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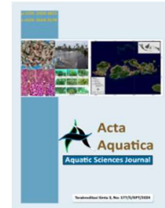

Optimasi produktivitas benur udang vaname (*Litopenaeus vannamei*) melalui pemberian Potassium Nitrat (KNO_3) pada budidaya *Skeletonema* sp

Optimizing the productivity of vaname shrimp fry (*Litopenaeus vannamei*) through giving Potassium Nitrate (KNO_3) to the cultivation of *Skeletonema* sp

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Abstrak

Kultur *Skeletonema* sp. dalam pertumbuhannya sangat dipengaruhi oleh unsur hara di lingkungan tempat tinggalnya, oleh karena itu diperlukan pupuk pada media kultur untuk mendukung ketersediaan unsur hara baik makro maupun mikro. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh pemberian KNO_3 terhadap pertumbuhan dan kepadatan *sel Skeletonema* sp. dan untuk mendapatkan dosis pupuk KNO_3 terbaik. Penelitian ini menggunakan desain acak lengkap (RAL), yang terdiri dari 4 perlakuan dan 3 ulangan. Perlakuan dosis pupuk KNO_3 meliputi A (100 ppm), B (200 ppm), C (300 ppm), dan kontrol K (500 ppm). Parameter yang diukur adalah tingkat pertumbuhan spesifik dan kepadatan sel *Skeletonema* sp. Hasil penelitian menunjukkan bahwa perlakuan terbaik untuk kepadatan dicapai pada perlakuan K 269,5 χ 10^4 sel/mL, diikuti oleh C 300 ppm (203,5 χ 104 sel/mL), B 200 ppm (126,08 χ 10^4 sel/mL), dan A 100 ppm (94,75 χ 10^4 sel/mL). Rata-rata laju pertumbuhan spesifik yang dihasilkan pada setiap perlakuan sesuai dengan pola kepadatan sel, yaitu K (0,434/hari); C (0,379/hari); B (0,279/hari); dan A (0,220/hari). Aplikasi pupuk KNO_3 dengan dosis yang berbeda memiliki efek yang signifikan pada pertumbuhan dan kepadatan sel *Skeletonema* sp. Semakin tinggi dosis KNO_3, semakin tinggi tingkat pertumbuhan dan kepadatan sel *Skeletonema* sp.

Kata kunci: Dosis Pupuk; Fitoplankton; Kalium Nitrat

Abstract

Skeletonema sp. culture its growth is strongly influenced by the nutrients in the environment where it lives, therefore fertilizer is needed in the culture media to support the availability of both macro and micro nutrients. The purpose of this study was to determine the effect of KNO_3 administration on the growth and density of Skeletonema sp. cells. and dosage to obtain the best KNO_3 fertilizer. This study used a completely randomized design (CRD), consisting of 4 treatments and 3 replications. KNO_3 fertilizer dosage treatments included A (100 ppm), B (200 ppm), C (300 ppm), and control K (500 ppm). Parameters measured were specific growth rate and cell density of *Skeletonema* sp. The results of the study on density showed that the best treatment was treatment K of 269.5 ˣ 104 cells/mL, followed by C 300 ppm (203.5 ˣ 10^4 cells/mL), B 200 ppm (126.08 ˣ 10^4 cells/mL), and A 100 ppm (94.75 ˣ 10^4 cells/mL). The average specific growth rate produced in each treatment corresponds to the cell density pattern, namely K (0.434/day); C(0.379/day); B(0.279/day); and A (0.220/day). The application of KNO_3 fertilizer with different doses had a significant effect on the growth and density of *Skeletonema* sp. cells. The higher the KNO_3 dose, the higher the growth rate and density of Skeletonema sp cells. high too.

Keywords: Fertilizer Dose; Kalium Nitrate; Phytoplankton

1. Introduction

Hatchery is the first step or key to the success of the fisheries cultivation business. The main factor that supports the success of seed management is the availability of suitable and sustainable natural feed. The availability of feed in aquaculture activities is needed to maintain the survival of aquaculture organisms (Junda, *et al.,* 2015). High-quality and adequate natural feeding is essential to maintain the larvae of various aquatic organisms such as fish and shrimp. The importance of providing natural feed is evident both in marine and freshwater hatcheries, as there is currently no artificial feed that can completely replace the role of natural feed (Erniati, *et al*., 2012).

