



Organic Feed Enrichment Effects toward Growth Performance and Egg Production of *Oithona similis*

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ABSTRACT

The optimum condition of culture media and feed diets which fit the necessities of *Oithona similis* life (ecophysiological) is very important for it to grow and develop in its maximum. The enrichment of *O. similis* feed can be done by adding fermented organic feed. Providing organic feed with optimum protein content is necessary to support the growth process and reproduction of *O. similis*. Osmoregulation process of water organism which is influenced by media salinity is related to bioenergetics process as the effort to use feed energy for the growth. This research aimed to examine the effect of different organic feed protein content and to determine the optimum point which may result in the best growth and egg production of *O. similis* in the optimum condition of culture media. The study used an experimental method with a completely random design consisting of 4 treatments and 4 repetitions each, i.e. *O. similis* culture with A. 25%; B. 30%; C. 35%; and D. 40% organic feed protein content. The results showed that the difference of protein content in organic feed affects the growth and production of *O. similis* eggs. The optimum point of protein content in the fermented organic feed is on protein content B, i.e. 30%, which resulted in the best growth performance (70.44 ± 0.43 ind.mL⁻¹ total density, 0.213 ± 0.001 .day⁻¹ population growth rate, and 27.38 ± 0.48 eggs.ind⁻¹ egg production. (tujuan dan pembahasan harus past tense), Tujuan di abstrak minimal harus menggambarkan atau sama dengan tujuan di pendahuluan)

Keywords: enrichment, *Oithona similis*, organic feed, protein, reproduction.

ABSTRAK

Kondisi optimum media kultur dan diet pakan yang sesuai dengan kebutuhan hidup (ekofisiologis) *Oithona similis* sangat penting agar dapat tumbuh dan berkembang secara maksimum. Pengkayaan pakan terhadap *O. similis* dapat dilakukan dengan penambahan pakan organik yang difermentasi. Kandungan protein pakan organik yang optimum sangat diperlukan untuk mendukung proses pertumbuhan dan reproduksi *O. similis*. Proses bioenergetika dalam upaya pemanfaatan energi pakan untuk pertumbuhan, erat hubungannya dengan proses osmoregulasi organisme air yang dipengaruhi oleh salinitas media kulturnya. Tujuan penelitian ini adalah mengkaji pengaruh perbedaan kandungan protein pakan organik dan menentukan titik optimum yang memberikan performa pertumbuhan dan produksi telur *O. similis* terbaik pada kondisi salinitas media kultur yang optimum. Penelitian ini menggunakan metode eksperimental dengan Rancangan Acak Lengkap yang terdiri dari 4 perlakuan dengan masing-masing 4 kali ulangan, yaitu: kultur *O. similis* dengan kandungan protein pakan organik A. 25%; B. 30%; C. 35%; dan D. 40%. Hasil penelitian menunjukkan bahwa perbedaan kandungan protein pada pakan organik berpengaruh terhadap pertumbuhan dan produksi telur *O. similis*. Titik optimum kandungan protein pada pakan organik yang difermentasi adalah pada kandungan protein 30%, yang memberikan performa pertumbuhan (kepadatan total $70,44 \pm 0,43$ ind./ml, laju pertumbuhan populasi $0,213 \pm 0,001$ /hari), produksi telur ($27,38 \pm 0,48$ telur/ind) terbaik.

Kata kunci: pengkayaan, *Oithona similis*, pakan organik, protein, reproduksi.

1. Introduction

Copepods of *Oithona* sp. is one of the zooplankton natural feed types which can be utilized as feed for larvae stadia in fish and shrimp hatchery (Molejon and Alvarez-Lajonchere, 2003). Copepod in marine waters ecosystem is categorized as a significant part of the food web and food chain in trophic levels in the sea (Sampey *et al.*, 2007; Drillet *et al.*, 2011). Copepods play a role as the first consumer in food chains at sea for the first producers of phytoplankton cells (Perumal *et al.*, 2009). The population of copepods is mainly influenced by its surrounding biotic conditions such as the availability of natural feed sources and phytoplankton cells (Noyon and Froneman, 2013), predators and competitors of copepods and other types of zooplankton, and certainly its biological environmental conditions like temperature, salinity, and dissolved oxygen (Beyrend-Dur *et al.*, 2011).

