Aquatic Food Studies 2(2), AFS112
https://doi.org/10.4194/AFS112

PROOF
RESEARCH PAPER

Effects of dietary inclusion of Seaweed in regular commercial fish feed on proximate composition, minerals and fatty acid profiling of Nile Tilapia *Oreochromis niloticus* (Linnaeus, 1758)

Nahid Sultana[1,*], Mumtahina Mohammad[2], Sharmin Akhter Lisa[1], Badhan Saha[1] Mahmuda Begum[1], M. Niamul Naser[2]

[1]Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka-1205, Bangladesh.
[2]Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh

How to cite

Sultana, N., Mohammad, M., Lisa, S.A., Saha, B., Begum, M., Naser, M.N. (2022). Effects of dietary inclusion of Seaweed in regular commercial fish feed on proximate composition, minerals and fatty acid profiling of Nile Tilapia *Oreochromis niloticus* (Linnaeus, 1758). *Aquatic Food Studies*, 2*(2), AFS112*. https://doi.org/10.4194/AFS112

Article History
Received 20 October 2022
Accepted 27 December 2022
First Online 28 December 2022

Corresponding Author
Tel.: +0001847235694
E-mail: nahid.bcsir@gmail.com

Keywords
Marine algae,
Hypnea sp.
Omega-3 fatty acids
BFRI
Mymensingh

Abstract

Feed formulation is important to improve the quality of feed by adding nutritive ingredients. The use of seaweed in fish feed is not usual in our country though these are cultivated in Cox's Bazar and Saint Martin's Island for human consumption. This study investigated the effects of seaweed (*Hypnea* sp.) as supplementation on the meat quality of Nile tilapia. The current study was continued for 3 months using 30 days old more or less similar-sized 30 tilapia fingerlings/aquarium (total of 10 aquariums). Commercial feed was selected based on its easy availability and is frequently used by farmers in rural areas. Collected seaweed was mixed with the formulated feed at four percentages. Finally, five different treatments were applied to the fishes where – T_1 (5% seaweed), T_2 (10% seaweed), T_3 (15% seaweed), T_4 (20% seaweed), and T_5 (Commercial feed as control) with twoa sets of replications for each. After 3 months of rearing, fish were harvested for proximate composition, minerals, and lipid extraction as well as for the determination of the quantity of different fatty acids in the sample with a trace amount of heavy metals. Heavy metals were detected in tilapia meat at a trace level which is not harmful for human consumption. The findings have a clear indication that a 10% seaweed mixed diet can be considered the optimum amount as supplementary feed for Nile tilapia to improve their nutrient composition

Introduction

Algae are more commonly used as animal feed ingredients rather than in animal diets. The suitability of various algae as a good supplementary feed has been proven by various nutritional and toxicological evaluations where algae have also been mentioned as substitutes for other conventional sources of protein (soy, fish meal, rice bran, etc.). (Becker, 2007). Seaweeds are rich in dietary minerals (Na, K, I), and fiber, which can improve the texture of foods.

Investigations are ongoing, in search of new protein sources for the fish farming industry, and a large number of algae are being considered as potential alternative protein sources, especially in tropical countries where some algae species are already used as supplements in commercial diets. (Nakagawa, 1985; Mustafa and Nakagawa, 1995).

Seaweed is a macroscopic, multicellular marine algae. It is very important because of its use for a variety of purposes. Seaweeds are a good source of minerals, vitamins, proteins, bioactive compounds, and indigestible carbohydrates (MacArtain *et al.,* 2007; Kalasariya *et al.,* 2016; Swarmalatha, 2018), used as a primary feed for different herbivorous fish (Tolentino-Pablico *et al.,* 2008; Siddik and Anh, 2015).

In Bangladesh, about 200 marine algal taxa have so far been found on the Saint Martin's Island, Chittagong, and Cox's Bazar coast where 98 taxa under 53 genera are red (Aziz and Islam, 2009; Islam et al., 2010). Among them, *Hypnea pannosa* and *Hypnea musciformis* have commercial significance. Around 100 households are involved in collecting seaweed manually or using push nets from St. Martin's Island. Collected seaweeds are sundried on shore.

In Europe, seaweeds have been used as animal feed since Roman times. In Iceland, France, and Norway pets were fed by algae to increase the nutritional value of the feed (Cunningham *et al.,* 2010 Swanson and Druehl 2002). In 2004, the use of algae as animal feed accounted for 1% of the global industry based on seaweed ($10MM in the U.S., mainly *Ascophyllum nodosum*) (Chopin *et al.,* 2009). In the case of microalgae used as feed additives, the value of the industry in the same year in the U.S. totaled $300MM (Pulz and Gross 2004). About 10,000 species of algae have been identified, and 5% of them are now used as human or animal food.

The significant role of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) in human health cannot be denied and freshwater fishes are rich in these nutrients. Especially, PUFAs are important for their ability to prevent cardiovascular disease, psychiatric disorders, and some other illnesses such as atherosclerosis, thrombogenesis, high blood pressure, and skin diseases (Gladyshev *et al*., 2012). Omega-3 fatty acids are the most important category of PUFAs which play a vital role in maintaining health conditions including alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and docosapentaenoic acid (DPA) and also omega-6 PUFAs include linoleic acid (LA) and arachidonic acid (ARA) (Abedi and Sahari, 2014). In the last decade, the impacts of different herbal extracts on the immune defense and growth of fish were researched. The products which are used for feed production, maintain the quality of fish and its health condition. It was observed that vitamin B, plant-based products improve the growth and health of salmon (Hemre *et al*. 2016) and fababean meal in Atlantic salmon usually helps in utilizing the feed efficiently (De Santis C. *et al*., 2015).

Tilapia is one of the most popular exotic fishes cultured in Bangladesh belonging "Cichlid" group, native to Africa. Nile tilapia *(Oreochromis niloticus)* has been introduced and cultured worldwide, with considerable benefits for the livelihoods of poor people in third-world countries, both in terms of household income and family consumption (Little, 2003). It is omnivorous and fast-growing with different supplementations (El- Sayed, 2010). It feeds on zooplankton, phytoplankton, and aquatic plants. Different types of aquatic plants have different dietary effects on Nile tilapia. Thyme, rosemary, and fenugreek as supplementary feed increase innate immune response and disease resistance of tilapia (Gultepe *et al.,* 2014).