Skeletonema sp. is a type of *phytoplankton* that is commonly used as natural feed in aquaculture activities. The advantage of *Skeletonema* sp. is that it has a high nutritional

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content in the form of protein of 21.63-33.30% (Herawati & Hutabarat, 2014; Wahyuni, *et al.,* 2020), fat 8.10%; and carbohydrates 11.60% (Fitriani, *et al*., 2017). *Skeletonema* sp. is also rich in unsaturated fatty acids, namely EPA (*eicosapentaenoic* acid) of 13.8 and HUFA of 15.5 which are very good for shrimp growth (Fitriani, *et al.,* 2017). The use of natural feed of *phytoplankton* of *the Skeletonema* sp. type in shrimp cultivation is highly recommended as food intake because the small cell size ranges from 4-15 µm which is suitable for the mouth opening of shrimp in the nauplius to zoea phase (Herawati & Hutabarat, 2014; Junda, *et al*., 2015). *This type of phytoplankton* is easy to breed and requires a relatively short maintenance time compared to other types of *phytoplankton*. *Skeletonema* sp. was able to achieve the highest population density faster on day 5 culture compared to *phytoplankton* of *the Porphyridium* sp. type on day 9 (Afriza, *et al.,* 2015) and *Chaetoceros muelleri* type on day 11 (Rahayu, *et al.,* 2022).

The need for *Skeletonema* sp. as a natural feed for vannamei shrimp fry is very high, so a mass culture with high density and maximum nutrient content is needed. The problem that occurs is the difficulty of producing *Skeletonema* sp. in large quantities due to the instability of production caused by the quality of *Skeletonema* sp. which is not the same for each culture period. The limited availability of natural feed *for Skeletonema* sp. is important to carry out a mass culture system in improving the quality and population of its cells (Rudiyanti, 2011). One of the mass culture techniques that can be used is fertilization to increase the fertility of culture media water. The culture of *Skeletonema* sp. in its growth is greatly influenced by the nutrients in the environment where it lives, therefore fertilizer is needed in the culture medium to support the availability of nutrients both macro and micro (Sambu, *et al.,* 2016). Complete nutrient composition and proper concentration determine biomass production and *phytoplankton* nutrient content (Christwardana and Hadiyanto, 2013).

One of the inorganic fertilizers that has nutrient content that is able to support the growth of *phytoplankton* is potassium nitrate or also called potassium nitrate (KNO_3). KNO_3 is a type of fertilizer that has been circulating on the market that contains a combination of elements N (nitrogen) and K (potassium) in the form of K_2O (Armawan, *et al.,* 2018). This fertilizer is very effective in use because the potassium (K_2O) content is quite large, which is 46% and the N content is 13% (Andayani, *et al.,* 2018; Ramadhani, *et al.,* 2021; Armawan, *et al*., 2022). Potassium in KNO_3 compounds can act as a catalyst that functions to convert proteins into amino acids, the preparation of carbohydrates, and can strengthen the cell body (Hutapea, *et al*., 2014). Nitrogen is required for cell division, vegetative growth, germination and absorption of nutrients (Ambarwati, *et al*., 2018; Dewanda, 2020). Potassium functions to activate enzymes, plays a role in photosynthesis, protein formation and sugar transport (Zuryanti, *et al*., 2016). The purpose of this study is to determine the effect of KNO_3 fertilizer application on the growth and density of *Skeletonema* sp. cells and to obtain the best KNO_3 fertilizer dosage for *Skeletonema* sp.

2. Materials and Methods

This research was carried out for 2 months at PT. Summa Benur, Kalianget Village, Banyuglugur District, Situbondo Regency, East Java. Research activities were carried out online and offline (*blended*). The online activities carried out were consultation with supervisors, data processing, and also the work on the final report. Offline activities carried out were preparation of materials and samples, *Skeletonema* sp. culture, and growth observation.

