalgae: a sustainable feed source for aquaculture. World J. Microbiol. Biotechnol. 27, 1737–1746.
Hodar, A., Vasava, R., Mahavadiya, D., Joshi, N., 2020. Fish meal and fish oil replacement for aqua feed formulation by using alternative sources: a review. J. Exp. Zool. India 23, 13–21.
Hoffman, J., Pate, R.C., Drennen, T., Quinn, J.C., 2017. Techno-economic assessment of open microalgae production systems. Algal Res. 23, 51–57.
Hussain, S., Afzal, M., Salim, M., Javid, A., Khichi, T., Hussain, M., Raza, S., 2011. Apparent digestibility of fish meal, blood meal and meat meal for Labeo rohita fingerlings. J. Anim. Plant Sci. 21, 807–811.
Indexmundi, 2021. Fishmeal yearly price. www.indexmundi.com. (accesed 27 August 2021).
Jaseera, K., 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, 736176.
Jeno, J.G.A., Viveka, R., Varjani, S., Nagappan, S., Nakkeeran, E., 2021. Current Trends and Prospects of Transforming Food Waste to Biofuels in India. Waste Biorefinery. Elsevier, pp. 391–419.
Jereos-Aujero, E., Millamena, O.M., 1981. Viability of frozen algae used as food for larval penaeids. Fish. Res. J. Philipp. 6, 63–69.
Jha, D., Jain, V., Sharma, B., Kant, A., Garlapati, V.K., 2017. Microalgae-based pharmaceuticals and nutraceuticals: an emerging field with immense market potential. ChemBioEng Rev. 4, 257–272.
Jiang, M., Zhao, H., Zai, S., Shepherd, B., Wen, H., Deng, D., 2019. A defatted microalgae meal (Haematococcus pluvialis) as a partial protein source to replace fishmeal for feeding juvenile yellow perch Perca flavescens. J. Appl. Phycol. 31, 1197–1205.
Jones, S.W., Karpol, A., Friedman, S., Maru, B.T., Tracy, B.P., 2020. Recent advances in single cell protein use as a feed ingredient in aquaculture. Curr. Opin. Biotechnol. 61, 189–197.
Ju, Z.Y., Davis, S., Ramm, K., Steck, M., Soller, F., Fox, B.K., 2017. Effects of microalgae-added diets on growth performance and meat composition of tilapia (Oreochromis mossambicus). Aquac. Res. 48, 5053–5061.
Jung, J.-Y., Damusaru, J.H., Park, Y., Kim, K., Seong, M., Je, H.-W., Kim, S., Bai, S.C., 2017. Autotrophic biofloc technology system (ABFT) using Chlorella vulgaris and Scenedesmus obliquus positively affects performance of Nile tilapia (Oreochromis niloticus). Algal Res. 27, 259–264.
Katerina, K., Berge, G.M., Turid, M., Aleksei, K., Grete, B., Trine, Y., Mats, C., John, S., Bente, R., 2020. Microalgal Schizochytrium limacinum biomass improves growth and filet quality when used long-term as a replacement for fish oil, in modern salmon diets. Front. Mar. Sci. 7, 57.
Katiyar, R., Arora, A., 2020. Health promoting functional lipids from microalgae pool: a review. Algal Res. 46, 101800.
Kaur, S., Sarkar, M., Srivastava, R.B., Gogoi, H.K., Kalita, M.C., 2012. Fatty acid profiling and molecular characterization of some freshwater microalgae from India with potential for biodiesel production. New Biotechnol. 29, 332–344.
Kim, G.-Y., Roh, K., Han, J.-I., 2019. The use of bicarbonate for microalgae cultivation and its carbon footprint analysis. Green Chem. 21, 5053–5062.
Kiron, V., Kulkarni, A., Dahle, D., 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, 57–66.
Knutsen, H.R., Ottesen, O.H., Palihawadana, A.M., Sandaa, W., Sørensen, M., Hagen, Ø., 2019. Muscle growth and changes in chemical composition of spotted wolffish juveniles (Anarhichas minor) fed diets with and without microalgae (Scenedesmus obliquus). Aquac. Rep. 13, 100175.
Kokou, F., Fountoulaki, E., 2018. Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. Aquaculture 495, 295–310.
Kousoulaki, K., Mørkøre, T., Nengas, I., Berge, R., Sweetman, J., 2016. Microalgae and organic minerals enhance lipid retention efficiency and fillet quality in Atlantic salmon (Salmo salar L.). Aquaculture 451, 47–57.
