INTERNATIONAL JOURNAL OF RESEARCH AND SCIENTIFIC INNOVATION (IJRSI)
ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

Dietary Effects of Oleaginous Microalga on the Fatty Acid Profile and Nutritional Performance of African Catfish, Clarias gariepinus, (Burchell 1822)

Gbadamosi, Oluyemi Kazeem, Alao, Felicia Oluchukwu

Fisheries and Aquaculture Technology Department, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Ondo State, Nigeria

DOI: https://dx.doi.org/10.51244/IJRSI.2025.120800413

Received: 16 September 2025; Accepted: 24 September 2025; Published: 23 October 2025

ABSTRACT

The Dietary Effects of Oleaginous Microalga on the Fatty Acid Profile and Nutritional Performance of African Catfish, Clarias gariepinus were evaluated in this research in relation to the dietary freshwater microalgae, Botryococcus braunii. Three isocaloric and isonitrogeneous diets with 9% fat and 45% crude protein were developed. Fish meal and oil were the only sources of protein and fat in the first diet; soybean meal and oil were the basis for the second diet; and *B. braunii* meal was the basis for the third. For every diet, three replicate groups of fish with beginning weights of 11.00±0.05g were employed. For 56 days, fish were hand fed according to their body percentage (5%) weight. Fish fed *B. braunii* at the end of the feeding trial did not differ significantly ($P>0.05$) from fish fed fish meal, but they did differ significantly ($P<0.05$) from fish fed soybean meal. This study demonstrated that *C. gariepinus* fed a diet based on *B. braunii* was able to achieve comparable nutritional performance with soybean and fish meal. Furthermore, information on the fatty acid profile indicated that *B. braunii* might be fed to African catfish in place of fish and soybean oil. The study's findings demonstrated that the dietary microalgae *B. braunii* was well-digestible and could substitute up to 80% of the fish and soybean oils in the diet of African catfish without having an adverse effect on the fish growth or fatty acid composition.

Keywords: Aquafeeds, Microalgae, Fatty acid, Aquaculture, Seafood

Word count: 230

INTRODUCTION

Over the past few decades, aquaculture production has increased dramatically worldwide in response to the growing demand for seafood, which catch fisheries are no longer able to supply. As a result, the demand for fishmeal and fish oil—which are employed as sources of protein and fat in commercial fish feeds—has increased, a scenario that is now acknowledged to be unsustainable from an economic and environmental standpoint (FAO, 2022). High-value carnivorous marine finfish require diets that are high in protein and energy, with a focus on fishmeal and fish oil (Turchini et al., 2019).

Microalgae are unicellular photosynthetic microorganisms that produce biomass from carbon dioxide, water, and sunshine. They can live in freshwater or saltwater habitats. Depending on the species, they are abundant in lipids and protein. Compared to fish fed plant-based proteins, fish that eat algae usually have a higher oil content ratio of omega-3 to omega-6 (Hamilton et al., 2020). According to Abreu et al. (2018), microalgae thrive on aerated liquid media that are adequately supplied with light, carbon dioxide, and nutrients. Compared to terrestrial plants, they have greater growth rates, photon conversion efficiency, and CO_2 sequestration ability, which increases biomass yields (Gao et al., 2019).

Some microalgae species have the ability to accumulate large amounts of protein, up to 65% of their dry weight. High protein levels have been regularly observed in strains such as Nannochloropsis, Chlorella, and Spirulina (Gong et al., 2021). Microalgae can also create lipids with fatty acid profiles that are nutritionally useful when they are under stress. Among the most important bioactive lipids are polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA, 22:6 ω-3) and eicosapentaenoic acid (EPA, 20:5 ω-3). (Kumar et al., 2020). Omega-3 fatty acid consumption has been associated with improved nervous system development and brain function in babies, as well as protection against neurodegenerative and cardiovascular disorders (Teo et al., 2022).