The research method carried out was the preparation of tools, including 3-liter plastic jars, aerators (aeration hoses, aeration stones), *haemocytometers, hand counters,* microscopes, dropper pipettes, erlenmeyer, TL lamps, air conditioners (*Air Conditioners*), digital scales, *refractometers,* measuring cups, thermometers, pH meters. Materials used include seawater, fresh water, *Skeletonema* sp., mixed fertilizer, KNO_3, silicate, lime, lime sulfate, CDR, bacteria, Na-thiosulfate, *chlorine*, soap, tissues. The equipment to be used is sterilized by washing and brushing first with soap then rinsed with fresh water, then soaking the equipment in *a chlorine* solution and neutralizing it with Na-thiosulfate, then rinsing with fresh water until *the chlorine smell* disappears. The fresh water used for the manufacture of KNO_3 mixed fertilizer is also sterilized using an autoclave with a temperature of 121°C for 30 minutes. The manufacture of mixed fertilizer with compositions such as Fecl 25 ppm, Na_2HPO_4 37 ppm, EDTA 53 ppm, and KNO_3 is weighed according to the dose, then mixed together and dissolved with 500 mL of sterilized fresh water at erlenmeyer. *Skeletonema* sp. seedlings were then cultured with 3,000 mL of seawater media that had been fertilized beforehand.

This study used a Complete Random Design (RAL) consisting of KNO_3 fertilizer administration treatment$_3$ with different dosages and control treatments. Control treatment using KNO_3 fertilizer in PT. Summa Benur. The study used 4 treatments with 3 repeats, including A (KNO_3 dose 100 ppm), B (KNO_3 dose 200 ppm), C (KNO_3 dose 300 ppm), K (ENT_3 dose 500 ppm). The observed parameter is cell density *Skeletonema* sp., specific growth rate and *doubling time* or the length of time the cell is duplicated *Skeletonema* sp.

Data analysis in empirical research by means of data obtained based on experimental. Data analysis was carried out statistically using the *Analysis of Variance* test. Data were analyzed using analysis of variance at a 95% confidence interval, and if there is an effect If it is real then a further LSD (Least Significant Difference) test is carried out. The data obtained was tabulated with MS Excel to determine the effect of dose on each parameter tested.

3. Results and Discussion

3.1. Cell density Skeletonema sp.

Observation results in each treatment of KNO_3 fertilizer application with dose A (100 ppm); B (200 ppm); C (300 ppm) and Control (500 ppm) showed different amounts of cell density during the culture period. Growth *Skeletonema* sp. Visually, it can be marked by a change in color in the culture medium due to differences in cell concentration.

Table 1
Parameters.

Parameter	Equation
Cell Density (10^4 salt/ml)	Kep.Fito = $\frac{nA+nC+nG+nI}{4}$ x 10^4
Specific Growth Rate (/day)	$\mu = \frac{\ln\,(Nt-N0)}{tt-t0}$
Doubling time (day)	$G = \frac{ln2}{\mu}$

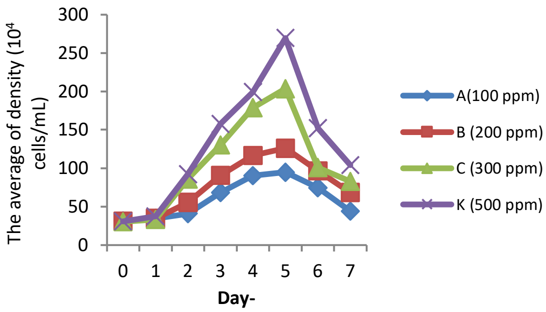

Figure 1. *Skeletonema* sp. density graph.

Skeletonema sp. cultures experienced an exponential peak phase on day 5 in each treatment with an initial stocking concentration of 30 χ 10^4 cells/mL. The highest average yield with the KNO_3 fertilizer treatment was found in treatment C of 203.5 χ 10^4 cells/mL, followed by treatment B of 126.08 χ 10^4 cells/mL and the lowest result in treatment A of 94.75 χ 10^4 cells/mL. The control treatment results were 269.5 χ 10^4 cells/mL. The control treatment resulted in a higher density of *Skeletonema* sp. cells by 25% than the best KNO_3 fertilizer treatment. The increase in the average cell density indicates that *Skeletonema* sp. cells can adapt and grow in the medium by applying KNO_3 fertilizer. This indicates that the nutrients in KNO_3 can be absorbed and utilized by *Skeletonema* sp. for its growth. The high density of *Skeletonema* sp. *cells is* due to the nutrients containing the element nitrate in sufficient quantities so that the nutritional needs of phytoplankton are met and their growth is maximized (Ambarwati, *et al.,* 2018).