Kroumov, A.D., Módenes, A.N., Trigueros, D.E.G., Espinoza-Quiñones, F.R., Borba, C.E., Scheufele, F.B., Hinterholz, C.L., 2016. A systems approach for CO_2 fixation from flue gas by microalgae—theory review. Process Biochem 51, 1817–1832.
Lähteenmäki-Uutela, A., Rahikainen, M., Camarena-Gómez, M.T., Piiparinen, J., Spilling, K., Yang, B., 2021. European Union legislation on macroalgae products. Aquac. Int. 29, 487–509.
Laporte, J., Woodgate, S., Davies, S., Serwata, R., Gouveia, A., Nates, S., 2009. Poultry meat meal: a valuable source of the partial replacement of fish meal in aquafeeds. Int. Aquafeed 12, 12–17.
Leyland, B., Leu, S., Boussiba, S., 2017. Are thraustochytrids algae? Fungal Biol. 121, 835–840.
Li, K., Liu, Q., Fang, F., Luo, R., Lu, Q., Zhou, W., Huo, S., Cheng, P., Liu, J., Addy, M., 2019. Microalgae-based wastewater treatment for nutrients recovery: a review. Bioresour. Technol. 291, 121934.
Liu, C., Li, Y., Chen, Z., Yuan, L., Liu, H., Han, D., Jin, J., Yang, Y., Hu, Q., Zhu, X., 2021. Effects of dietary whole and defatted Arthrospira platensis (Cyanobacterium) on growth, body composition and pigmentation of the yellow catfish Pelteobagrus fulvidraco. J. Appl. Phycol. 1–9.
Liu, W.-Y., Fang, L., Feng, X.-W., Li, G.-M., Gu, R.-Z., 2020. In vitro antioxidant and angiotensin I-converting enzyme inhibitory properties of peptides derived from corn gluten meal. Eur. Food Res. Technol. 246, 2017–2027.
Lu, Q., Li, H., Xiao, Y., Liu, H., 2021. A state-of-the-art review on the synthetic mechanisms, production technologies, and practical application of polyunsaturated fatty acids from microalgae. Algal Res. 55, 102281.
Madeira, M.S., Cardoso, C., Lopes, P.A., Coelho, D., Afonso, C., Bandarra, N.M., Prates, J. A., 2017. Microalgae as feed ingredients for livestock production and meat quality: a review. Livest. Sci. 205, 111–121.
Madhumathi, M., Rengasamy, R., 2011. Antioxidant status of Penaeus monodon fed with Dunaliella salina supplemented diet and resistance against WSSV. Int. J. Eng. Sci. Technol. 3, 7249–7260.
Maehre, H.K., Edvinsen, G.K., Eilertsen, K.-E., Elvevoll, E.O., 2016. Heat treatment increases the protein bioaccessibility in the red seaweed dulse (Palmaria palmata), but not in the brown seaweed winged kelp (Alaria esculenta). J. Appl. Phycol. 28, 581–590.
Makridis, P., Costa, R.A., Dinis, M.T., 2006. Microbial conditions and antimicrobial activity in cultures of two microalgae species, Tetraselmis chuii and Chlorella minutissima, and effect on bacterial load of enriched Artemia metanauplii. Aquaculture 255, 76–81.

Maliwat, G.C., Velasquez, S., Robil, J.L., Chan, M., Traifalgar, R.F., Tayamen, M., Ragaza, J.A., 2017. Growth and immune response of giant freshwater prawn Macrobrachium rosenbergii (De Man) postlarvae fed diets containing Chlorella vulgaris (Beijerinck). Aquac. Res. 48, 1666–1676.
Marketwatch, 2020. Aquaculture Market Size 2020. https://www.marketwatch.com/ (accesed 27 August 2021).
Marques, A., Dhont, J., Sorgeloos, P., Bossier, P., 2004. Evaluation of different yeast cell wall mutants and microalgae strains as feed for gnotobiotically grown brine shrimp Artemia franciscana. J. Exp. Mar. Biol. Ecol. 312, 115–136.
Martínez-Fernández, E., Southgate, P.C., 2007. Use of tropical microalgae as food for larvae of the black-lip pearl oyster Pinctada margaritifera. Aquaculture 263, 220–226.
