Dietary Microalgae on Fish Health and Fillet Quality

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An increase in the consumption of food fish, combined with a decrease in the harvest of fish, is driving the aquaculture industry at a fast pace. In parallel with the growth in the aquaculture sector and resulting stresses, the prevalence of diseases in farmed fish can increase.

aquaculture microalgae feed fish

1. Introduction

The fish farming industry has turned into one of the most sustainable tactics to provide food for humans [1]. However, fish farming is altered by various interconnected factors, including the fish environment, diet, and farmed stock [2]. Boosting fish farming conditions via appropriate and healthy diets is considered as an important approach, causing improvement in fish growth, feed utilization, health, and welfare as well as carcass quality [3][4][5][6]. Among different feed additives, natural feed additives, such as microalgae, are preferable as they contain high nutritional value, are inexpensive, readily available, and environmentally safe [7][8][9][10]. The incorporation of these additives into fish feed can provide vitamins, polyunsaturated fatty acids, some amino acids, polysaccharides, minerals, and pigments [11]. The most important microalgae in fish farming are *Spirulina*, *Chlorella*, *Dunaliella*, and *Haematocussus*, which are available commercially [12]. In fish feed, microalgae are viable as a partial replacement for fish meal and positively affect the fish growth, disease resistance, skin coloration, and physiological activity [13][14]. Meanwhile, the influence of dietary microalgae on fish meat quality traits is well documented in different studies.

2. Effects of Microalgae on Fish Fillet Proximate Composition

In light of the fact that lipids are critical to meeting energy needs and offering essential fatty acids, they are important nutrients included in aquafeeds for marine fishes. These fatty acids are necessary for sustaining health, reproduction, and growth [15]. Fish oil is an excellent digestible source of energy that also contributes to a significant amount of essential fatty acids, particularly omega-3 (n-3) highly unsaturated fatty acids, such as EPA and DHA that are widely acknowledged as the primary lipid sources used in the production of aquafeeds. These fatty acids are widely valued for their health advantages in the development of the nervous system and the prevention of inflammatory illnesses, as well as neurological and cardiovascular problems, and thus are recommended as an integral part of natural medications for improving human health. Due to issues with sustainability, economics, and the environment, the production of aquafeeds that rely on fish oil is constrained [15]. The capture fisheries are a major source of essential fatty acids and protein for the aquaculture of finfish. In 2018, 19% of capture fishery production was converted to fish oil and fish meal for aquafeeds. Finding compositions of alternative and sustainable sources of oil and protein, especially DHA and EPA, has become a top challenge for the aquaculture industry due to the limited supply of these compositions and the expansion of fish farming [16]. For the aquaculture business to continue to expand and be sustainable, fewer fish meals must be used in aquaculture diets in favor of other affordable and environmentally-friendly foods [17]. Fish meal and fish oil have been lowered, but not entirely eliminated, by aquafeed makers, who are now looking for alternatives that are less expensive [18]. In recent years, various studies have been carried out to replace fish meal with diverse sources, like plant-derived protein sources and microalgae [13][19][20][21][22]. Microalgae contain many biological compounds such as n-3 LC-PUFA, polysaccharides, and carotenoids [23]. DHA and EPA have significant effects on human health by reducing cardiovascular diseases and mitigating inflammatory diseases [23]. A fish fillet (a dark muscle), rib meat (a light muscle), mesenteric fat, and liver are a few of the tissues where lipids are stored. These tissues have several purposes for storing and digesting lipids. Light and dark muscles serve as greater short-term storage for localized energy requirements, the liver processes lipids, and mesenteric fat normally offers long-term lipid storage [24].

Atlantic cod (*Gadus morhua*) growth, body composition, and feed intake were studied after fish were fed with diets containing fish meal protein partially replaced with microalgae. For instance, the fish fed isocaloric diets containing protein substituted by a mixture of dried *Isochrysis* sp. and *Nannochloropsis* sp. (15 and 30%) for 84 days exhibited no changes, in terms of feed conversion ratios, survival, viscero-somatic indices, or n-3 and n-6 fatty acids in muscles compared to the treatment groups. No change was seen in fatty acid profiles of fish muscle in the control and fish fed with 15% replacement; however, the eicosenoic acid 20:1(n-9) level was increased in the treated fish. The growth and feed intake, which were related to algal incorporation level, considerably decreased in algae-fed fish, most likely due to palatability issues. The authors assumed that the amount of specific fatty acids could not guarantee the level in the cod fillet since differential metabolism of fatty acids took place in response to energy requirement. Therefore, a higher amount of EPA in the cod diet could not increase the quantity in fish muscle [17].