The adaptation phase can occur due to the response of *phytoplankton* to utilize nutrients and environmental factors that support the media (Pradana, *et al.,* 2017). The analysis of the growth of *Skeletonema* sp. in this study showed that there was no adaptation phase. This happens because the adaptation phase lasts for less than 24 hours so it is not observed at the time of observation which is carried out every 24 hours. The lag phase (adaptation) in *Skeletonema* sp. occurs at the 0th to 18th hour (Kurniawan, *et al*., 2017). Armanda (2013), stated that the time of the adaptation phase is highly dependent on the viability of the diatom cell. If the inoculum cells used are young cells (meaning taken from cultures that are in an exponential phase, not a stationary or death phase), it means that these cells are still viable to divide. Viable cells will adapt faster to the new environment, so this adaptation phase becomes shorter.

The exponential phase of *Skeletonema* sp. in this study occurred starting from day 1 to day 4 of the culture period, where the number of *Skeletonema* sp. cells continued to increase and the peak of the exponential phase occurred on day 5. The occurrence of increased *phytoplankton growth* is characteristic of the exponential phase. In this phase*, Skeletonema* sp. continues to divide so that cell density increases and the mortality rate is very small. Cell division can occur in this phase because the nutrients, light and space needed for phytoplankron growth are still sufficient (Kawaroe, *et al*., 2012). After going through the exponential phase, *Skeletonema* sp. will go through a stationary phase.

In the stationary phase, the reproductive rate is equal to the death rate, in the sense that the addition and subtraction of cells are relatively equal or balanced so that the density of phytoplankton tends to remain constant (Tjokorde, *et al*., 2013). In this study, *Skeletonema* sp. was not observed to have a stationary phase, due to the vulnerability of observation time that was carried out every 24 hours while the stationary phase *of Skeletonema* sp. occurred less than 24 hours. According to Rudiyanti (2011), the stationary phase *of Skeletonema* sp. occurs for 18 hours. On the 5th day, *Skeletonema* sp. experienced an exponential peak phase, then cell density decreased on the 6th day and cell density continued to decline until the end of the culture period. The phase of growth decline occurs due to cell division that slows down due to nutrient and other environmental factors that begin to limit growth (Muchammad, *et al*., 2013). This shows that *the culture of Skeletonema* sp. is undergoing a phase of death. This is due to the fact that the available nutrients are decreasing and insufficient to support growth, as well as the aging of the culture. In this phase of death, the shading effect occurs which is a phenomenon when cells in a population do not get light due to being blocked by a pile of other cells that have already died. This indirectly affects the ability of cells to perform photosynthesis (Muchammad, *et al.,* 2013). Theoretically, the density of microalgae cells is influenced by specific growth rates and doubling times (Armanda, 2013).

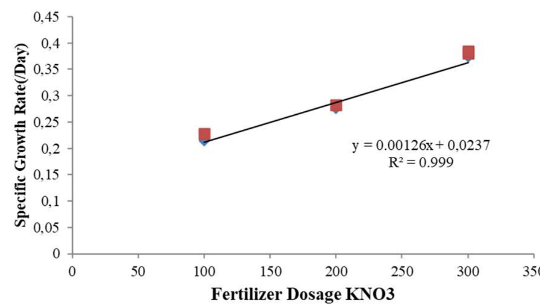

Figure 2. Specific Growth Rate graph'

3.2. Specific Growth Rate Skeletonema sp.

The relationship between different doses of KNO_3 fertilizer to the specific growth rate of *Skeletonema* sp. showed a curve with the quadratic equation y = 0.00126 + 0.0237 with R^2 = 0.999. The results of the variety analysis showed that the use of different doses of KNO_3 fertilizer had a real effect on the growth *of Skeletonema* sp. with F_{count}>F_{table}. The average growth rate of *Skeletonema* sp. in the treatment of applying KNO_3 fertilizer was the highest of 0.379/day with the best dose of 300 ppm. The control treatment had a higher specific growth rate of 13%, which was 0.434/day. The specific growth rate decreased at doses of 200 ppm and 100 ppm. Based on these results, it is proven that the addition of KNO_3 has a great effect on the growth of *Skeletonema* sp. This is in accordance with Agustini (2017), who stated that the appropriate application of N to cultures in the form of KNO_3 will increase the growth *of* phytoplankton optimally. Ambarwati, et al., (2018) also stated that element N is an element that has a great influence on growth. The main role of this element is as a vegetative growth stimulator, and increases the number of cells/individuals. According to Fauziah and Hatta (2015), growth is a process of change that occurs in organisms, whether it is an increase in length or weight or an increase in the number of *cells of Skeletonema* sp. Factors that affect the growth of *Skeletonema* sp. are the environment and food. Another important factor that affects the specific growth rate is the nutrient content contained in the culture media (Zainuddin, et al., 2017).