Matos, Â.P., Feller, R., Moecke, E.H.S., de Oliveira, J.V., Junior, A.F., Derner, R.B., Sant'Anna, E.S., 2016. Chemical characterization of six microalgae with potential utility for food application. J. Am. Oil Chem. Soc. 93, 963–972.
McCausland, M.A., Brown, M.R., Barrett, S.M., Diemar, J.A., Heasman, M.P., 1999. Evaluation of live microalgae and microalgal pastes as supplementary food for juvenile Pacific oysters (Crassostrea gigas). Aquaculture 174, 323–342.
Medina-Félix, D., López-Elías, J.A., Martínez-Córdova, L.R., López-Torres, M.A., Hernández-López, J., Rivas-Vega, M.E., Mendoza-Cano, F., 2014. Evaluation of the productive and physiological responses of Litopenaeus vannamei infected with WSSV and fed diets enriched with Dunaliella sp. J. Invertebr. Pathol. 117, 9–12.
Mekonnen, M.M., Hoekstra, A.Y., 2011. The green, blue and grey water footprint of crops and derived crop products. Hydrol. Earth Syst. Sci. 15, 1577–1600.
Mélo, R.C.S., Santos, L.Pd.S., Brito, A.P.M., Gouveia, Ad.A., Marçal, C., Cavalli, R.O., 2016. Use of the microalga Nannochloropsis occulata in the rearing of newborn longsnout seahorse Hippocampus reidi (Syngnathidae) juveniles. Aquac. Res. 47, 3934–3941.
Miglietta, P.P., De Leo, F., Ruberti, M., Massari, S., 2015. Mealworms for food: a water footprint perspective. Water 7, 6190–6203.
Mo, W.Y., Man, Y.B., Wong, M.H., 2018. Use of food waste, fish waste and food processing waste for China's aquaculture industry: Needs and challenge. Sci. Total Environ. 613, 635–643.
Mohebbi, F., Hafezieh, M., Seidgar, M., Hosseinzadeh Sahhafi, H., Mohsenpour Azari, A., Ahmadi, R., 2016. The growth, survival rate and reproductive characteristics of Artemia urmiana fed by Dunaliella tertiolecta, Tetraselmis suecica, Nannochloropsis oculata, Chaetoceros sp., Chlorella sp. and Spirolina sp. as feeding microalgae. Iran. J. Fish. Sci. 15, 727–737.
Moheimani, N.R., Vadiveloo, A., Ayre, J.M., Pluske, J.R., 2018. Nutritional profile and in vitro digestibility of microalgae grown in anaerobically digested piggery effluent. Algal Res. 35, 362–369.
Montoya, L.N.F., Martins, T.P., Gimbo, R.Y., Zanuzzo, F.S., Urbinati, E.C., 2017. β-Glucan-induced cortisol levels improve the early immune response in matrinxã (Brycon amazonicus). Fish. Shellfish Immunol. 60, 197–204.
Montoya-Camacho, N., Marquez-Ríos, E., Castillo-Yáñez, F.J., Cárdenas López, J.L., López-Elías, J.A., Ruíz-Cruz, S., Jiménez-Ruíz, E.I., Rivas-Vega, M.E., Ocaño-Higuera, V.M., 2019. Advances in the use of alternative protein sources for tilapia feeding. Rev. Aquac. 11, 515–526.
Muller-Feuga, A., 2000. The role of microalgae in aquaculture: situation and trends. J. Appl. Phycol. 12, 527–534.
Mustafa, M.G., 1995. A review: dietary benefits of algae as an additive in fish feed. Isr. J. Aquac. 47, 155–162.
Nagappan, S., Kumar, G., 2021. Investigation of four microalgae in nitrogen deficient synthetic wastewater for biorefinery based biofuel production. Environ. Technol. Innov. 23, 101572.
Nagappan, S., Nakkeeran, E., 2020. Biorefinery: a concept for co-producing biofuel with value-added products. Environmental Biotechnology. Springer, pp. 23–52.
Nagappan, S., Verma, S.K., 2018. Co-production of biodiesel and alpha linolenic acid (omega-3 fatty acid) from microalgae, Desmodesmus sp. MCC34. Energy Sources Part A Recovery Util. Environ. Eff. 40, 2933–2940.
Nagappan, S., Devendran, S., Tsai, P.-C., Jayaraman, H., Alagarsamy, V., Pugazhendhi, A., Ponnusamy, V.K., 2019b. Metabolomics integrated with transcriptomics and proteomics: Evaluation of systems reaction to nitrogen deficiency stress in microalgae. Process Biochemistry.
