Review

# Nutrients and Energy Digestibility of Microalgal Biomass for Fish Feed Applications

Senthil Nagappan Annamalai, Probir Das, Mahmoud I. A. Thaher, Mohammad Abdul Quadir, Shoyeb Khan, Chandan Mahata and Hareb Al Jabri

Special Issue

*Review*

# Nutrients and Energy Digestibility of Microalgal Biomass for Fish Feed Applications

**Senthil Nagappan Annamalai, Probir Das \*, Mahmoud I. A. Thaher, Mohammad Abdul Quadir, Shoyeb Khan** [ID] **, Chandan Mahata and Hareb Al Jabri**

Algal Technology Program, Center for Sustainable Development, College of Arts and Sciences, Qatar University, Doha P.O. Box 2713, Qatar; senthil.a@qu.edu.qa (S.N.A.); mahmoud.t@qu.edu.qa (M.I.A.T.); m.abdulquadir@qu.edu.qa (M.A.Q.); shoyeb.khan@qu.edu.qa (S.K.); chandan.mahata@qu.edu.qa (C.M.); h.aljabri@qu.edu.qa (H.A.J.)

\* Correspondence: probir.das@qu.edu.qa

**Abstract:** Aquafeed accounts for at least 75–90% of aquaculture's operating costs. Traditional aquafeed ingredients such as fishmeal, fish oil, and soybean meal are unsustainable; further, their increasing cost necessities developing alternative feed ingredients. Microalgae-based aquafeed is not only environmentally friendly, but it can also be cost-effective with proper optimization. In addition, the nutrition profile of microalgae is similar to that of many fishes. The digestibility of a feed is one of the most important factors to consider in feed formulation. A highly digestible feed can lower production costs, reduce feed waste, and reduce the risk of eutrophication. This review discusses the digestibility of various nutrients such as protein, lipid, carbohydrate, amino acids, and fatty acids (including omega-3 fatty acids), dry matter, and energy of various microalgae in fish. Other commonly used aquafeed ingredients were also compared to microalgae in terms of nutrient and energy digestibility in fish. The intrinsic characteristics of microalgae, biomass pretreatment, and feed preparation methods are all discussed as factors that contribute to the nutrient and energy digestibility of microalgae in fish. Furthermore, methods for increasing the digestibility of microalgal biomass in fish are suggested. Finally, the review concludes with the challenges and prospects of using microalgae as a fish feed in terms of digestibility.

**Keywords:** microalgae; aquafeed; protein; digestibility; lipid

## 1. Introduction

Microalgae biomass is a promising feed ingredient in aquaculture. Various studies have reported success in the partial replacement of fishmeal, soybean meal, and the complete replacement of fish oil in the fish diet by microalgal biomass [1,2]. The inclusion of microalgae in feed improves weight gain, increases the protein and lipid content in the fish muscle, improves the disease resistance and stress tolerance in fish, and enhances the texture and taste of fish fillet [3].

Microalgae are a nutrition-rich ingredient that could be used in fish feed. However, the nutritional content of microalgae is of limited use in feed formulation because it does not provide information on the hydrolytic products available for growth after digestion [4]. This could be known through digestibility studies. The digestibility study provides information on the amount of digestible nutrients and energy in ingredients, including microalgae. The digestibility of nutrients in the diet indicates the proportion of food consumed to be absorbed, with the undigested portion eliminated as feces [5]. Therefore, the portion of undigested food is a waste of resources.

The addition of highly digestible ingredients to feed can help to improve feed conversion ratios, lower production costs, and reduce environmental impact by lowering eutrophication potential [6–8]. Enhanced digestibility would ultimately result in higher growth and nutrient retention rates in muscle, thus increasing productivity [9]. Therefore,

one of the first steps in formulating a novel feed will be the determination of the nutrient and energy digestibility of the individual ingredients in the aquatic organism [5,10].

One of the major ingredients in any aquafeed is fishmeal because it is the ingredient best used by most fish species [6,9,11–19]. This is perhaps due to the high digestibility of nutrients and energy of fishmeal in various fishes. But fishmeal prices are steadily increasing over the years, and it is considered an unsustainable ingredient. Apart from fishmeal, commonly found ingredients in aquafeed are plant-based ones, including soybean meal, groundnut cake, rice bran, wheat gluten, wheat middlings, corn starch, and palm oil. These plant-based ingredients are either used as whole or used as extracts. Extracts are the leftover biomass after oil extraction. In many fishes, whole and extracts of plant-based substances have been shown to be comparable to fishmeal in terms of nutrient and energy digestibility [20]. If any novel biomass, such as microalgae or its specific metabolite, is to be used in the feed, it must have digestibility similar or higher than that of fishmeal and the other conventional ingredients currently used to replace it.

In recent years, microalgae biomass has attracted attention as a sustainable ingredient. Although many microalgal species have yet to be tested for digestibility in fish, preliminary results show a wide range of digestibility values [9,11,12,21–23]. Hence, microalgae's true potential as a feed ingredient has yet to be realized in aquaculture. It is widely accepted that the best way to compensate for faecal losses is to formulate diets on a digestibility basis. However, diets are formulated based on crude energy and nutrients due to a lack of proper information about a new ingredient's digestibility. From this perspective, this review summarizes the nutrient and energy digestibility of various microalgae in fish. Furthermore, factors affecting microalgae digestion in fish as well as methods to improve microalgae digestibility in fish are discussed.

## 2. Factors Contributing to Digestion of Microalgae

Many microalgal species ranging from prokaryote to eukaryote contribute to the rich diversity. Even intra-species variation in biochemical profiles has been reported. Each of these microalgal species differs in chemical composition and physical structure, leading to the difference in the nutrient and energy digestibility values. In particular, the digestion of microalgae by fish is shown to be mainly affected by the composition and rigidness of microalgae's cell wall [24]. The prokaryotic microalgae (i.e., cyanobacteria) have a peptidoglycan layer in their cell walls, whereas the eukaryotic green microalgae have a cellulosic layer in their cell walls [17,25,26]. A study has shown that the microalgae with peptidoglycan (murein) layered cell walls are easier to digest by fish than microalgae with cellulosic layered ones [17]. The rigidness of the cell wall was also found to affect the digestibility of microalgae. Studies show that thick-walled microalgae exhibit poor digestibility compared to species with thin cell walls or which lack cell walls [6,21,25,27–31]. Microalgae belonging to genera including *Nannochloropsis, Chlorella, Haemotococcus,* and *Desmodesmus* have thick cell walls, whereas species like *Isochrysis galbana, Porphyridium cruentum*, and *Dunaliella salina* lack cell walls.

Despite cell wall disruption, the digestibility of microalgae still could be affected by certain proteins that inhibit digestive enzyme activity. For instance, poor digestion of *Nannochloropsis* sp. could be attributed to the high amount of trypsin inhibitor, an adverse enzyme that prevents proteolytic enzyme activity. Furthermore, some marine microalgae are reported to contain lipase inhibitors which could affect the digestibility of lipids [32–34]. Other factors that affect digestibility are the presence of non-starch polysaccharides and fibers in microalgae [35]. Non-starch polysaccharides, found typically in cell walls, are mostly indigestible, including cellulose, gums, pectins, and hemicelluloses [25,36–39]. Some fish species, like Nile tilapia, lack digestive enzymes to break the beta glycosidic bond present in non-starch polysaccharides [40]. These undigested carbohydrates rapidly pass through the digestive tract, but not before absorbing proteins, thus reducing the protein digestibility [41,42]. A negative correlation was established for fiber content with the digestibility of organic matter, protein, and carbohydrate [24,43]. However, another

study reported no such correlation between fiber content and protein digestibility [24]. Therefore, more studies are required to establish the relationship between fiber content and nutrient digestibility.

Due to the low amount of non-starch polysaccharides and fibers, the digestibility of nutrients for *Isochrysis* sp. was found to be better than *Nannochloropsis* sp. in rainbow trout [35]. It is also reported that fiber and other anti-nutrient factors negatively affect proteolytic and amylase activity, decreasing digestibility [31,44]. There are other factors, which can affect the digestibility of a microalgae-based diet. Exopolysaccharides can inhibit protein digestion, since these can form stable complexes with protein preventing proteolysis [24,45]. Several strains (e.g., *Porphyridium* sp.) are known to produce exopolysaccharides, which are either secreted in the algal culture or remain attached to the cells [24]. Proteins can be precipitated by phenolic compounds generally associated with plant and seaweed ingredients [4,46–48]. Even though the phenolic content in microalgae is very low (0–20 mg gallic acid equivalents $g^{-1}$ DW), the microalgal protein digestibility could still be affected by plant phenolic compounds present in the feed [4].

The use of feed preparation techniques involving high-temperature processes (e.g., extrusion) can damage certain amino acids such as lysine [49,50]. This damage occurs due to heat, which can cause cross-linkage and denaturation of protein [20]. Thus, amino acid digestibility could be affected. Moreover, the differences in the physiology of each fish species contribute to variation in the digestibility of the same microalgal species [17]. In addition to physiology, fish species show variation in the profile of digestive enzymes. For example, only selected fishes such as Rohu (*Labeo rohita*) have enzymes needed for cellulose degradation [51].

## 3. Methods to Improve the Digestibility of Microalgae

The microalgal cells harvested from the growth reactor are usually spray dried to preserve the biomass quality. In addition, on a large scale, microalgal biomass is sun, drum, or oven-dried. However, in these cases, the microalgae cell wall remains intact, which is related to poor digestibility [9,22,34]. Nutrient digestibility can be improved by the pre-treatment/processing of biomass, including bead milling, pasteurization, freeze-drying, high pressure homogenization, pulse electric field, ultrasound, microwave, chemical and enzymatic treatment [21,52]. This is possible since biomass processing/pre-treatment disrupts the rigid cell wall of microalgae and thus releases intracellular nutrients for the fish digestive system and subsequent absorption. Literature reports suggest that biomass processing of selected microalgae is attributed to higher digestibility for various fishes [1,34,53].

In several studies, bead milling was found to be efficient among pre-treatments in improving microalgae digestibility in fish. For example, in juvenile Nile tilapia (*Oreochromis niloticus*), the diet containing bead *milled Nannochloropsis gaditana* had the highest protein, lipid, and energy digestibility when compared to other diets that contained the same algae but were treated with pasteurization, freezing, or freeze-drying methods [18]. Physical treatment of *Nannochloropsis* sp. and *Chlorella* sp. using bead milling improved protein digestibility by 3–8% compared to that of unprocessed ones in European seabass [34]. In particular, bead milling of *Tetraselmis* sp. improved protein digestibility by 20% compared to unprocessed cells in European seabass [34]. The digestibility of amino acids like phenylalanine, and aspartic acid in European seabass was improved by bead milled microalgae inclusion in diet rather than the whole biomass inclusion; however, essential amino acid digestibility was not improved by the pre-treatment of microalgae [34]. It was observed that 10–39% of the cell wall of *Nannochloropsis* sp. and *Chlorella* sp. could be broken by 10 min of bead milling and, in turn, may liberate nutrients and thus improve digestibility [1,17,54,55]. In some cases, the enzymatic processing also significantly improved the protein digestibility of *Nannochloropsis* sp. and *Chlorella* sp. and energy digestibility of *Nannochloropsis* sp., *Chlorella* sp., and *Tetraselmis* sp. by 14%, 11%, and 40% for European seabass [34]. The

extent of cell disruption differs from one species to another; thus, nutrient accessibility could differ despite the same processing conditions [56].

