Original Research Article

EFFECTIVENESS OF UREA FERTILIZER WITH DIFFERENTCONCENTRATIONS ON *T.chuii*CULTURE ON LABORATORY CONDITIONS

Abstract

T.chuii is a species of phytoplankton that has a high nutritional content, namely 70,287% protein, 0,795% lipid and 2.180% carbohydrate. The objective of this study was to determine the best concentration of urea fertilizer on the density of *T.chuii* cells on a laboratory scale. The research method used in this research was Completely Randomized Design (CRD) consisting of ^{4} treatments with ^{4} replications. The treatments in this study consisted of treatment A, namely KW21 fertilizer 1 ml/L (control), treatments B, C and D using Urea fertilizer 30 mg/L, ^{4}5 mg/L, 60 mg/L with a concentration of ZA ^{4}0 mg/L and TSP 5 mg/L. the observations were conducted for 2^{4} hours. Parameters observed included population density of *T.chuii*, growth rate, generation time and air quality.The findings of the study showed that treatment D gave the best results with a population density of 232,75 x 10^{4} cells/ml, a growth rate of 0.31 cells/ml/day and the fastest generation time of 15,41 hours.

Keywords :*T.chuii*, Urea Fertilizer, Population Density, Growth Rate, Generation Time

INTRODUCTION

The use of natural feed brings about several advantages, particularly in farming operations. One of these advantages is its economical pricing (Raharjo *et al.* 2016), this feed is appropriate for fish with different mouth structures and offers a comprehensive nutritional profile to satisfy the organism's nutritional demands(Rihi 2019). A wide variety of natural foods, such as plankton, specifically zooplankton and phytoplankton are essential for meeting the nutritional requirements of larvae throughout their life cycle (Arif 2014).

Phytoplankton or plant plankton are microscopic organisms in the form of plants because they can photosynthesize to produce organic or autotrophic materials, their life floats and drifts following the movement of water(Nontji 2008). Microalgae that have high nutritional content and are commonly found in Indonesia are *Chlorella vulgaris*, *Chaetoceroscalcitrans*, *Nannochloropsisoculata* and *T.chuii*(Suminto dan Susilowati 2017).

T.chuii is a species of phytoplankton that has a high nutritional content, namely 70.287% protein, 0.795% lipid and 2.180% carbohydrates(Hermawan *et al.* 2017). *T.chuii*possesses delicate cell walls and self-digesting properties, making it highly digestible

for larvae(Costa *et al*. 2004) it has a relatively small body size so that it fits the mouth opening of fish and shrimp larvae (Harun *et al.* 2010).

T.chuii necessitates nutrients for its growth, which are typically derived from inorganic fertilizers. These fertilizers, produced by industrial processes, encompass various types of inorganic chemicals, including urea, ZA, TSP, and specialized formulations such as KW21 fertilizers(Amini dan Syamsidi 2006).

According toMukhlis *et al.*(2017)KW21 fertilizer is a professional-grade fertilizer that is presently utilized in laboratory-scale cultures of T. *chuii*. This fertilizer is in liquid form, facilitating the easy absorption of its nutrients. The application method involves direct administration, which guarantees both safety and convenience for users.Furthermore, KW21 makes the growth of T. *chuii* stable because it has complete content with 49 g/L nitrogen, 4 g/L phosphoric acid, boron, manganese, iron, zinc, cobalt, EDTA, complex amino acids and a mixture of vitamins (B1, B12, biotin and others) which are quite reliable on a laboratory scale. Furthermore, KW21 is anticipated to sustain its peak density for an extended period following the development of T. *chuii*. Nevertheless, the high cost of KW21 and the challenges associated with its procurement, as it must be imported from Japan, necessitate careful consideration of its usage. Consequently, there is a need for research into alternative fertilizers that can either replicate the effects of KW21 or achieve a density level that is comparable.

