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Influence of microalgae on the fatty acid composition of Artemia cysts in the conditions of lakes of Northern Kazakhstan

Zhanara Mazhibaeva[1], Saltanat Orazova[2*], Tynysbek Barakbayev[3], Moldir Aubakirova[1], Kuanysh Isbekov[1], Moldrakhman Aidana[1]

1. Laboratory of Hydrobiology, Fisheries Research and Production Center, Almaty, Kazakhstan, https://orcid.org/0000-0002-5013-0503
2.Laboratory of Biochemistry, Al-Farabi Kazakh National University, Almaty, Kazakhstan, https://orcid.org/0000-0003-4380-2460
3. Laboratory of Hydrobiology, Aral branch Fisheries Research and Production Center, Kyzylorda, Kazakhstan

**Corresponding author email: saltanat.orazova@edu.kaznu.kz*

ABSTRACT
Artemia is widely used in aquaculture as a live feed. The presence of highly unsaturated fatty acids is a key determinant of nutritional value. The purpose of this study was to study the fatty acid composition of total lipids of Artemia cysts (*Artemia* sp.), taken from two lakes in the North Kazakhstan region in different seasons of the year, and the influence of phytoplankton composition on the fatty acid profile. Phytoplankton sampling was carried out by the sedimentation method, and a camera analysis of the sample was carried out using a microscope. Extraction of total lipids was carried out by a binary mixture of organic solvents chloroform-ethanol (2:1). Gas chromatographic separation of fatty acids was carried out after methanolysis of lipids to obtain methyl esters of fatty acids. The seasonal dynamics in the content of both the main groups of fatty acids and individual fractions are shown, which is associated with abiotic and biotic conditions for the growth of natural populations. The values of the unsaturation coefficient were twice as high in the spring samples of Artemia cysts as compared to the autumn samples for the populations of Lake Stanovoye. In the composition of polyunsaturated acids, such acids as 18:3n3 linolenic acid were found (from 4.13% to 23.37%); 20:5n3 eicosapentaenoic acid (from 8.24% to 16.27%); and 18:2n6t linoleidic acid (from 3.84% to 13.53%).

Keywords: Cysts, *Artemia* sp., Fatty acid, Seasonal dynamics, Phytoplankton, Algoflora

INTRODUCTION

Artemia is considered to be the most common live food base for rearing fish and crustacean larvae due to its physical characteristics: size, activity, high nutritional value, and presence of PUFAs (Mugwanya *et al*. 2021; Dawood 2021). Among PUFAs, such fatty acids as eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and arachidonic acid (ARA, 20:4n-6) play an important role (Nieves-Soto *et al*. 2021). The importance of EPA and DHA has also been described as promoting growth, stress tolerance, and pigmentation in aquaculture (Lavens *et al*. 1995). Therefore, most attention is currently paid to increasing the content of PUFAs, especially EPA and DHA in Artemia (Francis *et al*. 2019; Lavens *et al*. 2018).

As for the nutritional characteristics of Artemia, the regulation of its nutritional value can be carried out with the help of natural diets. Artemia is a continuous, non-selective, obligate phagotroph that begins to consume food at

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the stage II instar using its larval antennae (Le *et al*. 2019). In addition, particles consumed by crustaceans have been shown to vary from 1 to 50 µm, with the optimal size being around 16 µm (Fernández 2001). Due to these characteristics, several methods for fortifying brine shrimp feed have been developed, such as fish oil supplementation (Nieves-Soto *et al*. 2021), n-3 PUFA methyl esters (Roo *et al.* 2019), and microalgae feeding (Paulo *et al*. 2020). Among the available methods, many researchers have recommended the use of microalgae with a high content of PUFAs. A study by Basford *et al*. (2020) showed that microalgae were better than synthetic lipid emulsion supplements at increasing fatty acid content in live diets. Several species of microalgae are reported to have high PUFA content, such as *Chaetoceros calcitrans* (Méndez-Martínez *et al*. 2018), *Tetraselmis* spp., and *Dunaliela* spp. (Dineshbabu *et al*. 2019). In addition, such types of microalgae as *C. calcitrans* (protein 43.00% a.d.w., lipids 23.80% a.d.w.), *Nannochloropsis oculata* (protein 34.11% a.d.w., lipids 12.51% a.d.w.; Banerjee *et al*. 2011), *Tetraselmis chuii* (protein 56% a.d.w., lipids 9.4% a.d.w., carbohydrates 16.6% a.d.w.; Arkronrat *et al*. 2016) and *Dunaliela salina* (protein 40.46% a.d.w., lipids 15.51% a.d.w., carbohydrates 20.44% a.d.w.; Dineshbabu *et al*. 2019), which, accordingly, affects the survival and growth of brine shrimp, increase the content of proteins and fats. To determine the most effective phytoplankton diet, a study comparing different types of microalgae is needed.