The extrusion process is preferred for feed making since it produces pellets that could reduce feed loss and water pollution. It involves high temperature, high pressure, long processing time, and shear force. Under these conditions, the recalcitrant microalgal cell walls can be disrupted, which in turn increases nutrient bioavailability and digestibility [21,57–59]. Furthermore, the extrusion process can denature the enzymes like trypsin inhibitors, which affects digestibility [38]. In a study, extruded feed containing defatted biomass of *Nannochloropsis* sp. and *Desmodesmus* sp. had higher digestibility of ash, dry matter, and protein than that of non-extruded diet [21]. Similarly, in Gibel carp fed with a microalgae-based diet, the digestibility of dry matter, protein, starch, and energy was higher than that of pellets prepared by cold pelletization technique [52]. In the cold pelletization technique, all ingredients are mixed with cold water and cold-pressed, usually through a meat mincer, then dried, crushed, and sieved to obtain pellets. Cold pelletization, unlike extrusion, does not use high temperatures or pressure, so the cell wall of microalgae is largely unaffected during the process.

The digestibility of feed ingredients, including microalgae, can be improved by the addition of enzymes in diet or by enzymatic processing of microalgal biomass [60,61]. For example, the addition of enzyme-like protease improved protein digestibility and degraded the anti-nutritional factors, including lectins, trypsin inhibitors, and antigenic proteins in diet [62,63]. Cellulase enzyme hydrolyzed 62% of the cellulose in *Chlorella pyrenoidosa*, resulting in a 75% increase in lipid extraction, indicating that enzymes have the potential to improve microalgae digestibility [64]. The addition of non-starch polysaccharide (NSP) enzymes to the diet containing defatted *Nannochloropsis* sp. biomass increased the protein digestibility in Nile tilapia [35]. Besides enzymes, the addition of organic minerals rather than inorganic minerals in the diet containing microalgae also improved the digestibility of fatty acids, especially polyunsaturated fatty acids in Atlantic salmon [60].

## 4. Nutrient Digestibility

### 4.1. Dry Matter Digestibility

The dry matter contains all the components of biomass except for water. Microalgae's dry matter consists of lipids, proteins, carbohydrates, chlorophyll, vitamins, carotenoids, and ash. Dry matter content varies between 91% and 98% in microalgae [24]. Tables 1 and 2 show the digestibility of the microalgae dry matter in fish. All the values shown in Tables 1 and 2 are the digestibility values reported for microalgae and not for the diet in fish. The digestibility of microalgae dry matter in fishes evaluated ranged from 13% to 97%. In particular, digestibility of dry matter of *Schizochytrium* sp. in rainbow trout (*Oncorhynchus mykiss*) was in the range of 91–97% [65]. Similarly, for Nile tilapia (*Oreochromis niloticus*), the *Schizochytrium* sp. had dry matter digestibility of 82% [6]. Dry matter digestibility higher than 70% was observed in rainbow trout for *Isochrysis* sp., Nile tilapia for *Spirulina* sp., *Chlorella* sp., *Spirulina maxima* and *Chlorella vulgaris*, and African catfish (*Clarus gariepinus*) for *Spirulina maxima* and *Chlorella vulgaris* (Table 1). The in vitro studies showed that *Spirulina platensis*, *Chlorella sorokiniana*, and *Chlorella vulgaris* had dry matter digestibility greater than 70% (Table 2). These microalgal species may have high digestible dry matter content due to their high amount of easily digestible nutrients and low anti-nutritional factors, as well as less rigid cell wall [66,67].

Microalgae-based diets typically include microalgae as well as other ingredients such as fish meal, plant-based ingredients, minerals, and vitamins. Studies have reported no significant difference in dry matter digestibility between a microalgae-based diet and a control diet lacking microalgae in fish. For instance, a diet containing *Chlorella* sp., and a diet containing *Nannochloropsis oceanica* had similar dry matter digestibility to control diet lacking microalgae in European seabass (*Dicentrarchus labrax*) juveniles [34]. Similarly, in another study, there was no difference in dry matter digestibility between the control and a diet containing 30% defatted *Nannochloropsis* sp. in Atlantic salmon (*Salmo salar*) [21]. However, studies also have reported that increasing the algal content in diet negatively affected

the dry matter digestibility in fish. For instance, increasing *Phaeodactylum tricornutum* content in the diet, the dry matter digestibility decreased in Atlantic salmon (*S. salar*) [12]. In another study, with increased content of whole-cell *Nannochloropsis gaditana* in diet, the dry matter digestibility decreased in African catfish and Nile tilapia [18]. Similarly, other studies revealed that higher inclusion levels of algae led to a decrease in dry matter digestibility in fish [8]. In some cases, a microalgae-based diet's decreased dry matter digestibility was compensated by a higher intake of feed by fishes [8]. However, in such cases it could result in an increase in the feed conversion ratio.

The disruption of algal cells using bead miller led to an increase in dry matter digestibility of *Nannochloropsis oceanica*, *Chlorella vulgaris,* and *Tetraselmis* sp. for juvenile European seabass (*D. labrax*) by nearly 21%, 14%, and 50%, respectively [34]. Bead milling of *Nannochloropsis gaditana* increased the dry matter digestibility for juvenile African catfish (*C. gariepinus*) and juvenile Nile tilapia by 16% and 18%, respectively [68]. Enzymatic treatment increased the dry matter digestibility of *Nannochloropsis oceanica*, *Chlorella vulgaris,* and *Tetraselmis* sp. for juvenile European seabass (*D. labrax*) by 27%, 14%, and 12%, respectively [34]. The above results suggest that efficient cell disruption techniques to improve dry matter digestibility differ from one algal species to another. Defatting of the microalgae *Nannochloropsis oculata* decreased the dry matter digestibility for juvenile Nile tilapia by 7% [38]. This is most likely due to a decrease in the content of easily digestible lipids, such as polyunsaturated fatty acid (PUFA), in fishes, which has a negative impact on lipid digestibility and, as a result, on dry matter digestibility [69]. The starvation of *Tetraselmis Suecica* increased the dry matter digestibility (estimated by in vitro method) by nearly 5% [24]. This could be due to the fact that nutrient deprivation can increase the amount of highly digestible lipid-like PUFA and starch in microalgae [69]. The pelletization technique used to prepare feed has an impact on dry matter digestibility. This was demonstrated in a study in which extruded pellets had higher dry matter digestibility than pellets made by cold pelletization for Atlantic salmon diets containing *Nannochloropsis* sp. [21]. The above results suggest that the starvation of microalgae, cell disruption technique, and pelletization method can be applied to improve dry matter digestibility of microalgae.

*4.2. Protein and Amino Acid Digestibility*

Fish require diets comprising of 30% to 55% crude protein and an amino acid supply focused on specific requirements for maximum growth [70]. If the diet includes easily digestible ingredients, the high protein demand of fish can be met. The protein digestibility of microalgae in different fishes ranged from 50% to 94% (Tables 1 and 2). The digestibility of protein for *Schizochytrium* sp., *Isochrysis* sp., *Spirulina* sp., *Chlorella vulgaris*, *Nannochloropsis oceanica*, and *Nannochloropsis oculata* was found to be higher than 80% in fish such as rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*), European seabass (*Dicentrarchus labrax*), and African catfish (*Clarus gariepinus*) (Table 1). In comparison, the commonly used ingredient fish meal has protein digestibility ranging from 72% to 92% for salmonids and tilapia [8,11,21,71–77]. Other popular ingredients in the fish diet include soybean meal and corn which have a protein digestibility of 91–96% and 53–83%, respectively, for salmonids and tilapia [70]. The wheat middling has a protein digestibility of 20–76% for tilapia [70]. The protein digestibility of seaweed in rainbow trout ranged from 66% to 80% [77]. Unlike seaweed, the microalgal biomass has lower total phenolic content (TPC) (<20 mg GAE g$^{-1}$ DW) [46–48,78,79]. Protein digestibility is indirectly correlated to TPC content [80]. Thus, in general, microalgal biomass has higher protein digestibility than seaweed. Overall, the protein digestibility of selected microalgal species was comparable to that of fishmeal and plant-based ingredients and was higher than that of seaweed.

In the case of microalgae-based diets, a wide range of protein digestibility values has been reported for different fishes. The wide range of results could be explained by differences in feed processing technique, the type of ingredients used in combination with microalgae in the diet, and the digestive systems of fishes. In general, studies have shown a

linear decrease in protein digestibility relative to the increase in the content of microalgae in the diet [8,12]. Protein digestibility is negatively affected by the fiber content of the diet, and microalgal biomass has a considerable amount of total fiber [61,81]. The fiber inhibits pepsin activity, which catalyses protein hydrolysis [24,81]. Furthermore, microalgal biomass has relatively high levels of soluble polysaccharide fibers that can entrap proteins in the cellular matrix, rendering them less bioavailable to enzymatic hydrolysis [82]. However, decreased protein digestibility values were compensated by high feed intake for sustaining the fish growth [8]. Overall, at an appropriate concentration of microalgae (less than 15%) in diet, the protein digestibility was not found to be affected [12].

**Table 1.** In vivo digestibility of dry matter, nutrients, and energy of individual microalgae for various fish.

| Microalgae | Biomass Processing/ Pre-Treatment | Aqua Species | Pellet | Dry Atter (%) | Protein (%) | Lipid (%) | Carbohydrate (%) | Energy (%) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *Chlorella* sp. | – | Nile tilapia (*Oreochromis niloticus*) | Meat grinder | 73.4 | 80 | 94.4 | – | 83.9 | [6] |
| *Chlorella vulgaris* | – | African catfish (*Clarias gariepinus*) | Extruded into sinking pellet | 70.7 | 80.7 | 78.1 | 84.6 | 71.6 | [17] |
| *Chlorella vulgaris* | – | Atlantic salmon (*Salmo salar* L.) | Steam pelleted | – | 79.5 | 69.9 | 45 | 59.6 | [83] |
| *Chlorella vulgaris* | High pressure homogenization | Atlantic salmon (*Salmo salar* L.) | Steam pelleted | – | 85.4 | 82.1 | 82.7 | 76.5 | [83] |
| *Chlorella vulgaris* | – | European seabass (*Dicentrarchus labrax*) | Twin-screw extruder (Clextral BC 45) | 41.2 | 85.5 | 84.9 | – | 81.5 | [34] |
| *Chlorella vulgaris* | Bead milling | European seabass (*Dicentrarchus labrax*) | Twin-screw extruder (Clextral BC 45) | 63.4 | 88.6 | 81.2 | – | 90.4 | [34] |
| *Chlorella vulgaris* | Enzymatic processing | European seabass (*Dicentrarchus labrax*) | Twin-screw extruder (Clextral BC 45) | 63.4 | 87.6 | 78.4 | – | 90.6 | [34] |
| *Chlorella vulgaris* | – | Nile tilapia (*Oreochromis niloticus*) | Extruded into sinking pellet | 73.7 | 80.9 | 84.3 | 70.4 | 73.9 | [17] |
| *Desmodesmus* sp. | Defatting | Atlantic salmon (*Salmo salar*) | Cold pelleted | 31.8 | 54.1 | – | – | – | [21] |
| *Desmodesmus* sp. | Defatting | Atlantic salmon (*Salmo salar*) | Twin-screw cooking extruder | 46.9 | 67.1 | – | – | 50.9 | [21] |
| *Isochrysis* sp. | – | Rainbow trout (*Oncorhynchus mykiss*) | Steam-pelleted | 77.1 | 86.5 | 62.8 | – | 72.6 | [35] |
| *Nannochloropsis gaditana* | – | African catfish (*Clarias gariepinus*) | Extruded into sinking pellet | 61.1 | 72.4 | 65.1 | 46.9 | 59.5 | [17] |
| *Nannochloropsis gaditana* | – | African catfish (*Clarias gariepinus*) | Extruded into sinking pellet | 48.3 | 59.3 | 40.3 | 31.7 | 46.6 | [68] |
| *Nannochloropsis gaditana* | Bead milling | African catfish (*Clarias gariepinus*) | Extruded into sinking pellet | 63.7 | 75.6 | 76.8 | 34.9 | 63.5 | [68] |
| *Nannochloropsis gaditana* | Commercial processing (Nutrispring® Liquid 40) | African catfish (*Clarias gariepinus*) | Extruded into sinking pellet | 60.3 | 67.7 | 47.2 | 45.4 | 53 | [68] |
| *Nannochloropsis gaditana* | Freeze-drying | African catfish (*Clarias gariepinus*) | Extruded into sinking pellet | 47 | 59.8 | 49.9 | 43.3 | 46.7 | [68] |
| *Nannochloropsis gaditana* | Frozen thawing | African catfish (*Clarias gariepinus*) | Extruded into sinking pellet | 50.2 | 65.2 | 41.2 | 28.1 | 48.8 | [68] |
| *Nannochloropsis gaditana* | Pasteurization | African catfish (*Clarias gariepinus*) | Extruded into sinking pellet | 45.2 | 55.5 | 44 | 48.7 | 43.7 | [68] |
| *Nannochloropsis gaditana* | – | Nile tilapia (*Oreochromis niloticus*) | Extruded into sinking pellet | 66.9 | 74.7 | 74.5 | 21.6 | 65.1 | [17] |
| *Nannochloropsis gaditana* | – | Nile tilapia (*Oreochromis niloticus*) | Twin-screw extruder (Clextral) into sinking pellets | 48.4 | 61.5 | 50.4 | 34.9 | 51 | [18] |

