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The optimal culture media for crude protein and polyunsaturated fatty acid production from *Isochrysis galbana* and *Nanochloropsis oculata* for livestock and aquatic species nutrition

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Abstract

This study aimed to investigate the effect of different culture mediums based on the Walne medium on the growth rate, chemical composition, and fatty acid (FA) profile in *I. galbana* and *N. oculata*. This experiment was done in a factorial design with two culture media (Walne and modified Walne media) and two microalgae species, including *I. galbana* and *N. oculata*. The results showed that the modified culture medium increased total and daily fresh and dried biomass production of *I. galbana* and *N. oculata*. Modified culture media increased crude fat (CF) and crude protein (CP) content and total and daily lipid production in both the studied species. However, in both the studied culture mediums, *N. oculata* had higher growth and production performance compared to *I. galbana*. Modified growth media also affects the FA profiles of the studied microalgae species. Total saturated and unsaturated FA content was not influenced by the growth medium but modified media increased poly unsaturated FA (PUFA) at the expense of mono unsaturated FA (MUFA). Omega- 3 FA content (linolenic acid, Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA)) was increased as a result of the medium modification in both species ($P<0.05$). However, linoleic acid content was affected differently in *I. galbana* and *N. oculata*. The linoleic acid concentration was reduced in modified medium grown *N. oculata* but increased in *I. galbana* ($P<0.05$). Palmitic acid and stearic acid contents were also decreased in both of the studied species in the modified medium ($P<0.05$). This study develops microalgal cultivation using a modified Walne medium for higher CP, CF, EPA, DHA contents, the ratio of omega3: omega6 FA, and biomass production in *N. oculata* and *I. galbana* microalgae.

Keywords: Microalgae; Modified Culture Media; Fatty Acids; PUFA; Feed Supplement

Introduction

Microalgae species can produce oil and protein in non-cultivable lands, reducing the need for defrosting and can have an important role in reducing carbon foot-print of animal production. One of the most valuable products of microalgae is their oil, which ranges from 20 to 50% of the dry weight of microalgae (Brennan and Owende 2010; Leonga et al. 2018). The *I. galbana* is often grown on farms to produce oils that contain large amounts of PUFA rich in omega-3 long chain FA, such as EPA and DHA (Gouveia et al. 2008). Species of the genus *N. oculata* are also known to be rich in EPA (Kagan et al. 2014; Borges et al. 2016).

Factors such as nutrient quantity and quality, light, pH, turbulence, salinity, and temperature are the most important parameters on which, the growth of microalgae depends (Lavens and Sorgeloos 1996; Converti et al. 2009; Emmanuel and Nelson 2016). Vitamins regulate biochemical reactions in microalgae (Hakalin et al. 2014) and the growth rate of some microalgae species is highly dependent on some the vitamins such as cobalamin, biotin and thiamine (Tandon et al. 2017). However, the effect of vitamins on the growth, diversity, and productivity of microalgae has been poorly studied (Arif et al. 2019).

The effects of different Nitrogen and phosphorus concentration as the main limiting nutrients on growth performance and biomass production of *I. galbana* and *N. oculata* had been evaluated previously (Andersen 2005; Zarrinmehr et al. 2020). However, there was not any report about effects of modifying the availability of culture medium sources of nitrogen and phosphorus. In the composition of the Walne medium, $No3^-$, $Po4^-$, and Cl^- are considered as anions, and Na^+, Mn^+, Co^+, Zn^+, and Cu^+ are considered as cations. Cations chelating anions and making them less available to microalgae. So, we hypothesize that changing the concentration of cations in the culture media, without increasing the concentration of N and P sources, will change the availability of anionic compounds for microalgae and consequently affects microalgal production and composition. Therefore, the effects of lower levels of cations including $ZnCl_2$, $CoCl_2$. $6H_2O$, $(NH_4)_6Mn_7O_{24}$, and $CuSo_4$. $5H_2O$ and higher levels of B_1 and B_{12} vitamin in Walne medium on the growth rate, biomass production performance, chemical composition, and fatty acid profile of *I. galbana* and *N. oculata*, were investigated in this study.

