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Effects of Nitrogen Sources on the Growth and Biochemical Composition of Diatom (*Amphora coffeaeformis*) Used for Shrimp Larviculture

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Abstract

Amphora coffeaeformis is a benthic diatom and serves as a rich nutritional feed for various aquaculture industries. The objective of this experiment was to compare the effects of different nitrogen sources on the growth and biochemical composition of the diatom (*A. coffeaeformis*) culture. Sodium nitrate in Guillard's f medium, used as a control treatment, was compared with sodium nitrite and ammonium sulfate. The results in the 3rd batch of culture showed that all treatments led to the highest growth on Day 6. The highest cell dry weight occurred in the ammonium sulfate treatment with significance (0.78±0.06 mg/mL). The cultures in each treatment were harvested at the exponential phase (Day 3) for biochemical composition analysis. *A. coffeaeformis* in the ammonium sulfate treatment significantly accumulated the highest protein content, at 44.82 % by dry weight, while the highest lipid content at 29.78 % by dry weight was significantly found in the sodium nitrate treatment. The cell size and structure showed no significant differences among the treatments. The experiment demonstrated the effects of different nitrogen sources on the growth and biochemical composition of *A. coffeaeformis*. After that, we selected *A. coffeaeformis* that was cultured with the nitrate and ammonia nitrogen sources for feeding to shrimp larvae. This experiment was conducted to evaluate the effects of *A. coffeaeformis* on the growth, survival rate, salinity stress test, and ammonia stress test for shrimp. The experiment was designed with four treatments, as follows: 1) the control feed with flake winner (no water exchange for 28 days), 2) Am-NO_3 supplement with *A. coffeaeformis* cultured using nitrates (no water exchange for 28 days), 3) Am-NH_3 supplement with *A. coffeaeformis* cultured using ammonia (no water exchange for 28 days), and 4) the positive control feed with flake winner (daily water exchange at 20 %). The results showed that the growth of shrimp fed with the diet supplemented with *A. coffeaeformis* cultured using nitrate and ammonia were not significantly different from the negative and positive controls ($P > 0.05$). However, the survival rate in the negative control was significantly lower than the other groups ($P < 0.05$). In this study, the stress test for white shrimp postlarvae utilized 2 methods: the ammonia or salinity stress tests. The survival rate of the shrimp after the ammonia stress test was not statistically and significantly different ($P > 0.05$). However, the results from the salinity stress test indicated that the highest survival rate was observed in the shrimp fed with the diet supplemented with *A. coffeaeformis* cultured using nitrate. *A. coffeaeformis* cultured with the sodium nitrate treatment had a positive effect on shrimp tolerance to salinity changes.

Keywords: Nitrogen sources, *Amphora coffeaeformis*, Protein, Lipid, Larviculture

Introduction

Diatoms are a group of phytoplankton that is distributed throughout the world and have great biological importance to the primary production of aquatic animals [1,2]. They are rich sources of vitamins, minerals, amino acids, essential fatty acids, and pigments for aquaculture [3]. For this reason, they can enhance the health, strength, and disease resistance of aquatic animals, as well as boost their survival rate [4]. Nevertheless, microalgae biomass and chemical composition production are affected by many factors, such as nutrients, light, temperature, pH, and salinity [5-7]. Particularly, nitrogen nutrition is the most critical nutrient for growth, since it is a constituent in all structural and functional proteins, such as peptides, enzymes, chlorophylls, energy transfer molecules, and genetic materials in algal cells [8].

A report from Kim *et al*. [9] found that the microalga *Tetraselmis* sp. used effective organic nitrogen sources, such as yeast extract, glycine, and urea, for cell growth. When comparing between nitrate and ammonium, it was found that nitrate helps cells grow more than ammonium, but a higher lipid content in cells was obtained from the ammonium culture than from the nitrate. Consistent with the study of Fidalgo *et al*. [10], the marine diatom *Phaeodaetylum trieornutum* was cultured and grown in nitrates, nitrites, ammonia, and urea. The dry biomass production of *Phaeodaetylum trieornutum* was found to be higher in nitrates than urea. Microalgae can grow under heterotrophic conditions with all 3 nitrogen sources (i.e., sodium nitrate, ammonium chloride, and urea), and their uptake can affect the pH of the cultivation medium. The specific growth rate of *Cyclotella cryptica* is highest when providing low levels of urea and ammonium chloride [11]. It has been reported that microalgae are able to absorb nitrogen in both inorganic and organic forms [12]. In addition to the nitrogen sources, the growth of different microalgae cells also results in different biochemical compositions. Fidalgo *et al*. [13] cultured *Isochrysis galbana* with different nitrogen sources which grew with nitrates or nitrites, showing that the fatty acids were very similar at any stage of growth, but were slightly different from the urea-grown cells; and *Isochrysis* cultured in the urea media had the highest saturated and monounsaturated fatty acids. The results from the study of Yilancioğlua *et al*. [14] showed that sodium nitrate, ammonium chloride, and urea could be successfully utilized for the growth of the green algae *Scenedesmus obliquus*, and found that ammonium chloride resulted in increased lipid accumulation when compared to alternative nitrogen sources such as sodium nitrate and urea.