Nagappan, S., Devendran, S., Tsai, P.-C., Dahms, H.-U., Ponnusamy, V.K., 2019a. Potential of two-stage cultivation in microalgae biofuel production. Fuel 252, 339–349.
Nagappan, S., Tsai, P.-C., Devendran, S., Alagarsamy, V., Ponnusamy, V.K., 2019b. Enhancement of biofuel production by microalgae using cement flue gas as substrate. Environ. Sci. Pollut. Res. 27, 17571–17586.
Nagappan, S., Bhosale, R., Nguyen, D.D., Pugazendhi, A., Tsai, P.-C., Chang, S.W., Ponnusamy, V.K., Kumar, G., 2020. Nitrogen-fixing cyanobacteria as a potential resource for efficient biodiesel production. Fuel 279, 118440.
Nath, P., Khozin-Goldberg, I., Cohen, Z., Boussiba, S., Zilberg, D., 2012. Dietary supplementation with the microalgae Parietochloris incisa increases survival and stress resistance in guppy (Poecilia reticulata) fry. Aquac. Nutr. 18, 167–180.
Nayak, S.K., 2010. Role of gastrointestinal microbiota in fish. Aquac. Res. 41, 1553–1573.
Neori, A., 2011. "Green water" microalgae: the leading sector in world aquaculture. J. Appl. Phycol. 23, 143–149.
Niccolai, A., Zittelli, G.C., Rodolfi, L., Biondi, N., Tredici, M.R., 2019. Microalgae of interest as food source: Biochemical composition and digestibility. Algal Res. 42, 101617.
Nidhina, N., Muthukumar, S., 2015. Antinutritional factors and functionality of protein-rich fractions of industrial guar meal as affected by heat processing. Food Chem. 173, 920–926.
Nimrat, S., Boonthai, T., Vuthiphandchai, V., 2011. Effects of probiotic forms, compositions of and mode of probiotic administration on rearing of Pacific white shrimp (Litopenaeus vannamei) larvae and postlarvae. Anim. Feed Sci. Technol. 169, 244–258.
Nur-E-Borhan, S.A., 1993. Pigmentation of cultured black tiger prawn by feeding with a spirulina-supplemented diet. J. Jpn. Soc. Fish. Sci. 59, 165–169.
Oostlander, P., van Houcke, J., Wijffels, R.H., Barbosa, M.J., 2020. Microalgae production cost in aquaculture hatcheries. Aquaculture 525, 735310.
Ozkan, A., Kinney, K., Katz, L., Berberoglu, H., 2012. Reduction of water and energy requirement of algae cultivation using an algae biofilm photobioreactor. Bioresour. Technol. 114, 542–548.
Palmer, P.J., 2010. Polychaete-assisted sand filters. Aquaculture 306, 369–377.
Palmer, P.J., Burke, M.J., Palmer, C.J., Burke, J.B., 2007. Developments in controlled green-water larval culture technologies for estuarine fishes in Queensland, Australia and elsewhere. Aquaculture 272, 1–21.
Pascon, G., Messina, M., Petit, L., Valente, L.M.P., Oliveira, B., Przybyla, C., Dutto, G., Tulli, F., 2021. Potential application and beneficial effects of a marine microalgal biomass produced in a high-rate algal pond (HRAP) in diets of European sea bass, Dicentrarchus labrax. Environ. Sci. Pollut. Res. 1–15.
Patterson, D., Gatlin III, D.M., 2013. Evaluation of whole and lipid-extracted algae meals in the diets of juvenile red drum (Sciaenops ocellatus). Aquaculture 416, 92–98.
Pekàr, F., Olah, J., 1997. Fish pond manuring studies in Hungary. Integrated Fish Farming, 163.
Pereira, H., Sardinha, M., Santos, T., Gouveia, L., Barreira, L., Dias, J., Varela, J., 2020. Incorporation of defatted microalgal biomass (Tetraselmis sp. CTP4) at the expense of soybean meal as a feed ingredient for juvenile gilthead seabream (Sparus aurata). Algal Res. 47, 101869.
Pick, U., 2002. Adaptation of the halotolerant alga Dunaliella to high salinity. Salinity: Environment-Plants-Molecules. Springer, pp. 97–112.
Posten, C., Schaub, G., 2009. Microalgae and terrestrial biomass as source for fuels—a process view. J. Biotechnol. 142, 64–69.