Materials and methods

Microalgae culture stocks were kindly prepared by the Urmia Lake Research Institute of Urmia University, primarily transferred to 250 mL Erlenmeyer flakes and then transferred to 10 L plastic containers for mass production in batch cultures. In preliminary study, nine modified culture mediums were prepared with different concentration of cations and vitamins and three culture replications were considered for each of the media and microalgae combinations. Modified culture mediums were compared for fresh and dry biomass production in a factorial design arrangement and the best performing medium was selected to continue the experiment (Table 1). In the main experiment five culture replications were considered for each of the media and microalgae combinations. The salinity of the both of culture mediums in this experiment was considered to be 24 g/l. The temperature of the culture room was 23-25 °C and 40-watt fluorescent lamps were used to provide 24 h of light for 2500-3000 lux light intensity. Aeration was continuous (24 h) throughout the cultivation period. After reaching the plateau phase (16 days), the microalgae biomass is harvested by centrifugation at 4000 rpm for 10 min, weighed for fresh biomass production, and then dried in a forced air oven at 35 ° C for 72 h and then weighed again for dry biomass production. After drying, the microalgae were ground to 1 mm using a laboratory mill and then were analyzed for ash (method 923.03) and CP (method 976.05) content according to AOAC (2000). Total fat content was measured according to the modified Folch et al. (1957) method (Bligh and Dyer 1959).

Table1- Chemical composition of growth mediums (g/L).

	Walne medium*	Modified Walne medium**
Solution: 1		
$ZnCl_2$	21g	4.2g
$CoCl_2.\ 6H_2O$	20g	4g
$(NH_4)_6Mn_7O_{24}$	9g	1.8g
$CuSo_4.\ 5H_2O$	20g	4g
Solution: 2		
$FeCl_3.6H_2O$	1.3g	1.3g
$MnCl_2.4H_2O$	0.36g	0.36g
H_3Bo_3	33.6g	33.6g
Na_2EDTA	45g	45g
$NaH_2Po_4.\ 2H_2O$	20g	20g
$NaNo_3$	20g	20g
Solution: 3		
Vitamin B_1	0.1g	2g
Vitamin B_{12}	0.1g	0.2g
Vitamin H_7(Biotin)	0. 02g	0.02g

Walne medium*: Consumption of solutions 1 and 2 was 1 ml / l and solution 3 was 0.1 ml / l every week (Laing, 1991)
Modified Walne medium**: Consumption of solutions 1, 2, and 3 were 1 ml / l every week

Three laboratory replications were considered for chemical analysis.