Many reports have mentioned the application of nitrogen sources for microalgae, both for cell growth and biochemical composition. However, *Amphora coffeaeformis* is a diatom with a high nutritional value, and it is of interest to compare the growth and biochemical composition of this culture in several nitrogen sources. Therefore, this research aimed to investigate the effects of different nitrogen sources on the growth and biochemical composition of *A. coffeaeformis*.

Materials and methods

Cultivation of *A. coffeaeformis*

A. coffeaeformis, from the Center of Excellence in Shrimp, Walailak University, Thailand, was cultivated in batch cultures with sterile seawater at a salinity of 25 psu, aerated, and illuminated continuously with 5,000 lux of fluorescent light. The temperature in the laboratory room was kept at 25±2 °C. The culture medium used was the Guillard's f medium. The cultures were transferred to the next batch at the exponential phase of growth [4].

Effects of nitrogen sources on growth and biochemical composition of *A. coffeaeformis*

Cultivation of *A. coffeaeformis* occurred in the following laboratory conditions: 1 g/L of innoculum (2 L working volume in plastic bottle), salinity of 25 psu, aeration, continuous illumination with 5,000 lux of fluorescent light, temperature kept at 25±2 °C, and sodium nitrate in Guillard's f medium used as a control treatment in comparison with sodium nitrite and ammonium sulfate (4 replicates). The molar concentration in stock solution $NaNO_3$ = 0.88M (final concentration of nitrogen in culture = 0.88M). The calculation of the molecular weight and the price of nitrogen (sodium nitrite, sodium nitrate, and

ammonium sulphate) are shown in **Table 1**. In the harvesting of the cultivation, aliquots of 10 mL *A. coffeaeformis* suspension were filtered by GF/C every 24 h. The microalgae cells were dried in an oven (Binder BD 115) at 105 °C for 24 h. After that, the dried microalgae cells were measured, and the growth of the microalgae was calculated based on the microalgae dry weight produced per milliliter (g/mL) [15].

Table 1 Calculation of molecular weight and price of nitrogen from different sources.

Chemical	Molecular Weight	Molar of Nitrogen	Stock Solution (g/L)	Using (g/mL)	Price (Baht/mL)
$NaNO_3$	84.99	0.88	74.79	0.07500	0.051
$NaNO_2$	69.00	0.88	60.72	0.06072	0.073
$(NH_4)_2SO_4$	132.13	0.88	58.14	0.05814	0.036

Proximate composition analysis

A. coffeaeformis was dried at 105 °C for moisture analysis and at 550 °C for total ash following AOAC (2005) [16]. Protein contents of the microalgae were analyzed following the Lowry method [17,18] by using an ultrasonicator (Elmasonic, S100H) and spectrophotometer (Hitachi, U-1800). Lipid and fatty acid contents of the microalgae were analyzed following the Bligh and Dyer method [19,20] by using an ultrasonicator (Elmasonic, S100H, refrigerator, vacuum concentrator centrifuge (LABCONCO®), and fatty acid analyzer with GC-FID (AGILENT®, 7890a).

Shrimp and feeding experiment

L. vannamei postlarvae (PL10) were obtained from a hatchery in NS farm, and then transported to the Research Center of Excellence for Shrimp, School of Agricultural Technology, Walailak University. The shrimp were acclimatized in an 800-L tank with aeration at 20 ppt salinity. The experiment was designed with 4 treatments as follows: 1.) the control feed with flake winner (no water exchange for 28 days), 2.) Am-NO_3 feed with flake + *A. coffeaeformis* cultured using nitrates (no water exchange for 28 days), 3.) Am-NH_3 feed with flake + *A. coffeaeformis* cultured using ammonia (no water exchange for 28 days), and 4.) the positive control feed with flake (daily water exchange at 20 %) [21]. Shrimp were fed 7 times daily at 07.00, 10.00, 13.00, 16.00, 19.00, 21.00, and 01.00 h to satiation [22] with feed following the experiment design for 4 weeks. The ammonia and nitrites were measured using a phenol hypochlorite method and NED method, respectively [23]. After 4 weeks, all white shrimp postlarvae were collected to assess their final length and survival rate [24].