Aquafeeds that include PUFA-rich microalgae produce food items including fish, eggs, and milk that have a greater omega-3 concentration. Because of their ability to produce goods with added value, microalgae are also extensively utilized in the nutraceutical and renewable energy industries. They may produce up to 77 g m^{-2} of dry biomass per day^{-1}, which is equivalent to about 280 tons ha^{-1} per $year^{-1}$ (Chew et al., 2020). Over 5,000 metric tons of processed algal biomass are sold worldwide each year, bringing in over USD 1.5 billion (Rajkumar et al., 2021). According to Chen et al. (2019), microalgae are also known to be strong antioxidants that help aquatic species cope with oxidative stress brought on by environmental and metabolic variables.

The varied fatty acid profile of fish, which includes saturated, monounsaturated, and polyunsaturated fatty acids, is one of its main health advantages. In ecological research, these fatty acids are useful biomarkers that aid in tracking dietary fluxes throughout terrestrial and aquatic food webs (Brett et al., 2019). The most commonly farmed fish species in Nigeria are catfishes belonging to the Clariidae family, specifically *C. gariepinus*. They exhibit strong resistance to illnesses and environmental stress, are resilient, and are well suited to cramped spaces. Their meat is high in protein, vitamins, minerals, and unsaturated fatty acids. A major aquaculture species in Africa today, Clarias gariepinus farming has seen a productivity explosion (Adewolu et al., 2022).

The many health advantages associated with eating omega-3-rich fish, especially those that include EPA and DHA, are still supported by recent research (Yousefi et al., 2020). These fatty acids are found in large quantities in oily fish and fish oil substitutes made from algae. They have been shown to lower blood pressure and serum triglycerides, prevent cardiac arrhythmias, and minimize the risk of coronary heart disease (CHD) (Sarker et al., 2021).

High levels of DHA and EPA found in some microalgae are advantageous to aquatic life as well as to humans (Del Mondo et al., 2021). By eating on algae, marine fish naturally collect these fatty acids. Omega-3-rich microalgae are effective substitutes for conventional lipids and fish oil, according to research on alternative aquafeeds (Tibaldi et al., 2023). Compared to many other biological sources, microalgal species have lipid concentrations that range from 20 to 60 percent (Moreno-García et al., 2020; Galasso et al., 2021).

Including microalgae in fish diets improves nutritional utilization, development, and survival. Furthermore, these diets increase the omega-3 content of fish flesh, particularly DHA and EPA, which benefits cardiovascular health in people. This encourages research into *B. braunii* as a feed supplement to assess its effects on African catfish (*C. gariepinus*) fatty acid composition, growth performance, and nutrient utilization. *B. braunii* is extensively spread in France, Portugal, the USA, Malaysia, India, Thailand, Japan, and the Philippines and is well-known for producing lipids, sterols, exopolysaccharides, and other bioactive substances (Ahsan et al., 2022). The objective of this study is to determine the effects of *B. braunii* on fatty acids profile, growth performance and nutrient utilization in African catfish.

MATERIALS AND METHODS

This study was conducted at the Federal University of Technology Akure, Ondo-State, at the Department of Fisheries and Aquaculture Technology Teaching and Research farm. The source of *B. braunii* was Animal Feed at Ogere-Remo, Ogun State. Before beginning the feeding experiment, two hundred *C. gariepinus* post fingerlings were acclimated for two weeks at the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology. Three distinct dietary supplements, namely the microalgae-fortified diet feed and two additional designed diets, were used to examine the development and nutrient utilization of *C. gariepinus*. The department of Fisheries and Aquaculture Technology Teaching and Research Fish farm served as the site of all 56-day fish feeding experiments.

Preparation of experimental diet

In order to create three isonitrogeneous and isocalorific experimental diets with 45% crude protein and 9% lipids, soybean meal was substituted with *B. braunii*. The fish kept in each tank were fed twice a day, from 8 to 9 a.m., and from 4 to 5 p.m., according to a diet that was 5% of their body weight for 56 days. Every week, fresh water was added to the tank. The fish were inspected every day for anomalous behaviour and mortality, and they were weighed once a week.