Fatty acid profiles of the dried microalgae samples were also determined. In this case, all of the chemical solvents and reagents utilized in lipid extraction and preparation of the fatty acid methyl esters (FAME) were of analytical grade, and solvents were redistilled before use. As described in Folch et al. (1957) to avoid FA oxidation, lipid extraction was carried out three times with chloroform/methanol (C/M, 2/1, v/v) to a final volume of 100 ml administered under the argon gas blanket in an ultrasonic bath (SONICA 2200ETH S3, Italy). The flasks, after each extraction step, were centrifuged (1,800 g for 10 min), and the organic fraction was separated and injected into a 100 ml volumetric flask. Afterward, they were treated with anhydrous Na-sulfate to be dry and then vaporized using a rotary evaporator (Büchi, Switzerland) at 40 ºC under a vacuum. Using mild methanolysis/methylation via methanolic hydrochloride acid (HCl/MeOH), FAME were prepared by a method explained in Ichihara and Fukubayashi (2010). Hexane was utilized as a solvent to extract, GC analysis was conducted after drying with anhydrous Na-sulfate, and nonadecanoic acid was utilized as an internal standard. For FA analysis, an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, California, United States) equipped with an autoinjector (Agilent 7683 series, Santa Clara, California, United States) and FID detector was used. Samples (1µl) were injected in split mode, 50:1, into a RESTEK column for FAME (Rtx®-2330, 105 m×250µm×0. 2µm; Cat#10729; Serial#1525353, Restek Corporation, U.S., 110 Benner Circle, Bellefonte, PA 16823). The detector and injector temperatures were set at 250 º C. N2 with a constant flow of 1 ml/min was the carrier gas. Based on the method described by Lee et al. (2005) the oven temperature was set at the gradient temperature rise with some modifications, and it was 70º C for 1 min, and then was increased from 5º C/min to 100º C and was kept for 2 min. Then, the column temperature was increased from 10º C/min to 175º C and was maintained for 35 min. Eventually, the temperature was increased from 4º C/min to 225º C and was kept for 35 min. Based on a FAME standard mix (GLC 463, Nu-Chek Prep Inc,. Elysian, MN; reference mixture 47 885, Supelco Inc., Bellefonte PAGLC 463 reference mixture, http://www.nu-chekprep. com/10 11 catalog.pdf), individual peaks were specified.

To compare the effect of different culture mediums on the growth, production efficiency, and chemical composition of *I. galbana* and *N. oculata*, a factorial experiment was used in a completely randomized design. Five culture replications were used and mean of laboratory

replications were used for statistical analysis. Statistical analysis of data was performed using Mixed Proc of the SAS statistical software (SAS 2002) and Least square means (LSM) were corrected using the Tukey test and were compared with the PDIFF option, the data were reported as LSM, and the corresponding standard error of the means in the tables.

$Y_{ijk} = \mu + C_i + S_j + CS_{ij} + \varepsilon_{ij}$ Where, Y_i: observation i, μ: mean of total observations, C_i: culture medium type, S_j: microalgae species, CS_{ij}= interaction of microalgae species and culture media, and ε_{ij}: experimental error.

Results and Discussion

As shown in table 2, the results showed that the composition of the medium culture had no effects on the dry matter (DM) and ash content of *I. galbana* and *N. oculata*. However, TF and CP content, as well as total fat production and fat production efficiency in *I. galbana* and *N. oculata* were affected by the medium culture ($p < 0.05$); Modified Walne medium increased CP and TF content in both of the studied species. However, *N. oculata* had higher CP and TF, CP production (g/l/day), irrespective of culture medium compared to *I. galbana* ($p < 0.05$).

The results of the present study showed that the composition of the culture medium affects the CP and TF content and biomass production efficiency of *I. galbana* and *N. oculata.* Nitrogen content of the culture medium and salinity stress had been reported to affect the amount of fat production and FA profile by microalgae. Zarrinmehr et al. (2020) reported that adding $NaNO_3$ to the Walne medium increased the protein content of the *I. galbana* (36.3% vs. 17.1%, for $NaNO_3$ enriched and conventional Walne medium, respectively). Mata et al. (2010) reported that the lipid content of *I. galbana* and *Nanochloropsis* species ranges between 7 to 40% and 12 to 53% of the dry matter, respectively. On the other hand, Lin and Lin (2015) declared that the amount of extracted oil from *I. galbana* cultured with Walne medium was affected by the extraction procedure. So, microwave assisted extraction using isopropanol and hexane resulted in higher oil content than the Soxhlet method (22 vs. 13 %, respectively). Researchers also report that solvent use in oil extraction can affect the amount of the oil extraction (Boselli et al., 2001; Widjaja et al., 2009; Ryckebosch et al., 2012; Lin and Lin, 2015). Ryckebosch et al. (2012), reported that the degree of polarity of the solvents used during the extraction procedure as well as the extraction method affects the amount of fat extracted from the microalgae. In this experiment higher levels of B vitamins such as Thiamine and Biotin in a modified Walne medium resulted in higher lipid content. Thiamine in the form of thiamine pyrophosphate plays an essential role in the oxidative conversion of pyruvate to acetyl coenzyme in the de novo synthesis of FA in chloroplasts of microalgae. Biotin also activates the acetyl CoA synthetase, converts acetyl CoA to malonyl CoA, and eventually produces palmitic and long-chain fatty acids (Jaworski et al., 1989; Arif et al., 2019). In this experiment, the amount of thiamine and biotin in the modified culture medium was higher than in the conventional Walne medium, which could be a potential explanation for the higher lipid content of both microalgae species grown in the modified Walne medium. Vitamin B_{12} has a functional role in adenosyl cobalamin and methyl cobalamin, which play a vital role in cell division and inter-conversion of amino acids. The effect of some non-biological factors such as salinity, nitrogen, phytohormones, vitamins, and fertilizers on the growth of algae and their compounds has been proven (Arif et al., 2019). Decreased phosphorus levels in medium culture had been reported to reduce the fat, protein, pigments, and carbohydrates in the *I. galbana* (Zarrinmehr et al., 2020).