Nitrogen is the primary compound essential for phytoplankton fertilization. Urea fertilizer, commonly utilized by farmers to promote plant growth and development in agricultural practices, is one of the sources of nitrogen for this purpose (Ramadhani *et al.* 2016). Urea fertilizer is a synthetic fertilizer used to promote the growth of phytoplankton, a chlorophyll-producing organism that enhances the green color of cells and serves as a building block for proteins and fats(Fery *et al.* 2020). The cost of the fertilizer is lower and it is readily accessible in the market. The objective of this research is to establish the optimal concentration of urea fertilizer for the density of *T.chuii* cells in a laboratory setting.

MATERIAL AND METHODS

The tools and materials used in the study include 500 ml glass bottles, blowers, TL lamps, dropper pipettes, microscopes, hand counters, litmus paper, thermometers, digital scales, refractometers, lux meters, label paper, stationery, cameras, aluminum foil, measuring pipettes, petri dishes, 15 L jars, Haemacytometers, cover glasses, aeration stones, aeration hoses, funnels, cotton, magnetic stirrers, 500 ml Erlenmeyer flasks, autoclaves, *T.*chuii

inoculum, 24well plates, urea fertilizers, ZA, TSP, KW21, 70% alcohol, 25-32 ppt sea water, chlorine, potassium permanganate, sodium thiosulfate and chlorine testers..

This study consisted of 4 treatments with 4 repetitions. The number of bottles used in the study was 16 bottles. The treatments tested were the use of KW21 fertilizer 1 ml (treatment A) as a control, urea fertilizer 30 mg/L (treatment B), 45 mg/L (treatment C), 60 mg/L (treatment D) and each treatment using urea fertilizer was given ZA fertilizer with a concentration of 40 mg/L and TSP fertilizer 5 mg/L. The fertilizer was crushed using a pestle and mortar.

Data analysis was conducted to determine the effect of treatment on the response of test parameters. Population density data, growth rate and generation time were analyzed using variance analysis or F test with a 95% confidence level. If there is a significant difference, the test is continued with Duncan's multiple range test, while water quality is analyzed descriptively.

Preparation of culture medium

Seawater media is collected in a 15 L container, given 60 ppm chlorine and left for 24 hours with strong aeration to make it homogeneous. If after 24 hours it still contains chlorine (known from the smell of chlorine and the results of the chlorine test), seawater is given 30 ppm sodium thiosulfate for 24 hours. Then put into each culture bottle as much as 500 ml and sterilized using an autoclave together with a solution of urea, ZA and TSP fertilizer media for 20 minutes at a temperature of 121^{o}C at a pressure of 1 atm.

***T.chuii* culture**

The initial inoculum density of *T.chuii* used was 5 x 105 cells/mL. The inoculum was inserted into a culture bottle containing sterile seawater and fertilizer according to the treatment. Cell density calculations using a hemocytometer under a microscope were carried out every day for 6 days. The initial density calculation used the formula (Ruliaty *et al.* 2019)as follows :

$$V_1 \times N_1 = V_2 \times N_2$$

Notes :

V_1 = Desired volume of *Tertraselmis*(ml)

N_1 = Amount of *Tertraselmis* per ml inoculated (sel/ml)

V_2 =Volume of Kultur Medium Water (ml/L)

N_2 = The desired amount of *T.* per ml (sel/ml)

Cell density

The population density calculation is calculated using the following formula based on the theory from (Isnansetyo & Kurniastuty 1995) as follows:

$$P = N \times 10^4$$

Notes :

P = Cell density

N = Average number of cells

Growth rate

The growth rate of phytoplankton is calculated using the formula according to (Fogg 1987) in (Hermawan *et al.* 2017)as follows:

$$k = \frac{lnWt - lnWo}{T}$$

Notes :

k = Growth rate (cell/ml/day)

Wt = Number of cells t after(cell/ml)

Wo = Total start cell(cell/ml)