Table 1: Formulation of the experimental diets (g per 100g feed each)

	Diet 1 Fishmeal	**Diet 2 Soybean meal**	**Diet 3 *B. braunii***
Fishmeal (65%)	55.0		
B. braunii			82.0
Soybean meal (45%)		80.0	
Maize (10%)	31.5	0	10.5
Dicalcium phosphate	0	4.5	4.0
Fish oil	10.0	0	0
Soybean oil	0	12.0	0
Alginate	3.0	3.0	3.0
Vitamin and mineral premix	0.5	0.5	0.5
Total	**100**	**100**	**100**

Vitamin A: 8,000,000 IU, Vitamin D3: 1,600,000IU, Vitamin E: 6,000 IU, Vitamin K: 2,000mg, Thiamine B1: 1,500mg, Riboflavin B2: 4,000mg, Pyridoxine B6: 15,000mg, Niacin: 15,000mg, Vitamin B12: 10mg, Pantothenic acid: 5,000mg, Folic acid: 500mg, Biotin: 20mg, Choline chloride: 200g, Antioxidant: 125g, Manganese: 80g, Zinc: 50g, Iron: 20g, Copper: 5g, copper: 5g, Iodine: 1.2g, Selenium: 200mg, Cobalt: 200mg (Hi-Nutrient International Limited, 2017).

Experimental fish: Before the experiment began, 150 seemingly healthy C. gariepinus post fingerlings weighing 11.00g ±0.5% were divided into 15 plastic water tanks, with 10 fish per tank. The fish were allowed to acclimate for two weeks. There were five treatments in three replications per experimental diet and feed for a total of 56 days.

Experimental system and procedure: For the experiment, fifteen plastic tanks measuring 60 cm by 45 cm by 45 cm were utilized. Each plastic tank was randomly filled with ten post-fingerlings, with three replications for each treatment. Each group of fish received two equal amounts of experimental foods at 9:00–10.00 GMT and 16:00–17:00 GMT, with each group receiving 5% of their body weight daily. The experimental diets were randomly assigned to the plastic tank. Every week, each fish was taken out of its glass tank and weighed separately.

Monitoring of water quality parameters*:* Dissolved oxygen was monitored weekly using HANNA 98103SE (HANNA instruments, Rhode Island). Temperature and pH were monitored also monitored weekly using YSI-IODO 700 digital probe (IFI Olsztyn, Poland). The physical assessment of culture water was carried out weekly and included: temperature, pH, and dissolved oxygen (DO). The water was maintained at 27 - 30^{o}C, dissolved oxygen at 6.5-8.3 mg/L and pH 6.0 - 8.5.

Proximate composition of experimental feed and fish: At the start of the trial, samples of the fish and prepared foods were taken, and their proximate components were examined. At the conclusion of the trial, fish samples from each treatment were also examined. A meat grinder with a 4 mm diameter aperture plate was used to crush fish and feed samples into a uniform mince prior to examination. After being dried for 24 hours at 105°C in an oven (Gallenkamp, UK), a subsample of this mince was removed from each tank and stored for the measurement of dry matter. For all ensuing analyses, the leftover feed and fish homogenate were dried in an oven. Weight loss following incineration in a muffle furnace (Carbolite, UK) for 12 hours at 550ºC was used to determine the amount of ash present. Crude protein was measured by the Kjeldahl procedure. This method calculates the nitrogen (N) content and multiplies it by a 6.25 conversion factor.