Survival rate (%) = 100×(final shrimp number) / (initial shrimp number) (1)

Effects of *A. coffeaeformis* on the salinity and ammonia stress test of white shrimp postlarvae

After being cultured for 28 days, 20 shrimp from each tank were sampled for salinity stress (3 replicates, n = 60 shrimps/treatment) with the stress test at 0 psu of salinity for 60 min. The number of shrimp was counted and the survival rate was calculated [25]. The ammonia stress test was applied in 3 replicates. Ten shrimp per replication (each treatment containing three replications) were exposed to ammonium chloride, which was adjusted to 50 ppm of total ammonia nitrogen (TAN) for 48 h [26].

Survival rate stress test (%) = 100×(final shrimp number) / (initial shrimp number) (2)

Statistical analysis

Statistical significance was considered at $P < 0.05$ by one-way ANOVA following Duncan's multiple range test by using IBM SPSS statistics 24.

Results and discussion

Growth performance of *A. coffeaeformis*

A. coffeaeformis was cultured with the Guillard's f medium using different nitrogen sources as follows: Sodium nitrate (nitrate), sodium nitrite (nitrite), and ammonium sulphate (ammonia). It was found that *A. coffeaeformis* with the 3 nitrogen sources had high cell growth on Day 6. *A. coffeaeformis* with ammonia had the highest cell growth by dry weight, and statistical analysis was significant ($P > 0.05$), as shown in **Figure 1**.

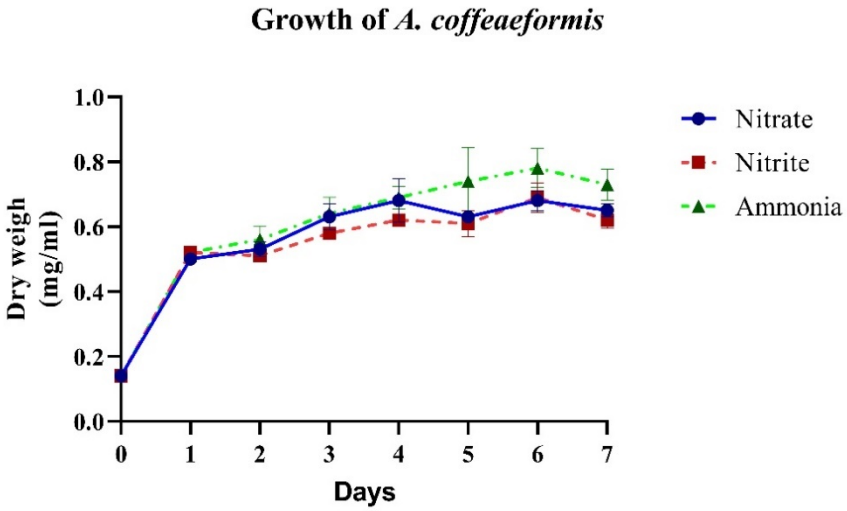

Figure 1 Dry weight of *A. coffeaeformis* grown in medium of different nitrogen sources.

Nitrogen assimilation in *A. coffeaeformis*

A. coffeaeformis could use nitrogen in the form of nitrates, nitrites, and ammonia. Considering the residue of nitrogen on Day 7, the results indicated that the best nitrogen source for *A.coffeaeformis* consumption was ammonia (**Figure 2**).

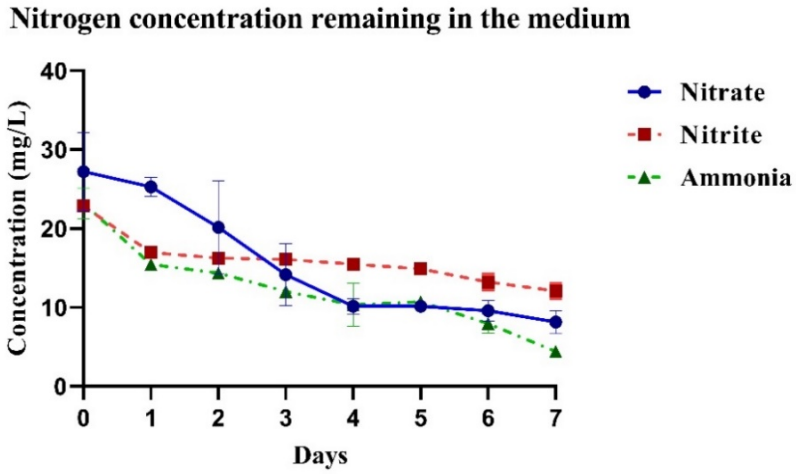

Figure 2 Nitrogen assimilation in *A. coffeaeformis* in Guillard f medium with different nitrogen sources.

Lipid, protein, and fatty acid composition of *A. coffeaeformis*

The lipid content of *A. coffeaeformis* cultured with sodium nitrate was significantly higher than all other treatments ($P > 0.05$) (**Figure 3**).