Prabha, S.P., Nagappan, S., Rathna, R., Viveka, R., Nakkeeran, E., 2020. Blue biotechnology: a vision for future marine biorefineries. Refining Biomass Residues for Sustainable Energy and Bioproducts. Academic Press, pp. 463–480.
Pugazhendhi, A., Shobana, S., Bakonyi, P., Nemestóthy, N., Xia, A., Kumar, G., 2019. A review on chemical mechanism of microalgae flocculation via polymers. Biotechnol. Rep. 21, 00302.
Pugazhendhi, A., Nagappan, S., Bhosale, R., Tsai, P.-C., Natarajan, S., Devendran, S., Al Haj, L., Ponnusamy, V.K., Kumar, G., 2020. Various potential techniques to reduce the water footprint of microalgal biomass production for biofuel—a review. Sci. Total Environ. 749, 142218.
Qiao, H., Hu, D., Ma, J., Wang, X., Wu, H., Wang, J., 2019. Analysis of the effects of different nutritional support methods on postoperative recovery in patients with gastric cancer. Zhonghua zhong liu za zhi 41, 378–383.
Raja, R., Coelho, A., Hemaiswarya, S., Kumar, P., Carvalho, I.S., Alagarsamy, A., 2018. Applications of microalgal paste and powder as food and feed: an update using text mining tool. Beni-Suef Univ. J. Basic Appl. Sci. 7, 740–747.
Regunathan, C., Wesley, S., 2004. Control of Vibrio spp. in shrimp hatcheries using the green algae Tetraselmis suecica. Asian Fish. Sci. 17, 147–158.
Ren, L.-J., Ji, X.-J., Huang, H., Qu, L., Feng, Y., Tong, Q.-Q., Ouyang, P.-K., 2010. Development of a stepwise aeration control strategy for efficient docosahexaenoic acid production by Schizochytrium sp. Appl. Microbiol. Biotechnol. 87, 1649–1656.
Reyes-Becerril, M., Guardiola, F., Rojas, M., Ascencio-Valle, F., Esteban, M.Á., 2013. Dietary administration of microalgae Navicula sp. affects immune status and gene expression of gilthead seabream (Sparus aurata). Fish. Shellfish Immunol. 35, 883–889.
Ribeiro, A.R., Gonçalves, A., Barbeiro, M., Bandarra, N., Nunes, M.L., Carvalho, M.L., Silva, J., Navalho, J., Dinis, M.T., Silva, T., 2017. Phaeodactylum tricornutum in finishing diets for gilthead seabream: effects on skin pigmentation, sensory properties and nutritional value. J. Appl. Phycol. 29, 1945–1956.
Rizwan, M., Mujtaba, G., Memon, S.A., Lee, K., Rashid, N., 2018. Exploring the potential of microalgae for new biotechnology applications and beyond: a review. Renew. Sustain. Energy Rev. 92, 394–404.
Robert, R., Trintignac, P., 1997. Substitutes for live microalgae in mariculture: a review. Aquat. Living Resour. 10, 315–327.
Rokey, G., 1994. Petfood and Fishfood Extrusion. The Technology of Extrusion cooking. Springer, pp. 144–189.
Ryckebosch, E., Bruneel, C., Muylaert, K., Foubert, I., 2012. Microalgae as an alternative source of omega-3 long chain polyunsaturated fatty acids. Lipid Technol. 24, 128–130.
Sales, R., de Souza-Santos, L.P., 2020. Production of concentrated inocula from the microalgae Nannochloropsis oculata. Aquac. Int. 28, 1609–1620.
Samuelsen, T.A., Oterhals, Å., Kousoulaki, K., 2018. High lipid microalgae (Schizochytrium sp.) inclusion as a sustainable source of n-3 long-chain PUFA in fish feed—effects on the extrusion process and physical pellet quality. Anim. Feed Sci. Technol. 236, 14–28.
Sarker, P.K., Kapuscinski, A.R., McKuin, B., Fitzgerald, D.S., Nash, H.M., Greenwood, C., 2020a. Microalgae-blend tilapia feed eliminates fishmeal and fish oil, improves growth, and is cost viable. Sci. Rep. 10, 1–14.
Sarker, P.K., Kapuscinski, A.R., Vandenberg, G.W., Proulx, E., Sitek, A.J., Thomsen, L., 2020b. Towards sustainable and ocean-friendly aquafeeds: evaluating a fish-free feed for rainbow trout (Oncorhynchus mykiss) using three marine microalgae species. Elem. Sci. Anthr. 8, 8.
