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Protein Content, Fucoxanthin and Growth of *Chaetoceros calcitrans* Using Liquid Organic Fertilizer from Agricultural and Fish By-Product

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ABSTRACT

In 2022, Indonesian aquaculture production reached 16.87 million tonnes, with *Chaetoceros calcitrans* contributing significantly as a source of protein (25%) and fucoxanthin, both crucial for aquatic growth and water color. However, excessive use of inorganic fertilizers raises environmental concerns, driving interest in organic alternatives like rice straw, water hyacinth, and tilapia offal. These materials are nutrient-rich: rice straw contains silica, water hyacinth offers protein and potassium, and tilapia offal is a source of protein and phosphorus. Through fermentation, their nutrients become more bioavailable. This study aimed to assess the effects of liquid organic fertilizer made from these wastes on the cell density, growth rate, protein content, and fucoxanthin level of *C. calcitrans*. Five treatments were tested: P0 (control), and P1–P4 (fertilizer doses of 6, 8, 10, and 12ml/ L, respectively). Data were analyzed using ANOVA and DMRT. Results showed peak cell density on day 6 for P1–P4 and day 7 for P0, with P4 yielding the highest cell density (190.08 × 10^4 cells/ml), growth rate (0.8746%/day), protein (34.79%), and fucoxanthin (8.308 ppm). These findings suggest that fermented organic waste-based fertilizers can effectively support *C. calcitrans* cultivation while offering an eco-friendly alternative to synthetic inputs.

INTRODUCTION

Aquaculture is a rapidly growing sector with significant potential in Indonesia. According to the Ministry of Marine Affairs and Fisheries (KKP), aquaculture production reached 16.87 million tons in 2022 **(Ministry of Maritime Affairs and Fisheries, 2022)**. One of the key factors influencing the success of aquaculture is the availability of high-quality natural feed, which directly affects production output **(Lestari *et al.*, 2019)**. Natural

feed refers to feed naturally present in aquatic environments **(Rihi, 2019)**, and one of the most widely used types in aquaculture is the microalga *Chaetoceros calcitrans*.

Chaetoceros calcitrans is a diatom microalga belonging to the class Bacillariophyceae. It has a thin silica cell wall, making it easily digestible by shrimp **(Sari & Ikbal, 2020)**. This species is characterized by rapid growth and high nutritional content, particularly protein levels of around 35%, making it ideal for larval development **(Prasetyo *et al.*, 2022)**. Research by **Miller *et al.* (2012)** demonstrated that *C. calcitrans* contains high levels of essential polyunsaturated fatty acids, such as EPA and DHA, especially during its logarithmic growth phase, enhancing its value for larval and juvenile aquaculture species. The alga also contains chlorophyll a and c, along with pigments such as fucoxanthin, β-carotene, and diatomin, which give it a golden-brown coloration. These pigments contribute to water quality improvements, aligning with **Utojo (2015)**, who noted that optimal shrimp pond water has a greenish-brown hue, a condition achievable by incorporating *C. calcitrans* as a natural feed.

The growth and availability of *C. calcitrans* depend on the nutrient composition of its culture medium, which must include both macronutrients (e.g., nitrogen, phosphorus, silicate) and micronutrients (e.g., iron, zinc, copper, and vitamins). Silicate, in particular, is essential for the development of the diatom's siliceous frustule (cell wall), supporting optimal cell division and growth **(Jannah *et al.*, 2019)**. These nutrients are typically provided through organic or inorganic fertilizers. Inorganic options such as urea, NPK, and silicate are commonly used to enhance microalgal growth **(Ramadhanty *et al.*, 2021)**. However, continuous use of inorganic fertilizers can disrupt nutrient balance and pose environmental risks **(Murnita & Taher, 2021)**. Additionally, the rising costs and limited government subsidies for these fertilizers have reduced their accessibility to many aquaculture producers **(Juwariyah *et al.*, 2020)**.

As an alternative, organic liquid fertilizer (OLF) derived from agricultural and aquaculture waste offers a more sustainable and cost-effective solution. This study explores the potential of OLF produced from rice straw, water hyacinth (*Eichhornia crassipes*), and the Nile tilapia (*Oreochromis niloticus*) entrails. *E. crassipes* has demonstrated high nutrient content and growth potential, making it suitable for enhancing plant productivity when used as fertilizer **(Kawet *et al.*, 2023)**.

Rice straw, an abundant agricultural by-product, is rich in essential nutrients. According to **Purwaningsih *et al.* (2012)**, it contains approximately 70.8% silicate, 36.5% cellulose, 12.3% lignin, and 33.8% hemicellulose—nutrients beneficial for microalgal development. However, the cellulose and hemicellulose components are indigestible to microalgae and must be pre-treated through fermentation using probiotic decomposer

microorganisms such as *Actinomycetes*, *Lactobacillus* sp., and *Saccharomyces* sp. **(Ponidi & Rizaly, 2023)**.