Fatty acid is a carboxylic acid with a long aliphatic chain. It is the building block of fat. At the time of digestion, fats are broken down into fatty acids, then absorbed into the blood. Each of the three fatty acid molecules is then linked together to form triglyceride. Saturated and unsaturated, these two types of fatty acids are mainly found. In the current study, both types of fatty acid composition were analyzed for different feeding trials where seaweed was used at different percentages as supplementary feed. So, the purpose of the present study was to estimate the proximate composition and fatty acid profiles of Nile tilapia (*Oreochromis niloticus*) reared on a laboratory scale supplemented with different proportions of marine algae and usual commercial feed as a major feed and also to evaluate the beneficial fatty acids in tilapia meat. On the other hand, it is very important to investigate whether seaweed increases heavy metal contents in fish or not when it is used as supplementary feed for fish. In this study, an initiative has been taken to evaluate the effects of *Hypnea* sp. as a supplementary feed on the mineral (Ca, Mg, P, Fe, and K) and heavy metal (Pb, Cr, Cd, and Ni) contents of Nile tilapia (*Oreochromis niloticus*).

Materials and Methods

Experimental Setup

These experiments were carried out in the wet laboratory of the Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. Tilapia fingerlings were brought from a hatchery of Bangladesh Fisheries Research Institute (BFRI), Mymensingh. The commercial feed of Mega feed limited was used as fundamental feed. Nursery feed was used for 1st seven days containing 35% crude protein (CP), 6% crude fat (CF), 5% crude fiber (Cf), and 12% moisture (M). For the rest of the days floating feed (containing 30% CP, 3% CF, 10% Cf, and 12% M) was used along with a kind of seaweed *Hypnea musciformis* as supplementary feed. *H. musciformis* was collected from Saint Martin's Island, Cox's Bazar, Bangladesh.

Tilapia fish was raised in glass aquaria (30 × 14 × 6 inches) at the Zoology Section of the Bangladesh Council of Scientific and Industrial Research (BCSIR) in Dhaka, Bangladesh. The aquariums were cleaned, filled with 60 L of clean tap water, and prepared with all other required facilities (aerator, water changing pipe). Then, more or less similar in size (in terms of length and weight), thirty tilapia fingerlings were kept in each aquarium with a triplicate for each experimental diet. Different water quality parameters such as water temperature, pH, dissolved oxygen, total dissolved solids (TDS), and conductivity were measured weekly with a thermometer, digital pH meter (Jenway, Model 3020, United Kingdom), dissolved oxygen meter (Model Oxi 3150i, Germany) and conductivity meter (Jenway, 4510) respectively.

Seaweed (Hypnea Sp.) Meal and Feeding Trial

Five different experimental feeds were used where four feeds contained different percentages of seaweed mixed (T_1 with 5% seaweed, T_2 with 10% seaweed, T_3 with 15% seaweed, and T_4 with 20% seaweed) and the last one was a commercial feed of Mega feed company as control (Table 1). For each treatment, the mixture was blended and some water was added to make a sticky ball-like form. Pellet-formed fish feed was produced with a pellet machine from this sticky dough. These were then dried and stored in airtight containers. Feed was given to the Tilapia fingerlings once a day at 5% of their body weight.

Sample Preparation

Fish from different aquariums were collected after three months of rearing, then measured, weighted, and euthanized. The dead fishes were taken for sample preparation and then for laboratory analysis to measure the percentages of water, protein, fat, ash, and minerals. Using a chemical tissue grinder, samples for each treatment were crushed separately and taken for moisture estimation. The weight of wet samples was recorded and after oven-drying at 100°C, weighed again. Proximate analysis was accomplished with the dry sample, later readjusted for weight wet.

Estimation of Proximate Composition

The moisture content of the fish sample was estimated by AOAC 1984 method, described by Gopalan 1976.

The modified Kjeldahl method A.O.A.C (1990) was used to measure the amount of nitrogen and then multiplied by 6.25 to get the amount of crude protein. The existing non-protein nitrogen (NPN) of the sample was also considered in the current experiment.

Lipid content was estimated using the modified method suggested by Folch *et al.,* 1957. Extraction of fat from the dry sample was performed using a mixture of anhydrous chloroform-methanol.

Estimation of Fatty Acid

Fat was extracted from fish by the hydroelectric method. Petroleum ether was added into the lipid extracted from the dry fish sample before and allowed to stay overnight and then allow to air dry and boil until getting the sticky fat content. The fat content was weighted. Then the fat content was methylated to fatty acids methyl esters (FAMEs). FAMEs were quantitatively measured by gas chromatography. By following the Chromatographic method, the profile of fatty acids was done (Nicholus *et al*. 1995). Fatty acids were obtained from fat through saponification using a NaOH dissolved in methanol H_2O mixture (hydrolysis with alkali).

Measurement of Minerals

Atomic Absorption Spectrometer (Model No.: AA-7000, Shimadzu, Japan) was used to estimate minerals

Table 1. Proximate composition of Sea weed (*H. musciformis*) and Commercial feed.

Nutrients	Hypnea musciformis	Saudi Bangla fish feed for grower
Protein (%)	13.73±1.12	12.52±0.3
Crude lipid (%)	0.34±0.4	24.00±0.5
Carbohydrate (%)	46.26±0.6	3.37±0.6
Fibre (%)	5.6±0.7	19.92±0.1
Ash (%)	9.76±1.4	40.19±0.36
Moisture (%)	24.314±0.5	6.85±0.41

Table 2. Proximate composition of Nile Tilapia fingerlings fed on seaweed mixed diet.

Treatments	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
T_1 (5% Seaweed)	74.39±0.57	14.33±0.19	6.08±0.90	4.28±0.30
T_2 (10% Seaweed)	74.52±0.12	14.34±0.10	5.13±0.18	5.00±0.25
T_3 (15% seaweed)	75.31±1.41	14.28±0.38	4.32±0.16	5.19±0.37
T_4 (20% seaweed)	75.25±0.88	14.14±0.12	3.49±0.87	6.13±0.13
T_5 (Control)	74.01±0.69	14.09±0.53	6.37±1.02	4.46±0.71

Values are mean ± SEM of duplicate groups of 30 fishes.

like Ca, Mg, and Fe. Calibration of all equipment was done using commercial chemical standard solutions, the samples were prepared using the reagents of analytical grade, and the water, used throughout the study was deionized. UV/VIS Spectrophotometer was used to estimate phosphorus content. The potassium in the sample was determined with a Flame photometer. The concentration of minerals was expressed by ppm.