Thus, the present study was carried out to identify the relationship between microalgae species included in the natural food base and the profile of fatty acids in the composition of lipids isolated from *Artemis* sp. cysts in the conditions of salt lakes of Northern Kazakhstan in seasonal dynamics.

MATERIALS AND METHODS

Artemia cysts, selected in the spring and autumn of 2021 from 2 reservoirs of Northern Kazakhstan: Stanovoye and Mengisor, served as the object of research. The geographic location and specification of sampling points are presented in Table 1.

Table 1. Hydrography of salt lakes in the North Kazakhstan region.

The reservoir	Reservoir coordinates	Altitude above sea level (mBS)	Lake area, sq. km	Mineralization (g L^{-1})	
				May, 2021	September, 2021
Stanovoye	54°45'201"N 068°22'868"E	118.0	38.0	116	147
Mengisor	54°29'769"N 067°55'552"E	119.0	32.8	73	77

Fig. 1 shows the sampling points for research. Phytoplankton sampling was carried out by the sedimentation method (Abakumov 1983). Water was taken into a 1-L plastic bottle and fixed with a few drops of a 40% formalin solution. Desk analysis of the sample was carried out using an MC 300 A microscope; for the identification of microalgae, determinants for individual groups and genera were used (Determinants of freshwater algae of the USSR, 1951-1982).

The selection of cysts was carried out in the presence of coastal discharges or from the bottom of reservoirs, using a bottom grab. The samples were preliminarily cleaned of impurities and, in the presence of excess water, dried with filter paper, and thoroughly ground in a porcelain mortar to a homogeneous, mushy state.

Extraction of total lipids was carried out with a binary mixture of organic solvents chloroform-ethanol (2:1), separation of solvents, and weight determination of lipid mass.

The method for determining fatty acids was carried out by the state industry standard of the Republic of Kazakhstan Measurement technique 1364-2000 Method for the gas chromatographic determination of fatty acids and cholesterol in food and blood serum. The principle of the method is based on the isolation of lipids by extraction with organic solvents, lipid methanolysis to obtain fatty acid methyl esters, gas chromatographic separation of the latter, and quantitative determination by the internal standard method using a calibration curve expressing the dependence of the ratios of the peak areas of fatty acid methyl esters to the internal standard on the concentration of the corresponding fatty acids.

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Lake Stanovoye

Lake Mengisor

Fig. 1. Points of sampling material for research.

A portion of 5 g was ground in a porcelain mortar with anhydrous sodium sulfate (20 g) until a crumbly homogeneous mass was obtained. The mixture was transferred quantitatively with 30 mL of extractant (chloroform-ethanol 2:1) and extracted for 20 min. The extract was filtered off, the solvent was distilled off to dryness on a rotary evaporator at 40°C. The extraction was repeated 3 times. A weighed portion of the fat was dissolved in 2 mL hexane solution of internal standards of methyl esters of pentadecanoic and margaric acids with the addition of a methanolic solution of sodium methoxide and shaken for 2 min. Immediately before analysis, 0.5 mL distilled water was added to the tube, shaken for 30 s, and left until the phases separated. An aliquot of 5 μL of the upper hexane layer was chromatographed 2 times. The desired components were identified by the retention times of fatty acid methyl esters.

