The Effect of Rumen’s Cattle Waste as Alternative Nutrient Sources for *Thalasiossira* sp Aquaculture

M B Santanumurti¹, M I Ogara², L Santoso³, S Hudaidah⁴

¹Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115 Indonesia
²Aquaculture Program, Faculty of Agriculture, University of Lampung, Jl. Prof. Sumantri Brojonegoro, Bandar Lampung, Lampung 35141, Indonesia
³Department of Fisheries and Marine Science, Faculty of Agriculture, University of Lampung, Jl. Prof. Sumantri Brojonegoro, Bandar Lampung, Lampung 35141, Indonesia
⁴Corresponding author: idahasan64@gmail.com

**Abstract.** The liquid from rumen’s cattle is expected to be used as nutrients in microalgae culture. This study aimed to examine the use of waste of rumen’s cattle as a source of nutrients with different doses in the culture of *Thalasiossira* sp. The research design used was a completely randomized design (CRD) with 5 treatments with 3 replications of (A) 1 ml conwey/l of seawater, (B) 1 ml of waste/l of sea water, (C) 10 ml of waste/l of seawater, (D) 1 ml of waste + 0.4 gr NaNO₃/l of seawater, and (E) 10 ml of waste + 0.4 gr NaNO₃/l of seawater. The calculated parameter were the population density of *Thalasiossira* sp., diatom diameter, protein content and water quality. The results showed that the waste of rumen’s cattle could be used as an alternative source of nutrients in the culture of *Thalasiossira* sp. and the peak of the population was reached on day 3. Measurement of protein content ranged from 9.9 to 11.8% and the diameter of the diatoms ranged from 5-10 m. The measurement of the water quality of the media was in the range of temperature 24-26 °C, pH value 7 and salinity 32-34 ppt.

1. Introduction
*Thalasiossira* sp culture requires sufficient nutrients as fertilizer for its growth [1]. Conway fertilizer is often used in microalga culture such as *Thalasiossira* sp. However, the high price of conwey fertilizer is an obstacle in microalgae production. Alternative fertilizers are needed that are more economical, one of which is the use of liquid cattle rumen contents. Liquid cattle rumen content has the potential as a liquid fertilizer because it contains high nutrients and has not been widely used. According to previous study, the provision of cattle rumen significantly affected the density of *Thalasiossira* sp [2]. The results of laboratory analysis showed that this waste was rich in nitrogen and phosphorus nutrients. The nitrogen (N) content functions to form protein, fat, and various other organic compounds, vegetative growth and cell formation. Phosphorus (P) functions for energy metabolism, as cell membrane stabilizer, regulation of algae metabolism, regulation of starch production, formation of carbohydrates, very important in energy transfer, protein, and amino acid synthesis [3]. The contents of the liquid cattle rumen contained 111.4 ppm nitrogen and 401.79 ppm phosphorus. However, it resulted in a small N/P ratio of 0.25:1. Therefore, in this study, the liquid cattle rumen fluid increased the N/P ratio to 10:1 with the addition of NaNO₃. According to Subarijunti [4] the ratio of N and P for good phytoplankton growth is 10:1. Because of the very high nutrient content, it was used in this study and will be applied to the culture of microalgae of the diatom type *Thalasiossira* sp with different doses.

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2. Material and methods

2.1 Sample preparation

Starter of *Thalasiossira* sp obtained from PT. Central Proteina Prima, Merak Belantung, Kalianda, South Lampung. Furthermore, *Thalasiossira* sp was cultured on conwey media. The culture was carried out for 2 days to reach the exponential phase. The contents of the cattle’s rumen were obtained from the slaughterhouse at the Lampung City. The liquid cattle rumen contents were steamed for 15-20 minutes then analyzed for the N and P content and the cattle rumen contents were ready to be used in this study. The research process uses procedures from previous studies [5]. This study was conducted using a completely randomized design (CRD), consisting of three treatments and three replications as follows:

1. Treatment A: Conway fertilizer (control) 1 mL/L seawater
2. Treatment B: Liquid cattle rumen of 1 mL/L seawater
3. Treatment C: Liquid cattle rumen of 10 mL/L seawater
4. Treatment D: Liquid cattle rumen of 1 mL+0.4 g NaN03/L seawater
5. Treatment E: Liquid cattle rumen of 10 mL+0.4 g NaN03/L seawater

*Thalasiossira* sp. was a diatom, 1 ml/L of seawater so all of the treatment was added to each treatment with silica.