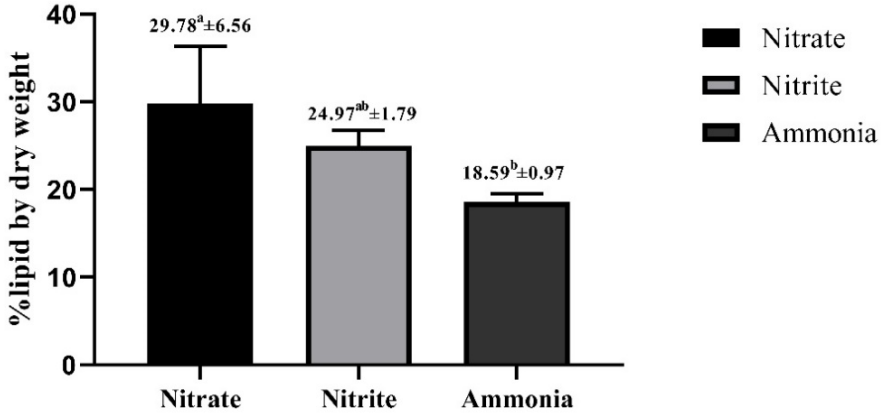

Figure 3. Lipid content of *A. coffeaeformis* cultured with different nitrogen sources

The protein content of *A. coffeaeformis* cultured with ammonium sulphate was significantly higher than all other treatments ($P > 0.05$) (**Figure 4**).

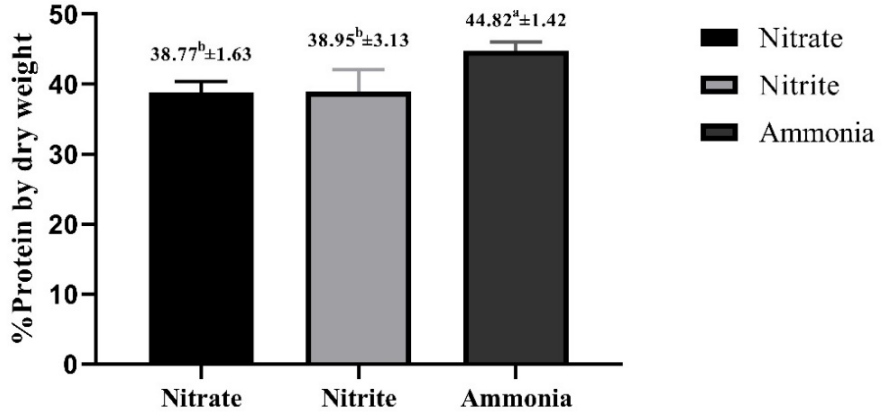

Figure 4 Protein content of *A. coffeaeformis* cultured with different nitrogen sources.

The fatty acid profile is presented in **Table 2** and indicated that *A. coffeaeformis* cultured with nitrate contained the highest fatty acids, such as palmitoleic, linoleic, arachidonic, and eiocosapentaenoic acid.

Table 2 Fatty acid consumption (% by weight of total fatty acids) of *A. Coffeaeformis*.

Fatty acid composition	FA	Nitrate	Nitrite	Ammonia
Capric acid	C10:0	0.02	0.12	0.02
Lauric acid	C12:0	0.30	0.27	0.20
Tridecanoic acid	C13:0	0.14	0.09	0.12
Myristic acid	C14:0	10.00	12.12	11.68
Pentadecanoic acid	C15:0	1.01	1.38	1.11
Palmitic acid	C16:0	21.01	37.96	27.73
Stearic acid	C18:0	0.33	0.77	0.51
Arachidic acid	C20:0	0.03	0.04	0.03
Heneicosanoic acid	C21:0	0.11	0.10	0.06
Behenic acid	C22:0	0.23	0.13	0.24
Lignoceric acid	C24:0	0.58	1.23	0.65
%Total saturated fatty acid		**33.76**	**54.20**	**42.33**
Myristoleic acid	C14:1	0.10	0.10	0.07
Palmitoleic acid	C16:1	45.38	35.27	42.17
cis-9-Oleic acid	C18:1n9c	1.58	2.72	1.75
cis-11-Eicosenoic acid	C20:1	0.00	0.05	0.03
%Total monounsaturated fatty acid		**47.06**	**38.14**	**44.01**
trans-Linolelaidic acid	C18:2n6t	0.00	0.04	0.00
Linoleic acid	C18:2n6c	3.67	1.48	3.15
γ -Linolenic acid	C18:3n6	3.07	0.76	2.84
cis-8,11,14-Eicosatrienoic acid	C20:3n6	0.06	0.06	0.05
Arachidonic acid	C20:4n6	3.64	1.33	2.05
Eiocosapentaenoic acid	C20:5n3	8.75	3.99	5.57
%Total polyunsaturated fatty acids		**19.18**	**7.66**	**13.65**

Growth performance and survival rate of white shrimp postlarvae

In this study, *A. coffeaeformis* cultured with two nitrogen sources, nitrates and ammonia, was chosen and considered because it had the highest lipid and protein content, respectively (**Table 3**).