The optimum condition of the culture media and feed diet containing protein which matches *Oithona* sp. life needs (ecophysiological) is fundamentally important for its maximum growth. Microalgae diets that contain nutrition needed by *Oithona* sp. is essentially decisive for successful *Oithona* sp. culture (Chilmawati and Suminto, 2016). One of various inputs in the natural feed cultivation system is enrichment of which the composition must be carefully concerned. This is due to the fact that it will cause nutritional deficiencies or even the excess of dangerous substances if the composition is not properly correct (Chilmawati and Suminto, 2010). Feed enrichment by giving the best microalgae and adding fermented organic feed is proven to improve the growth and egg production performance of *Oithona* sp. (Suminto *et al.*, 2018).

Protein is main biochemical component besides fats and carbohydrates in metabolic process of copepods (Santhanam and Perumal, 2012^b). Thus, protein content in feed affects copepods' growth and egg production (Holmborn and Elena, 2008). Furthermore, protein content functions as fuel used for RNA/DNA ratio in Copepod growth. Meanwhile, DNA is a factor related to molting process and RNA has an important role in protein synthesis. This is a complex process in metabolism involving the interaction of nucleic acids and proteins (Yebara *et al.*, 2011). It has been studied that growth is correlated with protein content in the consumed feed (Gorochova, 2003; Gorokhova *et al.*, 2007).

Protein content in feeding should possess similar nutritional profile with the phytoplankton (Wang *et al.*, 2014).

The studies concerning organic feed with optimum protein content for *Oithona* sp. has not been found until recently. Therefore it is necessary to conduct a research focusing on feed enrichment using microalgae diet and fermented organic feed with different protein contents, which provides the best growth and egg production of *O. similis* in the optimum condition of culture media. This research aimed to examine the effect of different organic feed protein content and to determine the optimum point which may result in the best growth and egg production of *O. similis* in the optimum condition of culture media.

2. Materials and Methods

2.1. Study area

This research was conducted at the Coastal Area Development Laboratory, Faculty of Fisheries and Marine Science, Diponegoro University, Jepara Campus, Central Java, Indonesia from July to September 2018.

2.2. Preparation of phytoplankton culture and fermented organic feed.

This study used *Chaetoceros calcitrans* as the best phytoplankton feed for *Oithona* sp. (Chilmawati and Suminto, 2016^a). This *C. calcitrans* was obtained from live feed laboratory of Brackish Water Culture Development Center (local term: *Balai Besar Pengembangan Budidaya Air Payau or BBPBAP*) located in Jepara, Central Java, Indonesia. This phytoplankton was cultured using seawater which has been sterilized at 25-28°C temperature, with 28-30 ppt salinity, and pH 8-9. The sterilized seawater was then added by a solution of sodium hypochlorite (NaClO) at a dose of 60 mg.L⁻¹ for 10-30 minutes. Moreover, it was added with a solution of 80 mg.L⁻¹ sodium thiosulphate (Na₂S₂O₃) for the process of dechlorination for 24 hours using aeration. The culture was done in a 60 L volume size plastic tube, which has been filled by 40 L of sterile seawater. The culture used a modified Walne media at a dose of 0.5 ml.L⁻¹ in 24 hours photoperiod light and 1500-1800 Lux light intensity. Besides, culture media also used aeration. The inoculation volume of *C. calcitrans* is 10% of the culture media volume (Lee *et al.*, 2006).

The peak of *C. calcitrans* cell growth is commonly on day 6. In addition, *C. calcitrans* culture can be harvested when it reaches the exponential phase because it at this point contains the highest nutrients (Creswell, 2010). The density of *C. calcitrans* cell is calculated daily by taking its cell samples under a microscope (Olympus CH20) and using 10x magnification with a hemocytometer (brand: Neubauer with 0.0025 mm³ volume).