Measurement of Heavy Metals

The heavy metals Cr, Pb, Cd, and Ni were also estimated using Atomic Absorption Spectrometer (Model No: AA-7000, Shimadzu, Japan) and the procedure was similar to mineral measurement. Heavy metal concentrations were expressed as ppm.

Statistical Analysis

All data were subjected to a one-way analysis of variance (ANOVA) test using SPSS version 20. Where ANOVA revealed a significant difference ($P<0.05$). Tukey's test was used to compare means.

Results

Proximate Composition of Nile Tilapia

The proximate composition of reared tilapia after three months of rearing with seaweed-supplemented feed is shown in Table 1.

The highest moisture (75.57±0.88%) and ash (6.13±0.13%) content was observed in T_4 (20% seaweed mixed feed). The value of protein content was observed highest (14.68±0.38) % in T_3 (15%).

Significant differences were found among all treatments ($P<0.05$) in lipid contents. Though the highest lipid value was measured in control fish T_5 (6.87±1.02%), treatments with 5% and 10% seaweed mixed feed had also more lipid content (6.18±0.90%) and (5.23±0.18%) respectively.

Fatty acid profiling of Nile Tilapia

A total of 16 fatty acids were detected from the reared tilapia for different treatments in the current study. Saturated fatty acids (SFA) are most abundantly present in tilapia fingerlings after three months rearing period. Which was followed by monounsaturated fatty acid (MUFA), n-6 polyunsaturated fatty acid (n-6 PUFA), and n-3 polyunsaturated fatty acid (n-3 PUFA) respectively (Figure 1).

Among the n-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is abundant in tilapia fingerlings treated with a 15% and 20% seaweed mixed diet. EPA is a polyunsaturated omega-3 fatty acid with 20 carbon chains and five cis double bonds. In the current study, the highest amount of EPA was found in T_3 (4.809±0.29%) followed by T_2, T_4, T_1, and T_5.

Docosahexaenoic acid (DHA) is another important n-3 fatty acid. The highest amount of DHA was found in T_2 (3.70±0.55) followed by T_3, T_4, T_1, and the lowest in T_5 (1.65±0.205). Docosapentaenoic acid (DPA) is a dietary omega-3 fatty acid. The highest amount of DPA was found in T_3 (3.13±0.03) followed by T_2, T_4, T_1, and the lowest one was in T_5 (1.63±0.17).

Arachidonic acid is a polyunsaturated omega-6 fatty acid 20:4 (ω-6). In this experiment, the highest amount of arachidonic acid was found in T_2 (1.17±0.39) followed by T_4, T_5, and T_1. In T_3 the acid was not traced.

Linoleic acid is a liquid, colorless polyunsaturated omega-6 fatty acid. It is a carboxylic acid with an 18-carbon chain and 2 *cis* double bonds. The highest amount of linoleic acid was found in T_2 (15.50±2.71) followed by T_3, T_5, and T_4, and the lowest one in T_1 (10.83±2.24). In this experiment, 10% and 15% seaweed mixed feed showed better performance in terms of enriching fatty acids in Tilapia meat. Though different fatty acids were high in amount for different treatments, the results of this experiment showed that seaweed as supplementary food had a positive impact on the fatty acid profile of tilapia.

Estimation of Minerals and Heavy Metals

As fishes are an important source of minerals specially calcium, magnesium, phosphorus, iron, and potassium, in this study, the amount of these minerals in the body of Nile tilapia which were fed different percentages of seaweed (*Hypnea* sp.) supplementation was analyzed. Again, the effects of seaweed supplementation on some common heavy metals like lead (Pb), chromium (Cr), Cadmium (Cd), and Nickel (Ni) concentration in the fish body were analyzed on a dry based. The minerals and heavy metals were calculated for the whole body including muscles, gills, and bones.

In Table 2 the highest amount of Ca is found in T_1 (6.66±0.09%) followed by T_2, T_3, and T_4, and the lowest one was found in T_5 (5.40±0.16 %). The mean difference is significant at the 0.05 level. The Ca content of T_1, T_2, T_3, and T_4 was significantly higher than T_5. These results are higher than the range of fish and seafood reported elsewhere (FAO/ IN FOODS, 2013). As would be expected, calcium content was much higher in species in which bones are commonly consumed and included in the edible parts, and in this analysis, the sample was prepared including bones. The maximum amount of Mg was found in T1 (0.138±0.001%) followed by T_4, T_2, and T_3, and the lowest one was found in T_5 (0.110±0.003%). This range was also high as the bones and scales were included in the sample. The highest amount of P was found in T_2 (3.46±0.07) % followed by T_4, T_1, and T_3, and the lowest one is found in T_5 (2.70±0.04%). There is no significant difference among all the treatments at 0.05 levels for Mg and P amount. In this experiment, Ca, Mg, and P were expressed in a form of percentages because of their presence in a high amount in the reared tilapia.

Iron is one of the important trace elements. The highest amount of Fe is found in T2 (13.35±0.36) mg/100g followed by T_1, T_4, T_5, and the lowest one is found in T_3 (8.83±0.39 mg/100g). The mean difference is significant at the 0.05 level. Fe content of T_2 and T_1 is significantly higher than T_5, T_3, and T_4. On the other hand, the highest amount of K is found in T_3 (391.63±35.45) mg/100g followed by T_1, T_5, T_4, and the lowest one in T_2 (285.23±0.06 mg/100g). There is no significant difference among all the treatments at 0.05 levels.

Overall 5%- 10% seaweed supplementation improves the minerals in the fish body mostly.

In this experiment, the highest amount of lead is found in T_2 (8.41±0.59mg/100gm) followed by T_4, T_1, and the lowest amount is found in (2.16±0.72 mg/100gm) and T_3 (2.16±0.44 mg/100gm). Here, T_2 is significantly higher than T_5, T_3, T_1, and T_4 at a 0.05 level. The maximum amount of Cr is found in T_2 (4.12±0.20 mg/100gm) followed by T_1, T_4, T_3, and the lowest one in T_5 (0.7±0.1) mg/100gm. Here, T_2 is significantly higher than T_5, T_3, T_4, and T_1. Again T_1 is significantly higher than T_5 at 0.05 level statistically (Table 3).