T = Culture time from Wo to Wt(day)

Generation time

Phytoplankton generation time is calculated using the formula according to (Isnansetyo & Kurniastuty 1995):

$$G = \frac{T}{3{,}3(logWt - logWo)}$$

Keterangan :

G = Generation time (hour)

Wt = Number of cells after time t (cell/ml)

Wo = Initial cell number (cell/ml)

T = Cultivation time from Wo to Wt(hour)

RESULT AND DISCUSSION

***T.chuii*Cell Density**

The adaptation phase of all treatments of this study occurred within 1 day. This was influenced by the environment of the previous T. *chuii* seedlings with the kultur process such as age, species, medium and kultur location still the same so that they had adapted. It is in line with the explanation from Pujiono (2013)that various factors influencing the duration of the adaptation phase encompass age and cell type. The time required for adaptation will be

extended in cases where the culture medium is deficient in nutrients, as cells need to synthesize enzymes that are compatible with the available nutrients.

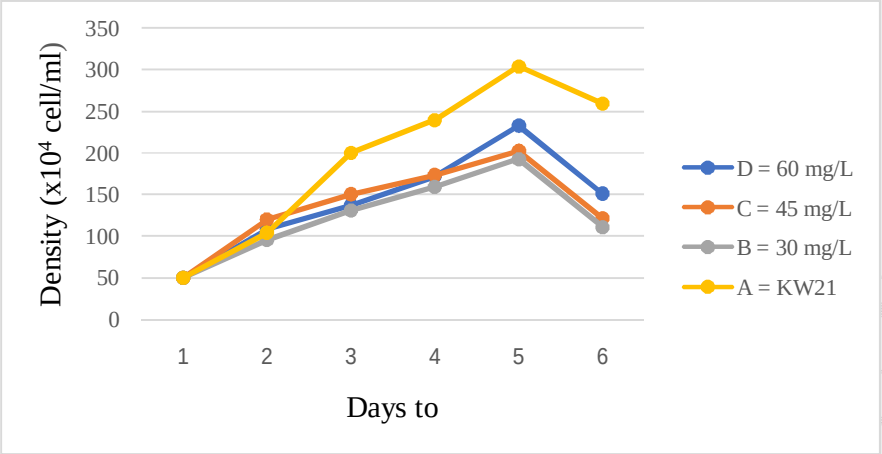

Fig 1. Cell Density of *T. chuii*

The exponential phase in this study is the increase in growth rate from various different concentrations. The average results of *T. chuii* cell density in treatment A reached peak density on the 5th day with a cell count of 303.75 x 10^4 cells/ml, for treatment B it reached peak density with a cell count of 192.5 x 10^4 cells/ml. The growth of the number of *T. chuii* cells in treatments C and D reached peak density with a cell count of 202.25 x 10^4 cells/ml and 232.75 x 10^4 cells/ml.

Treatment A produces the highest cell density because it uses KW21 fertilizer which contains more complete nutritional elements, thus producing the highest population density. It is supported by Regista *et al.*(2017) that the growth of microalgae is affected not only by the presence of essential macronutrients like carbon, nitrogen, and phosphorus, but also by the presence of micronutrients such as iron, manganese, zinc, cobalt, and copper, which must be present in adequate amounts and in accordance with the requirements.

Micronutrients are actually only needed in small amounts, but they play an important role in the growth of *T. chuii.* As stated by Isnansetyo & Kurniastuty (1995)that microalgae require various types of inorganic compounds, both macronutrients (N, P, K, S, Na, Si and Ca) and micronutrients (Cu, Mg, Fe, Mn, Zn, Mo, Co, B and others). Every nutrient in the fertilizer media is actually said to be complete because in the statement from Leksono *et al.*(2017)that Urea and ZA fertilizers are rich in nitrogen, whereas TSP fertilizers are high in phosphate content. These fertilizers are beneficial for phytoplankton culture due to their high solubility in water. It is in line with study conducted by Wirabumi *et al.*(2017) thatphytoplankton directly utilize nutrients dissolved in water bodies to fuel their growth,

leading to an increase in both their population and abundance. According to Kawaroe *et al.* (2010) the phase where the number remains constant or does not increase can be recognized as the stationary phase. Nevertheless, in this study, it is believed that this phase lasts for less than 24 hours, making it impossible to observe the production of *T. chuii.*