Water hyacinth, although considered an invasive aquatic weed that can disrupt freshwater ecosystems, is also rich in nutrients, including 11.2% protein and 0.0011% total phosphorus **(Famuntamah *et al.*, 2021; Nandiyanto *et al.*, 2023)**. Its fast growth and availability make it a promising organic input for fertilizer production.

The Nile tilapia entrails, often discarded during fish processing, are nutrient-dense, containing between 36–57% protein **(Zahroh *et al.*, 2018)** and 14.91% fat **(Suseno *et al.*, 2023)**. Incorporating these entrails into organic fertilizer not only reduces waste but also adds valuable nutrients that support microalgal growth.

These three organic materials—rice straw, water hyacinth, and fish entrails—contain essential nutrients that can support the cultivation of *C. calcitrans*. Their utilization not only addresses challenges related to fertilizer shortages and high costs but also promotes waste valorization and sustainability in aquaculture practices.

This study aimed to investigate the effects of organic liquid fertilizer derived from rice straw, water hyacinth, and the Nile tilapia entrails on the specific growth rate, protein content, and fucoxanthin concentration of *Chaetoceros calcitrans*.

MATERIALS AND METHODS

Tools and materials preparation

All equipment was sterilized to ensure aseptic conditions. The sterilization process involved washing with soap, rinsing with clean water, soaking in a 60ppm chlorine solution for 24 hours, followed by rinsing, air drying, spraying with 70% alcohol, and placement in a UV cabinet for 15 minutes. Sterilization of seawater (30ppt salinity) was performed by adding 60ppm chlorine for 24 hours. Afterward, the water was aerated and neutralized using 30ppm sodium thiosulfate **(Jannah *et al.*, 2019)**.

Production of liquid organic fertilizer (OLF)

Organic waste materials—rice straw, water hyacinth (*Eichhornia crassipes*), and tilapia (*Oreochromis niloticus*) entrails—were used for OLF preparation. The rice straw and water hyacinth were dried under indirect sunlight for 4–5 days, while the fish entrails were used fresh **(Kawet *et al.*, 2023)**.

A probiotic solution was activated by mixing 50mL molasses, 50mL probiotic, and 1L distilled water, and allowed to ferment for 24 hours **(Kartika *et al.*, 2021)**. To produce the fertilizer, 500g of rice straw, 500g of water hyacinth, and 1kg of tilapia entrails were added to a fermentation container. Then, 1L of molasses, 100mL of activated probiotic, and 10L of distilled water were added. The mixture was thoroughly stirred, covered with a

plastic trash bag and sealed with black duct tape, and fermented for 14 days under ambient conditions **(Kawet *et al.*, 2023)**.

Production of *Chaetoceros calcitrans* control fertilizer (Technical fertilizer)

The control treatment used a technical fertilizer composed of KNO_3, NaH_2PO_4, $FeCl_3$, Na_2SiO_4, EDTA, Vitamin B_{12}, and Na_2SiO_3. These ingredients were weighed, dissolved in 1L of distilled water using a magnetic stirrer on a hot plate, and then sterilized using an autoclave at 121°C for 15 minutes to eliminate microbial contamination **(Prafanda *et al.*, 2020)**.

***Chaetoceros calcitrans* cultivation**

The experiment consisted of five treatments, each with three replications. The treatments included one control (1mL/ L technical fertilizer) and four concentrations of liquid organic fertilizer: 6, 8, 10, and 12mL/ L.

Culture containers were 1L glass bottles (15 units total), each filled with 800mL of sterile seawater (30ppt). An aeration system was installed for continuous air supply. Each bottle was inoculated with *C. calcitrans* intermediate culture at an initial density of 1×10^4 cells/mL. The cultures were monitored daily for growth and potential contamination.

Harvesting was conducted by filtering the culture through Whatman filter paper moistened with distilled water to prevent cell adhesion. Filtration was assisted using a suction pump until the filter was blocked **(Dewi *et al.*, 2023)**.