Table 3 Protein, lipid, saturated, monosaturated, and polysaturated content of *A. coffeaeformis* used as supplemented diet for white shrimp postlarvae.

Treatment	%Protein	%Lipid
Am-NO_3	38.77 ± 1.63^a	29.78 ± 6.56^b
Am-NH_3	44.82 ± 1.24^b	20.58 ± 1.76^a

[1]Values are expressed as means±SD (4 replicates), with different superscripts (a-b) indicating significance ($P < 0.05$) between treatments.

The results from **Table 4** indicated that the growth of the shrimp fed with the supplemented diet of *A. coffeaeformis* cultured with nitrates and ammonia was not significantly different between the negative and positive controls ($P > 0.05$). However, the survival rate in the negative control was significantly lower than all other groups ($P < 0.05$).

Table 4 Effects of *A. coffeaeformis* on growth and survival rate of white shrimp postlarvae.

Treatment	Initial length (cm)	Final length (cm)	Survival (%)	%CV
Control (negative control)	0.74±0.07	2.88±0.37[a]	42.50±1.32[b]	12.8
Am-NO_3	0.74±0.07	2.89±0.05[a]	64.67±4.31[a]	1.7
Am-NH_3	0.74±0.07	2.87±0.09[a]	60.83±13.73[a]	3.1
PC (positive control)	0.74±0.07	2.68±0.12[a]	75.83±6.21[a]	4.4

[1]Values are expressed as means±SD (4 replicates), with different superscripts (a-b) indicating significance ($P < 0.05$) between treatments.

Effects of stress test with ammonia and salinity on white shrimp postlarvae

In this study, the stress test for the white shrimp postlarvae was performed using 2 methods: the ammonia and salinity stress tests. The survival rate of the shrimp after the ammonia stress test had no statistically significant differences ($P > 0.05$). However, the results from the salinity stress test indicated that the highest survival rate was observed in the shrimp fed with the supplemented diet of *A. coffeaeformis* cultured with nitrates (**Table 5**).

Table 5 Effects of *A. coffeaeformis* on white shrimp postlarvae stress test.

Treatment	Survival rate (%)	
	50 mgNH_4Cl/L	0 ppt
Control (negative control)	76.67[a]	95.00[a]
Am-NO_3	80.00[a]	100.00[a]
Am-NH_3	76.67[a]	78.33[b]
PC (positive control)	80.00[a]	85.00[b]

[1]Values are expressed as means±SD (3 replicates), with different superscripts (a-b) indicating significance ($P < 0.05$) between treatments.

Discussion

Nitrogen sources can help microalgae with high growth depending on the nitrogen concentration and species of microalgae [27]. In this study, *A. coffeaeformis* using the Guillard's f medium with 3 nitrogen sources showed high cell growth on Day 6, and *A. coffeaeformis* with ammonia had the highest cell growth by dry weight but with no significance ($P > 0.05$). This is in accordance with Altın *et al*. [28], who reported on the effects of different nitrogen sources on *Chlorella variabilis* microalgae growth and lipid content, as investigated by culturing *C. variabilis* with ammonium, nitrite, and urea; the maximum growth of *C. variabilis* occurred with the urea form. It is well known that ammonium is essential for microalgae, more than nitrates or nitrites, since ammonium is a condensed form of nitrogen. Ammonium is assimilated directly with the amino acids inside the cell, while nitrates or nitrites must be reduced to ammonium before use [29].

However, Yilancioğlua *et al.* [14] reported that *Scenedesmus obliquus* cultured with nitrates grew higher than when cultured with ammonia or nitrites. Moreover, a study on the effects of different nitrogen sources in 10 species of phytoplankton found that 2 species (*Hillea* sp. and *Prorocentrum minimum*) failed to grow with ammonium-N because of the toxic effect of ammonia in high concentrations [30]. Simsek and Cetin [31] studied *Chlorella vulgaris* using ammonium and nitrate as a nitrogen source from ammonium sulfate and sodium nitrate, which resulted in a high concentration of lipid content. Similar

results were shown by Sánchez-García *et al*. [32], who found that nitrates affected the biomass production and composition of lipid content in all 3 algae (*Monoraphidium contortum*, *Tetraselmis suecica*, and *Chlorella minutissima*).

