IJPAS | INTERNATIONAL JOURNAL PURE AND APPLIED SCIENCES
ISSN: 2149-0910

Vol. 10(1); 276-282 (2024)
DOI: 10.29132/ijpas.1477244

Research Article

Growth, fatty acid and amino acid composition of *Nannochloropsis* sp. (D.J. Hibberd, 1981) used in the feeding trials of crab *Callinectes sapidus* larvae

Övgü GENCER

Received: 07.05.2024
Accepted: 03.06.2024

Ege University, Faculty of Fisheries, Aquaculture Department, Izmir, Türkiye; ovgu.gencer@ege.edu.tr

Abstract: In aquaculture hatcheries, microalgae, including *Nannochloropsis* sp. (D.J. Hibberd, 1981), are utilized as live food sources, The development of algal cultures employed in inoculation can satisfy the need for Nannochloropsis. This process begins with stock cultures on a laboratory scale and advances to intensive algal cultures in higher volume structures set up in both indoor and outdoor environments. The aim of the study was (1) to determine the specific growth rate (μ) of *Nannochloropsis* sp. grown under laboratory conditions as a live feed source to feed crab larvae of *Callinectes sapidus* (Rathbun, 1896) (Brachyura: Portunidae), and (2) to determine the fatty acid and amino acid compositions of *Nannochloropsis* sp. According to the study's findings, Nannochloropsis sp. had a specific growth rate (μ) of 0.553 ± 0.004 when grown in Guillard F/2 culture medium with aeration, 25 ppt salinity, and 25 0C room temperature. Out of all the necessary fatty acids, DHA, at 22:6 (ω-3) made up 0.5 ± 0.07 percent, and EPA, at 20:5 (ω-3) made up 24.25 ± 3.45 percent. The total amino acid content of Nannochloropsis was observed to range from 0.00 ± 0.00% (taurine) to 16.35 ± 3.56% (proline).

Keywords: Microalgae; *nannochloropsis* sp.; amino acids; crab larvae

Yengeç, *Callinectes sapidus*, larvalarının beslenme denemelerinde kullanılan *Nannochloropsis* sp. (D.J. Hibberd, 1981)'nin büyüme, yağ asidi ve amino asit kompozisyonu

Özet: Nannochloropsis sp. (D.J. Hibberd, 1981) Su ürünleri kuluçkahanelerinde doğal besin kaynağı olarak kullanılan bir mikroalg türüdür. Çalışmanın amacı, Yengeç, Callinectes sapidus (Rathbun, 1896) (Brachyura: Portunidae) larvalarını beslemek için canlı yem kaynağı olarak laboratuvar koşullarında yetiştirilen Nannochloropsis sp.'nin (1) spesifik büyüme oranının (μ) belirlenmesi, ve (2) Nannochloropsis sp.'nin yağ asidi ve amino asit kompozisyonlarının tespit edilmesiydi. Çalışma sonuçları, havalandırmanın uygulandığı, Guillard F/2

kültür ortamının kullanıldığı, 25 ppt tuzluluğun ve 25 0C oda sıcaklığının uygulandığı kültür koşullarında Nannochloropsis sp.'nin spesifik büyüme oranının (μ) 0,553 ± 0,004 olduğunu bulundu. Esansiyel yağ asitlerinden EPA, 20:5 (ω-3), toplam yağ asitlerinin yüzde 24,25± 3,45 ünü oluştururken, DHA, 22:6 (ω-3) yüzde 0,5 ± 0,07'si kadardı. Nannochloropsis'teki amino asitler, toplam amino asitlerde %0,00 ± 0,00 (Taurin için) ile %16,35 ± 3,56 (Prolin için) arasında değiştiği tespit edildi.