Cell density measurement

Cell density was measured daily using a light microscope at 10× magnification, a haemocytometer, and a hand counter (tally). Calculations followed the method described by **Satyantini and Masithah (2008)** using the formula for the large square ("big block") on the haemocytometer:

$$\text{Cell Density (cells/mL)} = \frac{sum\ of\ block\ A\ cells + sum\ of\ block\ B\ cells + sum\ of\ block\ C\ cells + sum\ of\ block\ D\ cells}{4}$$

Specific growth rate analysis

The specific growth rate of *C. calcitrans* was calculated by the formula used in the study of **Becker (1994)**:

$$\mu = \frac{\text{In Nt} - \text{In No}}{Tt - T0}\ x\ 100\%$$

Where,

μ = Specific growth rate of microalgae (%/day)

N0 = Cell density on day 0 (cells/mL)

Nt = Cell density on day t (cells/mL)

T = Time (days) from N0 to Nt

Protein content measurement of *C. calcitrans*

Protein content in *Chaetoceros calcitrans* was determined using the semi-micro Kjeldahl method. A 0.2-gram dried sample was placed into a Kjeldahl digestion flask along with a catalyst mixture of sodium sulfate (Na_2SO_4) and mercuric oxide (HgO), followed by the addition of concentrated sulfuric acid (H_2SO_4). The mixture was heated for 2.5 hours to digest the sample and convert nitrogen-containing compounds into ammonium sulfate. Following digestion, ammonia was separated through distillation and collected in a receiving solution. The distillate was then titrated with 0.2 N hydrochloric acid (HCl) until a persistent pink endpoint was observed. The volume of HCl used was recorded to determine the nitrogen content of the sample.

The nitrogen concentration was then converted to protein content using the Suda formula, as follows

$$\% \text{ N} = \frac{\text{mL HCl x N HCl x } 14{,}008}{Material\ Weight}\ x\ 100\%$$

$$\% \text{ Protein Content} = \%\text{N x Conversion factor}$$

Fucoxanthin content measurement of *C. calcitrans*

The fucoxanthin content in *Chaetoceros calcitrans* was extracted using 90% acetone under dark conditions to prevent pigment degradation. The extracted solution was filtered and then centrifuged at 4000rpm for 30 minutes. The resulting supernatant, containing the fucoxanthin pigment, was used for spectrophotometric analysis.

Quantification of fucoxanthin was performed by measuring the absorbance of the supernatant. A standard curve was prepared using known concentrations of fucoxanthin (1, 2, 4, 6, 8, and 10ppm) dissolved in methanol, with absorbance measured at a wavelength of 480nm. Sample absorbance was measured at 420nm using methanol p.a. as the blank. The fucoxanthin concentration in the samples was then calculated using the linear regression equation derived from the standard curve **(Nuraini *et al.*, 2021)**.

The absorbance values of the fucoxanthin standards used to construct the calibration curve are presented in Table (1).

Table 1. Fucoxanthin standardized result

Fucoxanthin (ppm)	Absorbance
1	0,056
2	0,098
4	0,142
6	0,193
8	0,239
10	0,301

A standard curve was constructed to determine the fucoxanthin content in *C. calcitrans* by plotting its concentration against absorbance using linear regression (y = ax ± b), as shown in Fig. (1).

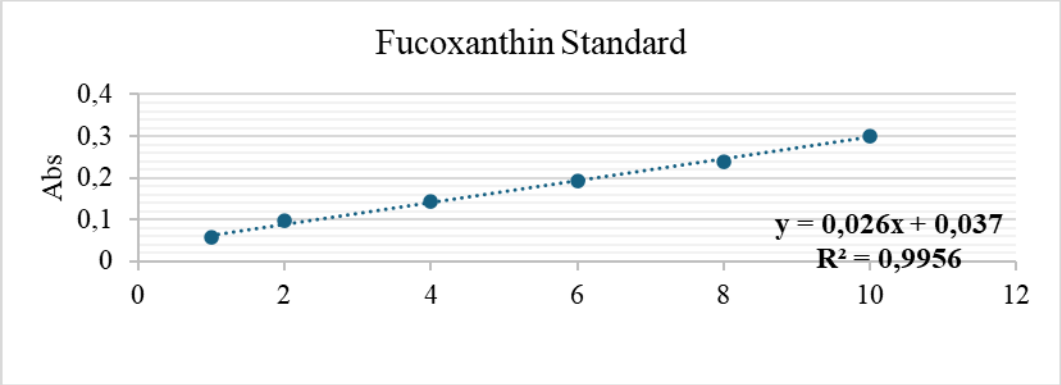

Fig. 1. Standard linear regression of fucoxanthin

Supporting parameters

Water quality parameters measured during the study included temperature, pH, and salinity. Temperature was measured using a mercury thermometer, pH was determined using a digital pH meter, and salinity was measured with a refractometer. These parameters were monitored regularly to ensure optimal growth conditions for *Chaetoceros calcitrans*.

Data analysis

The hypothesis testing for specific growth rate was conducted using analysis of variance (ANOVA). If significant differences were found, further comparison was carried out using Duncan's Multiple Range Test (DMRT) at a 95% confidence level.

Protein and fucoxanthin contents were analyzed quantitatively based on standard methods described previously. Water quality data were evaluated using a comparative descriptive approach, referencing values from relevant literature to assess suitability for *C. calcitrans* cultivation.