**Table 1.** *Cont.*

| Microalgae | Biomass Processing/ Pre-Treatment | Aqua Species | Pellet | Dry Atter (%) | Protein (%) | Lipid (%) | Carbohydrate (%) | Energy (%) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *Nannochloropsis gaditana* | Bead-milling | Nile tilapia (*Oreochromis niloticus*) | Twin-screw extruder (Clextral) into sinking pellets | 66.3 | 78 | 82 | 56.7 | 69.2 | [18] |
| *Nannochloropsis gaditana* | Commercially processed (nutrispring® Liquid 40) | Nile tilapia (*Oreochromis niloticus*) | Twin-screw extruder (Clextral) into sinking pellets | 61.2 | 72.9 | 66.4 | 46.6 | 60.6 | [18] |
| *Nannochloropsis gaditana* | Freeze-dried | Nile tilapia (*Oreochromis niloticus*) | Twin-screw extruder (Clextral) into sinking pellets | 50.6 | 60.6 | 57.8 | 38.5 | 53.1 | [18] |
| *Nannochloropsis gaditana* | Frozen thawed | Nile tilapia (*Oreochromis niloticus*) | Twin-screw extruder (Clextral) into sinking pellets | 55.2 | 66.2 | 53 | 40.5 | 57.1 | [18] |
| *Nannochloropsis gaditana* | Pasteurized | Nile tilapia (*Oreochromis niloticus*) | Twin-screw extruder (Clextral) into sinking pellets | 50.2 | 60.7 | 56.1 | 38 | 53.1 | [18] |
| *Nannochloropsis oceanica* | – | European seabass (*Dicentrarchus labrax*) | Twin-screw extruder (Clextral BC 45) | 32 | 81.6 | 63.1 | – | 76.2 | [34] |
| *Nannochloropsis oceanica* | Bead milling | European seabass (*Dicentrarchus labrax*) | Twin-screw extruder (Clextral BC 45) | 53.6 | 81 | 56.1 | – | 76.6 | [34] |
| *Nannochloropsis oceanica* | Enzymatic processing | European seabass (*Dicentrarchus labrax*) | Twin-screw extruder (Clextral BC 45) | 59.4 | 87.9 | 63.8 | – | 87 | [34] |
| *Nannochloropsis oculata* | Defatting | Nile tilapia (*Oreochromis niloticus*) | Meat grinder | – | 73.5 | 60.6 | – | 72.8 | [38] |
| *Nannochloropsis oculata* | – | Nile tilapia (*Oreochromis niloticus*) | Meat grinder | – | 81.1 | 64.2 | – | 80 | [38] |
| *Nannochloropsis* sp. | Defatting | Atlantic salmon (*Salmo salar*) | Twin-screw cooking extruder | 63.1 | 72.4 | – | – | 60.5 | [21] |
| *Nannochloropsis* sp. | Defatting | Atlantic salmon (*Salmo salar*) | Cold pelleted | 47.9 | 72.9 | – | – | – | [21] |
| *Nannochloropsis* sp. | Defatting | European seabass (*Dicentrarchus labrax*) | Dry pelleted at 50°C using pellet press | – | 85.4 | – | – | 68 | [32] |
| *Nannochloropsis* sp. | – | Rainbow trout (*Oncorhynchus mykiss*) | Steam-pelleted | 56.7 | 69.3 | 60.1 | – | 62.1 | [35] |
| *Scenedesmus dimorphus* | – | African catfish (*Clarias gariepinus*) | Extruded into sinking pellet | 58.2 | 68.3 | 68.3 | 62.3 | 61.4 | [17] |
| *Scenedesmus dimorphus* | – | Nile tilapia (*Oreochromis niloticus*) | Extruded into sinking pellet | 55.8 | 67 | 65.1 | 56.9 | 58.5 | [17] |
| *Schizochytrium* sp. | – | Nile tilapia (*Oreochromis niloticus*) | Meat grinder | 81.8 | 81.7 | 97.9 | – | 86.5 | [6] |
| *Schizochytrium* sp. | – | Rainbow trout (*Oncorhynchus mykiss*) | California Pellet Mill (model CPM CL-5) | 90.8 | 90.8 | 85.9 | – | 84.3 | [65] |
| *Schizochytrium* sp. | – | Rainbow trout (*Oncorhynchus mykiss*) | California Pellet Mill (model CPM CL-5) | 97.8 | 88.2 | 85.8 | – | 81.9 | [65] |
| *Spirulina maxima* | – | African catfish (*Clarias gariepinus*) | Extruded into sinking pellet | 73.1 | 81.4 | 89.1 | 66.3 | 75.3 | [17] |
| *Spirulina maxima* | – | Nile tilapia (*Oreochromis niloticus*) | Extruded into sinking pellet | 74.7 | 82.5 | 82.4 | 68.2 | 75.8 | [17] |
| *Spirulina* sp. | – | Nile tilapia (*Oreochromis niloticus*) | Meat grinder | 79.7 | 86.1 | 94.5 | – | 86.3 | [6] |

**Table 1.** *Cont.*

| Microalgae | Biomass Processing/Pre-Treatment | Aqua Species | Pellet | Dry Atter (%) | Protein (%) | Lipid (%) | Carbohydrate (%) | Energy (%) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *Tetraselmis* sp. | – | European seabass (*Dicentrarchus labrax*) | Twin-screw extruder (Clextral BC 45) | −19.1 | 69.7 | −92.4 | – | 48.9 | [34] |
| *Tetraselmis* sp. | Bead milling | European seabass (*Dicentrarchus labrax*) | Twin-screw extruder (Clextral BC 45) | 51.4 | 83.6 | −101.2 | – | 81.1 | [34] |
| *Tetraselmis* sp. | Enzymatic processed | European seabass (*Dicentrarchus labrax*) | Twin-screw extruder (Clextral BC 45) | 12.5 | 73.7 | −795.0 | – | 68.3 | [34] |

**Table 2.** In vitro digestibility of dry matter and nutrients of individual microalgae for various fish.

| Microalgae | Biomass Processing/Pre-Treatment | In Vitro Conditions | Dry Matter (%) | Protein (%) | Carbohydrate (%) | Reference |
|---|---|---|---|---|---|---|
| *Chlorella pyrenoidosa* | – | In vitro (pepsin-pancreatic system) | – | 75.3 | – | [61] |
| *Chlorella sorokiniana* F&M-M49 | – | In vitro (pepsin-pancreatic system) | 55 | 50 | 60 | [24] |
| *Chlorella sorokiniana* IAM C-212 | – | In vitro (pepsin-pancreatic system) | 72 | 70 | 72 | [24] |
| *Chlorella vulgaris* Allma | – | In vitro (pepsin-pancreatic system) | 70 | 75 | 70 | [24] |
| *Klamath* | – | In vitro (pepsin-pancreatic system) | 68 | 70 | 70 | [24] |
| *Nannochloropsis granulata* | – | In vitro pH-Stat using pyloric caeca enzyme extract of rainbow trout | – | 79.1 | – | [67] |
| *Nannochloropsis granulata* | Super Critical Fluid 70 °C extracted cell | In vitro pH-Stat using pyloric caeca enzyme extract of rainbow trout | – | 86.2 | – | [67] |
| *Nannochloropsis granulata* | Super Critical Fluid 90 °C extracted cell | In vitro pH-Stat using pyloric caeca enzyme extract of rainbow trout | – | 87.9 | – | [67] |
| *Nannochloropsis oceanica* F&M-M24 | – | In vitro (pepsin-pancreatic system) | 55 | 50 | 60 | [24] |
| *Nannochloropsis sphaeroides* F&M-C117 | – | In vitro (pepsin-pancreatic system) | 65 | 80 | 68 | [24] |
| *Porphyridium purpureum* F&M-M46 | – | In vitro (pepsin-pancreatic system) | 48 | 70 | 50 | [24] |
| *Phaeodactylum tricornutum* F&M-M40 | – | In vitro (pepsin-pancreatic system) | 50 | 70 | 55 | [24] |
| *Spirulina pacifica* | – | In vitro (pepsin-pancreatic system) | – | 85.6 | – | [61] |
| *Spirulina platensis* | – | In vitro (pepsin-pancreatic system) | – | 85 | – | [84] |
| *Spirulina platensis* | – | In vitro (pepsin-pancreatic system) | – | 94.3 | – | [61] |
| *Spirulina platensis* F&M-C256 | – | In vitro (pepsin-pancreatic system) | 78 | 80 | 80 | [24] |
| *Tisochrysis lutea* F&M-M36 | – | In vitro (pepsin-pancreatic system) | 65 | 60 | 65 | [24] |
| *Tetraselmis suecica* F&M-M33 | – | In vitro (pepsin-pancreatic system) | 50 | 65 | 55 | [24] |
| *Tetraselmis suecica* F&M-M33 | Nutrient starvation of cell | In vitro (pepsin-pancreatic system) | 55 | 70 | 58 | [24] |

Physical and enzymatic processing of microalgae was found to increase the protein digestibility of microalgal species. In comparison to whole-cell *Tetraselmis* sp., the bead milling processed biomass had 14% higher protein digestibility for European seabass [34]. Similarly, bead milling improved the digestibility of *Nannochloropsis gaditana* protein by 16% and 17% in African catfish (*C. gariepinus*) and Nile tilapia (*O. niloticus*), respectively [17,68]. Compared to whole-cell *Nannochloropsis oceanica*, the enzymatically processed microalgae

had 6% higher protein digestibility [34]. The addition of organic minerals improved the protein digestibility of a diet containing *Schizochytrium* sp. [60].