The fatty acid composition of total lipids was determined using gas-liquid chromatography on a Kristall-5000 capillary chromatograph with a flame ionization detector. A capillary column with an internal diameter of 0.32 mm and a length of 50 m with a stationary phase FFAP, 0.50 μm thick, was used. The analysis was carried out in isothermal mode at 225 °C. The concentration of the components was calculated using the Chromatech-analyst software. Fatty acids were identified by comparing the retention times of available markers and by matching the calculated equivalent chains of molecules with tabular data. The significance of differences was assessed by the requirements of the normalizing standards.

RESULTS AND DISCUSSION

In 2021, the phytoplankton of Lake Stanovoye was represented by only 3 taxa of diatoms and green algae (Table 2). In spring, only diatoms of the genus *Amphora* were recorded in the plankton of the lake. In autumn, a number of taxa were supplemented by green *Chlamydomonas* sp. and diatom *Navicula* sp. Quantitative indicators of microalgae of Lake Stanovoye were characterized by low values. In spring and autumn, the basis of abundance and biomass was represented by diatoms: 100% and 93% by number, and 100% and 98% by biomass. According to the trophic scale of S.P. Kitayev for the entire period under study, the biomass is estimated as a “very low” trophic class of the α-oligotrophic type of the reservoir (Kitayev, 2007).

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Table 2. Taxonomic composition and quantitative parameters of phytoplankton organisms in the conditions of lakes in Northern Kazakhstan.

Reservoir	Taxa	Number ($\times 10^6$ cells m^{-3})	Biomass (mg m^{-3})	Number ($\times 10^6$ cells m^{-3})	Biomass (mg m^{-3})
		May, 2021		September, 2021	
Lake Stanovoye	Bacillariophyta – diatom				
	Amphora coffeiformis (C.Agardh) Kützing	33.33	86.67	13.33	34.67
	Navicula sp.	0.00	0.00	133.33	644.75
	Chlorophyta – green				
	Chlamydomonas sp.	0.00	0.00	10	8.72
	Total: 3	33.33	86.67	156.67	688.14
Lake Mengisor	Bacillariophyta – diatom				
	Navicula sp.	0.00	0.00	30	15.83
	Chlorophyta – green				
	Mougeotia sp.	1736.67	1729.72	1106.67	1102.24
	Zygnema sp.	2183.33	1235.24	1270	747.05
	Total: 3	3920	2964.96	2406.67	1865.12

Phytoplankton of Lake Mengisor was represented by 3 taxa of diatoms and green algae. In the spring plankton of the lake, large-sized filamentous green algae of the Zignem family were noted. By autumn, the ranks of the greens were supplemented by representatives of the genus *Navicula.* In spring, in the plankton of the lake, *Zygnema* sp. was 56%, and in terms of biomass *Mougeotia* sp. was 58%. By September, in phytoplankton, there was a decrease in quantitative indicators by an average of 1.5 times. Despite the decrease in abundance and biomass, the leading position of *Zygnema* sp. and *Mougeotia* sp. survived. According to the trophic scale of S.P. Kitayev, the value of the spring biomass of microalgae is estimated by the "average" trophic level, and the autumn mass by the "moderate" class (Kitaev 2007).

Table 3 shows the fatty acid composition of lipids isolated from spring and autumn samples of Artemia cysts from lakes in the North Kazakhstan region. It was found that the lipids of cysts were in the conditions of Lake Stanovoye are rich in polyunsaturated fatty acids (PUFAs), where their content ranged from 40.94% to 54.95%. A relatively low content was typical for saturated fatty acids (SFAs) from 14.28% to 26.64%, and the content of monounsaturated fatty acids (MUFA) in the range from 30.77% to 32.43%. The composition of fatty acids isolated from lipids of cysts of autumn selection from Lake Mengisor was distinguished by a high content of monounsaturated fatty acids (MUFAs: 42.16%) and an average amount of polyunsaturated fatty acids (PUFAs: 33.83%). Such a distribution of FAs by type can be explained by the predominance of diatom microalgae *Amphora coffeiformis* and *Navicula* sp. in phytoplankton biomass. Characteristic fatty acid markers of diatoms (*Bacillariophyta*) are short-chain myristic acid (14:0), as well as C16 acids, namely 16:1n-7 and C16 PUFA families n-7, n-4, and n-1 (Li *et al*. 2014). Unlike cyanobacteria and green algae, FAs of diatoms contain almost no C18 acids (Abdo et al., 2015). At the same time, diatoms accumulate long-chain PUFAs: 20:4n-6, 22:6n-3, and especially 20:5n-3 (Mitani *et al*. 2017). According to some authors, diatoms are the main producers of EPA in both marine and freshwater ecosystems (Goedkoop *et al*. 2000). The content of eicosapentaenoic acid in different representatives of this division varies from 3 to 30%, which indicates a different efficiency of the synthesis of this acid in diatom taxa (Nielsen *et al*. 2018).