RESULTS

1. Analysis results of N, P, and Si in control fertilizer and liquid organic fertilizer

The results of the analysis of N, P, and Si in the control fertilizer (*C. calcitrans* technical fertilizer) and liquid organic fertilizer in each treatment can be seen in Table (2).

Table 2. Concentration of N, P, and Si in control and liquid organic fertilizer

No.	Parameter	Concentration of N, P, and Si in Fertilizer (mg/L)				
		P0	**P1**	**P2**	**P3**	**P4**
1	Nitrogen (N)	13,86	9,54	12,72	15,9	19,08
2	Phosphorus (P)	2,58	0,39	0,52	0,65	0,78
3	Silcate (Si)	9,19	27,6	36,8	46	55,2

Description: P0 = 1 ml/L *C. calcitrans* technical fertilizer as control, P1 = 6 ml/L Liquid organic fertilizer, P2 = 8 ml/L Liquid organic fertilizer, P3 = 10 ml/L Liquid organic fertilizer, P4 = 12 ml/L Liquid organic fertilizer.

2. Cell density of *C. calcitrans*

Based on the results of this study, the cell density of *C. calcitrans* (Table 3) in treatment P0 was 99.66 x 10^4 cells/mL, P1 was 110.41 x 10^4 cells/mL, P2 was 128 x 104 cells/mL, P3 was 146 x 10^4 cells/mL, and P4 was 190 x 10^4 cells/mL.

The results showed that the cell density of *C. calcitrans* with the addition of liquid organic fertilizer varied with each dose. The peak cell density for P1, P2, P3, and P4 occurred on day 6, while P0 reached its peak on day 7. ANOVA showed that liquid organic fertilizer had a significant effect on cell density (P<0.05). DMRT test confirmed the significant difference between treatments (P<0.05). The highest cell density was achieved by P4 with 190.08 x 10^4 cells/mL.

Table 3. Cell density of *C. calcitrans* (x 10^4 cells/mL)

Day-	Cell Density (x 10^4 sel/mL)				
	P0	**P1**	**P2**	**P3**	**P4**
0	1,00±0,00	1,00±0,00	1,00±0,00	1,00±0,00	1,00±0,00
1	7,66±0,52^{a}	8,5±0,75^{b}	9,5±0,25^{c}	9,5±0,25^{c}	9,08±0,14^{bc}
2	9,67±0,52^{a}	14,3±2,26^{b}	18,25±0,50^{c}	24,5±0,66^{d}	24,91±0,38^{d}
3	16,58±0,72^{a}	21,33±0,52^{b}	22±0,43^{b}	26,41±0,38^{c}	32±0,75^{d}
4	24,33±0,52^{a}	44,16±0,52^{b}	45,33±0,38^{c}	46,66±0,38^{d}	54,6±0,62^{e}
5	45,91±0,76^{a}	74,58±0,52^{b}	76,66±0,38^{c}	83,41±0,52^{d}	100,25±0,66^{e}
6	64,41±0,38^{a}	**110,41±0,52^{b}**	**128±0,25^{c}**	**146±0,80^{d}**	**190,08±1,04^{e}**
7	**99,66±0,62^{d}**	74,58±0,62^{a}	78,41±0,38^{b}	83,83±0,62^{c}	111,58±0,62^{e}
8	70,25±0,66^{d}	56,91±0,38^{a}	58,75±0,50^{b}	62,5±0,66^{c}	91,5±0,52^{e}

Notes:

Different superscripts in each row indicate significant differences (P<0.05)

P0 = Application of *C. calcitrans* Technical Fertilizer as much as 1 ml/L as control, P1 = Application of liquid organic fertilizer as much as 6 ml/L, P2 = Application of liquid organic fertilizer as much as 8 ml/L, P3 = Application of liquid organic fertilizer as much as 10 ml/L, P4 = Application of liquid organic fertilizer as much as 12 ml/L.