Table 2- The effect of culture medium composition on the chemical composition of *I. galbana* and *N. oculata* microalgae.

Parameters	*I. galbana*		*N. oculata*		SEM	P-value		
						Microalgae species	Culture medium	Microalgae species * Culture medium
	Walne Medium	Modified Medium	Walne Medium	Modified Medium				
DM%	96.65	96.4	96.35	96.55	0.248	0.9	0.053	0.2
Ash%	12.75	12.9	12.5	12.2	0.304	0.85	0.6	0.95
CP%	28.5^{c}	32^{b}	32.5^{b}	37^{a}	0.35	0.33	0.006	0.009
Fat%	32.05^{d}	36.3^{b}	34^{c}	39.15^{a}	0.055	0.0006	0.0001	0.0215

Dissimilar letters in each row indicate a significant difference at the level of $P < 0.05$.

In the present study, different levels of $ZnCl_2$, $CoCl_2.\ 6H_2O$, $CuSo_4.\ 5H_2O$, $(NH_4)_6Mn_7O_{24}$, and vitamins B_1 and B_{12} were used. In the Walne medium, the amounts recommended by previous researchers were used, and in the modified Walne medium, the amount of cation supplementing minerals was reduced by 5 times less than the recommended amount, and the amount of vitamins was increased. One of the main roles of minerals in culture medium is to create cations and anions. Cations chelating the Anions and make them inaccessible. It seems that due to lower cations levels in modified culture medium, anions are more available to microalgae growth. Accordingly, although phosphorus and nitrogen levels were constant in both of tested culture mediums, nitrogen and phosphorus were more available to microalgae in modified culture medium and had positive effect on growth rate, biomass production, lipid, and CP content of microalgae. The amount of fresh and dried biomass (g/l) was affected by the type of culture medium and the interaction between culture medium and microalgae species were statistically significant (Table 3). The modified Walne medium increased total fresh, dried biomass production, and biomass production efficiency in both of the studied species. However, *N. oculata* had higher biomass production and biomass production efficiency in both culture media compared to *I. galbana* ($p < 0.05$) (Table 3). Studies have shown that light, aeration, ambient temperature, and the chemical composition of the culture medium are the main factors affecting the microalgal biomass production, as well as the chemical composition of the produced biomass including lipid and protein content and its fatty acid profile (Tamburic et al., 2014). Vitamin B_1 is a water-soluble vitamin and has been shown to have great effects on the growth of microalgae (Croft et al., 2006). Thiamine is activated after phosphorylation and plays a vital role in the production of carbohydrates in glycolysis (Monteverde et al., 2015). Biotin (Vitamin H) is also a water-soluble vitamin that plays an important role as a common agent in the metabolism of CO_2 for various carboxylases in a variety of biochemical reactions such as citric acid cycle, gluconeogenesis, fatty acid biosynthesis, branched amino acid catabolism and gene expression regulation (Cui et al., 2012). In the present study, it was observed that changes in the Walne medium did have a significant effect on the biomass production of *I. galbana* and *N. oculata* and their chemical components. Changing the culture medium to increase anions availability and some the B vitamins increased the levels of fat and CP alongside the increased biomass production. As a result, an increase in the g CP/day (total period), g CP/day (L), g fat/day (total period), and g fat/day (L) was expected.