Anahtar Kelimeler: mikroalgler; nannochloropsis sp.; amino asitler; yengeç larvaları

1. Introduction

There are six known species of the algae genus Nannochloropsis. D.J. Hibberd first named the genus in 1981 [1]. These species are frequently found in freshwater and brackishwater environments, as well as marine environments [2]. These are tiny, immobile spheres that lack any distinguishable morphological characteristics that can be seen with an electron or light microscope. One important feature that distinguishes Nannochloropsis from other microalgae is that it has chlorophyll a but not chlorophyll b or c. They can also accumulate other pigments such as canthaxanthin, zeaxanthin, and astaxanthin [3]. The diameter of these algae is usually between 2 and 3 microns [4].

Because Nannochloropsis can retain large amounts of polyunsaturated fatty acids, including biodiesel, it is highly valued for its potential in industrial applications [5, 6]. For fish larvae and rotifers, it is currently mostly used as a nutrient-rich food source [4,7].

Nannochloropsis cells have an oil content of almost 30% of their dry weight when grown under normal growth conditions in Guillard's F/2 medium [8], a common algal culture growth medium, supplemented with nitrogen [4, 9]. However, it was found that different culturing conditions increased the average oil content per cell, sustaining only sluggish rates of culture growth and lowering total output. Nitrogen deficiency is one of these circumstances that has been examined the most extensively.

Research has looked at how cultures behave under nitrogen stress in a variety of culturing configurations as well as how the cells react physiologically and molecularly to nitrogen deficiency [4, 9]. When nitrogen is limited, it has been demonstrated that certain species of Nannochloropsis may store up to 60–70% of their total biomass as lipids [4, 9]. In this scenario, the composition as well as the amount of lipids vary. It was shown that whereas other lipids, primarily polar glycerolipids, free fatty acids, and diacylglycerols, somewhat decreased in nitrogen depletion, triacylglycerols increased it by a significant amount. The idea that the cells actively synthesize new triacylglycerols rather than merely converting the existing lipids into triacylglycerols is supported by the fact that the increase in triacylglycerols during nitrogen depletion is significantly greater than the reduction in the other glycerolipids [10].

Researchers present a measurement of the abundance of several proteins in cultures of Nannochloropsis grown without nitrogen and with a CO2 supplement [11]. The proteomic analysis appears to corroborate the hypothesis that the accumulation of triacylglycerols results from an increase in metabolic flux via the fatty acid biosynthesis pathway, even in the face of differing experimental settings.

The authors propose the theory that the increase in substrates across the route is caused by the breakdown of stored carbohydrates and the upregulation of glycolysis under their experimental conditions [11].

The study aimed to ascertain two things: (1) the specific growth rate (μ) of Nannochloropsis sp. cultivated in a lab as a live feed source for crab larvae; and (2) the composition of fatty and amino acids in Nannochloropsis sp.

2. Material and Method

Algal culture conditions

For this current study, *Nannochloropsis* sp. was chosen as a microalgal species, and it was obtained from laboratories in the United States. Prior to the experiment, *Nannochloropsis* sp. was grown in Guillard's F/2 algal culture medium [8]. Logarithmic stage cultures were used as inoculum.

20 mL of each algal stock culture was transfered to 200 mL of enriched F/2 medium in a 250 mL Erlenmeyer flask using three cultures grown separately. Cultures were cultivated at 25°C while under a 16/8 hours light/dark cycle by using 120 $\mu mol.m^{-2}.s^{-1}$ fluorescent white lights 4000Lux with constant shaking.

Algal growth conditions and determination of Specific growth rate

Each 200 mL *Nannochloropsis* sp. stock culture was transfered to 800 mL of enriched F/2 medium in a 1000 mL erlenmeyer flask with three replicates. Cultures were cultivated at 25°C during a 16/8 hours light/dark cycle by using 120 $\mu mol.m^{-2}.s^{-1}$ fluorescent white lights at 4000 Lux with constant air bubbling. A binocular microscope was used to count algal cells with a haemocytometer every day to determine the growth rate. A Thoma hemocytemeter counting chamber, under a microscope at 400X magnification, was used to count *Nannochloropsis* cells.