Organic feed fermentation was done by using a combination of materials in the form of organic flour consisting of tofu, rice bran, and fish flour to make powder feed with average diameter of 50-100 µm (Suminto *et al.*, 2018). The plastic tube was filled with 10 mL EM4 of probiotics containing *Lactobacillus casei*, *Saccharomyces cerevisiae*, and *Bacillus* sp. Then, the mixture is filled with 200 mL of purified water and 25 mL of molasses for 1 kg of mixed organic feed. The incubation was done for 48 hours before it is used.

2.3. Culture condition of *Oithona similis*.

Copepods were obtained from an axenic culture in the live food Laboratory of Marine Culture Development Research Center (MCDRC) in Lampung, Indonesia. The copepods species used was *Oithona similis* as the findings of Chilmawati *et al.* (2019). *O. similis* culture was carried out in 250 mL of Erlenmeyer volume with 100 mL of sterile seawater, 19.44 ppt optimum salinity (Chilmawati *et al.*, 2019), at 27-28°C water temperature, pH 8, and 1 ind.mL⁻¹ from the initial density (Lee *et al.*, 2006) for 20 days. The method used for feeding was libitum. The number of *C. calcitrans* given to *O. similis* was 0.01 mg.ind⁻¹ (Lee *et al.*, 2006). Based on Suminto *et al.*'s (2018) study, the dose of fermented organic feed is 0.5 g.L⁻¹ culture medium with a combination of 50% phytoplankton cells and 50% organic fermentation feed (Chilmawati and Suminto, 2016^b; Suminto *et al.*, 2019). The population of *O. similis* on Nauplii, Copepodite, and adult stages was observed under a microscope (Olympus CYK41). Each stage is determined by viewing its morphology (Takahashi and Uchiyama, 2007).

2.4. Design of the experiment

This study conducted a completely randomized design with 4 treatments and 4 repetition (replications). Treatment A involved *O. similis* cultured with fermented organic feed

containing 25% protein. Treatments B, C, and D were done by culturing *O. similis* with fermented organic feed containing 30%, 35%, and 40% protein. Cell density of *O. similis* was calculated once every 4 days to obtain total population, growth performance, and egg production.

2.5. Data collection and statistical analysis.

The density of *O. similis* was counted once every 4 days during 20 days of observations. 30 mL of sub-sample was taken from the culture media to calculate the total number of *O. similis*, specific population growth rate (r), and egg production.

Growth performance was calculated using Cheng *et al.* (2011) equation:

$$r = \frac{\ln N_t - \ln N_0}{t}$$

Where r is population growth (.day⁻¹), N_t is the final density of *O. similis*, N₀ is the initial density of *O. similis* and t is days needed to achieve *O. Similis* maximum growth.

Egg production is the average number of eggs produced by the female *O. similis* during their lifetime according to Zamora-Terol *et al.* (2014):

$$\text{Egg production} = \frac{\sum s \times e}{\sum n}$$

Where s is the amount of egg sac, e is the average amounts of egg per sac and n is the number of ovigerous females (ind).

The data were then analyzed by One Way Analysis of Variance (ANOVA) to determine the effect of different protein content in organic feed on growth performance and egg production of *O. similis*. Least Significant Different (LSD) test (α=0.05) by using SPSS 16 was conducted when the treatment showed a significant effect.

3. Results and Discussion

3.1. *O. similis* population

Different protein content in organic feed significantly affects (p. < 0.05) on the growth performance of *O. similis*. Total density is the total number of naupli, copepodite and adult copepods, including adult copepods with eggs. According to the results of each *O. similis* stage on the last day of culture (day 20), it showed that the highest density was on the treatment of organic feed with 30% protein content (Figure 1). The observation results on day 20 indicated that 40% protein had the lowest density.