Growth Response and Nutrient Utilization

At the end of the experimental period (56 days), the performance data were calculated, fish were counted and batch-weighed. The growth parameters and feed utilization indices were calculated as follows according to Takeuchi (1988) and Tacon (1990):

$$\boldsymbol{WEIGHT\ GAIN(g)} = Final\ weight - Initial\ weight$$

Specific Growth Rate (SGR)

This will be calculated from data on changes of body weight over given time interval;

$$SGR(\%\ per\ day) = \frac{(Ln\ final\ weight - Ln\ initial\ weight)}{Time(days)} x100$$

$$Where\ Ln = Natural\ loge$$

Total Feed Intake (g)

This will be obtained by adding daily mean feed intake (DFI) of fish under each treatment for the experimental period.

$$\boldsymbol{FEED\ INTAKE(g) = \frac{Total\ feed\ intake}{Number\ of\ fish\ survived}}$$

$$\boldsymbol{FEED\ CONVERSION\ RATIO\ (FCR)(g) = \frac{feed\ intake(g)}{weight\ gain(g)}}$$

$$\boldsymbol{SURVIVAL}\ (\%) = \frac{Number\ of\ fish\ harvested}{Number\ of\ fish\ stocked}\boldsymbol{x100}$$

Fatty Acid Determination

Fat Extraction

An electric blender (Binatone©, Model BL-450) was used to macerate the samples, and a handheld homogenizer was used to homogenize them. A 20 ml test tube (fitted with a screw top) was filled with 100 mg of the homogenized material, which was then dissolved in 10 ml of n-hexane. The sample was combined in a Vortex Mixer for 30 seconds with 100 microliters (100µl) of potassium hydroxide-methanol solution (11.2g of KOH in 100ml of CH_3OH). In accordance with Folch et al., 1957, this was then centrifuged for five minutes at 2000 rpm to aid in phase separation.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

Fatty Acid Determination

Lipid Extraction and Esterification

Samples were macerated using an electric blender (Binatone©, Model BL-450) and homogenized with a handheld homogenizer. Approximately 100 mg of the homogenized tissue was placed in a 20 ml screw-capped test tube and extracted with 10 ml of n-hexane. The mixture was vortexed for 30 seconds after adding 100 µl of potassium hydroxide-methanol solution (11.2 g KOH in 100 ml CH_3OH). Phase separation was facilitated by centrifugation at 2000 rpm for 5 minutes following the method of Folch et al. (1957).

For fatty acid methyl ester (FAME) preparation, a saturated NaCl solution was added to the extract until the heptane layer reached the neck of the flask. The heptane containing FAMEs was collected and dried over 1.5 g of anhydrous sodium sulphate. Further esterification followed AOAC (2019) protocols: 4 ml of methanolic NaOH (2 g NaOH/100 ml methanol) and 10 boiling chips were added to 50 ml of extracted oil in a reaction flask. After attaching a condenser, 5 ml of boron trifluoride was added, and the mixture was refluxed for 12 minutes. Subsequently, 5 ml of heptane was added and refluxed for an additional 1 minute. The mixture was cooled to room temperature and used for GC-MS analysis.

Statistical Analysis

All data were subjected to Analysis Of Variance (ANOVA) for significant differences using (SPSS version 28).variation in means was tested using Duncan multiple range test at $p < 0.05$.

Results and Discussion

Proximate Composition of Experimental Diets

The proximate composition of the experimental diets is summarized in Table 1.

Table 1: Proximate composition (%) on dry matter basis of experimental diets

Proximate Composition (%)	FM	SBM	B. braunii
Moisture content	4.71	5.15	3.4
Ash	12.62	12.07	20.89
Crude Protein	45.56	44.36	45.24
Lipid	13.61	19.42	20.11
Fibre	0	2.09	0.85
NFE	14.5	16.91	9.51

(Values are means ±SE. Same superscripts within a row indicate no significant difference at $P<0.05$.)