Table 3- The effect of culture medium composition on the biomass production and biomass production efficiency amount of *I. galbana* and *N. oculata.*

Parameters	*I. galbana*		*N. oculata*		SEM	P-value		
						Microalgae species	Culture medium	Microalgae species * Culture medium
	Walne medium	Modified medium	Walne medium	Modified medium				
Fresh Biomass (g/l)	1.01^{c}	1.46^{b}	1.39^{b}	2.16^{a}	0.064	0.0001	0.0001	0.037
Dried Biomass (g/l)	0.262^{d}	0.391^{b}	0.368^{c}	0.522^{a}	0.008	0.0001	0.0001	0.0001
Fresh Biomass (g/day in total period)	6.22^{d}	9.4^{b}	8.59^{c}	12.49^{a}	0.001	0.0001	0.0001	0.0001
Dried Biomass (g/day in total period)	1.6^{d}	2.41^{b}	2.23^{c}	3.24^{a}	0.022	0.0001	0.0001	0.002
CP (g/day in total period)	1.72^{c}	2.1^{ab}	2.05^{b}	2.35^{a}	0.0604	0.013	0.048	0.385
CP(g/day/L)	0.075^{c}	0.12^{b}	0.125^{b}	0.195^{a}	0.004	0.001	0.009	0.037
Fat (g/day in total period)	0.55^{c}	0.81^{b}	0.86^{b}	1.11^{a}	0.006	0.001	0.0001	0.05
Fat (g/day/L)	0.09^{d}	0.138^{b}	0.125^{c}	0.175^{a}	0.002	0.01	0.048	0.048

Dissimilar letters in each row indicate a significant difference at the level of P <0.05.

Table 4 shows the FA profile of *I. galbana* and *N. oculata*, grown in two different types of medium. The results of this experiment showed that the interaction between the type of Walne medium and microalgae had a significant effect on the FA profiles of the two microalgae species (p <0.05). Total saturated and unsaturated FA content was not influenced by the growth medium but modified media increased PUFA at the expense of MUFA. Omega- 3 FA content (Linolenic acid, EPA, DHA) was increased as a result of the medium modification in both species. However, linoleic acid content was affected differently in *I. galbana* and *N. oculata*. The linoleic acid concentration was reduced in modified medium grown *N. oculata* but increased in *I. galbana*. Palmitic acid and stearic acid contents were also decreased in both of the studied species in the modified medium. Microalgal fats contain a large proportion of PUFAs such as EPA, DHA, and Arachidonic acid (Rose and Connolly, 1999; Judé et al., 2006). The results of some research show that different methods of fat extraction affect the amount and profile of PUFA in the microalgae. Past studies have shown that different species of microalgae show fluctuations in the profile of FA, which are mainly related to their culture medium characteristics (Fernandez - Reiriz et al., 1989; Volkman et al., 1989; Dunstan et al., 1993; Ren et al., 2022). The *I. galbana* and *N. oculata* are rich in omega-3 FA such as EPA and DHA (Chini-Zitelli et al., 1999; Rocha et al., 2003; Gouveia et al., 2008). Hosseini Shekarabi et al. (2019) had been reported the fatty acid composition of *I. galbana* cultured with Guillard (F2) medium was mostly saturated (52.41%) followed by PUFA and MUFA (27.58 and 16.32%, respectively). As we used the same pH and salinity in culture conditions, differences in FA profile with that reported by Hosseini Shekarabi et al. (2019), can be related to culture medium composition. Hu et al. (2008) proved that some nutrients in the aquatic environment can affect fat metabolism in phytoplankton. For example, nitrogen deficiency in the water causes the accumulation of triglycerides, or a lack of silicon in the water causes an increase in SFA and MUFA in the diatoms. In the present study, increasing B vitamins and reduced

levels of cations changed the amount of omega-3 and omega-6 FA and the omega-3: omega-6 ratio.