A. coffeaeformis cultured with ammonium sulphate had the highest protein content but was not significant when compared with the other treatments ($P > 0.05$). However, *A. coffeaeformis* cultured with nitrates had the highest lipid content and saturated and monounsaturated fatty acids. On the other hand, Fidalgo *et al*. [33] reported that *Isochrysis galbana* cultured with nitrates or nitrites in urea culture medium contained the highest saturated and monounsaturated fatty acids. Regarding the dietary lipid component, several studies have demonstrated the essentiality of linoleic (LA; 18:2n-6) and linolenic (LNA; 18:3n-3) acids for the normal growth and survival of penaeid shrimp [34,35]. In this study, the growth of white shrimp postlarvae, fed with *A. coffeaeformis* cultured with both ammonia and nitrates, was not different from the control, but the survival rate was better due to the high level of linoleic and linolenic acids. The results from **Table 4** indicated that the growth of the shrimp fed with the diet supplemented with *A. coffeaeformis* cultured with nitrates and ammonia was not significantly different than the control ($P > 0.05$), and the negative control was lowest in terms of the survival rate when compared with all other groups with significance ($P > 0.05$).

Although arachidonic acid (ARA) is also considered an essential fatty acid (EFA) for different shrimp species [36], ARA-supplemented diets for *F. chinensis* significantly enhanced growth [37], whereas there was a negative dose-response effect on the growth of *P. monodon* [38]. This was similar to the present study, where *A. coffeaeformis* cultured with both ammonia and nitrates contained high levels of ARA but did not affect shrimp growth. However, there was an effect on the salinity tolerance of the shrimp postlarvae, because ARA is an essential fatty acid for penaeid larval and regulates cortisol synthesis in stress tests and improves the physiological response to abrupt salinity changes [39].

The salinity stress test indicated that the highest survival rate (100 %) was observed in the shrimp fed with the diet supplemented with *A. coffeaeformis* cultured with nitrates. This indicated that *A. coffeaeformis* cultured with sodium nitrate had a positive effect on shrimp health, especially in terms of tolerance to salinity changes. Even though the negative control and positive control had no difference in the survival rate of shrimp, there was a lower survival rate in the negative control than in the positive control which, according to the report of Allan and Maguire [40], showed that the daily water exchange may not necessarily increase shrimp growth, but should not be raised for spread periods with zero water exchange. The positive control had a significantly lower survival rate ($P > 0.05$) after the salinity stress test when compared to the negative control, which was inconsistent with research indicating that the survival rate and growth of shrimp reared with biofloc treatments were not expressed in the tolerance to salinity changes with the control (no biofloc) [41]. No water exchange in a closed aquaculture system to make a microorganism was also acceptable in being productive and successful as a bioremediation, such as nitrifying bacteria, algae grazers, and pathogenic bacteria (*Vibrio* spp.) [42].

Conclusions

Nitrogen has an effect on the growth and quality of *A. coffeaeformis*. High cell growth for *A. coffeaeformis* occurred on Day 6 for all treatments, and the highest cell growth occurred with the ammonium sulfate treatment. The cultures of *A. coffeaeformis* were harvested at the exponential phase (Day 3), and biochemical composition analysis found that *A. coffeaeformis* accumulated the highest protein content in the ammonium sulfate and the highest lipid content in the sodium nitrate treatment ($P > 0.05$). In this study, nitrate was considered to be suitable for the production of high-quality *A. coffeaeformis*. Although the *A. coffeaeformis* supplementations had no difference in their effect on survival rate, *A. coffeaeformis* cultured with the sodium nitrate treatment had a positive effect on shrimp tolerance to salinity changes.

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References

[1] PW Boyd, R Strzepek, S Chiswell, H Chang, J De Bruyn, M Ellwood, S Keenan, AL King, EW Maas, S Nodder, SG Sander, P Sutton, BS Twining, SW Wilhelm and DA Hutchins. Microbial control of diatom bloom dynamics in the open ocean. *Geophys. Res. Lett*. 2012; **39**, L18601.

[2] CB Field, MJ Behrenfeld, JT Randerson and P Falkowski. Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* 1998; **281**, 237-40.

[3] T Takeuchi, J Lu, G Yoshizakiand and S Satoh. Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw Spirulina. *Fish. Sci*. 2002; **68**, 34-40.

[4] PM Pimolrat. 2009, Production of high cell density *Chaetoceros gracilis* in laboratory for nursing marine shrimp larvae. Master Thesis. Walailak University, Nakhon Si Thammarat, Thailand.

[5] G Kim, CH Lee and K Lee. Enhancement of lipid production in marine microalga *Tetraselmis* sp. through salinity variation. *Kor. J. Chem. Eng*. 2014; **33**, 230-7.