Based on the orthogonal polynomial test on *Oithona similis* culture density with different

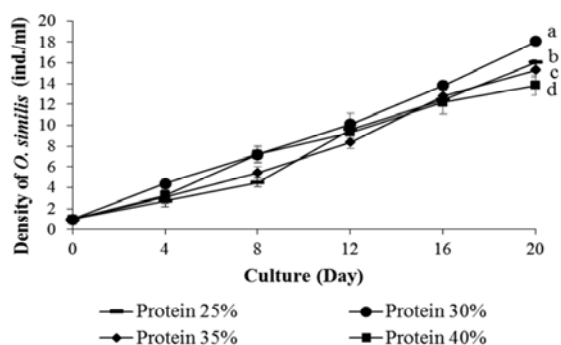


Figure 1. The density of *O. similis* at different protein content during 20 days culture period. Values not sharing the same letter are different significantly from one another ($p < 0.05$) by Least Significant Difference Test

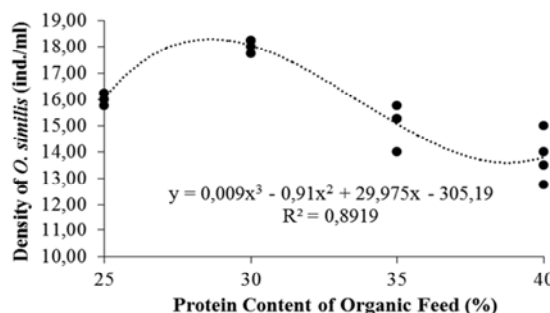


Figure 2. Relation model between protein content in organic feed and density of *Oithona similis*

protein content, it obtained a cubic patterned relation (Figure 2) with a regression equation, i.e. $y = 0,009x^3 - 0,91x^2 + 29,975x - 305,19$ and $R^2 = 0,8919$ and the optimum point of protein content was at 30%. The R^2 value indicated that 89.19% of *O. similis* total density was influenced by organic feed protein content while the remaining 10.81% was influenced by other factors.

3.2. Specific growth rate of *Oithona similis* population

The difference in protein content indicated a significant effect ($p < 0.05$) on the specific growth rate of *O. similis* population (Figure 3). The highest value of the population-specific growth rate (r) ($0.145 \pm 0.001 \text{ ind.day}^{-1}$) was indicated in *O. similis* culture given with organic feed containing 30% protein. Organic feed containing 25% and 35% protein respectively resulted in R-

value at 0.139 ± 0.001 and $0.136 \pm 0.002 \text{ ind.day}^{-1}$. The lowest population-specific growth rate ($0.131 \pm 0.003 \text{ ind.day}^{-1}$) was shown in culture of *O. similis* given with organic feed containing 40% protein.

The curve of *O. similis* population-specific growth rate during this study with protein content differences (Figure 4) indicated that there was a cubic patterned relation showed by equation $y = 3E-05x^3 - 0.0032x^2 + 0.1038x - 0.977$ with $R^2 = 0.9557$. This described that 95.57% of the population-specific growth rate was influenced by protein content difference in organic feed and 4.43% was influenced by other factors. The optimum point was reached when providing organic feed containing 30% protein.

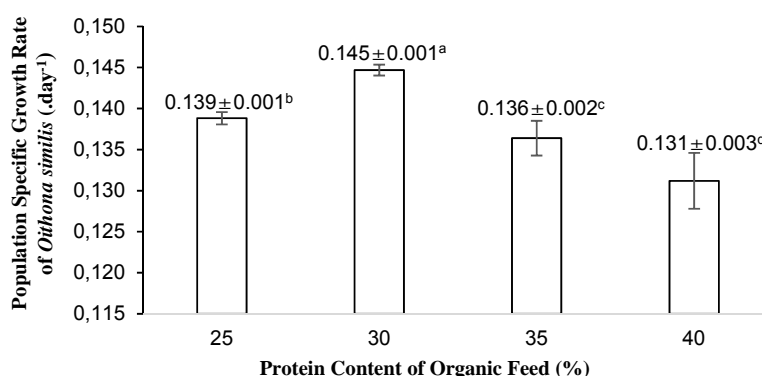


Figure 3. Specific growth rate of *O. similis* population at different protein content during 20 day culture period. Values not sharing the same letter are different significantly from one another ($p < 0.05$) by Least Significant Difference Test