Table 4- Effect of different levels of some compounds in culture medium on the pattern of fatty acids of *I. galbana* and *N. oculata* (g/100 g FA)

Parameters	*I. galbana*		*N. oculata*		SEM	P-value		
						Microalgae species	Culture medium	Microalgae species * Culture medium
	Walne medium	Modified medium	Walne medium	Modified medium				
C12	ND	ND	0.60^{b}	0.82^{a}	0.007	<0.0001	0.0006	0.0006
C12:1	4.83^{a}	0.915^{c}	0.96^{c}	1.10^{b}	0.0175	<0.0001	<0.0001	<0.0001
C14	4.03^{c}	4.68^{b}	4.08^{c}	5.21^{a}	0.0169	<0.0001	<0.0001	0.0001
C16	15.72^{c}	14.82^{d}	20.03^{a}	18.09^{b}	0.0167	<0.0001	<0.0001	<0.0001
C16:1 trans	ND	4.19^{b}	2.76^{c}	4.30^{a}	0.0134	<0.0001	<0.0001	<0.0001
C16:1cis	6.77^{d}	12.16^{c}	17.87^{b}	19.69^{a}	0.0152	<0.0001	<0.0001	<0.0001
C16:2	8.12^{a}	ND	2.16^{b}	2.20^{b}	0.0150	<0.0001	<0.0001	<0.0001
C18:0	4.06^{b}	5.81^{a}	2.06^{d}	3.33^{c}	0.0180	<0.0001	0.0001	0.0056
C18:1	21.53^{a}	6.61^{b}	5.16^{c}	4.71^{d}	0.0091	<0.0001	<0.0001	<0.0001
C18:2	15.62^{d}	21.65^{b}	25.64^{a}	16.22^{c}	0.0152	<0.0001	<0.0001	<0.0001
C18:3	3.73^{d}	6.29^{a}	4.05^{c}	5.72^{b}	0.0198	0.0035	<0.0001	<0.0001
C18:4	1.25^{b}	2.19^{a}	ND	ND	0.007	<0.0001	<0.0001	<0.0001
C20:3	2.46^{c}	6.42^{a}	2.36^{d}	5.82^{b}	0.0219	<0.0001	<0.0001	0.0004
C20:0	1.28^{a}	ND	0.7^{b}	0.75^{b}	0.015	0.0048	<0.0001	<0.0001
C20:5 EPA	1.35^{d}	2.74^{c}	6.22^{b}	9.31^{a}	0.0154	<0.0001	<0.0001	<0.0001
C22:3	3.47^{a}	1.12^{c}	1.11^{c}	1.30^{b}	0.0143	<0.0001	<0.0001	<0.0001
C22:6 DHA	4.46^{b}	9.07^{a}	0.35^{d}	0.54^{c}	0.0158	<0.0001	<0.0001	<0.0001
Other fatty acid	1.32^{c}	1.43^{b}	3.9^{a}	0.88^{d}	0.0193	<0.0001	<0.0001	<0.0001
SFA†	25.09^{d}	25.31^{c}	27.47^{b}	28.21^{a}	0.038	<0.0001	0.0002	0.0024
UFA††	73.59^{a}	73.26^{b}	68.63^{d}	70.91^{c}	0.0193	<0.0001	<0.0001	<0.0001
MUFA	33.14^{a}	23.86^{d}	26.74^{c}	29.8^{b}	0.055	0.0139	<0.0001	<0.0001
PUFA	40.45^{c}	49.4^{a}	41.89^{b}	41.11^{c}	0.0556	<0.0001	<0.0001	<0.0001
ω3	16.72^{c}	27.83^{a}	14.09^{d}	22.7^{b}	0.0658	<0.0001	<0.0001	<0.0001
ω6	15.62^{d}	21.65^{b}	25.64^{a}	16.22^{c}	0.0190	<0.0001	<0.0001	<0.0001
ω3/ω6	1.07^{b}	1.28^{a}	0.55^{c}	1.4^{a}	0.0418	0.0088	0.0002	0.0016