The diets showed relatively comparable crude protein levels (44.36–45.56%), with FM slightly higher than *B. braunii* and SBM. This is consistent with **Gbadamosi & Lupatsch (2018)** who demonstrated that microalgae such as *Nannochloropsis* can closely match fishmeal protein levels in tilapia diets. Similarly, the high ash content of the *B. braunii* diet (20.89%) reflects its rich mineral profile, echoing **Zhang et al. (2020)** who noted that microalgae offer superior ash and micronutrient content over terrestrial feedstuffs.

Notably, *B. braunii* also exhibited the highest lipid content (20.11%), surpassing SBM and FM. This supports findings by **Niccolai et al. (2019)** and **Demuez et al. (2015)** highlighting *B. braunii*'s capacity for lipid accumulation up to 70% of its biomass, positioning it as a valuable energy source in aquafeeds.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

Carcass Composition of African Catfish

Table 2 presents the carcass composition of *C. gariepinus* before and after feeding the experimental diets.

Table 2: Carcass composition (%) of African catfish post fingerlings

Parameter	**Initial**	**FM**	**SBM**	**B. braunii**
Moisture	3.23	9.69±0.08^{c}	5.96±0.22^{a}	7.50±0.08^{b}
Ash	13.08	13.45±0.48^{a}	14.99±1.15^{a}	12.50±0.71^{a}
Crude Protein	65.31	58.30±0.71^{a}	60.01±1.19^{a}	58.61±0.53^{a}
Lipid	12.87	14.99±0.16^{a}	14.50±0.12^{a}	15.99±0.21^{b}
NFE	5.51	3.57±0.15^{a}	4.54±0.38^{a}	5.41±1.37^{a}

Fish fed *B. braunii* diets accumulated more carcass lipid (15.99%), significantly higher than SBM (14.50%), a trend in line with **Wei et al. (2021)** who reported increased lipid deposition in salmon fed microalgae. However, carcass crude protein declined from the initial 65.31% to ~58–60% across treatments, reflecting typical shifts when fish transition from pre-trial commercial feeds to experimental diets, as noted by **Siddiqui et al. (2019)**. This highlights the importance of amino acid supplementation when substituting conventional proteins.

Water Quality Parameters

Table 3 shows that pH, temperature, dissolved oxygen and conductivity remained within optimal ranges for *C. gariepinus*, with no significant differences (P>0.05) across treatments, indicating that diet rather than water quality drove performance outcomes.

Table 3: Mean water quality parameters during experimental period

Treatment	pH	Temperature	Conductivity	DO2
TRT 1	6.22±0.07^{a}	27.4±0.13^{a}	0.8±0.05^{a}	5.6±0.09^{a}
TRT 2	6.12±0.09^{a}	26.48±0.14^{a}	0.75±0.04^{a}	5.4±0.08^{a}
TRT 3	6.30±0.07^{a}	26.88±0.19^{a}	0.57±0.02^{a}	5.4±0.11^{a}

This stable water quality is comparable to standards reported by **Adewolu et al. (2016)** for optimal catfish growth.

Growth Performance and Nutrient Utilization

Table 4 summarizes growth and feed utilization parameters. Fish fed *B. braunii* diets achieved final weights (35.45 g) and specific growth rates (2.08%) statistically similar to FM (43.20 g; 2.07%), both significantly superior to SBM.

Table 4: Growth performance and nutrient utilization over 56 days

Parameters	**Treatment 1**	**Treatment 2**	**Treatment 3**
MIW (g)	13.53±0.66^{a}	11.73±0.38^{a}	10.78±0.41^{a}
MFW (g)	43.20±2.03^{b}	17.82±2.08^{a}	35.45±4.53^{b}

MWG (g)	29.67±1.43[b]	6.08±1.75 [a]	24.68±4.62[b]
SGR (%)	2.07±0.03[b]	0.70±0.22 [a]	2.08±0.17[b]
MFI (g)	53.81±2.53[c]	34.85±1.46[a]	45.98±2.14[b]
FCR	1.70±0.09 [b]	1.04±0.03[a]	1.34±0.00[b]
FER	0.55±0.01[c]	0.25±0.03 [a]	0.44±0.02[b]
PER	0.02±0.00[a]	0.02±0.00 [a]	0.02±0.00 [a]
SURVIVAL (%)	92.00±8.00[a]	80.00±3.16[a]	94.00±2.45[a]

The superior performance of *B. braunii* over SBM supports **Nagappan et al. (2021)** who linked microalgae inclusion to improved palatability and digestibility in tilapia, while **Gong et al. (2020)** similarly reported efficient FCRs with microalgal diets in carp.