Haas et al. [25] conducted a research study that compared the effectiveness of using microalgae *Pavlova viridis* and *Nannochloropsis* sp. as n-3 PUFA sources in the diets of juvenile European sea bass (*Dicentrarchus labrax* L.). The study involved administering different isonitrogenous and isoenergetic diets, which were prepared using a fish oil diet, plant oil (a combination of linseed, rapeseed, and sunflower oil), and a basal diet consisting of fish oil and *Pavlova viridis* and *Nannochloropsis* sp. lipids. The total PUFA was the highest in the *Pavlova* 100% group, followed by *Pavlova* 50%, *Nannochloropsis* 100%, and *Nannochloropsis* 50%, due to the high content of linolenic and linoleic acids coming from microalgal products and plant oils, whereas the lowest levels were observed in the fish oil group. The fish oil diet had the highest amounts of total fatty acids and DHA, but not significantly higher than that in the *Pavlova* 50% and *Pavlova* 100% groups. Also, the highest content of EPA was in the *Pavlova* 100% diet. The results demonstrated that replacing 50% of the fish oil in the diet with *Nannochloropsis* sp. meal and 100% of it with *Pavlova viridis* meal could be implemented without posing a detrimental impact on the nutrient utilization and growth performance of juvenile sea bass [25].

Sarker et al. [26] showed that the complete substitution of fish oil with microalga *Schizochytrium* sp. in juvenile Nile tilapia feeds improves growth and the deposition of n3 -LC PUFA content in tilapia fillets. Higher contents of DHA were observed in fillets which led to high DHA: EPA ratios in fillets compared to controls. Saturated fatty acid (SFA) was higher in *Schizochytrium*-fed fish and in the SFA profile, the composition of 15:0, 18:0, 20:0, and 22:0 did not significantly differ in dietary treatments; despite this, palmitic acid (16:0) content increased, and the highest concentration was observed in the *Schizochytrium*-fed fish. Fish fed a control diet showed the highest content of mono-unsaturated fatty acids (MUFA), which was related to MUFA amount in the tested diets. Meanwhile, the 100% substitution of fish oil with *Schizochytrium* resulted in n3: n6 and DHA: EPA ratios of 1.4:1 and 34.2:1, respectively, in fish fillets, suggesting the possible inclusion of this microalga in tilapia feed with tailoring the n3 PUFA composition of tilapia muscles. The researchers assumed that fish feed with fish oil makes the fish fillet susceptible to lipid oxidation, producing the adverse effects of fillet quality. In return, the advantage of administrating DHA-rich whole cell *Scizochytrium* sp. biomass in fish feeds is the natural encapsulation provided by the cell wall that can protect valuable fatty acids from oxidation [26].

The efficacy of the defatted biomass of marine microalga, *Desmodesmus* sp., in *Atlantic salmon* (*Salmo salar*), was assessed for 70 days. In the treatment diets, the algal mass replaced a portion of the fish meal, with the results indicating that the growth indices, such as specific growth rate, condition factor, and survival of the *Desmodesmus*-fed (10 and 20%) fish were not different from the values of the control-fed fish. The whole-body proximate composition of fish, and the lipid and protein digestibility from all diets did not significantly differ. Fish fed 10% *Desmodesmus* in their diet demonstrated lower lipid amounts in their fillets and less digestibility of energy than the control fish. Authors concluded that the inclusion of *Desmodesmus* did not significantly change the Atlantic salmon growth, health, and fillet quality in comparison with the fish meal [27].