Dissimilar letters in each row indicate a significant difference at the level of P <0.05
ND :Not detected.
†: Saturated fatty acids
††: Unsaturated fatty acids

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محیط کشت بهینه برای تولید پروتئین خام و اسیدهای چرب غیراشباع از ریزجلبک‌های *آیزوکرایسیس گالبانا* و *نانوکلروپسیس اکولاتا* برای تغذیه دام و آبزیان

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چکیده

زمینه مطالعاتی: امروزه اهمیت استفاده از ریز جلبک‌ها از نظر قابلیت آنها در تولید ویتامین‌ها، پروتئین‌ها، اسیدهای چرب غیر اشباع و انواع آنتی اکسیدان‌ها روز به روز در حال افزایش است. لذا با توجه به اینکه یکی از مهمترین اهداف کارشناسان تغذیه معرفی و شناسایی مکمل های خوراکی ارزشمند مورد استفاده در خوراک دام است و از طرفی محدودیت منابع آب و خاک به دلیل تغییر اقلیم بیش از پیش مورد توجه قرار گرفته است؛ لذا معرفی مکمل‌های ارزشمند از نظر تغذیه ای کار چندان راحتی نیست. **هدف**: بنظر می‌رسد با توجه به اینکه گونه های ریزجلبکی *آیزوکرایسیس گالبانا* و *نانوکلروپسیس اکولاتا* در محیط آبی شور و زمین های غیر زراعی قادر به رشد کردن و تولید ترکیبات ارزشمند پروتئینی واسیدهای چرب هستند. لذا کشت این دوگونه در محیط های کشت مختلف به منظور تولید زیتوده با چربی و پروتئین بالا و الگوی اسیدهای چرب غیر اشباع مناسب جهت مصرف در خوراک دام مورد بررسی قرار گرفت. **روش کار**: به منظور بررسی تاثیر محیط کشت بر تولید زیتوده، چربی و پروتئین و الگوی اسیدهای چرب از محیط کشت والنه با ترکیبات متفاوت مواد معدنی و ویتامینی استفاده شد. ریز جلبک‌های برداشت شده از نظر زیتوده، چربی، پروتئین، الگوی اسیدهای چرب مورد بررسی قرار گرفتند. **نتایج**: محیط کشت والنه تغییر یافته توانست پروتئین، چربی، ایکوزاپنتانوئیک اسید، دکوزاهگزانوئیک اسید و نسبت اسیدهای چرب امگا۳ به امگا۶ را در *آیزوکرایسیس گالبانا* و *نانوکلروپسیس اکولاتا* افزایش دهد ($P<0/05$). علاوه بر این، تولید زیست توده و راندمان تولید زیست توده نیز افزایش یافت ($P<0/05$). **نتیجه گیری نهایی**: محیط کشت تغییر یافته والنه بر تولید ترکیبات ارزشمند ریز جلبک های *آیزوکرایسیس گالبانا* و *نانوکلروپسیس اکولاتا* تاثیر زیادی دارد.

واژگان کلیدی: ریز جلبک؛ محیط کشت تغییر یافته؛ اسیدهای چرب، اسیدهای چرب غیر اشباع با چند پیوند دوگانه؛ مکمل خوراک دام