In this study, only 0.015 mg/100g Cd is found in T_4 and only 0.007 mg/100g Cd is found in T_2. In T_5, T_1, and T_3, no Cd is found. Ni is absent for all treatments (Table 4).

Estimation of Fatty Acids

Total 16 fatty acids were detected from the reared tilapia for different treatments in the current study. Saturated fatty acids (SFA) are most abundantly present in tilapia fingerlings after three months rearing period. Which was followed by mono unsaturated fatty acid (MUFA), n-6 polyunsaturated fatty acid (n-6 PUFA) and n-3 polyunsaturated fatty acid (n-3 PUFA) respectively (Figure 1).

Among the n-3 polyunsaturated fatty acids eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) is abundant in tilapia fingerlings treated with 15% and 20% seaweed mixed diet. EPA is a polyunsaturated omega-3 fatty acid with 20 carbon chain and five cis double bonds. In the current study, the highest amount of EPA was found in T3 (4.8091±0.29) % followed by T2, T4, T1 and T5. (Figure 3)

Docosahexanoic acid (DHA) is another important n-3 fatty acid. Highest amount of DHA was found in T2 (3.703±0.55) followed by T3, T4, T1 and the lowest in T5 (1.65±0.205). Docosapentaenoic acid (DPA) is a dietary omega-3 fatty acid. Highest amount of DPA was found in T3 (3.13±0.03) followed by T2, T4, T1 and the lowest one was in T5 (1.63±0.17). (Figure3)

Arachidonic acid is a polyunsaturated omega-6 fatty acid 20:4 (ω-6). In this experiment, the highest amount of arachidonic acid was found in T2 (1.17±0.39) followed by T4, T5 and T1. In T3 the acid was not traced. (Figure 2)

Linoleic acid is a liquid, colorless polyunsaturated omega-6 fatty acid. It is a carboxylic acid with an 18-carbon chain and 2 *cis* double bonds. Highest amount of linoleic acid was found in T2 (15.50±2.71) followed by T3, T5, T4 and the lowest one in T1 (10.83±2.24). In this experiment, 10% and 15% seaweed mixed feed showed better performance in terms of enriching fatty acids in Tilapia meat. Though different fatty acids were high in amount for different treatments, results of this experiment showed that seaweed as supplementary food had positive impact on fatty acid profile of tilapia. (Figure2)

Discussions

In this experiment, moisture content increases with the increasing amount of seaweed supplement. The range of moisture content of *Oreochromis niloticus* was (74.01±0.69-74.52±0.88%). The highest moisture

Table 3. Amount of different minerals in Nile tilapia for seaweed supplementation.

Treatments	Calcium (%)	Magnesium (%)	Phosphorus (%)	Iron ($mg.100g^{-1}$)	Potassium ($mg.100g^{-1}$)
T1 (5% Seaweed)	6.66±0.09^{a}	0.138±0.001	3.05±0.41	12.20±0.21^{a}	356.25±0.29
T2 (10% Seaweed)	6.60±0.02^{a}	0.117±0.002	3.46±0.07	13.35±0.36^{a}	285.23±0.06
T3 (15% seaweed)	6.18±0.17^{a}	0.115±0.002	2.99±0.005	8.83±0.39^{b}	391.63±35.45
T4 (20% seaweed)	6.12±0.02^{a}	0.125±0.012	3.19±0.21	9.59±0.50^{b}	320.41±35.70
T5 (Control)	5.40±0.16^{b}	0.110±0.003	2.70±0.04	8.99±0.36^{b}	320.49±35.55

Table 4. Amount of different heavy metals in Nile tilapia for seaweed supplementation.

Treatments	Lead ($mg.100g^{-1}$)	Chromium ($mg.100g^{-1}$)	Cadmium ($mg.100g^{-1}$)	Nickel ($mg.100g^{-1}$)
T_1 (5% Seaweed)	4.14±0.55^{b}	2.01±0.41^{c}	BDL	BDL
T_2 (10% Seaweed)	8.41±0.59^{a}	4.12±0.20^{a}	0.007	BDL
T_3 (15% seaweed)	2.16±0.44^{b}	1.16±0.06^{b,c}	BDL	BDL
T_4 (20% seaweed)	4.85±0.02^{b}	1.91±0.11^{b,c}	0.015	BDL
T_5 (Control)	2.16±0.72^{b}	0.7±0.1^{b}	BDL	BDL

Values are mean ± SEM of duplicate groups of 30 fishes.
BDL= Below Detection Limit

was in T_4 (75.57±0.88%), whereas the lowermost one was in T_5 (74.01±0.69%). There is no significant difference among all treatments at the 0.05 level. Experiments on moisture amount for the freshwater fish (27 species), done by Rubbi *et al*. (1987) suggested the presence of 72.18-83.65% moisture normally, which supports the result of the current study. Another finding by Dresosier (1977) on fish moisture also supports our results where he suggested 70-80% moisture content in general fishes. Here, the percentage varies depending on different factors such as seasons, sex, age, etc. Overall, the amount of moisture found in this study was similar to many of the previous studies.

According to Basemir *et al*. (2004) and Nakagawa and Montgomery (2007), lipids, extracted from macroalgae, contain different types of fatty acids. Among them, long-chain polyunsaturated fatty acids have great importance for neural function and health.

In *Ulva* sp. (green seaweed), crude protein amounts may vary between 10-26% of dry weight, whereas, for red seaweed, the amount may be up to 47% which indicates their potential as human and animal food (Fleurence, 1999).