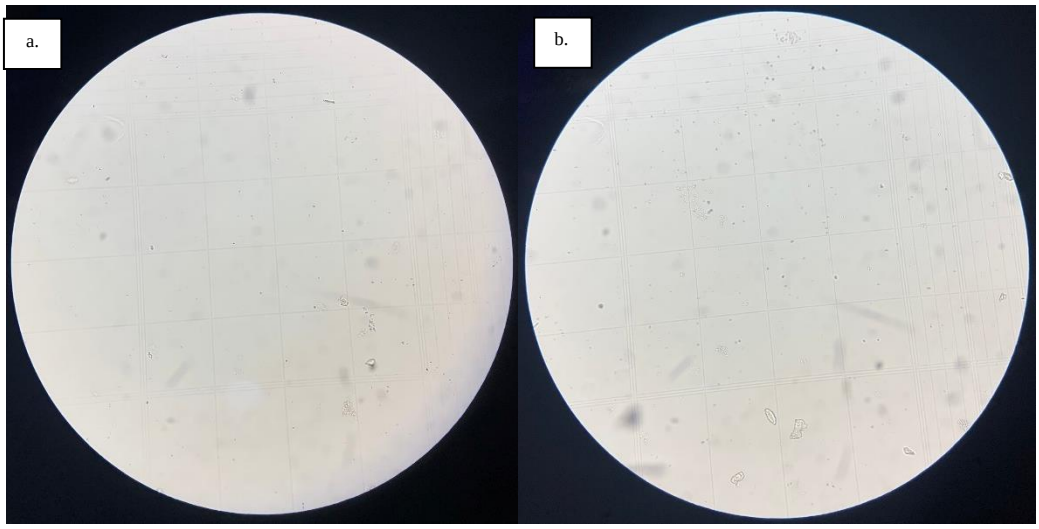

Fig. 1. Comparison of *Chaetoceros calcitrans* density on day 3 observed under a microscope at 100x magnification. a. Control treatment; b. P4 treatment (addition of 12 ml/L Liquid organic fertilizer)

3. Specific growth rate

The average specific growth rate of *C. calcitrans* (Table 4) for 8 days with different treatments were: P0: 0.6574%/day, P1: 0.7840%/day, P2: 0.8087%/day, and P4: 0.8746%/day. ANOVA analysis showed that the application of liquid organic fertilizer with different doses had a significant effect on the specific growth rate of *C. calcitrans* ($P < 0.05$). Duncan's test confirmed the significant differences between treatments.

Table 4. Specific growth rate of *C. calcitrans* (%/Day)

Specific Growth Rate (%/Day)				
P0 (1 mL/L)	**P1 (6mL/L)**	**P2(8 ml/L)**	**P3 (10 mL/L)**	**P4 (12 mL/L)**
0,6574±0,0009[a]	0,7840±0,0007[b]	0,8087±0,0003[c]	0,8310±0,0009[d]	0,8746±0,0009[e]

Notes: Letter notations (a, b, c, d, and e) indicate that the treatment of liquid organic fertilizer provides a significant difference in the specific growth rate of *C. calcitrans* ($P< 0.05$).

Duncan's test results showed that the specific growth rate of *C. calcitrans* was significantly different between treatments ($P<0.05$). Treatment P4 showed the highest specific growth rate, followed by P3, P2, P1, and P0 respectively.

4. Protein content of *C. calcitrans*

The results of protein content analysis are presented in tabular form and explained descriptively. Data on the protein content of *C. calcitrans* can be seen in Table (5).

Table 5. Results of protein content analysis of *C. calcitrans*

Protein Content of *C. calcitrans*	
Treatment	**Protein (%)**
P0	33,54
P1	33,71
P2	33,95

P3	34,37
P4	34,79

The results of the protein content analysis showed the value of protein content in the P0 treatment of 33.54%, P1 of 33.71%, P2 of 33.95%, P3 of 34.37%, while the P4 treatment had a protein content of 34.79%.

5. Fucoxanthin content of *C. calcitrans*

The results of the analysis of fucoxanthin content are then presented in tabular form and described descriptively. Data on the fucoxanthin content of *C. calcitrans* can be seen in Table (6).

Table 6. Analysis result of fucoxanthin content of *C. calcitrans*

Fucoxanthin content of *C. calcitrans*		
Treatment	**Absorbance Result**	**Fucoxanthin (ppm)**
P0	0,075	1,462
P1	0,194	6,062
P2	0,219	7,036
P3	0,229	7,412
P4	0,253	8,308

The analysis results showed the fucoxanthin content in *C. calcitrans* as follows: P0 at 1.462 ppm, P1 at 6.062 ppm, P2 at 7.036 ppm, P3 at 7.412 ppm, and P4 at 8.308 ppm.

6. Water quality parameters

During the 8 days of *C. calcitrans* culture, water quality (temperature, pH, and salinity) showed minimal changes. Water quality parameter data for each treatment are presented in Table (7).

Parameter	**Treatment**					**Based on Literature (Isnansetyo and Kurniastuty, 1995).**
	P0	**P1**	**P2**	**P3**	**P4**	
Temperature (°C)	26-27,5	26-27,5	26-27,5	26-27,5	26-27,5	20-30
pH	7,21-8,19	6,5-7,2	7,13-8,3	7,09-8	6,5-7,2	6,5-8,5
Salinity (ppt)	30-31	30-31	30-31	30-31	30-31	17-35

During the 8 days of observation, water temperature ranged from 26 to 27.5°C, pH between 6.5 to 8.3, and salinity of the culture medium between 30 to 31. Although these values varied, they were all within the optimum range **(Isnansetyo & Kurniastuty, 1995)**.