Amino acid digestibility of various microalgae ingredients is shown in Table 3. The amino acid digestibility of *N. oceanica* and *C. vulgaris,* for European seabass (*D. labrax*), and *C. vulgaris* for Atlantic salmon (*S. salar*) were higher than 90% [34,83]. *Tetraselmis* sp. had relatively lower amino acid digestibility for European seabass (*D. labrax*) juveniles (Table 3). Among individual amino acids of microalgae, arginine, isoleucine, and lysine were generally more digestible than other amino acids. Processing of biomass by physical and enzymatic methods did not increase the essential amino acid digestibility except in cases of threonine and phenylalanine in physically processed *Nannochloropsis* sp.; however, the digestibility of protein for physically processed *Chlorella* sp., and *Tetraselmis* sp. was enhanced by 11−19% [34]. The reason for the increase in amino acid digestibility is that larger size protein would have been cleaved into peptides and individual amino acid during the pretreatment process.

**Table 3.** In vivo digestibility of individual amino acids of microalgae in fish.

| Microalgae | Biomass ProCessing/ Pre-Treatment | Aqua Species | Arginine (%) | Histidine (%) | Lysine (%) | Threonine (%) | Isoleucine (%) | Leucine (%) | Valine (%) | Methionine (%) | Phenylalanine (%) | Tryptophan (%) | Refrence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chlorella vulgaris* | – | Atlantic salmon (*Salmo salar* L.) | 83.3 | 77.9 | 97.3 | 73.3 | 79.4 | 79.2 | 78 | 83.4 | 81.8 | 90 | [83] |
| *Chlorella vulgaris* | High pressure homogenization | Atlantic salmon (*Salmo salar* L.) | 94.6 | 93.1 | 92.7 | 91.5 | 90.5 | 92.4 | 92.2 | 89.6 | 89.2 | 68.8 | [83] |
| *Chlorella vulgaris* | – | European seabass (*Dicentrarchus labrax*) | 93.2 | 74.4 | 73.8 | 90.3 | 83.4 | 89.1 | 86.7 | 97.1 | 88.7 | – | [34] |
| *Chlorella vulgaris* | Bead milling | European seabass (*Dicentrarchus labrax*) | 92.2 | 88.2 | 71.7 | 92.3 | 91.9 | 92.6 | 92.7 | 95.8 | 92.3 | – | [34] |
| *Chlorella vulgaris* | Enzymatic processed | European seabass (*Dicentrarchus labrax*) | 92.8 | 50.3 | 73.8 | 89 | 89.7 | 89.2 | 90.5 | 67.3 | 88.3 | – | [34] |
| *Chlorella* sp. | – | Nile tilapia (*Oreochromis niloticus*) | 96.7 | 94.1 | 68.9 | 90.5 | 86.5 | 93.4 | 91.5 | 93.9 | 92.3 | 95.5 | [6] |
| *Isochrysis* sp. | – | Rainbow trout (*Oncorhynchus mykiss*) | 99.2 | 93.2 | 101.4 | 94 | 92.1 | 94.5 | 98.5 | 94.9 | 94.4 | 84.4 | [35] |
| *Nannochloropsis oceanica* | – | European seabass (*Dicentrarchus labrax*) | 86.1 | 92 | 87.5 | 86.2 | 90.5 | 88.5 | 91.1 | 61.4 | 87.4 | – | [34] |
| *Nannochloropsis oceanica* | Bead milling | European seabass (*Dicentrarchus labrax*) | 94.6 | 83.6 | 90.5 | 90.5 | 88.4 | 88.1 | 88.6 | – | 88.4 | – | [34] |
| *Nannochloropsis oceanica* | Enzymatic processed | European seabass (*Dicentrarchus labrax*) | 90.6 | 78.8 | 86.4 | 88.7 | 86.7 | 86 | 87.5 | 91.2 | 89.9 | – | [34] |
| *Nannochloropsis oculata* | – | Nile tilapia (*Oreochromis niloticus*) | 71.4 | 74 | 75.8 | 66.6 | 79.8 | 78.3 | 77.7 | 88.1 | 72.5 | 86.5 | [38] |
| *Nannochloropsis* sp. | – | Rainbow trout (*Oncorhynchus mykiss*) | 74.5 | 74.1 | 72.6 | 67.4 | 63.1 | 71.8 | 58.9 | 69.8 | 64.8 | 11.8 | [35] |
| *Schizochytrium* sp. | – | Nile tilapia (*Oreochromis niloticus*) | 100 | 93.1 | 90.9 | 93.3 | 91.9 | 100 | 99 | 100 | 100 | 89.6 | [6] |
| *Spirulina* sp. | – | Nile tilapia (*Oreochromis niloticus*) | 94 | 100 | 100 | 95.3 | 94.9 | 99.7 | 93.2 | 100 | 100 | 96.2 | [6] |
| *Spirulina* sp. | Defatted | Nile tilapia (*Oreochromis niloticus*) | 83 | 76.7 | 81.5 | 60.5 | 73.6 | 81.3 | 73.4 | 64.1 | 74 | 56.1 | [38] |
| *Tetraselmis* sp. | – | European seabass (*Dicentrarchus labrax*) | 81.4 | 59.4 | 84.2 | 74.8 | 76.5 | 71.1 | 69.1 | 73.9 | 74.4 | – | [24] |
| *Tetraselmis* sp. | Bead milling | European seabass (*Dicentrarchus labrax*) | 90.9 | 78.7 | 76.2 | 87.8 | 93.9 | 85.6 | 93.3 | 89.9 | 85 | – | [24] |
| *Tetraselmis* sp. | Enzymatic processed | European seabass (*Dicentrarchus labrax*) | 95 | 49.3 | 76.8 | 87.5 | 78 | 77.4 | 86.1 | 86.5 | 83.7 | – | [24] |

### 4.3. Lipid and Fatty Acid Digestibility

Lipids are an excellent source of energy for fish [14,85,86]. Fish use the beta-oxidation process to break down lipids in the mitochondria of the cell to generate energy. The lipid content of microalgae varies from 1 to 70% of their dry weight. Table 1 shows the digestibility of microalgae lipids in different fish. In Nile tilapia (*Oreochromis niloticus*), a digestibility of *Schizochytrium* sp. lipids as high as 98% has been reported [6]. Lipid digestibility higher than 80% was observed in juvenile European seabass (*Dicentrarchus labrax*) for *Chlorella vulgaris*, Nile tilapia (*O. niloticus*) for *Chlorella vulgaris*, *Schizochytrium* sp., *Spirulina* sp., and *Chlorella* sp., and African catfish (*Clarus gariepinus*) for *Chlorella vulgaris* and *Spirulina maxima* [6,17,34]. However, in some cases, lower lipid digestibility values were reported, as in the case of juvenile African catfish (*C. gariepinus*) for *Nannochloropsis gaditana*, which was only 40% [68].

The wide range of lipid digestibility by fishes could be due to the type of lipid and fatty acid present in the microalgae, as well as the fish to which algae is fed. Various studies have reported that an increase in algal content in the diet decreased the lipid digestibility in fish [12,29,32,34,83]. In addition, the presence of lipase inhibitors in microalgae at higher concentrations can decrease lipid digestibility [32]. Many marine microalgae are known to contain lipase inhibitors [32]. Lipase inhibitors inhibit lipase, which is an enzyme responsible for lipid digestion. An example of a lipase inhibitor present in microalgae is terpene caulerpenyene [33]. In addition, the form of lipid could also affect lipid digestibility. For example, polar lipids such as phospholipids were found to be better digested by Nile tilapia than neutral lipids such as triglycerides [6,7].

The digestibility of fatty acids varies with the melting point. Fatty acids with higher melting point have lower digestibility [87–89]. The digestibility of fatty acid for fish is also dependent on carbon length and degree of saturation of fatty acid [90]. With an increase in the carbon length of fatty acids, the digestibility of fatty acids decreased [91]. However, an increase in the degree of unsaturation increased the digestibility of fatty acids [87,92,93]. Typically, polyunsaturated fatty acid (PUFA) is highly digestible compared to monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA), as shown for several fishes listed in Table 4. Studies involving other biomass also showed PUFA to be more digestible and absorbed than MUFA and SFA for rainbow trout, cod, and Atlantic salmon [6,12,35,88,93–96]. One of the most important fatty acids in fish nutrition is omega-3 fatty acid. Higher digestibility of omega-3 fatty acids, including docosahexaenoic acid (DHA), were observed in *Schizochytrium* sp., a species rich in omega-3 fatty acid, particularly DHA [6,7,65].

**Table 4.** Digestibility of individual fatty acids of microalgae in fish.

| Microalgae | Biomass Processing/ Pre-treatment | Aqua Species | Pellet | Total SFA (%) | Total MUFA (%) | 20:5n3 EPA (%) | 22:6n3 DHA (%) | Total PUFA (%) | Refrence |
|---|---|---|---|---|---|---|---|---|---|
| *Chlorella* sp. | – | Nile tilapia (*Oreochromis niloticus*) | Meat grinder | 74.7 | 69.6 | – | – | 90.9 | [6] |
| *Isochrysis* sp. | – | Rainbow trout (*Oncorhynchus mykiss*) | Steam-pelleted | 58.9 | 72.2 | 87.7 | 91 | 91.7 | [35] |
| *Nannochloropsis oculata* | – | Nile tilapia (*Oreochromis niloticus*) | Meat grinder | 39.6 | 57.1 | 94 | – | 74.1 | [38] |
| *Nannochloropsis oculata* | Defatted | Nile tilapia (*Oreochromis niloticus*) | Meat grinder | 82.2 | 54.8 | 96.9 | – | 58.1 | [38] |
| *Nannochloropsis* sp. | – | Rainbow trout (*Oncorhynchus mykiss*) | Steam-pelleted | 55.9 | 44.7 | 69.4 | – | 61.8 | [35] |
| *Schizochytrium* sp. | – | Nile tilapia (*Oreochromis niloticus*) | Meat grinder | 52 | 84.8 | – | – | 97.5 | [6] |
| *Schizochytrium* sp. | – | Rainbow trout (*Oncorhynchus mykiss*) | California Pellet Mill (model CPM CL-5) | 70.6 | 92.1 | 98.7 | 98.5 | 98.5 | [65] |
| *Schizochytrium* sp. | – | Rainbow trout (*Oncorhynchus mykiss*) | California Pellet Mill (model CPM CL-5) | 77.4 | 87.5 | 98.4 | 99.1 | 98.7 | [65] |
| *Spirulina* sp. | – | Nile tilapia (*Oreochromis niloticus*) | Meat grinder | 75.5 | 76.1 | – | – | 79.1 | [6] |

### 4.4. Carbohydrate Digestibility

Digestibility of microalgal carbohydrates depends on the type of carbohydrates present in microalgal species, carbohydrate content in the biomass, and fish species [41]. Therefore, there is a wide variation in the digestibility values reported for carbohydrates of microalgae. The carbohydrate digestibility of individual microalgal species for different fishes ranged from 22% to 83% in Tables 1 and 2. Higher carbohydrate digestibility (greater than 70%) was observed for species including *Spirulina maxima* and *Chlorella vulgaris* for Nile tilapia (*Oreochromis niloticus*) [17]. This can be attributed to the presence of starch-like, easily digestible carbohydrates in these microalgae. Moreover, species including *Chlorella sorokiniana*, *Klamath*, and *Nannochloropsis sphaeroides* displayed higher carbohydrate digestibility as estimated by in vitro studies [24]. *Chlorella vulgaris* had a higher carbohydrate digestibility value than other algal species in Nile tilapia, African Catfish, and in vitro studies (Table 1).