This experiment showed that protein content for treatments was almost similar and no significant difference among all the treatments at 0.05 levels. The range of protein content was (14.09±0.53-14.48±0.10%). The highest protein content was observed in T_3 (14.48±0.10) % followed by T_2, T_1, and T_4, whereas the lowest one was in C (14.09±0.53%). This result was fresh- based. Dresosier *et al.* (1977) reported that protein content in fish remains in a range of 13-

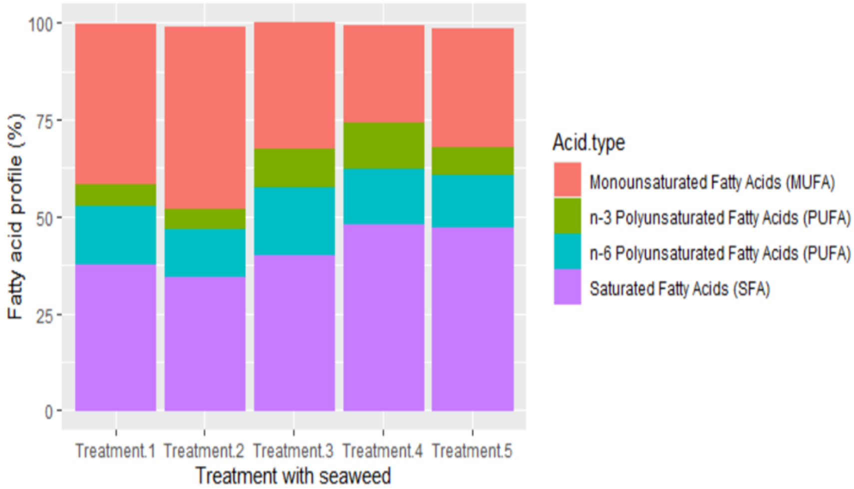

Figure 1. Fatty acid profiling of Nile Tilapia treated with seaweed supplemented feed (T_1- 5% seaweed, T_2- 10% seaweed, T_3- 15% seaweed, T_4- 20% seaweed, and T_5- control).

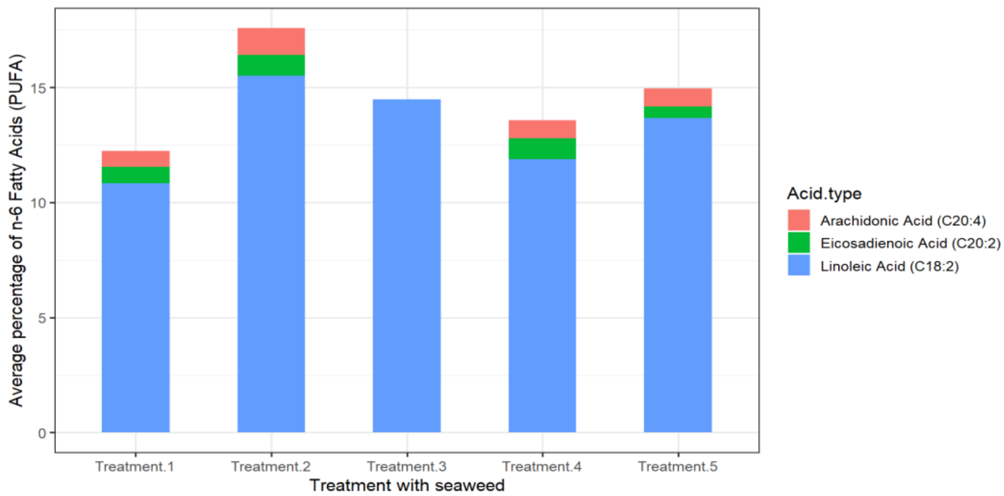

Figure 2. n-6 polyunsaturated fatty acids of tilapia treated with seaweed supplemented feed (T_1- 5% seaweed, T_2- 10% seaweed, T_3- 15% seaweed, T_4- 20% seaweed, and T_5- control).

20%. On the other hand, 9-25% protein (both for marine and freshwater fishes) was suggested by Govindan (1985). According to literature, fish may contain a wide range of proteins.

In this study, the ash content increases with the increasing amount of seaweed supplement. Here, the range of ash content of *Oreochromis niloticus* was (4.28±0.30-6.13±.13%) where the highest amount was in T_4 (6.13±0.13%) and the lowest one in C (4.16±0.71%). No significant difference among all the treatments at 0.05 levels. The result for ash was fresh-based. Ash content indicates the presence of different minerals including Ca, Mg, P, Fe, and Zn, which have a vital role in maintaining the body structure of various organisms. Jim *et al.* (2017) found ash content for Nile tilapia ranged between 1.76- 3.30%.

A high amount of fatty acid indicates a high nutritional value of the fish. The high level of Oleic, Palmitic acid, myristic and stearic acids for saturated fatty acids, palmitoleic and oleic acids for monounsaturated fatty acids, eicosapentaenoic and docosahexaenoic acids are the most abundant omega-3 fatty acids found in tilapia.

In this experiment, the highest amount of arachidonic acid was found in T_3 (1.17±0.39) followed by T_4, T_5, and T_1. In T_3 the acid was not traced. Normally in freshwater fishes, Arachidonic acid ranges between 3.35-10.67% (Suloma *et al.,* 2008). Here, the amount is less than the normal value. The highest amount of DHA was found in T_2 (3.70±0.55) followed by T_3, T_4, and T_1, and the lowest in T_5 (1.65±0.205). Normally in freshwater fishes, docosahexaenoic acid ranges between 4.21- 20.71 % (Suloma *et al.,* 2008). Here, the amount is also less than the normal value. In the current study, the highest amount of EPA was found in T_3 (4.52±0.29) followed by T_2, T_4, T_1, and T_5. Here, the amount of EPA in T_3 is significantly higher than T_5 (1.55±0.05) and T_1 (1.61±0.56) at 0.05 levels. Normally in freshwater fishes, eicosapentaenoic acid ranges between 0.42- 4.74% (Suloma *et al.,* 2008). The report supports our findings and indicates that the fish of this experiment contain a comparatively higher amount of EPA within range. In this study, the highest amount of oleic acid was found in T_1 (38.39±5.38) followed by T_5, T_2, and T_4 and the lowest one was in T_3 (20.76±0.07). Normally in freshwater fishes, oleic acid ranges between 9.50-48.76% (Suloma *et al.,* 2008) which strongly supports the findings of recent work and also indicates that fish contain a comparatively higher amount of oleic acid within the range. The highest amount of palmitoleic acid in this study was found in T_3 (4.62±0.32) followed by T_5, T_4, and T_2, and the lowest one was found in T_1 (2.68±0.01). According to Suloma *et al. (*2008), the normal range of palmitoleic acid for freshwater fish ranges between 4.24-11.96%. The report supports our findings and indicates that the fish of the current study contain a comparatively lower amount of palmitoleic acid. In this study, the highest amount of palmitic acid was found in T_3 (33.66±1.15) followed by T_4, T_5, and T_2 and the lowest one was in T_1 (22.47±4.23). Again Stanceby (1982) reported that 20-25% of total fatty acids in fish were palmitic acids. The above reports support the findings of this study and indicate that fish contain a comparatively higher amount of palmitic acid. According to this experiment, the highest amount of myristic acid was found in T_3 (3.39±0.21) followed by T_4, T_5, T_2, and the lowest one in T_1 (1.64±0.41). The highest amount of Linoleic acid was found in T_2 (15.50±2.71) followed by T_3, T_5, T_4, and the lowest one in T_1 (10.83±2.24) and, the highest amount of stearic acid was found in T_3 (12.19±0.29) followed by T_2, T_4, T_1 and the lowest one was found in T_5 (8.42±0.01). Suloma *et al.* (2008) observed that the amount of myristic, linoleic, and stearic acids ranges between 1.56-4.67%, 0.86-6.52%, and 8.51-12.74% respectively. The normal values indicate that the fish of this experiment contains