The next phase is the death phase which occurs on the sixth day with each treatment having a decreasing population density. Diminished nutrient availability and deteriorating water quality parameters hinder the optimal growth and development of cells, resulting in a decrease in cell density (Prihastama *et al.* 2016).

Table 1. The Effect of Fertilizer Concentration on *T. chuii* Density ($x10^4$ cells/ml) at Peak Population.

Treatment	Time to Reach Peak Population (day)	Maximum Population Density ($x10^4$ cells/ml)
A	5	303,75 ± 17,21 [c]
B	5	192,50 ± 11,73 [a]
C	5	202,25 ± 21,42 [ab]
D	5	232,75± 42,30 [bc]

Note : The mean values followed by the same letter are not significantly different at the 5% level.

Growth Rate of*T.chuii*

Treatment D is an agricultural fertilizer medium that results in the highest daily growth rate of 0.31 cells/ml/day, a rate that is not markedly different from treatment A. This is believed to be due to the fertilizer composition in treatment D meeting the requirements of *T.chuii*, thereby enhancing growth rate and population size. Furthermore, the study indicates that the greater the concentration of urea fertilizer, the higher growth of *T.chuii.*

Treatment B exhibited the lowest growth rate of 0.27 cells/ml/day, indicating a notable difference compared to treatment D as shown in Table 2. This disparity can be attributed to the lower nitrogen content, despite its essential role in chlorophyll formation, a crucial component in the photosynthesis process.According toAmanatin & Nurhidayati (2013)decreasing the nitrogen element concentration hampers the production of chlorophyll, leading to the inhibition of photosynthesis. Consequently, the growth of *T.chuii* is also hindered.

Table 2. Effect of Fertilizer Concentration on Daily Growth Rate of *T.chuii* (cells/ml/day) at Peak Population.

Treatment	Daily Growth Rate (cells/ml/day)
A	$0,36 \pm 0,011^{c}$
B	$0,27 \pm 0,012^{a}$
C	$0,28 \pm 0,020^{ab}$
D	$0,31 \pm 0,034^{bc}$

Note :The mean values followed by the same letter are not significantly different at the 5% level.

Generation Time

The generation time refers to the rate at which phytoplankton divides its cells into two. Once the cell splits into two, each part becomes a cell that will then divide once more, continuing the process. According toBoleng (2015) several growth factors, including nutrient availability, light intensity, pH, oxygen, and genetics, impact the generation time. When these factors are present in optimal levels for cell division in phytoplankton, the population will increase within a specific timeframe.

Based on the calculation results on table 3, treatment D has the fastest generation time, which is 15.41 hours, which is not significantly different from treatment A, which is 14.65 hours. This can occur because the nutrient content in the media can meet the growth needs

and accelerate the division of *T. chuii* cells(Pujiono 2013). Meanwhile, treatment B experienced a slower time, namely a generation time of 15.92 hour.

Table 3. The Effect of Fertilizer Concentration on Generation Time (Hours) of *T. chuii* at Peak Population

Treatment	Generation time (hour)
A	14,65 ± 0,14[a]
B	15,92 ±0,19 [b]
C	15,79 ±0,29 [b]
D	15,41 ±0,47 [a]

Note :Mean values followed by the same letter indicate no significant difference at the 5% level.