Scholz, M.J., Weiss, T.L., Jinkerson, R.E., Jing, J., Roth, R., Goodenough, U., Posewitz, M.C., Gerken, H.G., 2014. Ultrastructure and composition of the Nannochloropsis gaditana cell wall. Eukaryot. Cell 13, 1450–1464.

Seong, T., Matsutani, H., Haga, Y., Kitagima, R., Satoh, S., 2019. First step of non-fish meal, non-fish oil diet development for red seabream,(Pagrus major), with plant protein sources and microalgae Schizochytrium sp. Aquac. Res. 50, 2460–2468.
Sfez, S., Van Den Hende, S., Taelman, S.E., De Meester, S., Dewulf, J., 2015. Environmental sustainability assessment of a microalgae raceway pond treating aquaculture wastewater: From up-scaling to system integration. Bioresour. Technol. 190, 321–331.
Shah, M.R., Lutzu, G.A., Alam, A., Sarker, P., Chowdhury, M.K., Parsaeimehr, A., Liang, Y., Daroch, M., 2018. Microalgae in aquafeeds for a sustainable aquaculture industry. J. Appl. Phycol. 30, 197–213.
Sharawy, Z.Z., Ashour, M., Abbas, E., Ashry, O., Helal, M., Nazmi, H., Kelany, M., Kamel, A., Hassaan, M., Rossi Jr., W., 2020a. Effects of dietary marine microalgae, Tetraselmis suecica, on production, gene expression, protein markers and bacterial count of Pacific white shrimp Litopenaeus vannamei. Aquac. Res. 51, 2216–2228.
Sharawy, Z.Z., Ashour, M., Abbas, E., Ashry, O., Helal, M., Nazmi, H., Kelany, M., Kamel, A., Hassaan, M., Rossi Jr, W., 2020b. Effects of dietary marine microalgae, Tetraselmis suecica, on production, gene expression, protein markers and bacterial count of Pacific white shrimp Litopenaeus vannamei. Aquac. Res. 51, 2216–2228.
Sharma, S.A., Surveswaran, S., Arulraj, J., Velayudhannair, K., 2021. Bromelain enhances digestibility of Spirulina-based fish feed. J. Appl. Phycol. 33, 967–977.
Sheikhzadeh, N., Mousavi, S., Hamidian, G., Firouzamandi, M., Oushani, A.K., Mardani, K., 2019. Role of dietary Spirulina platensis in improving mucosal immune responses and disease resistance of rainbow trout (Oncorhynchus mykiss). Aquaculture 510, 1–8.
Shi, Z., Li, X.-Q., Chowdhury, M.K., Chen, J.-N., Leng, X.-J., 2016. Effects of protease supplementation in low fish meal pelleted and extruded diets on growth, nutrient retention and digestibility of gibel carp, Carassius auratus gibelio. Aquaculture 460, 37–44.
Skalli, A., Firmino, J.P., Andree, K.B., Salomón, R., Estévez, A., Puig, P., Sabater-Martínez, M., Hechavarria, T., Gisbert, E., 2020. The inclusion of the microalga Scenedesmus sp. in diets for rainbow trout, Onchorhynchus mykiss, juveniles. Animals 10, 1656.
Skrede, A., Berge, G., Storebakken, T., Herstad, O., Aarstad, K., Sundstøl, F., 1998. Digestibility of bacterial protein grown on natural gas in mink, pigs, chicken and Atlantic salmon. Anim. Feed Sci. Technol. 76, 103–116.
Skrede, A., Mydland, L., Ahlstrøm, Ø., Reitan, K., Gislerød, H., Øverland, M., 2011. Evaluation of microalgae as sources of digestible nutrients for monogastric animals. J. Anim. Feed Sci. 20, 131–142.
Snow, A., Ghaly, A.E., 2007. The nutritive value of wastewater grown barley and its utilization in fish feed. Am. J. Agric. Biol. Sci. 2, 168–183.
Sørensen, M., Morken, T., Kosanovic, M., Øverland, M., 2011. Pea and wheat starch possess different processing characteristics and affect physical quality and viscosity of extruded feed for Atlantic salmon. Aquac. Nutr. 17, e326–e336.
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.
Sørensen, M., Gong, Y., Bjarnason, F., Vasanth, G.K., Dahle, D., Huntley, M., Kiron, V., 2017. Nannochloropsis oceania-derived defatted meal as an alternative to fishmeal in Atlantic salmon feeds. PloS One 12, 0179907.