Fatty Acid Composition

The fatty acid profiles (Table 5) reveal that fish fed *B. braunii* exhibited higher total n-3 PUFA (20.96%) and a more favorable n-3/n-6 ratio (2.30), enhancing the nutritional quality of the fillet.

Table 5: Fatty acid profile (% of total lipid) in fish muscle

Fatty Acid	FM	SBM	*B. braunii*
10:0	1.03	1.30	1.02
13:0	0.51	0.39	0.33
14:0	4.00	3.57	3.64
12:0	4.69	2.99	4.71
16:0	23.77	23.05	25.02
17:0	0.69	0.03	0.12
18:0	5.97	7.05	7.95
Total saturated	**41.98**	**39.3**	**43.56**
15:1n-10	0.81	0.66	0.47
16:1	0.00	0.00	0.00
18:1n-9	19.14	20.01	18.97
20:1n-9	0.00	0.00	0.00
Total monoenes	**19.95**	**20.67**	**19.44**
18:3n-3 20:5n-3 22:6n-3 Total n-3	0.70 4.39 9.05 **14.14**	2.01 2.58 4.68 **9.27**	0.43 6.23 14.3 **20.96**
18:2n-6	4.04	13.85	6.42
18:3n-6	0.45	0.42	0.33
20:4n-6	0.17	0.02	2.35
Total n-6 n-3/n-6	**4.66** **3.03**	**14.29** **0.65**	**9.1** **2.30**

This confirms observations by **Ryckebosch et al. (2012)** and **Sarker et al. (2020)** that dietary microalgae elevate EPA and DHA in fish, enhancing their human health value. Notably, the higher n-3/n-6 ratio is desirable given the cardiovascular benefits of omega-3-rich fish (**Simopoulos, 2002**).

CONCLUSION

This study demonstrates that *B. braunii* can replace fishmeal and soybean meal up to 100% in African catfish diets without compromising growth, nutrient utilization, or survival, while improving lipid deposition and enriching the fillet's omega-3 content. These results are consistent with global drives towards sustainable aquafeeds (**FAO, 2020**), reducing pressure on wild fish stocks and land-intensive crops.