DISCUSSION

Chaetoceros calcitrans is one type of diatom microalgae that is used as natural food in aquaculture. The density of *C. calcitrans* is very important because it is used as a reference in monitoring microalgae growth **(Nisa *et al.*, 2022)**. In general, *C. calcitrans* has a growth period of 6 to 10 days, which consists of the adaptation phase (lag), exponential phase, stationary phase, and death phase **(Prasetyo *et al.*, 2022)**. In the adaptation phase (lag), the density of *C. calcitrans* is relatively low because it is adapting to its environment. **Isrokhatun *et al.* (2017)** explained that the lag phase is characterized by low cell density, in line with the low cell growth rate. The adaptation phase is influenced by nutrient composition, temperature, and culture environmental conditions **(Johan *et al.*, 2020)**. In this study, the lag phase occurred from day 0 to day 3 in all treatments.

The exponential phase in this study occurred on day 4 and reached the peak point of the exponential phase on day 6 in treatments P1, P2, P3, and P4, while P0 experienced the exponential phase from day 4 and reached the peak point of the exponential phase on day 7. Based on the results, the treatment with the highest cell density of *C. calcitrans* was P4 (190.08×10^4 cells/mL), followed by P3 (146×10^4 cells/mL), P2 (128×10^4 cells/mL), P1 (128×10^4 cells/mL), and P0 (99.66×10^4 cells/mL). In this study, the P4 treatment achieved the highest density which exceeded the results of previous research by **Lestari (2019)**, which recorded the density of *C. calcitrans* at the peak of the exponential phase of 111×10^4 cells/mL.

During this exponential phase, *C. calcitrans* has adapted to its environment, resulting in faster nutrient absorption. This phase is characterized by cell division and population increase **(Prasetyo *et al.*, 2022)**. According to **Tewal *et al.* (2021)**, the exponential phase is influenced by light, temperature, and nutrients contained in the culture medium.

Nutrients such as nitrogen, phosphorus, and silicate generally affect the growth of *C. calcitrans*. To determine the relationship between nutrients and cell density, a regression correlation test was conducted. In the regression correlation test, the relationship coefficient (r) is in the range $-1 \leq r \leq +1$. If $r = -1$, the correlation is negative; $r = 0$, there is no correlation; and $r = 1$, the correlation is positive **(Rahmawati *et al.*, 2014)**. Regression correlation analysis showed that the correlation between nitrogen and *C. calcitrans* density was $r = 0.6646$ and $R^2 = 0.4417$. This means that the effect of nitrogen on growth rate is 44.17%. Thus, it can be concluded that the concentration of nitrogen in fertilizer is moderately positively correlated with the cell density of *C. calcitrans*.

In phosphorus, the regression correlation showed a value of $r = 0.6570$ and $R^2 = 0.4317$. This means that the effect of phosphorus on growth rate was 43.17%. Thus, it can be concluded that the concentration of phosphorus in fertilizer is moderately negatively correlated with the cell density of *C. calcitrans*. Meanwhile, the correlation between silicate showed a value of $r = 0.9858$ and $R^2 = 0.9719$. This means that the effect of silicate

on growth rate is 97.19%. Thus, it can be concluded that the silicate concentration in fertilizer is moderately negatively correlated with the cell density of *C. calcitrans*.

The ratio of total nitrogen and total phosphorus can affect the density of *C. calcitrans*. P4, as the treatment with the highest cell density value, had an N content of 19.08mg/ L and P of 0.624mg/ L, indicating an N:P ratio of 30:1. This ratio is within the optimal range for the growth of *C. calcitrans*, in accordance with the research of **Nguyen *et al.* (2020)**, which states that the optimal condition for the N:P ratio of *C. calcitrans* ranges from 4:1 to 44:1. Further explained by **Putri *et al.* (2014)**, if the N:P ratio is more than 12:1, phosphorus can be a limiting factor in microalgae density.

Based on the results of the regression correlation test analysis in this study, silicate is the nutrient that most affects the cell density of *C. calcitrans*. This is in line with research conducted by **Umiatun *et al.* (2017)**, which indicates that silicate is positively correlated with diatom density. This is thought to be because silicate is the main nutrient needed by diatoms for growth and cell wall formation. Silicate has a small particle size and is easily soluble in water, so it is easily absorbed by diatom cells **(Nasuki *et al.*, 2022)**.