Souza, F.Pd, Lima, E.C.Sd, Urrea-Rojas, A.M., Suphoronski, S.A., Facimoto, C.T., Bezerra Júnior, Jd.S., Oliveira, T.E.Sd, Pereira, Ud.P., Santis, G.W.D., Oliveira, C.A.Ld, 2020. Effects of dietary supplementation with a microalga (Schizochytrium sp.) on the hemato-immunological, and intestinal histological parameters and gut microbiota of Nile tilapia in net cages. PloS One 15, 0226977.
Sui, Y., Jiang, Y., Moretti, M., Vlaeminck, S.E., 2020. Harvesting time and biomass composition affect the economics of microalgae production. J. Clean. Prod. 259, 120782.
Sun, X., Chang, Y., Ye, Y., Ma, Z., Liang, Y., Li, T., Jiang, N., Xing, W., Luo, L., 2012. The effect of dietary pigments on the coloration of Japanese ornamental carp (koi, Cyprinus carpio L.). Aquaculture 342, 62–68.
Tacon, A.G., 2020. Trends in global aquaculture and aquafeed production: 2000–2017. Rev. Fish. Sci. Aquac. 28, 43–56.
Taelman, S.E., De Meester, S., Roef, L., Michiels, M., Dewulf, J., 2013. The environmental sustainability of microalgae as feed for aquaculture: a life cycle perspective. Bioresour. Technol. 150, 513–522.
Takagi, S., Murata, H., Goto, T., Endo, M., Yamashita, H., Ukawa, M., 2008. Taurine is an essential nutrient for yellowtail Seriola quinqueradiata fed non-fish meal diets based on soy protein concentrate. Aquaculture 280, 198–205.
Tavakoli, S., Regenstein, J.M., Daneshvar, E., Bhatnagar, A., Luo, Y., Hong, H., 2021. Recent advances in the application of microalgae and its derivatives for preservation, quality improvement, and shelf-life extension of seafood. Crit. Rev. Food Sci. Nutr. 1–14.
Teuling, E., Wierenga, P.A., Agboola, J.O., Gruppen, H., Schrama, J.W., 2019. Cell wall disruption increases bioavailability of Nannochloropsis gaditana nutrients for juvenile Nile tilapia (Oreochromis niloticus). Aquaculture 499, 269–282.
Tibaldi, E., Zittelli, G.C., Parisi, G., Bruno, M., Giorgi, G., Tulli, F., Venturini, S., Tredici, M., Poli, B., 2015. Growth performance and quality traits of European sea bass (D. labrax) fed diets including increasing levels of freeze-dried Isochrysis sp.(T-ISO) biomass as a source of protein and n-3 long chain PUFA in partial substitution of fish derivatives. Aquaculture 440, 60–68.
Tibbetts, S.M., Yasumaru, F., Lemos, D., 2017. In vitro prediction of digestible protein content of marine microalgae (Nannochloropsis granulata) meals for Pacific white shrimp (Litopenaeus vannamei) and rainbow trout (Oncorhynchus mykiss). Algal Res. 21, 76–80.
Tomás-Almenar, C., Larrán, A., de Mercado, E., Sanz-Calvo, M., Hernández, D., Riaño, B., García-González, M., 2018. Scenedesmus almeriensis from an integrated system waste-nutrient, as sustainable protein source for feed to rainbow trout (Oncorhynchus mykiss). Aquaculture 497, 422–430.
Tulli, F., Zittelli, Chini, Giorgi, G., Poli, G., Tibaldi, B., Tredici, M, E., 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. Aquat. Food Prod. Technol. 21, 188–197.
Ullah, Z., Ahmed, G., un Nisa, M., Sarwar, M., 2016. Standardized ileal amino acid digestibility of commonly used feed ingredients in growing broilers. Asian-Austral J. Anim. Sci. 29, 1322–1330.
Valente, L.M.P., Custódio, M., Batista, S., Fernandes, H., Kiron, V., 2019. Defatted microalgae (Nannochloropsis sp.) from biorefinery as a potential feed protein source to replace fishmeal in European sea bass diets. Fish. Physiol. Biochem. 45, 1067–1081.
Van Den Hende, S., Beelen, V., Bore, G., Boon, N., Vervaeren, H., 2014. Up-scaling aquaculture wastewater treatment by microalgal bacterial flocs: from lab reactors to an outdoor raceway pond. Bioresour. Technol. 159, 342–354.