Several experimental diets containing n-3 fatty acids from fish oil (1%, 3%, and 5%), algal meal (1.75%, 5.26%, and 8.77%), or a control diet (with 6.3% corn oil) were fed to tilapia. With an increase in fish oil and algal meal levels in the diets, all tissues showed better fatty acid profiles, higher n-3 content, lower n-6, and n-6: n-3 rates. Diet with algal meal 8.77% was the most effective for enhancing the lipid profile in fish fillets. Total n-3 level in the fillet increased from 151.2 mg to 438.7 mg, while the n-6: n-3 ratio decreased from 5.19 mg to 1.29 mg, suggesting such formulated diets as a workable solution for increasing the n-3 content of tilapia fillets. The treated fish also showed the capacity to lengthen and desaturate shorter-chain PUFAs into longer-chain PUFAs. Authors indicated that *Schizochytrium* sp. and fish oil can boost n-3 fatty acids in tilapia fillets over a period of four weeks, which is of high nutritional benefit to consumers [23]. Five isonitrogenous and isolipidic feed diets were prepared by replacing fish oil with *Isochrysis galbana* in pompano (*Trachinotus ovatus*). Results demonstrated that the inclusion of microalga *Isochrysis galbana* (4.5–5.0 wt %) in the fish diet enhanced growth performance, lipid deposition, DHA, EPA, and total n-3 fatty acid contents in lipids of fish muscles [28]. Feeding spotted wolfish (*Anarhichas minor*) with isocaloric and isonitrogenous diets containing 7.5% or 15% microalgae *Nannochloropsis oceanica* and replaced with wheat and fish meal in the diets for 12 weeks exhibited an increase in omega-3 fatty acid EPA value in fish fed microalgae, which could be explained by the high content of EPA in the microalga. On the other hand, muscle protein level decreased in the algal-fed groups, indicating a lower ability of fish to absorb and then utilize protein due to the complex carbohydrates in the cell walls of this microalgae [29].

A combination of two microalgae, a protein-rich defatted biomass of *Nannochloropsis oculata* replaced with fish meal and a whole cell of DHA-rich *Schizochytrium* sp. as a substitute for fish oil, was used to produce a high-performing fish-free feed for Nile tilapia (*Oreochromis niloticus*) [30]. Fish-free feeds revealed a higher growth, specific growth rate, weight gain, and a better feed conversion ratio than the control diets. Higher levels of fillet lipid, protein, and DHA were also observed in fish fed fish-free meals than control diets although the data were insignificant. Furthermore, in vitro protein digestibility and protein hydrolysis were higher in fish-free meals than the control diets. The median feed cost for experimental diets was marginally higher than the control diet. However, the median economic conversion ratio of the fish-free feed was lower than the reference diet. This research provides evidence of a cost-competitive microalgae-based tilapia diet that enhances growth performance and reduces dependency on fish oil and fish meal [30].

A 6-week feeding *Totoaba macdonaldi* juvenile diets containing 33%, 66%, and 100% lipid of soybean oil and microalgae (*Schizochytrium limacinum*) sources replaced with fish oil exhibited higher levels of weight gain, with the thermal growth coefficient being higher in fish fed *S. limacinum* meals than in fish fed soybean oil. The proximate composition of liver was influenced by lipid source; apart from that, crude fat was higher in fish-fed soybean oil than in fish-fed *S. limacinum*. Also, soybean oil increased the concentrations of total n-6 FA and 18:2n-6 FA in fish fillet, whereas it decreased growth, n-3/n-6 ratios, total n-3, 20:5n-3, and 22:6n-3 FA. In contrast, *S. limacinum* meals elevated n-3/n-6 ratios, total n-3 FA, and 22:6n-3 FA, which is beneficial in foods destined for human consumption [15]. Considering the daily need of 200 mg DHA+EPA for the prevention of coronary heart disease, the authors assumed that consuming 200 g totoaba fillet administrated with *S. limacinum* meals, with the intake of nearly 380 mg DHA+EPA, can satisfy the daily consumption for providing the health benefit to consumers [15].