Table 3. Comparative content of saturated, monounsaturated, and polyunsaturated fatty acids in Artemia cyst lipids from populations of lakes in Pavlodar region, % of the total amount

Reservoir	Saturated fatty acids		Monounsaturated fatty acids		Polyunsaturated fatty acids		Σ PUFAs / Σ SFAs	
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
Stanovoye	14.28	26.64	30.77	32.43	54.95	40.94	3.85	1.54
Mengisor	-	24.01	-	42.16	-	33.83	-	1.41

The influence of the change of seasons on the content of the main groups of higher fatty acids in the total lipids of Artemia cysts can also be noted. Autumn samples taken from Lake Stanovoye were distinguished by an almost twofold increase in the content of saturated fatty acids from 14.28% to 26.64% and a drop in the amount of

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polyunsaturated fatty acids from 54.95% to 40.94% (Table 2), which affected the value of the unsaturation coefficient (the ratio of the sum polyunsaturated to the amount of saturated higher fatty acids). The values of this ratio were higher in spring samples of Artemia cysts compared to autumn samples. According to the hypothesis of homeoviscosal adaptation, a change in the ratios of short-chain/long-chain and unsaturated/saturated acids in the composition of lipids makes it possible to stabilize the fluidity (viscosity) of cell membranes at different temperatures (Kolomiytseva 2011).

Among SFAs, the highest content was found for 16:0 palmitic acid from 9.01% to 14.28%. Among the MUFAs, a high content is characteristic of 18:1 (cis-9) oleic acid (from 12.74% to 27.12%) and 16:1 (cis-9) palmitoleic acid (from 10.09% to 15.22 %). Among PUFAs, the profile can be expressed as follows: 18:3n3 linoleic acid (from 4.13% to 23.37%) > 20:5n3 eicosapentaenoic acid (from 8.24% to 16.27%) > 18:2n6t linoleidic acid (from 3.84% to 13.53%). In the seasonal dynamics of the Artemia population in the conditions of Lake Stanovoye, changes in the qualitative composition of SFAs can be noted, in particular, in the autumn samples there were no such acids as 8:0 caprylic, 10:0 capric, 12:0 lauric, 13:0 tridecanoic, 15:0 pentadecanoic, 17:0 margaric, 21:0 behen (Table 4).

Table 4. Fatty acid composition of lipids extracted from samples of Artemia cysts taken in the spring and autumn of 2021 on Lake Stanovoe and Lake Mengisor of the North Kazakhstan region.