The average specific growth rate (μ) of *Nannochloropsis* from the triplicate findings from each algal sample counts were calculated from the formula mentioned below:

$\mu = 100 \times In (N_2/N_1)/ (t_2-t_1)$, where N_1 and N_2 represent the biomass at time 1 (t_1) and time 2 (t_2), respectively, and μ is the specific growth rate [12]. The sign μ indicates the specific growth rate (μ), which is the cell's exponential growth rate per unit of cell content.

Fatty acid composition study

A dry *Nannochloropsis* sample of 5–10 mg was added to 2% H_2SO_4 methanol [13]. Fatty acid methyl esters (FAMEs) were extracted To separate and identify FAMEs, gas chromatography (GC) using a DB-23 capillary column (30 m × 0.32 mm × 0.25 μm) and flame ionization detection (FID) was performed on an Agilent 7890 (Agilent Technologies Inc., Santa Clara, California, USA). The split ratio was 10:1, and the injection temperature was 270 °C. The process of temperature rise was the same [14].

Amino acid composition study

A dry *Nannochloropsis* sample powder (100 mg) was used for the analysis [15]. A combination of tandem mass 2 Phycologia spectrometry (API 3200 Q TRAP, AB Sciex, Framingham, Massachusetts, USA; HPLC-MS/MS) and high-performance liquid chromatography (Ultimate 3000, Dionex, Sunnyvale, California, USA) was used to identify derivatives of amino acids (AAs). An MSLab50AA-C18 column (150 × 4.6 mm; 5 μm) was used to separate AAs at 50 °C. Water with 1

$ml.min^{-1}$ formic acid (A) and acetonitrile with 1 $mL.min^{-1}$ formic acid (B) served as the mobile phase. An electrospray ion source (EIS) was used as the ion source, and the injection volume was 5 µl [15].

Statistical analysis

Algal Growth, fatty acid and amino acid composition data were analyzed using the MEAN Procedure using using SPSS Statistics Software (SPSS 16.0). All data were expressed as means ± standard deviation.

3. Results and Discussions

Algal Growth

Daily specific growth rate (µ) of *Nannochloropsis* sp. was found to be 55.3 ± 4.00 % at the culture conditions where bubbling aeration was applied, Guillard's F/2 medium was used at 25 ppt salinity and 25 ^{0}C.

Fatty acid composition

The results on fatty acid composition of *Nannochloropsis* sp. was summarized in Table 1.

Table 1. *Percent of total fatty acid composition of Nannochloropsis sp*.

Faty acids	Percent of total fatty acids
12:0	0.3± 0.02
14:0	2.6± 0.14
15:0	0.2± 0.05
16:0	0.7± 0.05
16:1 (ω-7)	19.0± 0.54
16:2 (ω-4)	0.4± 0.02
17:0	0.5± 0.03
16:3 (ω-4)	0.6± 0.03
18:0	0.6± 0.05
18:1 (ω-9)	3.6± 0.17
18:1 (ω-7)	2.0± 0.10
18:2 (ω-6)	4.2± 0.08
18:3 (ω-6)	0.4± 0.05
18:3 (ω-4)	0.2± 0.03
18:3 (ω-3)	1.4± 0.08
18:4 (ω-3)	0.3± 0.05
20:1 (ω-9)	0.0± 0.00
20:2 (ω-6)	0.0± 0.00
20:3 (ω-6)	0.5± 0.07
20:4 (ω-6)	3.8± 0.53
20:3 (ω-3)	0.3± 0.06
20:4 (ω-3)	0.2± 0.06
20:5 (ω-3)	24.25± 3.45
22:5 (ω-6)	0.0± 0.00
22:5 (ω-3)	0.1± 0.04
22:6 (ω-3)	0.5 ± 0.07

As can be seen from the Table 1, 24-25% of the oil is calculated as EPA and 0.5% as DHA. These results are similar to previous studies of [4, 5, 6, 7, 16-21].