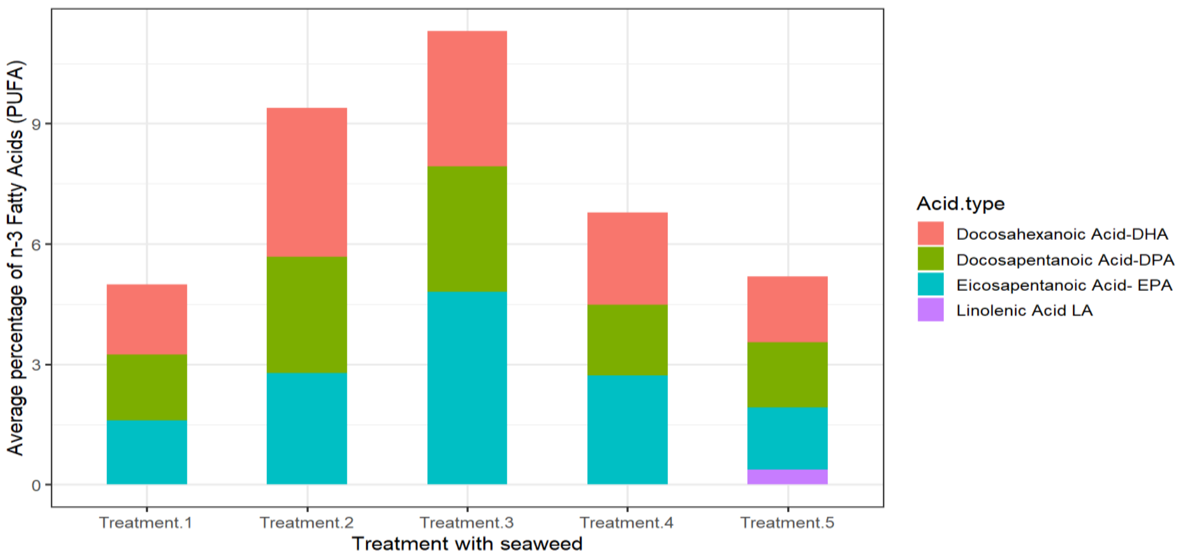

Figure 3. n-3 polyunsaturated fatty acids of tilapia treated with seaweed supplemented feed (T_1- 5% seaweed, T_2- 10% seaweed, T_3- 15% seaweed, T_4- 20% seaweed, and T_5- control).

a comparatively higher amount of myristic and stearic acid within range and a high amount of linoleic acid. The fatty acids amount among different treatments were almost similar. No significant difference among all the treatments at 0.05 levels for any fatty acids except EPA.

The current study showed that the lipid contents of Nile tilapia decrease in a linear way with the increase of the amount of seaweed as supplements. Here, the range of lipid content of *Oreochromis niloticus* was (6.87±1.02-3.89±0.87%). The highest lipid content was observed in T_5 (6.87±1.02%) and the lowest one in T_4 (3.89±0.87%). No significant difference was found among all the treatments at 0.05 levels. This result was fresh-based. Rubbi *et al*., (1987) found from the experiment that the lipid content of 27 species of freshwater fish in Bangladesh varied from 0.89-15.1%. Overall, the findings of the previous study support the result of the current study. Fish lipid is mainly a combination of glycerides, polar lipids, and some lesser components including sterol, sterol ester, free fatty acids, and so on and it varies in different species during different seasons (Woyewode *et al.* 1986). Different fatty acids are high in amount for different treatments. Again, the results of this experiment showed that for 15% seaweed (T_3) and 10% seaweed (T_2) supplements, the amount of different fatty acids was comparatively higher than other treatments which means 10%-15% seaweed supplement was the most suitable amount for Nile tilapia.

In this study, calcium content ranged considerably from 5.40±0.16-6.66±0.09%, which is more the maximum edge of the range for fish and seafood (FAO/ IN FOODS, 2013). The highest amount of Ca was found in T_1 (6.66±0.09%). In this study, it was expected because of the inclusion of bones during sample preparation for analysis and considered bone as an edible portion. Although no studies have been conducted to estimate the total impact of Ca deficiency in Bangladesh, from data on the development of rickets, it is predicted that 550,000 children were affected in 2008. (Craviari *et al.,* 2008; Fischer *et al*., 1999; ICDDR'B 2009). One study with two rural sub-districts showed that 0% of women or children had a diet with the required amount of calcium and the likely reasons were low food intake and lack of dietary variety (Arsenault *et al*., 2013). According to Belton *et al*., 2014, whereas, as a regular food item, dairy products have become the key source of dietary calcium in developed countries; In Bangladesh, dairy products are not included in the regular diet of most people. The range of magnesium is 0.11±0.003 to 0.13±0.001% which is high in amount as the bones and scales were included in the sample. The highest amount of Mg was found in T_1 (0.13±0.001%).

Phosphorus content ranged from 2.70±0.04 to 3.46±0.07% and this higher composition was due to the inclusion of bone as an edible part. Nevertheless, our findings have been supported by values reported elsewhere (FAO/INFOODS 2013). The highest amount of P was found in T_2 (3.46±0.07%).