Complex carbohydrates (e.g., non-starch polysaccharides/fibers) are difficult to be digested by fishes; hence, these compounds affect dry matter, and energy digestibil-

ity [20,25,32,62,97]. The fiber content of microalgae ranges from 5% to 18% [6,25,35]. The fiber digestibility of individual microalgal species, including *Spirulina* sp., *Chlorella* sp., and *Schizochytrium* sp. for Nile tilapia were 83%, 58%, and 71%, respectively [7]. Fiber digestibility of *Isochrysis* sp. and *Nannochloropsis* sp. in rainbow trout were 96% and 38%, respectively [35]. Even though *Isochrysis* sp. had higher fiber content than *Nannochloropsis* sp., the latter had a higher fiber digestibility than the former, indicating the importance of fiber type (soluble and insoluble) in digestibility. Compared to other nutrients, starch is a well-digested nutrient by fish and crustaceans [29]. For instance, the starch digestibility in microalgae including *Spirulina maxima*, *Chlorella vulgaris*, and *Scenedesmus dimorphus* for tilapia and African catfish were greater than 85% [17]. Typically, microalgae biomass has more starch compared to other plant-based ingredients [98]. The starch content of microalgal species varies between 7% and 49% [3]. In comparison to other microalgae, *Tetraselmis subcordiformis*, *Chlorella vulgaris*, and *Chlamydomonas reinhardtii* have a significant starch concentration in their biomass (30–49%) [3].

*4.5. Ash (Mineral) Digestibility*

The ash represents the mineral matter in the feed, which typically contains phosphorus, calcium, potassium, magnesium, and other micronutrients required for fish survival and growth. The ash content of microalgae ranges from 3% to 30%, depending on microalgae habitat [12,22,56]. Fresh water habitat species have less ash content than sea water species. Ash digestibility of microalgae in fish has been reported only in a few studies [12,22,56]. Ash digestibility of individual microalgae ranged from 23% to 83% for different fishes (Figure 1). An increase from 3% to 6% in the inclusion level of *Phaeodactylum tricornutum* in the diet of Atlantic salmon did not affect the digestibility of ash [12]. The digestibility of ash in the diet fed to seawater fishes has sometimes been reported to be a negative value [99]. This is due to the consumption of seawater by fishes [56,100]. As mentioned earlier, the microalgae-based diet also contains plant-based ingredients. In plant-based ingredients, phytate stores 80% of the total phosphorus content; the phytate chelates minerals and amino acids and is poorly digested by fish [101]. Supplementing the diet with phytases can help to achieve optimal fish growth. In a recent report, a phytase-expressing cell-wall deficient *Chlamydomonas reinhardtii* strain was developed to solve poor phosphorus digestibility and decreased mineral availability [101]. Since some microalgae have well-developed genetic tools, methods similar to those described above can be used to over express digestion-related enzymes in microalgae.

One of the important minerals for fish growth and survival is phosphorus. However, data is scarce for the phosphorous digestibility of microalgae. Based on limited reports, the digestibility of microalgal phosphorus in fish was found to range from 38% to 100% [17,18]. The phosphorus digestibility of *Nannochloropsis gaditana* for juvenile Nile tilapia (*Oreochromis niloticus*) and juvenile African catfish (*Clarias gariepinus*) was reported to be 92% and 77%, respectively [18]. The phosphorus digestibility of *Spirulina maxima*, *Chlorella vulgaris*, and *Scenedesmus dimorphus* for Nile tilapia was 93%, 86%, and 39%, respectively [17]. Similarly, phosphorus digestibility values of *Spirulina maxima*, *Chlorella vulgaris*, *Scenedesmus dimorphus* for African catfish (*Clarus gariepinus*) was 92%, 84%, and 45%, respectively [17]. The phosphorus digestibility of selected microalgae is higher than that of soy meal in different fishes, including European seabass, Senegalese sole, and rainbow trout [102–105]. The form of phosphorus found in plants is organic phytic acid, which is indigestible and considered anti-nutritional for fish, whereas the form of phosphorus found in algae is not clearly established [105–107]. But some studies suggest that phosphorous in microalgae is found in the polyphosphate granules, whose digestibility is yet to be determined [106].
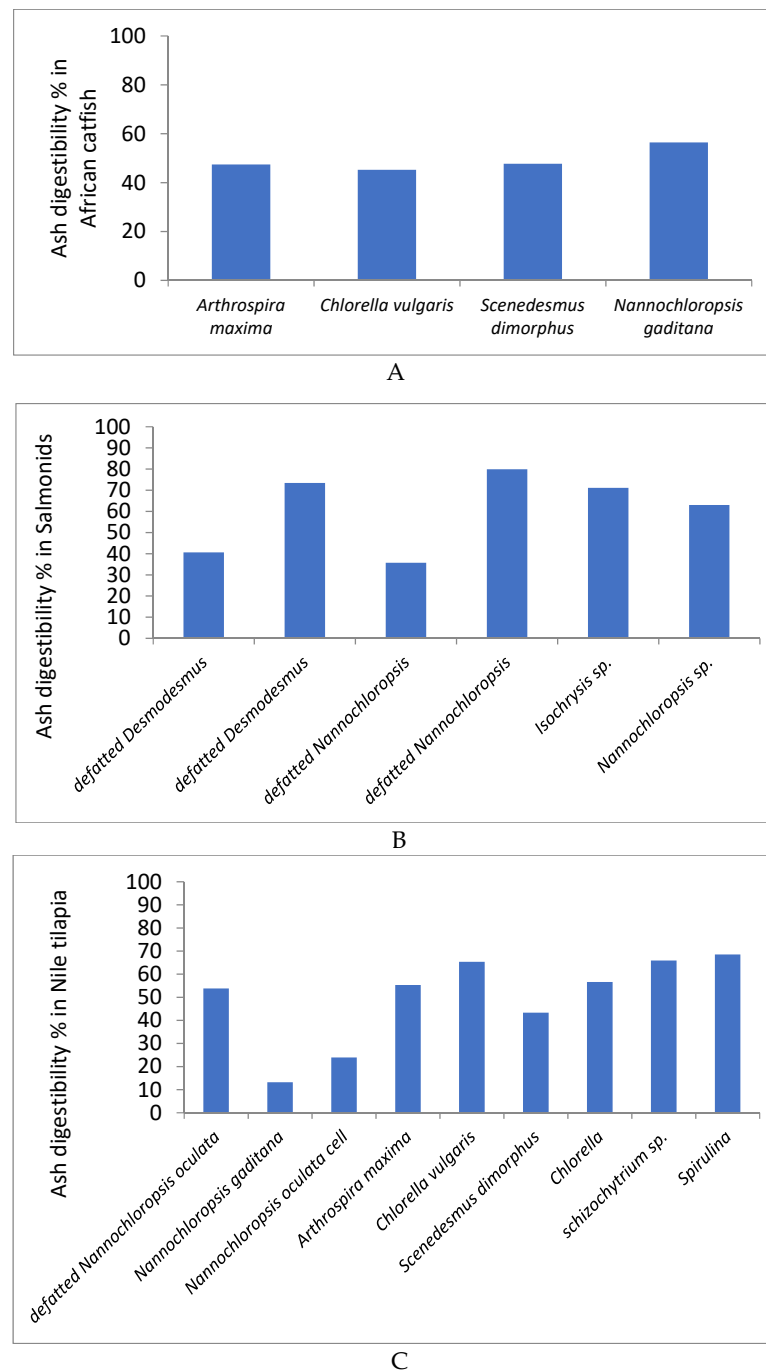
**Figure 1.** Digestibility of ash of microalgae in different fish (**A**) African catfish (**B**) Salmonids (**C**) Nile tilapia.

## 5. Energy Digestibility

Energy is required by fish to sustain normal body functions, as well as to grow and reproduce. The energy digestibility of microalgae in fishes evaluated ranged from 44% to 90% (Table 1). Energy digestibility higher than 80% was reported in rainbow trout and Nile tilapia for *Schizochytrium* sp. [6,65], juvenile European seabass for *Chlorella vulgaris* [34], juvenile Nile tilapia for *Nannochloropsis oculata*, *Spirulina* sp., and *Chlorella* sp. [7,38]. The reported energy digestibility values of microalgae were similar to that of plant-based ones, fishmeal, animal by-products, seaweed for fishes [8,11,72,77,104,108–110]. Energy digestibility in juvenile European seabass (*Dicentrarchus labrax*) was improved by 5% upon bead milling of the *Chlorella vulgaris* cell [34]. Bead milling of *Nannochloropsis gaditana* was also found to be the most efficient technique compared to other cell disruption methods to

improve energy digestibility in juvenile African catfish (*Clarias gariepinus*) and juvenile Nile tilapia, as it improved the value by nearly 17% and 18%, respectively [68]. However, in another study, the enzymatically processed *Nannochloropsis oceanica* had 11% higher energy digestibility than the same microalgae that had been treated with bead milling for European seabass juveniles (*Dicentrarchus labrax*) [34]. A significant increase in dry matter digestibility of over 40% was observed for *Tetraselmis* sp. by both bead milling and enzymatic methods in European seabass juveniles (*D. labrax*) [34]. High-pressure homogenization improved the energy digestibility of *Chlorella vulgaris* from 60% to 77% for juvenile Atlantic salmon (*Salmo salar* L.) [83]. Other than the cell disruption technique, the removal of lipid from the microalgae was found to negatively affect the energy digestibility. For instance, in juvenile Nile tilapia, the energy digestibility of defatted *Nannochloropsis oculata* was 7% less than that of the whole cell [38]. The removal of energy-rich lipid from biomass may have resulted in a reduction in energy digestibility. The above results suggest that the energy digestibility of microalgae in fish can be improved by careful selection of cell disruption techniques and avoiding lipid extraction of microalgae.

## 6. Digestibility of Individual Microalgal Species

Among all the microalgae, the nutrients and energy of *Spirulina* sp. are the most easily digested by fishes evaluated in the study (Tables 1 and 2). For instance, *Spirulina* sp.' nutrients and energy were well digested by Nile tilapia [7]. Similarly, the protein digestibility of *Spirulina platensis* and *Spirulina pacifica* estimated by in vitro (pepsin-pancreatic system) method was reported to be 94% and 86%, respectively [61]. The cell wall of *Spirulina* sp. lacks complex polysaccharides, which attributes to its higher digestibility. The dry matter, protein, lipid, and energy digestibility of *Schizochytrium* sp. were higher in rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*; 82–91%) when compared to many other microalgae [6,65]. Despite the presence of complex carbohydrates such as pectin, *Schizochytrium* sp. biomass was found to be more digestible by fish, indicating the need for further research in the future. Also, *Isochrysis* sp. in rainbow trout had higher protein, moderate lipid, and moderate energy digestibility, with values of 87%, 63%, and 73%, respectively [35]. *Isochrysis* sp. is a diatom that lacks a rigid cell wall and offers better digestibility. Although dry matter digestibility was lower, the protein digestibility was found to be higher (82%) for *Chlorella vulgaris* and *Nannochloropsis oceanica* in juvenile European seabass (*Dicentrarchus labrax*) [34]. Furthermore, in spite of poorer dry matter digestibility, the *Nannochloropsis oculata* for Nile tilapia and *Nannochloropsis sphaeroides* F&M-C117 (estimated by in vitro method) had a higher protein digestibility of 80% and 81%, respectively [24,38]. The studies mentioned above show that certain microalgae are an excellent source of digestible nutrients and readily available energy for fish.