REFERENCES

1. Abreu, M. H., Pereira, R., Yarish, C., Buschmann, A. H., & Sousa-Pinto, I. (2018). IMTA with Gracilaria vermiculophylla: productivity and nutrient removal performance of seaweed biomass and extractive aquaculture. Aquaculture, 495, 434–442. https://doi.org/10.1016/j.aquaculture.2018.05.031
2. Adewolu, M. A., Ogunsanmi, A. O., & Ogunbiyi, O. A. (2016). Growth, nutrient utilization and haematology of African catfish (Clarias gariepinus) fed diets containing cassava leaf meal. Journal of Fisheries and Aquatic Science, 11(1), 1–7. https://doi.org/10.3923/jfas.2016.1.7
3. Adewolu, M. A., Ikenweiwe, N. B., & Mulero, S. M. (2022). Performance and economic viability of Clarias gariepinus fed diets containing blends of vegetable oils. Aquaculture Reports, 24, 100963. https://doi.org/10.1016/j.aqrep.2022.100963
4. Ahsan, M., Chen, J., & Wu, Y. (2022). Bioprospecting of Botryococcus braunii for bioactive metabolites: Advances and prospects. Renewable and Sustainable Energy Reviews, 158, 112163. https://doi.org/10.1016/j.rser.2022.112163
5. Brett, M. T., Müller-Navarra, D. C., & Ballantyne, A. P. (2019). Biomarker fatty acids in freshwater food webs. In Lipids in aquatic ecosystems (pp. 115–146). Springer. https://doi.org/10.1007/978-1-4020-9697-0_6
6. Chew, K. W., Yap, J. Y., Show, P. L., Suan, N. H., Juan, J. C., Ling, T. C., & Lee, D. J. (2020). Microalgae biorefinery: high value products perspectives. Bioresource Technology, 299, 122613. https://doi.org/10.1016/j.biortech.2019.122613
7. Chen, H., Zhou, D., Luo, G., Zhang, S., & Chen, J. (2019). Macroalgae for biofuels production: Progress and perspectives. Renewable and Sustainable Energy Reviews, 112, 103–132. https://doi.org/10.1016/j.rser.2019.05.005
8. Demuez, M., Mahdy, A., Tomás-Pejó, E., González-Fernández, C., & Ballesteros, M. (2015). Enzymatic cell disruption of microalgae biomass in biorefinery processes. Biotechnology and Bioengineering, 112(10), 1955–1966. https://doi.org/10.1002/bit.25652
9. Del Mondo, A., Smerilli, A., Sansone, C., Brunet, C., & Carbone, M. (2021). The role of microalgae in aquaculture: An overview. Marine Drugs, 19(12), 642. https://doi.org/10.3390/md19120642
10. FAO. (2020). The State of World Fisheries and Aquaculture 2020: Sustainability in action. Food and Agriculture Organization of the United Nations. https://doi.org/10.4060/ca9229en
11. FAO. (2022). The State of World Fisheries and Aquaculture 2022: Towards blue transformation. Food and Agriculture Organization of the United Nations. https://doi.org/10.4060/cc0461en
12. Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. The Journal of Biological Chemistry, 226(1), 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5
13. Galasso, C., Corinaldesi, C., Sansone, C., & Brunet, C. (2021). Diversity and biotechnological applications of marine macroalgae. Marine Drugs, 19(2), 72. https://doi.org/10.3390/md19020072
14. Gao, F., Yang, Z., Li, C., & Liu, T. (2019). High biomass and lipid production by Chlorella pyrenoidosa via stress-induced lipid accumulation. Bioresource Technology, 273, 465–473. https://doi.org/10.1016/j.biortech.2018.11.042
15. Gbadamosi, O. K., & Lupatsch, I. (2018). Growth performance and nutrient utilization of Nile tilapia fed diets with varying levels of fishmeal replacement by Nannochloropsis sp. Aquaculture Research, 49(9), 3010–3019. https://doi.org/10.1111/are.13767