[6] E Suyono, W Haryadi, M Zusron, M Nuhamunada, S Rahayu and A Nugroho. The effect of salinity on growth, dry weight and lipid content of the mixed microalgae culture isolated from Glagah as biodiesel substrate. *J. Life Sci*. 2015; **9**, 229-33.

[7] M Bartley, WJ Boeing, D Daniel, BN Dungan and T Schaub. Optimization of environmental parameters for *Nannochloropsis salina* growth and lipid content using the response surface method and invading organisms. *J. Appl. Phycol*. 2016; **28**, 15-24.

[8] T Cai, SY Park and Y Li. Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renew. Sustain. Energ. Rev*. 2013; **19**, 360-9.

[9] G Kim, G Mujtaba and K Lee. Effects of nitrogen sources on cell growth and biochemical composition of marine chlorophyte *Tetraselmis* sp. for lipid production. *Algae* 2016; **31**, 257-66.

[10] JP Fidalgo, A Cid, E Torres, A Sukenik and C Herrero. Effects of nitrogen source and growth phase on proximate biochemical composition, lipid classes and fatty acid profile of the marine microalga *Isochrysis galbana*. *Aquaculture* 1998; **166**, 105-16.

[11] LP Stephen, DM Lewis, KD King and F Chen. Heterotrophic growth and nutritional aspects of the diatom *Cyclotella cryptica* (Bacillariophyceae): Effect of nitrogen source and concentration. *J. Appl. Phycol*. 2012; **24**, 301-7.

[12] T Jauffrais, B Jesus, V Méléder, V Turpin, PG Russo, P Raimbault and VM Jézéquel. Physiological and photophysiological responses of the benthic diatom *Entomoneis paludosa* (Bacillariophyceae) to dissolved inorganic and organic nitrogen in culture. *Mar. Biol*. 2016; **163**, 115.

[13] JP Fidalgo, A Cid, J Abalde and C Herrero. Culture of the marine diatom *Phaeodactylum tricornutum* with different nitrogen sources: Growth, nutrient conversion and biochemical composition. *Cah. Biol. Mar.* 1995; **36**, 165-73.

[14] K Yilancioğlua, HO Tekinb and S Cetiner. Nitrogen source, an important determinant of fatty acid accumulation and profile in *Scenedesmus obliquus*. *In*: Proceedings of the 2nd International Conference on Computational and Experimental Science and Engineering. Kemer, Turkey. 2016, p. 428-33.

[15] ZY Ju, IP Forster and WG Dominy. Effects of supplementing two species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp (*Litopenaeus vannamei*). *Aquaculture* 2009; **292**, 237-43.

[16] Association of Official Analytical Chemists (AOAC). *Official method of analysis of analysis of Association of Official Analytical Chemists*. Gaithersburg.