Iron content varied extensively between 8.83±0.39 to 13.35±0.36 mg/100g and the highest amount of Fe was found in T_2 (13.35±0.36 mg/100g). Our results showed more significant variations in iron content compared to values reported in the global FAO/INFOODS database on fish and shellfish (excluding mollusks) (FAO/IN FOODS, 2013). This may be partly attributable to sampling variability, the use of seaweed as a supplementary feed, differences in the analytical methods, or various environmental conditions. The true nature and magnitude of these differences should be further investigated. Here, the data indicated that the use of seaweed supplements for tilapia may have a noteworthy effect on dietary iron consumption in Bangladesh which is of high bioavailability as an animal-source food (FAO and WHO, 2004). This policy may prove to be efficient in public health management to reduce iron deficiency in Bangladesh, as a report by ICDDR'B, 2013 showed that 10.7% of preschool-aged children and 7.1% of adult women were suffering from problems in different physical and cognitive development, pregnancy outcomes, morbidity, and mortality due to iron deficiency.

Potassium (285.23±0.06 to 391.63±35.45 mg/100 g) content was broadly consistent with ranges for other fish and seafood reported elsewhere (FAO/IN FOODS 2013). The highest amount of K is found in T_3 (391.63±35.45mg/100g).

Analysis of the mineral contents indicated that 5%-10% seaweed supplement resulting a higher amount of minerals than other treatments.

As different levels of seaweed were used as supplementary feed with commercial feed, there was a chance of high heavy metal accumulation in fish in this study. The amounts of four heavy metals (Pb, Ni, Cd, Cr) were analyzed, within which nickel (Ni) and cadmium (Cd) were absent. Lead (Pb) and chromium (Cr) were found in a little amount that does not cross the upper limit of the range which could be harmful to the fish species as well as to humans.

Conclusion

The observations in the experiment substantiate the conclusion that an appropriate proportion of macroalgal species in fish meals may form a promising strategy to enhance food quality. The most important and beneficial PUFA (EPA and DHA) were increased with the proper use of *Hypnea sp*. these two fatty acids are important for the development of the nervous system and the prevention of cardiovascular diseases. And the ultimate target of this experiment was to enhance the meat of tilapia fish using locally available commercial feed with the supplementation of this particular seaweed but the improvement of fatty acid profile depends on the amount of seaweed used for supplementation. In this experiment, different fatty acids were high in different amounts for the treatment profile of Nile tilapia. After the rearing of tilapia best performances were shown with the supplementation of 10% seaweed (T_2) followed by 15% seaweed (T_3) and 5% seaweed (T_1). After analyzing the whole result, we can come to the conclusion that T_2 (10% seaweed supplementation) was moderate. 10% seaweed supplementation can be considered optimum for the improvement of the fatty acid profile of Nile tilapia. Considering the mentioned experimental views seaweed has a great prospect in our country and the farmers can easily use this nutrient-enriched marine macrophyte in fish feed farming.

Ethical Statement

The research work was conducted following the ethical rules and regulations of the society.

Funding Information

This study was supported by the funding from Research and Development Project of Bangladesh Council of Scientific and Industrial Research (BCSIR) under the Ministry of Science and Technology.

Author Contribution

Nahid Sultana- Conceptualization, supervision and investigation, writing and editing the manuscript; Mumtahina Mohammad- Writing the draft of the manuscript and data prcessing; Sharmin Akhter Lisa-Analyzing the wet sample and data processing; Badhan Saha- Investigation and analysis; Mahmuda Begum-Data processing and editing the manuscript; M Niamul Naser- Supervision, investigation and reviewing the manuscript. All authors read the manuscript and finalized for submission.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgements

We are thankful to Bangladesh Fisheries Research Institute, Cox's Bazar for supplying seaweed to be used in fish feed. We also thank BCSIR for logistic support in wet laboratory.

References

Abedi, E., Sahari, M.A. (2014). Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. Food Science & Nutrition **2**(5): 443-463. https://doi.org/10.1002/fsn3.121

AOAC (1884). Association of Official Analytic Chemist, 14th edition, Eds. Willim's Publiturs.

A.O.A.C. (1990). Official Methods of Analysis. 15th Edition, Association of Official Analytical Chemist, Washington DC.

Arsenault, J.E. et al. (2013). Very low adequacy of micronutrient intakes by young chidren and women in rural Bangladesh is primarily explained by food intake and limited diversity. Journal of Nutrition 143: 197-203. https://doi.org/10.3945/jn.112.169524

Aziz, A., Islm, S. (2009). Marine algae of St. Martin's Island, Bangladesh. VII. Acrochaetium nurulislmaii sp. nov. and new records of *Acrochaetium nurulislamii.* Bangladesh Journal of Botany 38: 145-15. https://doi.org/10.3329/bjb.v38i2.5139

Basemir, A., Just, N., Michalik, M., Lalk, M., Lindequist, U. (2004). Extracts and Sesquiterpens derivatives from red alga *Laurencia chondrioiodes* with antibacterial activity against fish and human pathogenic bacteria. Chem Biodiv 1: 463-467. https://doi.org/10.1002/cbdv.200490039

Becker, E.W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*. **25**: 207-210. Belton, B. and Thilsted, S.H. 2014. Fisheries in transition: Food and nutrition security implications for the global South. Global Food Security 3:59–66. https://doi.org/10.1016/j.biotechadv.2006.11.002

Chopin, T., Sawhney, M. In: Steele J.H., Thorpe S.A., Turekian K.K., Eds, Encyclopedia of ocean sciences. Elsevier, Oxford (2009), pp. 4477–4487. https://doi.org/10.5670/oceanog.2002.08

Craviari, T., Pettifor, J.M., Thacher, T.D., Meisner, C., Arnaud, J., Fischer, P.R. (2008). Rickets: an overview and future directions, with special reference to Bangladesh. Journal of Health Population and Nutrition 26:112–121.

Cunningham S., Joshi L. In: Kole C, Ed. Transgenic crop plants. (2010). Springer Verlag Berlin Heidelberg. pp. 343–357.

De Santis, C., *et al*. (2015). Replacement of dietary soy with air classified faba bean protein concentrate alters the hepatic transcriptome in Atlantic salmon (*Salmo salar*) parr. Comp. Biochem. Physiol. Part D Genomics Proteomics 16:48-58. https://doi.org/10.1016/j.cbd.2015.07.005

Dresosier, N.W. (1977). Element of food technology. *The Avi. Pub. Co. Inc*. pp.384.

El-Sayed, A.F. *et al.* (2010). Effects of L- carnitine on growth performance of Nile tilapia (*Oreochromis niloticus)* fingerlings fed basal diet or diets containing decreasing protein levels. Vol. 6(5):165-172.