## 7. Perspective and Future Direction

The composition and structure of microalgae cell walls affect digestibility. This underlines the importance of screening for potential commercial strains that could be easily processed for cell disruption by conventional methods. So far, only a handful of microalgal species have been tested for digestibility. Still, numerous microalgae, including *Dunaliella* sp., *Botryococcus* sp., and many cyanobacteria like *Anabaena* sp. and *Nostoc* sp., are yet to be tested for digestibility in fish and other aquatic species. It is worth mentioning that some strains (e.g., *Dunaliella salina*) lack a cell wall, which could improve the digestibility of the cellular metabolites in fish. Moreover, the environmental conditions and stress application that can change the biochemical composition of microalgae need to be explored. For instance, nitrogen starvation can lead to the accumulation of PUFA and starch, which are easily digestible by fishes. However, the biochemical response to stress like nitrogen starvation by microalgae varies from one species to another. For instance, nitrogen starvation can suppress the protein content while enhancing the carbohydrate content in several strains while no such difference can be observed for other species [69]. Therefore, optimization

of stress conditions and selection of the right species are essential to obtain a suitable biochemical profile with desired digestibility.

So far, screening of microalgal species for digestibility has been tested in limited fishes including salmonids, tilapia, sea-bass, and African catfish. As the aquaculture industry expands rapidly, more novel fish species are introduced, for which the digestibility of microalgae must be tested. The feed screening studies should include the determination of anti-nutritional factors like digestive enzyme inhibitors (eg., terpene caulerpenyene) and factors that decrease nutrient bioavailability in microalgae. Until now, microalgae digestibility studies have focused on determining the digestibility of macronutrients, including lipids, carbohydrates, protein, and energy. However, the individual classes of these macromolecules have been demonstrated to affect digestibility. Therefore, the digestibility and content of fiber, starch, non-starch polysaccharide, amino acids, fatty acids, polar lipids like phospholipids, and neutral lipids like triglycerides in microalgae must be estimated for targeted fishes.

Microalgae biomass generation involves high production cost, which is due to the energy-intensive harvesting step. Microalgal biorefinery routes could be adopted to reduce the production cost of microalgal feed ingredients. In the biorefinery route, the high-value biochemical components like lipids, carotenoids, etc., are separated from biomass, and left-over biomass is used as fish/animal feed. So far, only a few studies have reported the effect of defatted biomass on digestibility in fishes. These studies have shown mixed results. In the biorefinery routes, supercritical fluid extraction and organic solvent extraction are typically employed to produce defatted biomass. Although digestibility studies on defatted biomass generated via the above-mentioned biorefinery routes have been published, no studies on biomass generated via the saponification-based biorefinery route have been reported. The saponification-based biorefinery is primarily used for the production of carotenoids and other value-added products from microalgae. In this approach, free fatty acids, sterols, squalene, and carotenoids are separated from the biomass, while the leftover biomass can be used as fish feed. Therefore, the left-over biomasses from such biorefinery routes also need to be tested for digestibility in fishes.

The use of cell disruption technique can be very useful in improving the digestibility of microalga species. So far, cell disruption-related studies on microalgal digestibility are minimal, therefore, necessitating further exploration. At the same time, there are some side effects of pre-treatment/processing. One of the side effects of processing the biomass to disrupt the cell wall is that it may increase the content of anti-nutrient factor-like fiber, hemicellulose, trypsin inhibitor, and lectin, as demonstrated in a study involving lipid extracted biomass of *Nannochloropsis oculata* [38]. Hence, the level of these anti-nutrient substances must be measured before and after pretreatment of microalgae to ensure the high digestibility of nutrients. Although cell disruption processes are useful to improve nutrient and energy digestibility of microalgae, these techniques utilize considerable energy, thus increasing the production cost of feed [111]. Therefore, cost-effective methods need to be identified to prepare algal feed and disrupt the cell wall of microalgae. A promising cost-effective route may be the utilization of cell-wall-less microalgae such as *Isochrysis* sp. and *Dunaliella salina* as promising candidates in future digestibility studies.

The selection of a high-pressure and high-temperature extrusion process to make microalgae-based feed pellets can further improve nutrient and energy digestibility. However, microalgal digestibility studies employed mostly cold pelletization or room-temperature extrusion processes. Future studies should focus on the effect of pellet-making processes (i.e., extrusion, cold pelletization, etc.) on the digestibility of different algae. The high-pressure-high-temperature process of extrusion can damage the digestive enzymes present in microalgae, but at the same time degrade anti-digestive factors. Therefore, it would be interesting to study the effect of the extrusion process on digestibility inducing and inhibiting factors in microalgae.

On the methodology aspect, the processing conditions of pellets are not appropriately described in many studies. For instance, in several studies, the information on temperature

and pressure at which the extrusion process is carried out and the type of pellet generated is missing. As mentioned earlier, these factors could significantly affect the digestibility of nutrients and energy of microalgae. Therefore, future studies should include such data. As microalgae are included only as an ingredient in the fish diet, many studies have determined the digestibility of the fish diet rather than individual microalgae. Therefore, more data on microalgae digestibility in fishes by direct and in-direct assessment is required in the future. Nevertheless, from the limited studies, the nutrient and energy digestibility of microalgae in fishes seems promising.

## 8. Conclusions

Microalgae are a promising aquafeed ingredient with the right blend of nutrients and functional components. Microalgae-based feed displays a wide variation in nutrient and energy digestibility, depending on several factors. This stresses the importance of digestibility estimation in microalgae-based feed formulation. *Spirulina* sp., *Isochrysis* sp., *Chlorella vulgaris*, and *Schizochytrium* sp. are some of the microalgae, which showed very high nutrient and digestibility values for the fishes. So far, the use of cell disruption techniques, pellet processing methods, selection of appropriate species, and optimization of environmental conditions to generate a suitable biochemical profile have been useful in improving the digestibility of microalgae for fishes. More screening of microalgae for digestibility in different fishes will further reveal its true potential.

**Author Contributions:** Conceptualization: P.D.; Reviewing and Editing: S.N.A.; Data curation, Writing, and Original draft preparation: M.A.Q., M.I.A.T., S.K.; Data collection, Investigation, validation: C.M. Supervision: H.A.J. All authors have read and agreed to the published version of the manuscript.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Becker, W. 21 Microalgae for Aquaculture. In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*; Wiley: Hoboken, NJ, USA, 2004; p. 380.
2. Fleurence, J.; Morançais, M.; Dumay, J.; Decottignies, P.; Turpin, V.; Munier, M.; Garcia-Bueno, N.; Jaouen, P. What are the prospects for using seaweed in human nutrition and for marine animals raised through aquaculture? *Trends Food Sci. Technol.* **2012**, *27*, 57–61. [CrossRef]
3. Nagappan, S.; Das, P.; AbdulQuadir, M.; Thaher, M.; Khan, S.; Mahata, C.; Al-Jabri, H.; Vatland, A.K.; Kumar, G. Potential of microalgae as a sustainable feed ingredient for aquaculture. *J. Biotechnol.* **2021**, *341*, 1–20. [CrossRef]
4. Tibbetts, S.M.; Milley, J.E.; Lall, S.P. Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. *J. Appl. Phycol.* **2015**, *27*, 1109–1119. [CrossRef]
5. Glencross, B.D.; Booth, M.; Allan, G.L. A feed is only as good as its ingredients–a review of ingredient evaluation strategies for aquaculture feeds. *Aquac. Nutr.* **2007**, *13*, 17–34. [CrossRef]
6. Sarker, P.; Gamble, M.; Kelson, S.; Kapuscinski, A. Nile tilapia (*Oreochromis niloticus*) show high digestibility of lipid and fatty acids from marine *Schizochytrium* sp. and of protein and essential amino acids from freshwater *Spirulina* sp. feed ingredients. *Aquac. Nutr.* **2016**, *22*, 109–119. [CrossRef]
7. Sarker, P.K.; Kapuscinski, A.R.; Lanois, A.J.; Livesey, E.D.; Bernhard, K.P.; Coley, M.L. Towards sustainable aquafeeds: Complete substitution of fish oil with marine microalga *Schizochytrium* sp. improves growth and fatty acid deposition in juvenile Nile tilapia (*Oreochromis niloticus*). *PLoS ONE* **2016**, *11*, e0156684.
8. Cardinaletti, G.; Messina, M.; Bruno, M.; Tulli, F.; Poli, B.; Giorgi, G.; Chini-Zittelli, G.; Tredici, M.; Tibaldi, E. 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* **2018**, *485*, 173–182. [CrossRef]
9. Kiron, V.; Sørensen, M.; Huntley, M.; Vasanth, G.K.; Gong, Y.; Dahle, D.; Palihawadana, A.M. Defatted biomass of the microalga, *Desmodesmus* sp., can replace fishmeal in the feeds for Atlantic salmon. *Front. Mar. Sci* **2016**, *3*, 67. [CrossRef]

10. Cho, C.; Kaushik, S. Nutritional energetics in fish: Energy and protein utilization in rainbow trout (*Salmo gairdneri*). *Asp. Food Prod. Consum. Energy Values* **1990**, *61*, 132–172.

11. Burr, G.; Barrows, F.; Gaylord, G.; Wolters, W. Apparent digestibility of macro-nutrients and phosphorus in plant-derived ingredients for Atlantic salmon, *Salmo salar* and Arctic charr, *Salvelinus alpinus*. *Aquac. Nutr.* **2011**, *17*, 570–577. [CrossRef]

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

13. McGoogan, B.B.; Reigh, R.C. Apparent digestibility of selected ingredients in red drum (Sciaenops ocellatus) diets. *Aquaculture* **1996**, *141*, 233–244. [CrossRef]

14. Sullivan, J.A.; Reigh, R.C. Apparent digestibility of selected feedstuffs in diets for hybrid striped bass (Morone saxatilis♀ x Morone chrysops♂). *Aquaculture* **1995**, *138*, 313–322. [CrossRef]

15. Wilson, R.P. Channel Catfish, *Ictalurus punctatus*. In *Handbook of Nutrient Requirements of Finfish*; CRC Press: Boca Raton, FL, USA, 2017; pp. 35–54.