Although treatment P4 was harvested at the peak of the exponential phase on day 6, the cell number was still higher than the cell density of P0, which reached its peak population on day 7. This is thought to be due to the different nutrient availability in each treatment for the diatom growth process. In the exponential phase, when nutrient availability is still sufficient, microalgae cells will develop to reach the maximum point of population growth. But when nutrient availability begins to decrease, microalgae cells can no longer maintain the same growth rate because protein synthesis and cell division require certain nutrients such as nitrogen. This aligns with the statement of **Dwirejeki and Ermavitalini (2019)**, which explains that if the nitrogen element is fulfilled, microalgae cells would have a high rate of reproduction or self-division so that the cell population formed also increases.

The high nitrogen content in liquid organic fertilizer is due to the long fermentation, so there is time for the decomposition process of organic waste. This is in line with research conducted by **Wardah *et al.* (2021)**, which explains that the longer the fermentation time of liquid organic fertilizer, the more the nitrogen content will increase. This is suspected to be caused by an increase in the effectiveness of the decomposition process by decomposing microorganisms contained in the probiotic, which progresses with fermentation time. The bacteria in the probiotic are able to break down complex nutrients such as protein into simpler elements, thereby increasing the growth rate of *C. calcitrans*. This is supported by **Anwar and Nurlina (2019)**, who stated that probiotics contain bacteria that produce decomposing enzymes, namely amylase, protease, lipase, and cellulose enzymes. These enzymes will break down nutrient complex molecules into simpler molecules that are easily absorbed by microalgae.

After passing the exponential phase, a decrease in cell density occurs, known as the death phase. In this study, the death phase occurred on day 7 for P1, P2, P3, and P4, while P0 experienced the death phase on day 8. This was due to the limited nutrient content in

the culture medium. **Nisa *et al.*, (2022)** added that the nutrients present at the beginning of the culture are utilized by the cells for growth until they reach the peak of the exponential phase. Growth then ceases as nutrient demand increases, but without additional nutrients supplied during the culture period, a rapid decline in cell numbers occurs.

Specific growth rate is one of the parameters that describes the growth rate of *C. calcitrans* per unit of time. **Anggraeni and Abdulgani (2013)** further explained that specific growth rate represents the daily growth rate of *C. calcitrans* cell density. The highest specific growth rate of *C. calcitrans* was observed in treatment P4 (0.87%/day), followed by P3 (0.83%/day), P2 (0.8%/day), P1 (0.78%/day), and P0 (0.65%/day), which had the lowest growth rate compared to other treatments. Compared to a previous study by **Sas *et al.* (2023)**, which recorded a growth rate of 0.26%/day, the results in this study were still higher due to the application of different doses of liquid organic fertilizer. This was suspected to be because the technical fertilizer used as the control for *C. calcitrans* contained a lower silicate concentration of 9.19mg/ L compared to P4, which contained 55.2mg/ L of silicate. Silicate is an essential component in the life of diatom microalgae, as diatoms utilize large amounts of silicate for cell wall formation **(Lestari *et al.*, 2019)**. Another factor suspected to influence the specific growth rate of diatoms is their ability to absorb silicate—optimal silicate absorption in diatoms leads to better cell formation **(Jati, 2012)**.

With an N:P ratio of approximately 30:1, the protein content analysis of *C. calcitrans* in this study showed that the protein content in P0 was 33.54%, in P1 was 33.71%, in P2 was 33.95%, in P3 was 34.37%, while P4 had the highest protein content at 34.79%. This indicates that the treatment applied to the diatoms in P4 successfully influenced protein production. The protein content in microalgae can be affected by nutrient conditions in the culture medium, particularly nitrogen availability **(Ulya *et al.*, 2018)**. Meanwhile, a study conducted by **Rasdi and Qin (2014)** also showed that the protein content in *Tisochrysis lutea* and *Nannochloropsis oculata* was higher at N:P ratios of 20:1 and 30:1. This highlights the importance of the nitrogen-to-phosphorus ratio in regulating protein production in microalgae, demonstrating that protein content can be influenced by nutrient conditions in the culture medium, especially nitrogen availability.

Trikuti *et al.* (2016) added that the addition of $FeCl_3$ in *C. calcitrans* technical fertilizer as a control fertilizer in P0 may be a contributing factor, since $FeCl_3$ has the ability to reduce nitrate to nitrite and then reduce nitrite to ammonium. Ammonium is an important nitrogen source for phytoplankton growth. However, it is important to note that the $FeCl_3$ added to the fertilizer remains in molecular form, making it difficult for microalgae to absorb. This may affect its effectiveness in increasing nitrogen availability for protein formation.