When rainbow trout was fed with a mixture of two microalgae meals, including *Nannochloropsis oceanic* and *Schizochytrium limacinum* in a 1:1 ratio (9% and 17%) replacing fish oil, the feed conversion ratio and growth performance were reduced at a higher level (17%) as compared to the fish-oil fed group [30]. The authors suggested that the current result might be related to the indigestible carbohydrates in the microalga cell wall, which decrease the digestibility of proteins and lipids. A reduction of 23% was also seen in EPA value in the muscle of fish fed 17% microalgae meal, but DHA+EPA content was similar among the dietary treatments. Authors indicated that the need for EPA might be covered by the available fish oil or fish meal or the trout capacity to metabolically retro-convert DHA into EPA as the dietary inclusion of *S. limacinum* increased [30]. Karapanagiotidis et al. [31] studied the effects of replacing fish meal with *Chlorella vulgaris* and fish oil using a mixture of *Microchloropsis gaditana* and *Schizochytrium* sp. on growth performance and the fatty acid composition of muscle in gilthead seabream (*Sparus aurata*). The fish meal protein of the control diet was replaced with *C. vulgaris* meal at 10%, 20%, and 30%; and the fish oil of the control diet was replaced with a mixture of *Microchloropsis* sp. and *Schizochytrium* sp. at 50% and 100%. The results exhibited that C. *vulgaris* meal at 30% and the mixture of *Schizochytrium* sp. and *M. gaditana* at 100% had no harmful effect on fish feed intake. Diets containing *C. vulgaris* revealed an increase in lipid deposition, especially in fish liver, while no other diet-related changes were seen in fish muscle and total body composition. *C. vulgaris* diets increased values of 18:3n-3 and 18:2n-6 in muscle, while an insignificant decrease was seen in DHA and EPA contents, probably due to their deposition by the dietary fish oil. The diet containing a mixture of *Microchloropsis* sp. and *Schizochytrium* sp. enhanced n-6 PUFA, especially 22:5n-6 and 20:4n-6, in fish muscle while maintaining similar DHA and EPA levels compared to the control group, suggesting that a biomass of *M. gaditana* and *Schizochytrium* sp. could replace dietary fish oil in a gilthead seabream diet [31]. Zhao et al. [32] assessed the growth performance and flesh quality of golden pompano (*Trachinotus ovatus*) fed with astaxanthin-rich *Oedocladium carolinianum* at 1% and 5% powder for 6 weeks which exhibited a significant increase in total MUFA, eicosenoic acid (C20:1), and oleic acid (C18:1) contents in fish fed *O. carolinianum* at 5%. Despite a decline in palmitoleic acid content, fish fed the *O. carolinianum* 5% diet exhibited lower levels of n-6 PUFA, PUFA, and linoleic acid (C18:2n6) compared to those fed the control and *O. carolinianum* 1% diets. No significant change was noted in total SFA profiles, total n-3 PUFA, n-3/n-6 ratio, and values of whole-body EPA and DHA, suggesting O. *carolinianum* at 5% could promote the synthesis of endogenous DHA and EPA in golden pompano and produce astaxanthin, which is attractive to consumers and contributes to the increase in sales price [32].

3. Effects of Microalgae on Organoleptic Parameters of Fish Fillet

Moreover, chemical and microbial properties, sensorial characteristics, including appropriate color, are important determinants in consumer's choice of food fish [33]. The impression of natural and bright colors often reflects high-quality fish food while pale color unconsciously is associated with inferior quality [34]. To acquire an appropriate color and a suitable appearance, the dietary administration of pigments in the optimized levels for fish species is necessary. Previous studies proved that microalgal biomasses have significant impacts on fish pigmentation [35][36] since they are rich in substances such as carotenoids, chlorophylls, and xanthophylls. Besides color, textural indices are also crucial for fillet quality [37]. Macroscopically, the *Phaeodactylum tricornutum* biomass diet caused a significantly lighter and more intense yellow coloration in the operculum of seabream. Also, the lightness of ventral skin coloration was affected by the *Phaeodactylum tricornutum* diet although this variation was not perceptually potent [35]. A three-week addition of a diet containing *Chlorella pyrenoidosa* at 2.5, 5, and 7.5 g/kg added into a common carp (*Cyprinus carpio*) diet significantly affected meat juiciness, color, and complete acceptability better than the control one [38]. In another study, Güroy et al. [39] showed that a four percent inclusion of *Spirulina* sp. in a trout diet increased the red/green tonality (*a**) fillet compared with the control diet. The luminosity (*L**) value of all fish fillets increased during the storage period [39], suggesting that *Spirulina* could induce pigmentation in trout fillet that is more acceptable by fish markets since this microalga contains pigments such as xanthophyll (yellow), carotene (orange), except chlorophyll (green) phycoerythrin (red) and phycocyanin (blue). In parallel, Sáez et al. [36], in their study showed that the quality characteristics of gilthead seabream (*Sparus aurata*) fillets fed *Nannochloropsis gaditana* (2.5 and 5%) in a 42-day feeding trial manifested a positive dose-dependent effect on fillet harness and skin color, suggesting an extended shelf life of gilthead seabream fillet using this dietary strategy.