Varelas, V., 2019. Food wastes as a potential new source for edible insect mass production for food and feed: a review. Fermentation 5, 81.
Venou, B., Alexis, M., Fountoulaki, E., Haralabous, J., 2009. Performance factors, body composition and digestion characteristics of gilthead sea bream (Sparus aurata) fed pelleted or extruded diets. Aquac. Nutr. 15, 390–401.
Vizcaíno, A., Rodiles, A., López, G., Sáez, M., Herrera, M., Hachero, I., Martínez, T., Cerón-García, M., Alarcón, F.J., 2018. Growth performance, body composition, and digestive functionality of Senegalese sole (Solea senegalensis Kaup, 1858) juveniles fed diets including microalgae freeze-dried biomass. Fish. Physiol. Biochem. 44, 661–677.
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. N. Am. J. Aquac. 73, 76–83.
Wan, A.H., Davies, S.J., Soler-Vila, A., Fitzgerald, R., Johnson, M.P., 2019. Macroalgae as a sustainable aquafeed ingredient. Rev. Aquac. 11, 458–492.
Wang, T., Cheng, Y., Chen, X., Liu, Z., Long, X., 2017a. Effects of small peptides, probiotics, prebiotics, and synbiotics on growth performance, digestive enzymes, and oxidative stress in orange-spotted grouper, Epinephelus coioides, juveniles reared in artificial seawater. Chin. J. Oceanol. Limnol. 35, 89–97.
Wang, Y., Li, M., Filer, K., Xue, Y., Ai, Q., Mai, K., 2017b. Evaluation of Schizochytrium meal in microdiets of Pacific white shrimp (Litopenaeus vannamei) larvae. Aquac. Res. 48, 2328–2336.
Watanabe, T., 1990. Effect of dietary Spirulina supplement on growth performance and flesh lipids of cultured striped jack. J. Tokyo Univ. Fish. 77, 231–239.
Wei, M., Parrish, C.C., Guerra, N.I., Armenta, R.E., Colombo, S.M., 2021. Extracted microbial oil from a novel Schizochytrium sp.(T18) as a sustainable high DHA source for Atlantic salmon feed: Impacts on growth and tissue lipids. Aquaculture 534, 736249.
Wickramasuriya, S.S., Yi, Y.-J., Yoo, J., Kang, N.K., Heo, J.M., 2015. A review of canola meal as an alternative feed ingredient for ducks. J. Anim. Sci. Technol. 57, 1–9.
Yamamoto, F.Y., Yin, F., Rossi Jr, W., Hume, M., Gatlin III, D.M., 2018. β-1, 3 glucan derived from Euglena gracilis and AlgamuneTM enhances innate immune responses of red drum (Sciaenops ocellatus L.). Fish. Shellfish Immunol. 77, 273–279.
Yao, C., Ai, J., Cao, X., Xue, S., Zhang, W., 2012. Enhancing starch production of a marine green microalga Tetraselmis subcordiformis through nutrient limitation. Bioresour. Technol. 118, 438–444.
Yarnold, J., Karan, H., Oey, M., Hankamer, B., 2019. Microalgal aquafeeds as part of a circular bioeconomy. Trends Plant Sci. 24, 959–970.
Yeganeh, S., Teimouri, M., Amirkolaie, A.K., 2015. Dietary effects of Spirulina platensis on hematological and serum biochemical parameters of rainbow trout (Oncorhynchus mykiss). Res. Vet. Sci. 101, 84–88.
Yu, R., Cao, H., Huang, Y., Peng, M., Kajbaf, K., Kumar, V., Tao, Z., Yang, G., Wen, C., 2020. The effects of partial replacement of fishmeal protein by hydrolysed feather meal protein in the diet with high inclusion of plant protein on growth performance, fillet quality and physiological parameters of Pengze crucian carp (Carassius auratus var. Pengze). Aquac. Res. 51, 636–647.
Zhou, R., Zhou, R., Zhang, X., Fang, Z., Wang, X., Speight, R., Wang, H., Doherty, W., Cullen, P., Ostrikov, K., 2019. High-performance plasma-enabled biorefinery of microalgae into value-added products. ChemSusChem 12, 4976–4985.
Zhou, Z., Ringø, E., Olsen, R., Song, S., 2018. Dietary effects of soybean products on gut microbiota and immunity of aquatic animals: a review. Aquac. Nutr. 24, 644–665.