Chaetoceros calcitrans is a diatom that contains the pigment fucoxanthin, which gives it a reddish-brown color. Based on analysis, the fucoxanthin content in P0 was 1.462 ppm, in P1 was 6.062 ppm, in P2 was 7.036 ppm, in P3 was 7.412 ppm, while in P4, the

fucoxanthin concentration reached 8.308 ppm. The fucoxanthin content in *C. calcitrans* from P4 was relatively high compared to a study by **Tokushima *et al.* (2016)**, which obtained a fucoxanthin content of 1.71 ppm from *Chaetoceros* cultured for 8 days.

The difference in fucoxanthin content is suspected to be related to several factors, one of which is environmental conditions that support the production and accumulation of this pigment, such as adequate nutrient availability **(Rahmawaty *et al.*, 2014)**. Furthermore, **Khaw *et al.* (2022)** explained that nitrogen, phosphorus, and silicate are key nutrients in regulating fucoxanthin production in microalgae. The optimal availability of each of these nutrients is required to support the growth and maximum accumulation of fucoxanthin in microalgae cells. Nitrogen is needed for microalgae protein synthesis and plays a role in stimulating growth and fucoxanthin accumulation. Phosphorus is essential for fucoxanthin production, while silicate is a crucial factor for diatoms in forming the outer shell of *C. calcitrans* and accumulating fucoxanthin.

In this study, the observed water quality parameters included temperature, pH, and salinity. During the 8-day cultivation period, temperature measurements ranged from 26°C to 27.5°C. These results are still within the optimal temperature range for *C. calcitrans* cultivation, as reported by **Isnansetyo and Kurniastuty (1995)**, which is between 20°C and 30°C. Temperature is one of the water parameters that can affect the metabolic activity of microalgae. When the culture medium temperature is below the optimal range, the specific growth rate of *C. calcitrans* will decrease, and exceeding the upper temperature limit will cause mortality in *C. calcitrans*.

Akiyoshi *et al.* (2005) further explained that temperature increases in the culture medium can affect diatoms in terms of photosynthesis, as temperature influences enzymatic reaction rates, including metabolism and photosynthesis in microalgae. This is supported by **Lupitasari *et al.* (2020)**, who stated that increased temperature results in higher energy or ATP production, making the photosynthesis process easier.

The pH measurements during the 8-day cultivation period ranged from 6.5 to 8.3. These values are still within the acceptable range, as stated by **Isnansetyo and Kurniastuty (1995)**, which is between 6.5 and 8.5. A study by **Astrini *et al.* (2014)** emphasized that pH significantly influences biochemical processes in water, affecting the solubility of chemical compounds, enzymatic activity, and microalgae growth.

Based on this study, P4 had the lowest pH range among the treatments. This is suspected to be due to the higher dosage of organic liquid fertilizer added to P4 compared to the other treatments. This is supported by **Ariany *et al.* (2021)**, who stated that the low pH level in organic liquid fertilizer (OLF) is due to the fermentation process during fertilizer production. During fermentation, dissolved CO_2 gas, which is acidic (H_2CO_3), is produced.

According to **Herve *et al.* (2012)**, intracellular pH regulation also plays a role in the silica metabolism process, which is susceptible to changes in external pH. Intracellular pH contributes to silica metabolism because maintaining an appropriate pH within the cell is necessary to balance ions and catalyze the chemical reactions required for silica deposition

in diatom cells. Disruptions in intracellular pH can interfere with the enzymatic activity involved in silica synthesis and deposition, as well as disturb the regulation of silica transport within the cell. Therefore, intracellular pH regulation is crucial to ensuring the smooth biochemical processes required for efficient silica metabolism in diatoms.

Salinity is one of the aquatic parameters that directly and indirectly affects the growth and activity of microalgae. The salinity measurements in this study, conducted using a refractometer, yielded values ranging from 30 ppt to 31 ppt. According to **Isnansetyo and Kurniastuty (1995)**, these values fall within the optimum range for the survival of *C. calcitrans*.

Microalgae, including *C. calcitrans*, exhibit different adaptability to salinity. They can be classified as halophilic or halotolerant, indicating varying tolerance levels to salinity fluctuations in their environment. In a study conducted by **Adenan *et al.* (2013)**, *C. calcitrans* cultures showed a progressive increase from the early cultivation period, reaching maximum density and biomass. The physiology of diatoms, such as *C. calcitrans*, can be directly or indirectly influenced by interactions with other growth factors, such as ion composition in saline systems. For example, low salinity can lead to a reduction in cell size, which may affect the growth and productivity of these microalgae.

CONCLUSION

Based on the results of the study, it can be concluded that the use of liquid organic fertilizer derived from rice straw waste, water hyacinth, and tilapia offal has a significant effect on the specific growth rate, protein content, and fucoxanthin in *C. calcitrans* ($P<0.05$). In addition, the optimum dose of liquid organic fertilizer to support specific growth, protein increase, and fucoxanthin in *C. calcitrans* was 12ml/ L.

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