Amino acid composition

The results on amino acid composition of *Nannochloropsis* sp. was summarized in Table 2.

Table 2. *Percent of total amino acid composition of Nannochloropsis sp.*

Amino acids	Percentage (%)
Aspartic Acid	8.35 ± 1.75
Serine	5.36 ± 1.68
Glutamic	14.14 ± 2.67
Glycine	6.20 ±1.34
Histidine	0.71 ± 0.03
Arginine	5.55 ± 1.15
Threonine	6.29 ± 1.45
Alanine	1.85 ± 0.35
Proline	16.35 ± 3.56
Tyrosine	1.37 ± 0.05
Valine	5.97 ± 1.24
Methionine	2.44± 0.53
Lysine	9.33 ± 1.75
Isolucine	1.59 ± 0.53
Leucine	12.55 ± 2.27
Phenylalanine	2.55 ± 0.74
Taurine	0.0 ± 0.00

Amino acids in *Nannochloropsis* were found to vary between 0.00 ± 0.00% (for Taurine) and 16.35 ± 3.56% (for Proline) in total amino acids and showed similarities to the previous studies [4,5,17,19,21].

4. Discussions

The aquaculture sector worldwide includes microalgae farming since several species, such as *Nannochloropsis* (Eustigmatophyceae), are used in aquaculture. Enhancing the nutritional content (prote-ins, carotenoids, fatty acids, etc.) of *Nannochloropsis* cultivated in mass cultures has the greatest aquaculture potential. This has a favorable impact on the physical and general health of the generated creatures, including the larvae of crabs (*Callinectes sapidus*). For the larvae of mollusks, crustaceans, fish hatchings, or rotifers in aquaculture—all sources of biomass—*Nannochloropsis* spp. are employed in a variety of ways. These include direct use as unprocessed cells (live feed), as a means of bolstering the corresponding food chain, or as processed biomass that can be supplemented with food. Aquaculturists frequently use open systems outdoors or a variety of closed photobioreactors to handle the in-house growing of *Nannochloropsis* for food.

In addition to its nutritional value, *Nannochloropsis* sp. has a positive environmental impact on fish, crustacean, and mollusc aquaculture. This includes improved oxygen production, CO_2 consumption, waste nutrient management, and antibacterial activity. The so-called "green-water technique," which involves introducing *Nannochloropsis* to rearing ponds, has resulted in significantly improved growth and survival rates for the larval and juvenile stages of many freshwater and marine species than the more conventional clear-water techniques.

Conflict of Interest

The author(s) declare that they have no conflicts of interest regarding this article.

Statement of Research and Publication Ethics

The author(s) declare that this study complies with research and publication ethics.

References

[1] Hibberd, D. J. (1981). Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae (synonym Xanthophyceae). *Botanical Journal of the Linnean Society*, 82(2), 93–119.

[2] Fawley (2007). Observations on the Diversity and Ecology of Freshwater *Nannochloropsis* (Eustigmatophyceae), with Descriptions of New Taxa. *Protist*., 158 (3), 325–336.

[3] Lubian, L. M.; et al. (2000). *Nannochloropsis (Eustigmatophyceae)* as source of commercially valuable pigments. *Journal of Applied Phycology*, 12 (3/5): 249–255.

[4] Boussiba, S., Vonshak, A., Cohen, Z., Avissar, Y., Richmond, A. (1987). Lipid and biomass production by the halotolerant microalga *Nannochloropsis salina*. *Biomass*, 12, 37–47.

[5] Canini, D., Ceschi, E., Perozeni, F. (2024). Toward the Exploitation of Sustainable Green Factory: Biotechnology Use of *Nannochloropsis* spp. *Biology*, *13*, 292.

[6] Demirbaş, A., and Demirbaş, M. F. (2011). Importance of algae oil as a source of biodiesel. Energy Conversion and Management. 52, 163-170.