FAO/WHO. (1991). Protein quality evaluation. Report of joint FAO/WHO expert consulation. Rome, Italy: Food and Agriculture Organization of Human United Nations.

Fischer, P., Rahman, A., Cimma, J., Kyaw-Myint, T., Kabir, A., Talukder, K., Hasan, N., Manaster, B., stab, d., Duxbury, J., Welch, R., Meisner, C., Haque, S., Combs, G. (1999). Nutritionnal rickets without vitamin D deficiency in Bangladesh. Journal of Tropical Pediatrics 45: 291-293. https://doi.org/10.1093/tropej/45.5.291

Fleurence, J. (1999). Seaweed proteins: biochemical, nutritional aspects and potential uses. Trends in food science and technology 10: 25-28. https://doi.org/10.1016/S0924-2244(99)00015-1

Folch, M.J, Sloane, Stanely, G.H. (1957). A simple method for isolation and purification of total lipids from animal tissue. Journal of Biological Chemistry 8p 26: 497.

Gladyshev, M.I., Lepskaya, N.N., Sushchik, *et al.,* (20120. Comparison of polyunsaturated fatty acids content in fillets of anadromous and Andlocked, Sockeya Salmon *Onorhynchus nerka*. Journal of Food Science Vol. 77 (12): 1307-1310.

https://doi.org/10.1111/j.1750-3841.2012.02998.

Gopalan, C., Sastri, B.V.R., Balasubrahanian, S.C. (1978). Nutritive value of Indian foods. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India.

Govindan, T.K. (1985). Fish Processing Technology. Oxford and IBH publishing Co. New Delhi, Bombay and Calcutta. 47-48: 180-198.

Gultepe, N., Bilen, S., Yilmaz, S., Guroy, D., Aydin, S. (2014). Effects of herbs and spice on health status of tilapia (*Oreochromis mossambicus*) challenged with *Streptococcus iniae.* Acta vet. Brano. 83:125-131.

Hemre, G.L. *et al*. (2016). Atlantic salmon (*Salmo salar*) require increased dietary levels of B-vitamins when fed diets with high inclusion of plant based ingredients. PeerJ 4: e2493. https://doi.org/10.7717/peerj.2493

ICDDR'B, UNICEF, GAIN, IPHN. (2009). National Micronutrients Status Survey 2011– 12. Final Report ICDDR'B, Dhaka, Bangladesh.

Islam, S., Aziz, A., Chowdhury, A.H. 2010. Marine algae of St. Martin's Island, Bangladesh. IX. Red algae (Rhodophyceae). Bangladesh Journal of Botany 39: 87-96. https://doi.org/10.3329/bjb.v39i1.5531

Jim, F., Garamumjango, P., Musara, C. (2017). Comprative analysis of nutritional value of *Oreochromis niloticus* (Linnaeus), Nile Tilapia, meat from three different ecosystems. Journal of Food Quality Article ID: 6714347. Pp. 8. https://doi.org/10.1155/2017/6714347

Kalasariya, H.S, Patel, R.V., Pandya, K.Y, Jasrai, R.T., Brahmbhatt, N.H. (2016). A review on nutritional facts of seaweeds. Int J Chem Sci Technol 1: 28-32. DOI: 10.20517/2394-4722.2020.134

MacArtain, P., Gill, C.I.R., Brooks, M., Campbell, R., Rowland, I.R. (2007). Nutritional value of edible seaweeds. Nutr Rev 65: 535-543. https://doi.org/10.1301/nr.2007.dec.535-543

Mustafa, M.G., Wakamatsu, S., Takeda, T., Umino, T., Nakagawa, H., Nakagawa, (1995a). Effect of algae as a food additive on growth performance in red sea bream, Pagrus major. Trace Nutrients Research **12**: 67-72.

Mustafa, M.G., Wakamatsu, S., Takeda, T., Umino, T., Nakagawa, H., Nakagawa, (1995b). Effects of algae meal as feed additive on growth, feed efficiency and body composition in red sea bream. Fisheries Science 61: 25-28.

Nakagawa, H. (1985). Usefulness of Chlorella extract for improvement of the physiological condition of cultured ayu, *Plecoglosus altivelis*, Tethys. 11: 328-334.

Nicholus, D.S., Nichols, P.D., Mcmeekin, T.A. (1995). Polyunsaturated fatty acids in Antarctica bacteria. Antarctica Science 5(2): 149- 160.

Ozogul, Y., Simsek, A., Balikci, E., Kenar, M. (2012). The effects of extraction methods on the contents of fatty acids, especially EPA and DHA in marine lipids. Int J Food Sci Nutr 63(3): 326-31. doi: 10.3109/0937486.2011.627844

Pulz, O., Gross W. (2004). Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology 65: 635–648.

Rubbi, S.F., Mujibar, M., Khan, A.R., Jahan, S. S., Majeda, B. (1987). Proximate composition and quality of some commercial species of fresh water fishes. Bangladesh J. Sci. Res 5(1): 1-20.

Siddik, M.A.B., Anh NTN. (2015). Preliminary assessment of the gut weeds. Ulva intestinalis as food for herbivorous fish. Int Aquat Res **7**: 41-46. DOI 10.1007/s40071-014-0091-5

Stansby, M.E. (1954). Composition of certain species of freshwater fish. Food Res 16: 231-234.

Suloma, A. et al. (2008). Fatty acid composition of Nile Tilapia *Oreochromis niloticus* muscles: a comparative study with commercially important tropical freshwater fish in Philippines. 8th Internatinal Symposium on Tilapia in Aquaculture. 921-931.

Swanson, A.K., Druehl, L.D. (2002). Induction, exudation and the UV protective role of kelp phlorotannins. Aquatic Botany 73: 241–253. http://dx.doi.org/10.1016/S0304-3770(02)00035-9

Swarmalatha, G. 2018. Comparisn of nutritive value of seaweed with other terrestrial foods- a review. IJFANS **7**: 61-70.

Tolentino-Pablico G, Bailly N, Froese R, Elloran C. (2008). Seaweeds preffered by herbivorous fish. J Appl Phycol 20: 933-938. DOI 10.1007/s10811-007-9290-4

Woyewode, A.D., Shaw, S.J., Ke. P.J., Burns, B.G. (1986). Recommend laboratory methods for assessmengt of fish quality. Can. Tech. Rep. Fish Aquat Sci. No. 14.