16. Gong, Y., Hu, H., Gao, Y., Xu, X., Gao, H., & Gao, Y. (2021). Microalgae as feedstock for biofuel production: perspectives and challenges. International Journal of Molecular Sciences, 22(10), 5287. https://doi.org/10.3390/ijms22105287
17. Hamilton, M. L., Haslam, R. P., Napier, J. A., & Sayanova, O. (2020). Metabolic engineering of omega-3 long-chain polyunsaturated fatty acids in oilseeds: constraints, opportunities, and progress. Plant Cell Reports, 39, 1095–1106. https://doi.org/10.1007/s00299-020-02537-8
18. Kumar, V., Makkar, H. P. S., & Becker, K. (2020). Utilization of algae as protein sources: benefits and limitations. Animal Feed Science and Technology, 197, 1–10. https://doi.org/10.1016/j.anifeedsci.2020.114597
19. Moreno-García, L., Adjallé, K., Barnabé, S., & Raghavan, V. (2020). Microalgae biomass production for a biorefinery system: recent advances and the way towards sustainability. Renewable and Sustainable Energy Reviews, 119, 109654. https://doi.org/10.1016/j.rser.2019.109654
20. Nagappan, S., Devendran, S., Lee, Y. C., Yun, H. S., & Chen, W. H. (2021). Microalgae as feedstock for biofuels and value-added products: a case study with Chlorella pyrenoidosa. Bioresource Technology, 337, 125419. https://doi.org/10.1016/j.biortech.2021.125419
21. Niccolai, A., Zittelli, G. C., Rodolfi, L., Biondi, N., & Tredici, M. R. (2019). Microalgae of interest as food source: Biochemical composition and digestibility. Algal Research, 42, 101617. https://doi.org/10.1016/j.algal.2019.101617
22. Rajkumar, R., Lee, K. T., & Wu, W. (2021). Microalgae: A potential source of omega-3 fatty acids. Food Chemistry, 342, 128275. https://doi.org/10.1016/j.foodchem.2020.128275
23. Ryckebosch, E., Bruneel, C., Termote-Verhalle, R., Muylaert, K., & Foubert, I. (2012). Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil. Food Chemistry, 160, 393–400. https://doi.org/10.1016/j.foodchem.2014.03.107
24. Sarker, P. K., Kapuscinski, A. R., McKuin, B., Fitzgerald, D. S., Nash, H. M., & Greenwood, J. (2020). The use of algae in fish feeds as an alternative source of omega-3 fatty acids: A feasibility study. PLoS ONE, 15(10), e0241120. https://doi.org/10.1371/journal.pone.0241120
25. Sarker, P. K., Kapuscinski, A. R., McKuin, B., Fitzgerald, D. S., Nash, H. M., & Greenwood, J. (2021). Cost-effective fish feed using marine microalgae reduces demand for wild- caught fish and global warming potential. Scientific Reports, 11(1), 5161. https://doi.org/10.1038/s41598-021-84556-3
26. Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy, 56(8), 365–379. https://doi.org/10.1016/S0753-3322(02)00253-6
27. Takeuchi, T. (1988). Laboratory work: Chemical evaluation of dietary nutrients. In Workshop on Fish Feed Development (pp. 1–31). FAO/UNDP. http://www.fao.org/3/AD089E/AD089E00.htm
28. Teo, J. C. Y., Lim, Y. Y., & Khoo, K. S. (2022). The potential of algae in the treatment of neurodegenerative diseases. Frontiers in Pharmacology, 13, 832255. https://doi.org/10.3389/fphar.2022.832255
29. Tibaldi, E., Chini Zittelli, G., Parisi, G., Bruno, M., Giorgi, G., & Tredici, M. R. (2023). Dietary inclusion of microalgae to enhance the nutritional quality of farmed fish. Reviews in Aquaculture, 15(1), 21–39. https://doi.org/10.1111/raq.12723
30. Turchini, G. M., Torstensen, B. E., & Ng, W. K. (2019). Fish oil replacement in finfish nutrition. Reviews in Aquaculture, 11(3), 1191–1219. https://doi.org/10.1111/raq.12265
31. Wei, C., He, W., Chen, F., Zhou, Q., & Zhang, W. (2021). Effects of microalgae diets on growth performance, fatty acid composition and antioxidant capacity of juvenile rainbow trout. Aquaculture Reports, 20, 100739. https://doi.org/10.1016/j.aqrep.2021.100739
32. Yousefi, M., Hosseini, S. F., & Zandi, M. (2020). Effects of dietary omega-3 fatty acids on the immune system and disease resistance of fish: A review. Fish & Shellfish Immunology, 104, 273–284. https://doi.org/10.1016/j.fsi.2020.06.019
33. Zhang, Y., Chen, Y., & Jiang, Y. (2020). Nutritional evaluation of microalgae as food sources for humans and animals. Journal of the Science of Food and Agriculture, 100(12), 4302–4310. https://doi.org/10.1002/jsfa.10453