[7] Assaf Sukenik, Y. C. T. B. (1989). Regulation of fatty acid composition by irradiance level in the Eustigmatophyte *Nannochloropsis* sp. *Journal of Phycology*. 25(4), 686–692.

[8] Guillard R.R.L. (1975). Culture of phytoplankton for feeding marine invertebrates. In: Culture of marine invertebrate animals (Ed. by W. L. Smith & M.H. Chanley), 29–60. Plenum Press, New York, USA.

[9] Rodolfi, L, Zittelli, Chini, et al. (2008). Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering*. 102(1), 100–112.

[10] Simionato, D., Block, M. A., Rocca, N. L, Jouhet, J., Maréchal, E., Finazzi, G., Morosinotto, T. (2013). The response of *Nannochloropsis gaditana* to nitrogen starvation includes de novo biosynthesis of triacylglycerols, a decrease of chloroplast galactolipids, and reorganisation of the photosynthetic apparatus. *Eukaryotic Cell*. 12(5), 665–676.

[11] Dong, H. P., Williams, E., Wang, D. Z., Xie, Z. X., Hsia, R. C, Jenck, A., Halden, R., Li, J., Chen, F., and Place, A. R. (2013). Responses of *Nannochloropsis oceanica* IMET1 to long-term nitrogen starvation and recovery. *Plant Physiology*, 162(2), 1110–1126.

[12] Gani, P., Sunar, N. M,, Matias-Peralta, H. et al (2016). Influence of initial cell concentrations on the growth rate and biomass productivity of microalgae in domestic wastewater. *Appl Ecol Environ Res* 14, 399–409. https://doi.org/10.15666/aeer/1402_399409 [7] El Gamal, A. A. (2010). Biological importance of marine algae. *Saudi Pharmaceutical Journal*, 18(1), 1-25.

[13] Liu, J., Liu, Y., Wang, H., and Xue, S. (2015). Direct transesterification of fresh microalgal cells. *Bioresource Technology*, 176, 284–287.

[14] Meng, Y., Jiang, J., Wang, H., Cao, X., Xue, S., Yang, Q., and Wang, W. (2015). The characteristics of TAG and EPA accumulation in *Nannochloropsis oceanica* IMET1 under different nitrogen supply regimes. *Bioresource Technology*, 179, 483–489.

[15] Gao, G., Clare, A. S., Rose, C., and Caldwell, G. S. (2017). Reproductive sterility increases the capacity to exploit the green seaweed *Ulva rigida* for commercial applications. *Algal Research*, 24,64–71.

[16] Ganuza, E., Etomi, E. H., Olson, M., and Whisner, C.M. (2024). Omega-3 eicosapentaenoic polar-lipid rich extract from microalgae *Nannochloropsis* decreases plasma triglycerides and cholesterol in a real-world normolipidemic supplement consumer population. *Front Nutr.*, 6, 11, 1293909.

[17] Khan, M. I., Shin, J. H., Kim, J. D. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories*, 17, 36.

[18] Abıshek, P., M., Patel, J., and Prem Rajan, A. (2014). Algae oil: a sustainable renewable fuel of future. *Biotechnology Research International*.

[19] Ünver Alçay, A., Bostan, K., Dinçel, E., Varlık, C. (2017). Alglerin insan gıdası olarak kullanımı. *Aydın Gastronomy*, 1(1), 47-59.

[20] Villarruel-López, A., Ascencio, F., Nuño, K. (2017). Microalgae, a potential natural functional food source – a review. *Polish Journal of Food and Nutrition Sciences*, 67(4), 251-263.

[21] Ye, Y., Liu, M., Yu, L., Sun, H., Liu, J. (2024). *Nannochloropsis* as an Emerging Algal Chassis for Light-Driven Synthesis of Lipids and High-Value Products. *Mar. Drugs*, 2024, *22*, 54.