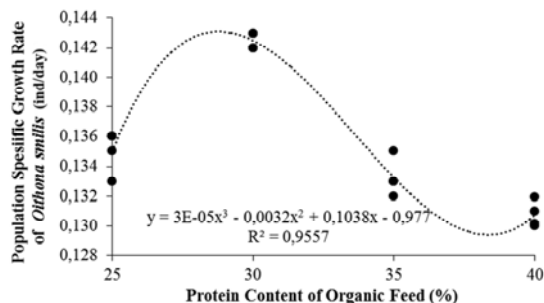


Figure 4. Correlation between protein content and the population specific growth rate of *O. similis*

3.3. Egg production of *O. similis*.

The histogram of *O. similis* average egg production at the end of the observation (Figure 5) showed that culture with 30 % protein content led to the highest egg production (26.75 ± 0.96 egg.ind⁻¹) which was significantly different ($p < 0.05$) with all three other treatments. Moreover, giving organic feed containing 25% and 35% protein generated egg production which was not significantly different ($p > 0.05$) while 40% protein content resulted significantly different ($p > 0.05$) egg production (20.50 ± 0.58 egg.ind⁻¹).

Based on the orthogonal polynomial test on egg production of *O. similis* fed by organic feed with different protein contents, it obtained a cubic patterned relation with the following equation $y = 0,0107x^3 - 1,095x^2 + 36,558x - 372,5$ and $R^2 = 0.9244$ and the optimum point was on protein content 30%. The R^2 value indicated that 92.44%

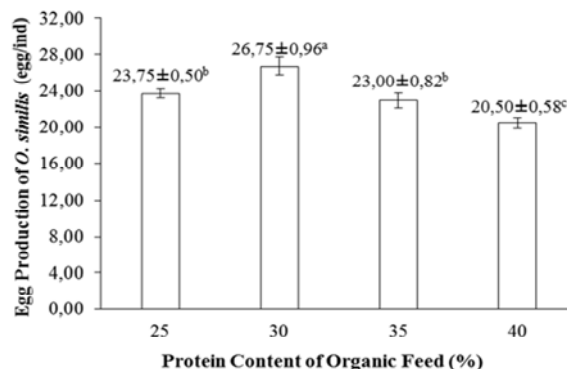


Figure 5. Egg production of *O. similis* at different protein content during 20 days culture period. Values not sharing the same letter are different significantly from one another ($p < 0.05$) by Least Significant Difference Test

of *O. similis* egg production was influenced by organic feed protein content while 7.56% of egg production is influenced by other factors.

The feeding of organic feed fermented with different protein contents and combined with the best microalgae diet has caused different influence on the growth performance and egg production of *O. similis* cultured at optimum media salinity. The feeding of organic feed in addition to phytoplankton cell feed has essential effect on the growth and egg production of *Oithona* sp. (Suminto *et al.*, 2018). The increase in organic feed containing protein is presumed to highly improve the quality and quantity of *Oithona similis*. Protein is an important component for growth (Lee *et al.*, 2006), population density (Drillet *et al.*, 2011), and copepods fertility ((Rajthilak *et al.*, 2014).