Water Quality

The water quality measurements throughout the study exhibited a notable degree of stability, attributed to the intensive nature of the culture process, which facilitated relatively uniform and manageable environmental conditions. The parameters monitored included temperature, salinity, and pH. Daily observations were conducted for each parameter, while light intensity was recorded at the outset of the study, measuring 5530 lux. The temperature of the culture media across the four treatments varied between 26.9°C and 28°C, salinity levels ranged from 29 to 34 ppt, and the pH was maintained at a value of 7.

The nutrient composition and concentration play a crucial role in influencing the growth of phytoplankton. Furthermore, salinity and light intensity can also have an impact on cell density and the rate of growth(Hotos & Avramidou 2021).The results of this research produced an average salinity value ranging between 29-34 ppt. This value is in line with the study from Rostini (2007) that phytoplankton can live in a fairly wide range of salinity values, namely 15-36 ppt with optimal conditions of 25-35 ppt. However, the results of the salinity values of this study have a very large salinity range. Therefore, a two-way ANOVA test was needed because urea fertilizer and salinity were two variable conditions. The results of the two-way ANOVA test show a calculated F value of 4.56> F table 3.287, so H_0 is rejected. The conclusion obtained is that there is a difference in the average salinity based on 4 fertilizer treatments, meaning that fertilizer concentration affects the difference in salinity levels.

The result of study from Noriko *et al.*(2015)showed thatthe density and growth rate of microalgae are influenced by salinity, leading to variations in microalgal population density

based on the salinity levels present in their environment. Acoording to Wardani *et al.*(2022)it is possible for the salinity value to fluctuate during the culture process without causing harm. The increase in salinity can occur as a result of respiration by aquatic organisms, leading to enhanced mineralization and a subsequent rise in salt content within the kultur system.

Organism growth is connected to pH due to its impact on enzyme activity. Moreover, pH can influence the metabolism and growth of microalgae, as well as alter nutrient availability, which can in turn affect the physiology of *T. chuii* cells (Afriza et al. 2015). The research findings show that the average pH value for each treatment was 7. As stated by Pratiwi et al. (2015) thatthe suitable pH for plankton growth is 7-8.5.

According toIndrastuti *et al.*(2014)light plays a crucial role in the cultivation of microalgae, as it is essential for the photosynthetic pigments that generate energy vital for their survival. Insufficient light can hinder the photosynthesis process, leading to suboptimal growth of microalgae. However, there is also another opinion that providing light intensity can be said to be optimal depending on the cultivation objective itself(Gonzalez *et al.* 2021).

This research used 65 watts of white light with a light intensity of 5530 lux. It is in line with theory from Isnansetyo & Kurniastuty (1995)that The optimal value for the growth of *T. chuii* ranges from 5,000-1,0000 lux. According toKusdarwati *et al.*(2011)the intensity of light is crucial, with requirements varying significantly based on the depth and density of the cultivation. Light sources can be natural or artificial, such as lamps. White light is ideal for this process due to its comprehensive range of light components, high intensity, and maximum energy content compared to other colors. The wavelength emitted is measured at 2500 lux. The relationship between light intensity and the process of photosynthesis is very close, but it is important to note that increasing light intensity does not always result in an increase in the process of photosynthesis. This is because each color of light has a different wavelength and varying penetration and absorption capabilities. The growth of microalgae can be supported by light energy to facilitate the process of photosynthesis, but this energy is contingent upon the intensity of light, photoperiod, and the quality of light(Muyassaroh *et al.* 2018).

CONCLUSION

The research findings indicate that the application of treatment D resulted in the highest T. chuii density, specifically 232.75 x 10^4 cells/ml, a growth rate of 0.31 cells/ml/day, and the shortest generation time of 15.41 hours.These findings indicate a significant population density close to that of KW21 fertilizer, with a density of 303.75 x 10^4 cells/ml, a growth rate

of 0.36 cells/ml/day, and a generation time of 14.65 hours. This suggests that, it may be a viable option for reducing production expenseseconomically.

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