16. Schmitz, O.; Greuel, E.; Preffer, E. Digestibility of crude protein and organic matter of potential sources of dietary protein for eels (*Anguilla anguilla*, L.). *Aquaculture* **1984**, *41*, 21–30. [CrossRef]

17. Teuling, E.; Schrama, J.W.; Gruppen, H.; Wierenga, P.A. Effect of cell wall characteristics on algae nutrient digestibility in Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarus gariepinus*). *Aquaculture* **2017**, *479*, 490–500. [CrossRef]

18. Teuling, E.; Wierenga, P.A.; Agboola, J.O.; Gruppen, H.; Schrama, J.W. Cell wall disruption increases bioavailability of *Nannochloropsis gaditana* nutrients for juvenile Nile tilapia (Oreochromis niloticus). *Aquaculture* **2019**, *499*, 269–282. [CrossRef]

19. Hossain, M.; Jauncey, K. Studies on the protein, energy and amino acid digestibility of fish meal, mustard oilcake, linseed and sesame meal for common carp (*Cyprinus carpio* L.). *Aquaculture* **1989**, *83*, 59–72. [CrossRef]

20. Allan, G.L.; Parkinson, S.; Booth, M.A.; Stone, D.A.; Rowland, S.J.; Frances, J.; Warner-Smith, R. Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. *Aquaculture* **2000**, *186*, 293–310. [CrossRef]

21. Gong, Y.; Guterres, H.; Huntley, M.; Sørensen, M.; Kiron, V. Digestibility of the defatted microalgae *Nannochloropsis* sp. and *Desmodesmus* sp. when fed to Atlantic salmon, *Salmo salar*. *Aquac. Nutr.* **2018**, *24*, 56–64. [CrossRef]

22. Sørensen, M.; Gong, Y.; Bjarnason, F.; Vasanth, G.K.; Dahle, D.; Huntley, M.; Kiron, V. *Nannochloropsis oceania*-derived defatted meal as an alternative to fishmeal in Atlantic salmon feeds. *PLoS ONE* **2017**, *12*, e0179907. [CrossRef]

23. Vizcaíno, A.; López, G.; Sáez, M.; Jiménez, J.; Barros, A.; Hidalgo, L.; Camacho-Rodríguez, J.; Martínez, T.; Cerón-García, M.; Alarcón, F. Effects of the microalga *Scenedesmus almeriensis* as fishmeal alternative in diets for gilthead sea bream, *Sparus aurata*, juveniles. *Aquaculture* **2014**, *431*, 34–43. [CrossRef]

24. Niccolai, A.; Zittelli, G.C.; Rodolfi, L.; Biondi, N.; Tredici, M.R. Microalgae of interest as food source: Biochemical composition and digestibility. *Algal Res.* **2019**, *42*, 101617. [CrossRef]

25. Scholz, M.J.; Weiss, T.L.; Jinkerson, R.E.; Jing, J.; Roth, R.; Goodenough, U.; Posewitz, M.C.; Gerken, H.G. Ultrastructure and composition of the *Nannochloropsis gaditana* cell wall. *Eukaryot. Cell* **2014**, *13*, 1450–1464. [CrossRef]

26. Palinska, K.A.; Krumbein, W.E. Perforation patterns in the peptidoglycan wall of filamentous cyanobacteria. *J. Phycol.* **2000**, *36*, 139–145. [CrossRef]

27. Marshall, R.; McKinley, S.; Pearce, C.M. Effects of nutrition on larval growth and survival in bivalves. *Rev. Aquac.* **2010**, *2*, 33–55. [CrossRef]

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

29. Skrede, A.; Mydland, L.; Ahlstrøm, Ø.; Reitan, K.; Gislerød, H.; Øverland, M. Evaluation of microalgae as sources of digestible nutrients for monogastric animals. *Anim. Feed Sci. Technol.* **2011**, *20*, 131–142. [CrossRef]

30. Nuño, K.; Villarruel-López, A.; Puebla-Pérez, A.; Romero-Velarde, E.; Puebla-Mora, A.; Ascencio, F. Effects of the marine microalgae Isochrysis galbana and Nannochloropsis oculata in diabetic rats. *J. Funct. Foods* **2013**, *5*, 106–115. [CrossRef]

31. Rodehutscord, M.; Borchert, F.; Gregus, Z.; Pfeffer, E. Availability and utilisation of free lysine in rainbow trout (*Oncorhynchus mykiss*): 2. Comparison of l-lysine· HCl and l-lysine sulphate. *Aquaculture* **2000**, *187*, 177–183. [CrossRef]

32. Valente, L.M.P.; Custódio, M.; Batista, S.; Fernandes, H.; Kiron, V. Defatted microalgae (Nannochloropsis sp.) from biorefinery as a potential feed protein source to replace fishmeal in European sea bass diets. *Fish Physiol. Biochem.* **2019**, *45*, 1067–1081. [CrossRef]

33. Bitou, N.; Ninomiya, M.; Tsujita, T.; Okuda, H. Screening of lipase inhibitors from marine algae. *Lipids* **1999**, *34*, 441–445. [CrossRef] [PubMed]

34. Batista, S.; Pintado, M.; Marques, A.; Abreu, H.; Silva, J.L.; Jessen, F.; Tulli, F.; Valente, L.M. 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.* **2020**, *32*, 3429–3446. [CrossRef]

35. Sarker, P.K.; Kapuscinski, A.R.; Vandenberg, G.W.; Proulx, E.; Sitek, A.J.; Thomsen, L. Towards sustainable and ocean-friendly aquafeeds: Evaluating a fish-free feed for rainbow trout (*Oncorhynchus mykiss*) using three marine microalgae species. *Elem. Sci. Anthrop.* **2020**, *8*, 5. [CrossRef]

36. Sinha, A.K.; Kumar, V.; Makkar, H.P.; De Boeck, G.; Becker, K. Non-starch polysaccharides and their role in fish nutrition–A review. *Food Chem.* **2011**, *127*, 1409–1426. [CrossRef]

37. Norambuena, F.; Hermon, K.; Skrzypczyk, V.; Emery, J.A.; Sharon, Y.; Beard, A.; Turchini, G.M. Algae in fish feed: Performances and fatty acid metabolism in juvenile Atlantic salmon. *PLoS ONE* **2015**, *10*, e0124042. [CrossRef]

38. Sarker, P.K.; Kapuscinski, A.R.; Bae, A.Y.; Donaldson, E.; Sitek, A.J.; Fitzgerald, D.S.; Edelson, O.F. Towards sustainable aquafeeds: Evaluating substitution of fishmeal with lipid-extracted microalgal co-product (*Nannochloropsis oculata*) in diets of juvenile Nile tilapia (*Oreochromis niloticus*). *PLoS ONE* **2018**, *13*, e0201315. [CrossRef] [PubMed]

39. Domozych, D.; Ciancia, M.; Fangel, J.U.; Mikkelsen, M.D.; Ulvskov, P.; Willats, W.G. The cell walls of green algae: A journey through evolution and diversity. *Front. Plant Sci.* **2012**, *3*, 82. [CrossRef] [PubMed]

40. Karapanagiotidis, I.T.; Bell, M.V.; Little, D.C.; Yakupitiyage, A. Replacement of dietary fish oils by alpha-linolenic acid-rich oils lowers omega 3 content in tilapia flesh. *Lipids* **2007**, *42*, 547–559. [CrossRef]

41. Wee, K. Aquaculture Nutrition Research in Australia. In Proceedings of the Aquaculture Nutrition Workshop Salamander Bay, New South Wales, Australia, 15–17 April 1991.

42. Falge, R.; Schpanof, L.; Jurss, K. Amylase, esterase and protease activity in the intestine content of rainbow trout *Salmo gairdneri* Rich., after feeding with feed containing different amounts of starch and protein. *J. Ichthyol.* **1978**, *18*, 283–287.

43. Becker, W. 18 Microalgae In Human and Animal Nutrition. In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*; Wiley Online Library: Hoboken, NJ, USA, 2004; Volume 312.

44. Encarnação, P.; de Lange, C.; Rodehutscord, M.; Hoehler, D.; Bureau, W.; Bureau, D.P. Diet digestible energy content affects lysine utilization, but not dietary lysine requirements of rainbow trout (*Oncorhynchus mykiss*) for maximum growth. *Aquaculture* **2004**, *235*, 569–586. [CrossRef]

45. Mišurcová, L. Seaweeds digestibility and Methods Used for Digestibility Determination. In *Handbook of Marine Macroalgae: Biotechnology and Applied Phycology*; John Wiley & Sons, Ltd: Hoboken, NJ, USA, 2012.

46. Duval, B.; Shetty, K.; Thomas, W.H. Phenolic compounds and antioxidant properties in the snow alga *Chlamydomonas nivalis* after exposure to UV light. *J. Appl. Phycol.* **1999**, *11*, 559–566. [CrossRef]

47. Goiris, K.; Muylaert, K.; Fraeye, I.; Foubert, I.; De Brabanter, J.; De Cooman, L. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *J. Appl. Phycol.* **2012**, *24*, 1477–1486. [CrossRef]

48. Li, H.-B.; Cheng, K.-W.; Wong, C.-C.; Fan, K.-W.; Chen, F.; Jiang, Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chem.* **2007**, *102*, 771–776. [CrossRef]

49. Carpenter, K.; Booth, V. In Damage to lysine in food processing: Its measurement and its significance. *Nutr. Abstr. Rev.* **1973**, *43*, 423–451.

50. Opstvedt, J.; Miller, R.; Hardy, R.W.; Spinelli, J. Heat-induced changes in sulfhydryl groups and disulfide bonds in fish protein and their effect on protein and amino acid digestibility in rainbow trout (*Salmo gairdneri*). *J. Agric. Food Chem.* **1984**, *32*, 929–935. [CrossRef]

51. Evans, D.; Claiborne, J. *The Physiology of Fishes*; CRC Press: Boca Raton, FL, USA, 2005.

52. Shi, Z.; Li, X.-Q.; Chowdhury, M.K.; Chen, J.-N.; Leng, X.-J. 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* **2016**, *460*, 37–44. [CrossRef]

53. Guedes, A.C.; Sousa-Pinto, I.; Malcata, F.X. Application of Microalgae Protein to Aquafeed. In *Handbook of Marine Microalgae*; Elsevier: Amsterdam, The Netherlands, 2015; pp. 93–125.

54. Halim, R.; Danquah, M.K.; Webley, P.A. Extraction of oil from microalgae for biodiesel production: A review. *Biotechnol. Adv.* **2012**, *30*, 709–732. [CrossRef] [PubMed]

55. Berge, G.; Hatlen, B.; Odom, J.; Ruyter, B. Physical treatment of high EPA *Yarrowia lipolytica* biomass increases the availability of n-3 highly unsaturated fatty acids when fed to Atlantic salmon. *Aquac. Nutr.* **2013**, *19*, 110–121. [CrossRef]

56. Gong, Y.; Bandara, T.; Huntley, M.; Johnson, Z.I.; Dias, J.; Dahle, D.; Sørensen, M.; Kiron, V. Microalgae *Scenedesmus* sp. as a potential ingredient in low fishmeal diets for Atlantic salmon (*Salmo salar* L.). *Aquaculture* **2019**, *501*, 455–464. [CrossRef]

57. Shene, C.; Monsalve, M.T.; Vergara, D.; Lienqueo, M.E.; Rubilar, M. High pressure homogenization of *Nannochloropsis oculata* for the extraction of intracellular components: Effect of process conditions and culture age. *Eur. J. Lipid Sci. Technol.* **2016**, *118*, 631–639. [CrossRef]

58. Maehre, H.K.; Edvinsen, G.K.; Eilertsen, K.-E.; Elvevoll, E.O. 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.* **2016**, *28*, 581–590. [CrossRef]

59. McMillan, J.R.; Watson, I.A.; Ali, M.; Jaafar, W. Evaluation and comparison of algal cell disruption methods: Microwave, waterbath, blender, ultrasonic and laser treatment. *Appl. Energy* **2013**, *103*, 128–134. [CrossRef]

60. Kousoulaki, K.; Mørkøre, T.; Nengas, I.; Berge, R.; Sweetman, J. Microalgae and organic minerals enhance lipid retention efficiency and fillet quality in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **2016**, *451*, 47–57. [CrossRef]

61. MišurCoVá, L.; KráčMar, S.; KLeJduS, B.; VaCeK, J. Nitrogen content, dietary fiber, and digestibility in algal food products. *Czech J. Food Sci.* **2010**, *28*, 27–35. [CrossRef]

62. Douglas, M.W.; Parsons, C.M.; Bedford, M.R. Effect of various soybean meal sources and Avizyme on chick growth performance and ileal digestible energy. *J. Appl. Poult. Res.* **2000**, *9*, 74–80. [CrossRef]