4. Effects of Microalgae on Fish Fillet Biochemical and Microbiological Parameters

Intensive fish culture can expose fish species to various factors, such as pollutants and drugs that can heighten the production of free radicals inside their body. The oxidation of lipid and protein by the produced free radicals will reduce fillet quality, induce an unpalatable flavor and odor, reduce the fillet shelf life, cause loss of nutritional values, and lead to unhealthy molecules. For ameliorating the deteriorative effects of arsenic in rainbow trout fillet, Sheykhkanlu Milan et al. [40] investigated the effects of dietary *Haematococcus pluvialis* at 0.28, 0.56, and 1.12% of their diets on fish fillet quality. The researchers discovered that when fish were fed with *H. pluvialis*, it resulted in reduced levels of pH, peroxide value, and the oxidation of proteins and lipids. Additionally, the expression of antioxidant genes (CAT, GPX, SOD, and GST) was significantly higher in the treated fish compared to the control group. This indicates that *H. pluvialis* can enhance the fish's ability to defend against reactive oxygen species by improving their antioxidant defense system. Therefore, dietary *H. pluvialis*, as a potent antioxidant, could protect fish fillets during the storage period. Protective effects of *Spirulina platensis* against the toxic impact of sodium sulfate in Nile tilapia muscle revealed a higher GPX level in fish muscle fed the microalga and challenged with sodium sulfate compared with challenged tilapia [41]. It was concluded that dietary *S. platensis* can be recommended to counteract the oxidation induced by sodium sulphate toxicity in fish species. A 12-week feeding Nile tilapia with microalgae mixture (*Nannochloropsis oculate*, *Schizochytrium* and *Spirulina* species) at 0.75%, 1.5%, and 3% exhibited a higher antioxidant capacity and reduced ROS, H2O2, and MDA levels especially at 3% inclusion [42], Meanwhile, contents of EPA and DHA in tilapia fillet were significantly enhanced with an increase in NSS (microalgae mix (NSS) containing *Nannochloropsis oculate* and *Schizochytrium* and *Spirulina* species) levels. Such antioxidant activity could be in part due to the availability of tocopherols, phenolic compounds, and carotenoids that can reduce the oxidative stress responses in different fish species [42].

Usually, fresh fish fillet is contaminated with different microorganisms available on skin, gill, and intestine tissues. However, specific spoilage organisms can penetrate the fish's muscle tissue, resulting in an unpleasant flavor and odor. Using natural additives in fish diets for improving the microbial characteristics of fillet quality is a novel approach [43]. In a study by Gulhan et al. [44] and Selamoglu et al. [45], rainbow trout and common carp administered with dietary propolis showed lower mesophilic and psychrophilic bacteria counts following a challenge with cypermethrin and arsenic. Similarly, Öz [46] showed that garlic-supplemented feed could decrease the number of total bacterial count as well as *Enterobacteriaceae* and psychotropic bacterial populations in rainbow trout fillet during storage at −18 °C. In contrast, no positive effects of dietary zeolite/chitosan and zeolite/nanochitosan composites on the rainbow fillet microbiological characteristics were observed [47]. The authors concluded that lower doses of these additives in fish diets might be the main reason for unchanged bacterial counts in fish fillets. Meanwhile, using alginate and carrageenan in the formulation of edible films showed antimicrobial properties against *L. monocytogenes*, e.g., in cold smoked salmon [48]. Despite the presence of few studies on the effects of some additives on fish fillet microbiological indices, in just one study, the effects of *Spirulina platensis* on rainbow trout microbiological aspects were assessed [39]. At the conclusion of the storage period, the total coliform measurements in fish that were given the PM/S diet were lower compared to all other diets.

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