Fatty acid	Lake Stanovoye		Lake Mengisor
	Spring	Autumn	Autumn
Saturated fatty acids			
C8:0 (%)	0.01 ± 0.001	-	-
C10:0 (%)	0.02 ± 0.001	-	-
C12:0 (%)	0.06 ± 0.003	-	-
C13:0 (%)	0.04 ± 0.002	-	-
C14:0 (%)	1.29 ± 0.06	3.13 ± 0.15	2.62 ± 0.13
C15:0 (%)	0.47 ± 0.03	-	0.80 ± 0.04
C16:0 (%)	9.01 ± 0.45	14.28 ± 0.71	12.61 ± 0.63
C17:0 (%)	0.39 ± 0.02	-	0.71 ± 0.04
C18:0 (%)	0.06 ± 0.003	8.24 ± 0.41	5.32 ± 0.27
C20:0 (%)	2.69 ± 0.01	0.47 ± 0.02	1.43 ± 0.07
C21:0 (%)	0.14 ± 0.007	-	-
C22:0 (%)	0.03 ± 0.001	0.52 ± 0.03	0.11 ± 0.01
C24:0 (%)	0.08 ± 0.004	-	0.42 ± 0.02
Monounsaturated fatty acids			
C14:1 (cis-9; %)	0.27 ± 0.01	0.38 ± 0.02	0.29 ± 0.02
C15:1 (cis-10; %)	0.05 ± 0.003	-	0.44 ± 0.02
C16:1 (cis-9; %)	11.72 ± 0.59	15.22 ± 0.76	10.06 ± 0.50
C17:1 (cis-10; %)	0.11 ± 0.01	-	1.89 ± 0.09
C18:1 (trans-9; %)	5.14 ± 0.26	0.37 ± 0.02	0.18 ± 0.01
C18:1 (cis-9; %)	12.74 ± 0.64	16.46 ± 0.82	27.12 ± 1.36
C20:1 (cis-11; %)	0.51 ± 0.03	-	0.99 ± 0.05
C22:1 (cis-13; %)	0.07 ± 0.003	-	-
C24:1 (cis-15; %)	0.17 ± 0.008	-	1.16 ± 0.06
Polyunsaturated fatty acids			
C18:3n3 (%)	23.37 ± 1.17	4.13 ± 0.21	13.41 ± 0.67
C20:3n3c (cis-11,14,17; %)	0.23 ± 0.01	-	-
C20:5n3 (%)	16.27 ± 0.814	10.57 ± 0.50	8.24 ± 0.41
Σ n3 of acids	**39.87**	**14.70**	**21.65**
C18:2n6t (%)	8.30 ± 0.42	13.53 ± 0.68	3.84 ± 0.19
C18:2n6c (%)	3.49 ± 0.18	-	5.69 ± 0.28
C18:3n6Y (%)	0.86 ± 0.04	-	1.33 ± 0.07
C20:2n6 (%)	0.08 ± 0.004	-	-
C20:3n6c (cis-8,11,14; %)	0.05 ± 0.002	-	-
C20:4n6 (%)	2.31 ± 0.12	1.42 ± 0.07	1.31 ± 0.07
Σ n6 of acids	**15.09**	**14.95**	**12.17**
Σ n3 / Σ n6	**2.64**	**0.98**	**1.78**

Polyenoic higher fatty acids of the linoleic series (n6 type) have a higher melting point in comparison with acids of the linolenic series (n3 type). As a result, an indicator of changes in the microviscosity of membrane lipids is

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the ratio of the sum of n3 fatty acids to that of n6. In our studies, the value of this coefficient was higher in spring samples in Lake Baikal. Stanovoye, amounting to 2.64 and 0.98 in May and September, respectively. As is known, a decrease in the n3/n6 ratio of PUFAs indicates an elevation in the viscosity of membrane lipids.

Table 5. The content of 20 fatty acids in lipids of Artemia cysts from the populations of lakes in Northern Kazakhstan (% of the total).

Brief designation of fatty acid	Systematic name of the fatty acid	Fatty acid content (%)	
		min	max
Saturated fatty acids			
C14:0 (%)	Myristic	1.29	3.13
C15:0 (%)	Pentadecanoic	0.47	0.80
C16:0 (%)	Palmitic	9.01	14.28
C17:0 (%)	Margarine	0.39	0.71
C18:0 (%)	Stearic	0.06	8.24
C20:0 (%)	Arachidic	0.47	2.69
C22:0 (%)	Behenic	0.03	0.52
C24:0 (%)	Lignoceric	0.08	0.42
Monounsaturated fatty acids			
C14:1 (cis-9) (%)	Myristoleic	0.27	0.38
C15:1 (cis-10) (%)	Pentadecene	0.05	0.44
C16:1 (cis-9) (%)	Palmitoleic	10.09	15.22
C17:1 (cis-10) (%)	Margarinoleic	0.11	1.89
C18:1 (trans-9) (%)	Oleic	0.37	5.14
C18:1 (cis-9) (%)	Oleic	12.74	27.12
C20:1 (cis-11) (%)	Eicosene	0.51	0.99
C24:1 (cis-15) (%)	Selaholic	0.17	1.16
Polyunsaturated fatty acids			
C18:3n3 (%)	Linolenic	4.13	23.37
C18:2n6t (%)	Linoeidine	3.84	13.53
C20:4n6 (%)	Arachidonic	1.31	2.31
C20:5n3 (%)	Eicosapentaenoic	8.24	16.27

Compounds of great aquaculture interest such as 20:5n3 (eicosapentaenoic acid) have been found in the populations, so these populations can be assessed as a "marine" type using the classification of Watanabe *et al.* (1978) who suggest the presence of mechanisms that promote accumulation, which deserves further study.