63. Cowieson, A.; Ravindran, V.; Selle, P. Influence of dietary phytic acid and source of microbial phytase on ileal endogenous amino acid flows in broiler chickens. *Poult. Sci.* **2008**, *87*, 2287–2299. [CrossRef]

64. Fu, C.C.; Hung, T.C.; Chen, J.Y.; Su, C.H.; Wu, W.T. Hydrolysis of microalgae cell walls for production of reducing sugar and lipid extraction. *Bioresour. Technol.* **2010**, *101*, 8750–8754. [CrossRef] [PubMed]

65. Bélanger, A.; Sarker, P.K.; Bureau, D.P.; Chouinard, Y.; Vandenberg, G.W. Apparent digestibility of macronutrients and fatty Acids from microalgae (*Schizochytrium* sp.) fed to rainbow trout (*Oncorhynchus mykiss*): A potential candidate for fish oil substitution. *Animals* **2021**, *11*, 456. [CrossRef]

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

67. Tibbetts, S.M.; Yasumaru, F.; Lemos, D. 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.* **2017**, *21*, 76–80. [CrossRef]

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

69. Nagappan, S.; Devendran, S.; Tsai, P.-C.; Jayaraman, H.; Alagarsamy, V.; Pugazhendhi, A.; Ponnusamy, V.K. Metabolomics integrated with transcriptomics and proteomics: Evaluation of systems reaction to nitrogen deficiency stress in microalgae. *Process Biochem.* **2020**, *91*, 1–14. [CrossRef]

70. Wilson, R.P.; John, E.H. Protein and amino acid requirements of fishes. *Annu. Rev. Nutr.* **1986**, *6*, 225–244. [CrossRef]

71. Guimarães, I.; Pezzato, L.E.; Barros, M.M. Amino acid availability and protein digestibility of several protein sources for Nile tilapia, Oreochromis niloticus. *Aquac. Nutr.* **2008**, *14*, 396–404. [CrossRef]

72. Reitan, K.I.; Rainuzzo, J.R.; Olsen, Y. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. *J. Phycol.* **1994**, *30*, 972–979. [CrossRef]

73. Sørensen, M.; Penn, M.; El-Mowafi, A.; Storebakken, T.; Chunfang, C.; Øverland, M.; Krogdahl, Å. Effect of stachyose, raffinose and soya-saponins supplementation on nutrient digestibility, digestive enzymes, gut morphology and growth performance in Atlantic salmon (*Salmo salar*, L). *Aquaculture* **2011**, *314*, 145–152. [CrossRef]

74. Refstie, S.; Storebakken, T.; Roem, A.J. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. *Aquaculture* **1998**, *162*, 301–312. [CrossRef]

75. Krogdahl, Å.; Sundby, A.; Olli, J.J. Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) digest and metabolize nutrients differently. Effects of water salinity and dietary starch level. *Aquaculture* **2004**, *229*, 335–360. [CrossRef]

76. Grisdale-Helland, B.; Helland, S. Replacement of protein by fat and carbohydrate in diets for Atlantic salmon (*Salmo salar*) at the end of the freshwater stage. *Aquaculture* **1997**, *152*, 167–180. [CrossRef]

77. Pereira, R.; Valente, L.M.; Sousa-Pinto, I.; Rema, P. Apparent nutrient digestibility of seaweeds by rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*). *Algal Res.* **2012**, *1*, 77–82. [CrossRef]

78. Mabeau, S.; Fleurence, J. Seaweed in food products: Biochemical and nutritional aspects. *Trends Food Sci. Technol.* **1993**, *4*, 103–107. [CrossRef]

79. Bobin-Dubigeon, C.; Hoebler, C.; Lognone, V.; Dagorn-Scaviner, C.; Mabeau, S. Chemical composition, physico-chemical properties, enzymatic inhibition and fermentative characteristics of dietary fibres from edible seaweeds. *Sci. Aliment.* **1997**, *17*, 619–639.

80. Wong, K.H.; Cheung, P.C. Nutritional evaluation of some subtropical red and green seaweeds Part II. In vitro protein digestibility and amino acid profiles of protein concentrates. *Food Chem.* **2001**, *72*, 11–17. [CrossRef]

81. Horie, Y.; Sugase, K.; Horie, K. Physiological differences of soluble and insoluble dietary fibre fractions of brown algae and mushrooms in pepsin activity in vitro and protein digestibility. *Asia Pac. J. Clin. Nutr.* **1995**, *4*, 251–255.

82. Marrion, O.; Fleurence, J.; Schwertz, A.; Guéant, J.-L.; Mamelouk, L.; Ksouri, J.; Villaume, C. Evaluation of protein in vitro digestibility of *Palmaria palmata* and *Gracilaria verrucosa*. *J. Appl. Phycol.* **2005**, *17*, 99–102. [CrossRef]

83. Tibbetts, S.M.; Mann, J.; Dumas, A. Apparent digestibility of nutrients, energy, essential amino acids and fatty acids of juvenile Atlantic salmon (*Salmo salar* L.) diets containing whole-cell or cell-ruptured *Chlorella vulgaris* meals at five dietary inclusion levels. *Aquaculture* **2017**, *481*, 25–39. [CrossRef]

84. Devi, M.A.; Subbulakshmi, G.; Devi, K.M.; Venkataraman, L.V. Studies on the proteins of mass-cultivated, blue-green alga (*Spirulina platensis*). *J. Agric. Food Chem.* **1981**, *29*, 522–525. [CrossRef]

85. Sargent, J.R.; Tocher, D.R.; Bell, J.G. The lipids. In *Fish Nutrition*; Academic Press: Cambridge, MA, USA, 2003; pp. 181–257.

86. Cho, C.; Slinger, S.; Bayley, H. Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. *Comp. Biochem. Physiol.* **1982**, *73B*, 25–41. [CrossRef]

87. Sigurgisladottir, S.; Lall, S.P.; Parrish, C.C.; Ackman, R.G. Cholestane as a digestibility marker in the absorption of polyunsaturated fatty acid ethyl esters in Atlantic salmon. *Lipids* **1992**, *27*, 418–424. [CrossRef]

88. Austreng, E.; Skrede, A.; Eldegard, Å. Digestibility of fat and fatty acids in rainbow trout and mink. *Aquaculture* **1980**, *19*, 93–95. [CrossRef]

89. Turchini, G.M.; Torstensen, B.E.; Ng, W.K. Fish oil replacement in finfish nutrition. *Rev. Aquac.* **2009**, *1*, 10–57. [CrossRef]

90. Merican, Z.O.; Shim, K. Apparent digestibility of lipid and fatty acids in residual lipids of meals by adult *Penaeus monodon*. *Aquaculture* **1995**, *133*, 275–286. [CrossRef]

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

92. Johnsen, R.; Grahl-Nielsen, O.; Roem, A. Relative absorption of fatty acids by Atlantic salmon *Salmo salar* from different diets, as evaluated by multivariate statistics. *Aquac. Nutr.* **2000**, *6*, 255–261. [CrossRef]

93. Ng, W.-K.; Codabaccus, B.M.; Carter, C.G.; Nichols, P.D. Replacing dietary fish oil with palm fatty acid distillate improves fatty acid digestibility in rainbow trout, *Oncorhynchus mykiss*, maintained at optimal or elevated water temperature. *Aquaculture* **2010**, *309*, 165–172. [CrossRef]

94.  Ng, W.K.; Campbell, P.J.; Dick, J.R.; Bell, J.G. Interactive effects of dietary palm oil concentration and water temperature on lipid digestibility in rainbow trout, *Oncorhynchus mykiss*. *Lipids* **2003**, *38*, 1031–1038. [CrossRef]

95.  Caballero, M.; Obach, A.; Rosenlund, G.; Montero, D.; Gisvold, M.; Izquierdo, M. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **2002**, *214*, 253–271. [CrossRef]

96.  Hansen, J.Ø.; Berge, G.M.; Hillestad, M.; Krogdahl, Å.; Galloway, T.F.; Holm, H.; Holm, J.; Ruyter, B. Apparent digestion and apparent retention of lipid and fatty acids in Atlantic cod (*Gadus morhua*) fed increasing dietary lipid levels. *Aquaculture* **2008**, *284*, 159–166. [CrossRef]

97.  Lech, G.P.; Reigh, R.C. Plant products affect growth and digestive efficiency of cultured Florida pompano (*Trachinotus carolinus*) fed compounded diets. *PLoS ONE* **2012**, *7*, e34981. [CrossRef]

98.  Roy, S.S.; Pal, R. Microalgae in aquaculture: A review with special references to nutritional value and fish dietetics. In *Proceedings of the Zoological Society*; Springer: Berlin/Heidelberg, Germany, 2015; pp. 1–8.

99.  Moheimani, N.R.; Vadiveloo, A.; Ayre, J.M.; Pluske, J.R. Nutritional profile and in vitro digestibility of microalgae grown in anaerobically digested piggery effluent. *Algal Res.* **2018**, *35*, 362–369. [CrossRef]

100.  Thodesen, J.; Storebakken, T.; Shearer, K.D.; Rye, M.; Bjerkeng, B.; Gjerde, B. Genetic variation in mineral absorption of large Atlantic salmon (*Salmo salar*) reared in seawater. *Aquaculture* **2001**, *194*, 263–271. [CrossRef]

101.  Erpel, F.; Restovic, F.; Arce-Johnson, P. Development of phytase-expressing *Chlamydomonas reinhardtii* for monogastric animal nutrition. *BMC Biotechnol.* **2016**, *16*, 1–7. [CrossRef]

102.  Dias, J.; Yúfera, M.; Valente, L.M.; Rema, P. Feed transit and apparent protein, phosphorus and energy digestibility of practical feed ingredients by Senegalese sole (*Solea senegalensis*). *Aquaculture* **2010**, *302*, 94–99. [CrossRef]

103.  Kaushik, S. Phosphorus requirements and intake in fish. *INRA Prod. Anim.* **2005**, *18*, 203–208. [CrossRef]

104.  Oliva-Teles, A. Apparent digestibility coefficients of feedstuffs in seabass (*Dicentrarchus labrax*) juveniles. *Aquat. Living Resour.* **1998**, *11*, 187–191.

105.  Pereira, H.; Sardinha, M.; Santos, T.; Gouveia, L.; Barreira, L.; Dias, J.; Varela, J. 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.* **2020**, *47*, 101869. [CrossRef]

106.  Feng, W.; Zhu, Y.; Wu, F.; He, Z.; Zhang, C.; Giesy, J.P. Forms and lability of phosphorus in algae and aquatic macrophytes characterized by solution 31 P NMR coupled with enzymatic hydrolysis. *Sci. Rep.* **2016**, *6*, 1–10. [CrossRef]

107.  Mukherjee, C.; Chowdhury, R.; Ray, K. Phosphorus recycling from an unexplored source by polyphosphate accumulating microalgae and cyanobacteria—A step to phosphorus security in agriculture. *Front. Microbiol.* **2015**, *6*, 1421. [CrossRef]

108.  Kaushik, S.; Coves, D.; Dutto, G.; Blanc, D. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture* **2004**, *230*, 391–404. [CrossRef]

109.  Bureau, D.; Harris, A.; Cho, C. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **1999**, *180*, 345–358. [CrossRef]

110.  Council, N.R. *Nutrient Requirements of Fish and Shrimp*; National Academies Press: Washington, DC, USA, 2011.

111.  Shields, R.; Lupatsch, I. 5 Algae for Aquaculture and Animal Feeds. In *Microalgal Biotechnology: Integration and Economy*; De Gruyter: Berlin, Germany, 2012; pp. 79–100.