The energy contained in KNO_3 is used for the growth and division of Skeletonema sp. cells. At lower doses of KNO_3 fertilizer, *Skeletonema* sp. has lower growth as well. This may be due to the concentration and completeness of the nutrient composition in the medium that is insufficient for the needs of *Skeletonema* sp. cells. A lack of the elements N and Mg (macronutrients) affects the formation of chlorophyll. Meanwhile, a lack of micronutrients such as Mn can affect the process of photosynthesis because Mn is an enzyme activator in the bright reaction of photosynthesis. This will affect the rate of photosynthesis. Carbohydrates produced by photosynthesis by phytoplankton are not only used for growth but also for cellular respiration. If the results of photosynthesis are reduced, the carbohydrates left after some use in the respiration process are insufficient for cell growth (Primadona, 2020).

3.3. Doubling time Skeletonema sp.

Based on the observation of the growth *of Skeletonema* sp., the results of the calculation *of the doubling time of Skeletonema* sp., are shown in Figure 3.

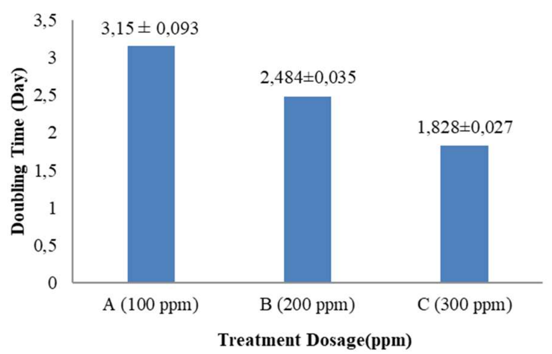

Figure 3. Chart *Doubling time Skeletonema* sp.

Doubling time Skeletonema sp. showed that the fastest cell doubling time was the treatment of 300 ppm KNO_3 fertilizer dosage with a time of 1.82 days, followed by the treatment of giving a dose of KNO3 fertilizer 200 ppm with a time of 2.48 days and the treatment of giving a dose of KNO_3 fertilizer 100 ppm with a time of 3.15 days. The cell doubling time in the control treatment was 1.59 days. The cell doubling time in the control treatment was 50% higher than that of the KNO_3 fertilizer treatment. Oktariflani, et al., (2019), stated that the faster it takes for phytoplankton to divide or multiply cells, the daily cell production will also increase. Armanda (2013), in his research stated that the *best doubling time* of *S. costatum* was obtained at 1.26 days in medium conway.

The growth rate of a microalgae can describe the rate at which cells of the microalgae grow or multiply per unit of time. If the specific growth rate is high, it produces a low *doubling time* value which means the cell multiplication time is fast, and vice versa if the specific growth rate value is low, the *doubling time* value is high which means the cell multiplication time will be longer (Simamora, et al., 2017). Nitrate and phosphate are important factors in the process of photosynthesis. The results of this photosynthesis will produce glucose and energy used in cell metabolism so that the growth of *Skeletonema* sp. increases (Robi, 2014). Nitrate and phosphate are elements that play a role in supporting the growth of microalgae both in protein formation and metabolic activity. Growth and biomass can be achieved well if these nutrients are sufficient (Herawati and Hutabarat, 2015).

4. Conclusion

The administration of KNO_3 fertilizer with different doses has a noticeable effect on the growth and density *of Skeletonema* sp. cells. The higher the dose of KNO_3 results in a higher growth rate and density of *Skeletonema* sp. cells. The best treatment during the study was a dose of 300 ppm with a specific growth rate of 0.379/day and the highest average density of 203.5 χ 10^4 cells/mL.

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