2.2 Research parameter calculation

The calculation of the density of *Thalasiossira* sp was carried out every day at 10.00 WIB until the end of the study. The tools used in the calculation were a microscope and a haemacytometer. The sample of diatoms was dripped on the surface of the haemacytometer and sticking it with a glass slide with a microscope magnification of 10x in calculations using 4 fields of view using the following formula modified from previous research [6]:

\[
\text{Density} = \frac{n}{\text{Field of view (4)}} \times 10^4
\]

\[n = \text{the average number of phytoplankton obtained from observations}\]

Proximate analysis of *Thalasiossira*’s protein from each treatment was carried out on the final day of the study. The method used was the Kjeldhal method by means of titration carried out at the Agricultural Product Technology Laboratory, Lampung State Polytechnic. Diatom diameter measurements were carried out on the final day of the study using a microscope and an ocular objective lens with a magnification of 10x. The ocular objective lens was installed and calibrated, after being calibrated, the sample was dropped onto the glass slide. conducted at the Laboratory of Aquaculture, Faculty of Agriculture, University of Lampung.

The water quality parameters measured were temperature, pH, and salinity. Temperature measurements were carried out using a thermometer, pH measurements using pH paper, and salinity measurements using a refractometer. Water quality measurements are carried out every day in the morning, afternoon and evening. Data on density of *Thalasiossira* sp. tested for normality and homogeneity. If the data was normally distributed and homogeneous, it is continued with the variance test (ANOVA) with a 95% confidence level. If there were significantly different results, then proceed with Duncan's test using the SPSS program.

3. Result and discussion

The results showed that there was a significant difference between each treatment from day 1 to day 7. The highest treatment was found in treatment A (control) where the treatment of liquid rumen contents of each treatment was not significantly different. However, on the 3rd day, the density of treatment D
and E was not significantly different from treatment A so that it could be harvested on day 3 and could be used as an alternative fertilizer for microalgae Thalasiossira sp.

Figure 1. Daily growth chart of Thalasiossira sp. in this study

Treatments D and E with the addition of NaNO₃ with an N/P ratio of 10:1 had a better density than treatments B and C without the addition of NaNO₃. There was an increase in the population in treatments D and E on day 3. This showed that the liquid cattle rumen waste enriched with NaNO₃ produced a good N/P ratio for the growth of Thalasiossira sp. Previous study showed that NaNO₃ (sodium nitrate) contained high concentration of nitrate and helped the growth of Thalasiossira sp [7].

Growth of Thalasiossira sp. in treatment A showed the highest density compared to other treatments. This study indicated that the provision of liquid cattle rumen has no effect on the growth of Thalasiossira sp. According to previous study fertilizer for microalgae culture had more complete elements than natural fertilizers because it contained higher nutrients [8]. The treatment with the highest density using liquid rumen contents was found in treatment D, while the lowest density was found in treatment E. The liquid rumen used had nitrogen content of 101.9 ppm and P of 400 ppm. The availability of liquid cattle rumen contents was not high enough compared to the N/P ratio of conwey fertilizer, causing a significant difference in growth. In this study, liquid cattle rumen was added as much as 0.4 mg of NaN0₃ so that the N/P ratio was 10:1. Diatoms will grow fast if the water contains sufficient N and P nutrients [9].

Table 1. Water quality parameters in this study

| Parameter       | Treatment | Optimum          |
|-----------------|-----------|------------------|
| Temperature (°C) | A: 24-26  | 19-32 [10]       |
|                 | B: 24-26  |                  |
|                 | C: 24-26  |                  |
|                 | D: 24-26  |                  |
|                 | E: 24-26  |                  |
| pH              | A: 7      | 7-9 [11]         |
|                 | B: 7      |                  |
|                 | C: 7      |                  |
|                 | D: 7      |                  |
|                 | E: 7      |                  |
| Salinity (ppt)  | A: 32-34  | 25-35 [10]       |
|                 | B: 33-34  |                  |
|                 | C: 32-34  |                  |
|                 | D: 33-34  |                  |
|                 | E: 32-33  |                  |
Based on the results of measurements, the temperature range was still in a good value. This was in accordance with the statement of previous study that the optimal temperature in the culture of diatom ranged from 19°C to 32°C [10]. The temperature in the culture was regulated in such a way because it is very dependent on the medium used. Temperatures below 16°C could cause growth rates to drop, while temperatures above 36°C causes death. The result of pH measurement during the study was 7. This range was still in the range that could be tolerated by *Thalasiossira* sp. According to Irianto [12], variations in pH in culture media would affect metabolism and growth in microalgae culture, among others, changing inorganic carbon balance, changing nutrient availability and affecting cell physiology. The pH range for algae culture was usually 7-9 [13].

The results of salinity measurements during this study was 32-34 ppt. This salinity range was still in a good salinity range for the growth of *Thalasiossira* sp. According to Irianto [12], the varying salinity range could affect the growth of microalgae. Some microalgae could grow in a high salinity range but not in optimum phase. The most optimum salinity range for micro-algae growth was 19-45 ppt [13].

### 4. Conclusion
The results showed that the waste of rumen’s cattle could be used as an alternative source of nutrients in the culture of *Thalasiossira* sp. since the density was lower than convey although another parameters such as protein, diameter of plankton and water quality showed same results.

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### 6. Acknowledgement
The authors gratefully acknowledge the collaboration from Universitas Airlangga and the University of Lampung.