The possibility of synthesizing unsaturated fatty acids with two or more double bonds in plant and animal organisms is determined by the presence of the corresponding desaturases. For instance, higher plants and algae have genes encoding desaturases Δ12 and Δ15, so they can synthesize FAs with double bonds in positions n-6 and n-3, respectively (Tocher *et al*. 2015). In turn, due to the lack of these desaturases, most invertebrates and all vertebrates cannot form n-6 linoleic acid (LA, 18:2n-6) and n-3 linolenic acid (ALA, 18:3n-3) from 18:1n-9. Therefore, LA and ALA are essential fatty acids and must be ingested by animals with food (Calder *et al*. 2018). With the help of elongase and n6 and n5 desaturases, essential fatty acids can be further converted into physiologically important C20 and C22 PUFAs. The main products of the conversion process in animals are a number of long-chain PUFAs that play an important role in metabolic processes: arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n -3).

Ramos-Llorens *et al.* (2023) showed that *A. franciscana* has a limited ability to biosynthesize long-chain PUFAs due to the absence of key desaturases in its genome, which confirms the effectiveness of the enrichment procedure, providing an exogenous supply of crustaceans with essential fatty acids, to prevent a deficit.

CONCLUSION

For the first time, a study was made on the fatty acid composition of Artemia cysts of Kazakh populations in the conditions of lakes in Northern Kazakhstan, and a relationship was established between the types of microalgae included in the natural food base and the profile of lipid fatty acids. Phytoplankton of Lake Stanovoye and Lake Mengisor were represented by only 3 taxa of diatoms (*Amphora coffeiformis* and *Navicula* sp.) and green microalgae (*Chlamydomonas* sp. and *Mougeotia sp., Zygnema* sp.). According to the trophic scale of S.P. Kitayev for the entire period under study, the biomass of Lake Stanovoye is rated as a “very low” trophic class of the α-

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oligotrophic type of the reservoir; Mengisor, the value of the spring biomass of microalgae is estimated by the "average" level of trophicity, and the autumn mass by the "moderate" class.

The results of the study showed the presence of seasonal dynamics in the content of both the main groups of fatty acids and individual fractions. In particular, by autumn, the synthesis of polyunsaturated fatty acids drops, while the amount of saturated fatty acids increases, affecting the value of the unsaturation coefficient. The values of this ratio were twice as high in the spring samples of Artemia cysts for the populations of Lake Stanovoye. Quantitative variations of polyunsaturated fatty acids in Artemia cysts are due to seasonal changes in the species and fatty acid composition of their food. Based on the data obtained, it can be assumed that in the diet of crustaceans in the summer period, a significant proportion is detritus, while in the autumn green microalgae. Differences in the values of the n3/n6 ratio of fatty acids depending on the salinity of the habitat were revealed in Artemia cysts.

Among saturated fatty acids, the highest content was found for 16:0 palmitic acid from 9.01% to 14.28%. This acid is the most commonly occurring saturated fatty acid (about 15-50%). Its main role is as an energy store and a substrate in the biosynthesis of fatty acids. Among monounsaturated fatty acids, a high content is characteristic of 18:1 (cis-9) oleic acid (from 12.74% to 27.12%) and 16:1 (cis-9) palmitoleic acid (from 10.09% to 15. 22%). Oleic acid is the main substrate in the biosynthesis of polyunsaturated fatty acids. Among PUFAs, the profile can be expressed as follows: 18:3n3 linoleic acid (from 4.13% to 23.37%) > 20:5n3 eicosapentaenoic acid (from 8.24% to 16.27%) > 18:2n6t linolenic acid (from 3.84% to 13.53%). The results obtained are recommended to be used in further experiments to improve the fatty acid profile and growth of brine shrimp in aquaculture.

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