[17] OH Lowry, NJ Rosebrough, AL Farr and RJ Randall. Protein measurement with the folin phenol reagent. *J. Biol. Chem*. 1951; **193**, 265-75.
[18] C Safi, AV Ursu, C Laroche, B Zebib, O Merah, PY Pontalier and C Vaca-Garcia. Aqueous extraction of protein from microalgae: Effect of different cell disruption methods. *Algal Res*. 2014; **3**, 61-5.
[19] EG Bligh and WJ Dyer. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol*. 1959; **37**, 911-7.
[20] O Işik, E Sarihan, E Kuşvuran, O Gül and O Erbatur. Comparison of the fatty acid composition of the freshwater fish larvae *Tilapia zillii*, the rotifer Brachionus calyciflorus, and the microalgae *Scenedesmus abundans*, *Monoraphidium minitum* and *Chlorella vulgaris* in the algaerotifer-fish larvae food chains. *Aquaculture* 1999; **174**, 299-311.
[21] LP Lage, EP Juan, SM Putrino, F Baron, D Weissman, A Guyonvarch, R Brugger, AJP Nunes and S Panserat. Ontogenesis of metabolic gene expression in whiteleg shrimp (*Litopenaeus vannamei*): New molecular tools for programming in the future. *Aquaculture* 2017; **479**, 142-9.
[22] S Xie, W Fang, D Wei, Y Liu, P Yin, J Niu and L Tian. Dietary supplementation of *Haematococcus pluvialis* improved the immune capacity and low salinity tolerance ability of post-larval white shrimp, Litopenaeus vannamei. *Fish Shellfish Immunol*. 2018; **80**, 452-7.
[23] W Jiwyam. *Introduction to water quality and water quality analysis in aquaculture ponds*, Chulalongkorn University Press, Bangkok, 2001.
[24] MY Karim. The effect of osmotic various medium salinity of vitality of female mud crab (*Scylla olivacea*). *J. Protein* 2007; **14**, 65-72.
[25] SR Wangsoontorn, CL Limsuwanand and NT Chuchird. Effect of density and transportation times on survival rate of Litopenaeus vannamei postlarvae. *In*: Proceedings of the Kasetsart University Annual Conference. Veterinary Medicine, Fisheries, Bangkok, Thailand. 2013, p. 198-206.
[26] T Wang, HW Shan, ZX Geng, P Yu and S Ma. Dietary supplementation with freeze-dried *Ampithoe* sp. enhances the ammonia-N tolerance of *Litopenaeus vannamei* by reducing oxidative stress and endoplasmic reticulum stress and regulating lipid metabolism. *Aquaculture Rep.* 2020; **16**, 100264.
[27] L Xin, H Hong-ying, G Ke, and S Ying-xue. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. *Bioresource Tech*. 2010; **101**, 5494-500.
[28] N Altın, T Kutluk, B Uyar and N Kapucu. Effect of different nitrogen sources on the growth and lipid accumulation of *Chlorella variabilis*. *J. Appl. Biol. Sci*. 2018; **12**, 38-40.
[29] M Podevin, DD Francisci, SL Holdt and I Angelidaki. Effect of nitrogen source and acclimatization on specific growth rates of microalgae determined by ahigh-throughput *in vivo* microplate autofluorescence method. *J. Appl. Phycol.* 2015; **27**, 1415-23.
[30] SO Lourenço, E Barbarino, J Mancini-Filho, KP Schinke and E Aidar. Effects of different nitrogen sources on the growth and biochemical profile of 10 marine microalgae in batch culture: An evaluation for aquaculture. *Phycologia* 2002; **41**, 158-68.
[31] GK Simsek and AK Cetin. Effect of nitrogen sources on growth, protein, total lipid amount and pigment content in *Chlorella vulgaris*. *Fresen. Environ. Bull*. 2019; **8**, 3065-72.
[32] D Sánchez-García, A Resendiz-Isidro, TL Villegas-Garrido, CM Flores-Ortiz, B Chávez-Gómez and E Cristiani-Urbina. Effect of nitrate on lipid production by *T. suecica*, *M. contortum*, and *C. minutissima*. *Cent. Eur. J. Biol*. 2013; **8**, 578-90.
[33] JP Fidalgo, A Cid, E Torres, A Sukeni and C Herreroa. Effects of nitrogen source and growth phase on proximate biochemical composition, lipid classes and fatty acid profile of the marine microalga *Isochrysis galbana*. *Aquaculture* 1998; **166**, 105-16.
[34] ZO Merican and KF Shim. Quantitative measurements of linolenic and docosahexaenoic acid for juvenile *Penaeus monodon*. *Aquaculture* 1997; **157**, 277-95.
[35] BD Glencross and DM Smith. The dietary linoleic and linolenic fatty acid requirements of the prawn *Penaeus monodon*. *Aquaculture Nutr*. 1999; **5**, 53-63.
[36] XL Xu, WJ Ji, JD Castell and RK O'Dor. Essential fatty acid requirement of the Chinese prawn, *Penaeus chinensis*. *Aquaculture* 1994; **127**, 29-40.

[37] BD Glencross and DM Smith. Optimizing the essential fatty acids, eicosapentaenoic and docosahexaenoic acids, in the diet of the prawn, *Penaeus monodon. Aquaculture Nutr.* 2001; **7**, 101-12.
[38] BD Glencross and DM Smith. A study of the arachidonic requirements of giant tiger prawn *Pennaeus monodon*. *Aquaculture Nutr.* 2001; **7**, 59-69.
[39] RDV Anholt, WM Koben, S Lutzky and SEW Bonga. Dietary supplementation with arachidonic acid alters the stress response of gilthead seabream (*Sparus aurata*) larvae. *Aquaculture* 2004; **238**, 369-83.
[40 GL Allan and GB Maguire. The effects of water exchange on production of *Metapenaeus macleayi* and water quality in experimental pools. *J. World Aquaculture Soc*. 1993; **24**, 321-8.
[41] M Emerenciano, ELC Ballester, RO Cavalli and W Wasielesky. Effect of biofloc technology (BFT) on the early postlarval stage of pink shrimp *Farfantepenaeus paulensis*: Growth performance, floc composition and salinity stress tolerance. *Aquaculture Int*. 2011; **19**, 891-901.
[42] H Manan, JHZ Moh, NA Kasan, S Suratman and M Ikhwanuddin.Identification of biofloc microscopic composition as the natural bioremediation in zero water exchange of Pacific white shrimp, *Penaeus vannamei*, culture in closed hatchery system. *Appl. Water Sci.* 2016; **7**, 2437-46.