The use of organic material from mixture of

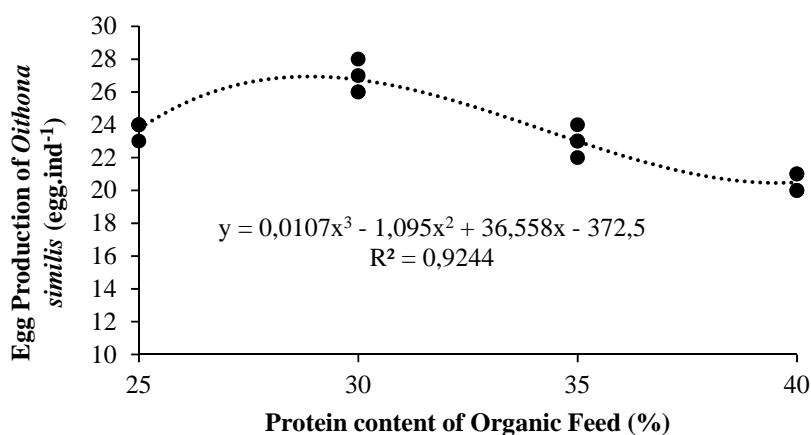


Figure 6. Correlation between protein content and egg production of *O. similis*

fish flour, pulp tofu and fermented bran flour can improve the quality and quantity of the cultured *O. similis*. In addition, these waste and dirt materials are rich in organic materials and some types of microbes which can convert them into carbohydrates, proteins, pigments, oils, alcohol, aldehydes, and so on that can be used by copepods to increase its population. Previous study by Molejon and Alvarez (2003) mentioned that the use of fermented organic material can trigger the growth of Ciliata, bacteria, and detritus, and does not give any negative effect on copepods since these organisms are also food sources for copepods. Fermentation is a unique process which can improve nutritional value of feed material (Felix and Berindo, 2008). Fermentation on organic materials of feed nutrient sources can improve the growth of copepod (Rajhtilak *et al.*, 2014). Fermentation also increases the availability of such vitamins as Riboflavin, Cyanocobalamin, thiamine, niacin, B₆, B₁₂, and folic acid levels in several feed material (Felix and Berindo, 2008). Some studies stated that the use of organic fermentation materials as copepod feed as one in Japan (Omori, 1973; Fukusho, 1980; Fukusho *et al.*, 1980 in Molejon *et al.*, 2013) could produce good result in culture.

Organic feed containing 30% protein provided the best growth performance than those of the other three treatments. Thus, it was assumed that organic feed containing 30% protein fit with the needs of *O. similis* for its metabolic and growth processes. Santhanam and Perumal (2012) mentioned that protein is the main biochemical component of *Oithona* sp. and is the major fraction rather than lipid and carbohydrates which function as spare energy and metabolic substrate. The protein needs in each species depend on the type, size, feed material, quality of protein, and environment.

Giving organic feed with 25% protein content resulted in lower growth performance and egg production than that containing 30% protein. This was presumed that the protein content still could not meet the needs of *O. similis* to grow and reproduce. Protein is the main source of nutrients in the feed playing a significant role to form the materials for growth, producing enzymes, and other substances (Steffens, 1989). Robinson and Li (2007) also mentioned that protein will be continuously synthesized and degraded in the body of cultivars. In addition, food supply in the form of non-specific amino acids and nitrogen is needed during its life for body maintenance and growth. Thus, the growth and reproduction of *O. similis* will also not be able to achieve its

maximum if the quantity and quality of feed are still inadequate.

This study also concerned Robinson and Li (2007) on several factors affecting feed protein requirements such as size, cultivars, water temperature, feeding, the amount of non-protein energy in the feed, the quality of protein, the availability of natural feed, and the practice of feed management. The results of this study showed that an increase of protein content in organic feed did not always increase the growth of *O. similis*. The use of limited volume and the absence of aeration on culture media was assumed to be the cause of growth performance and egg production of *O. similis* fed with organic feed containing 35% and 40% protein to be lower than those with 30% and 25% protein content. Thus, it is necessary to do a study concerning the influence of aeration and culture volume differences on the growth reproduction performance of *O. similis*.

The highest egg production was also produced by *O. similis* fed with organic feed containing with 30% protein. Moreover, high protein levels do not guarantee that egg production will also be high. It may be because the nutritional quality of organic feed fermentation is still insufficient even though it is much higher in quantity. This result is supported by (Peterson *et al.*, 1991) stating that the low production of *Oithona* sp. can also be affected by the concentration of feed and the limited feed.

The difference in protein content in organic feed has significant effect on the growth and egg production of *O. similis*. Organic feed containing 30% protein and microalgae diet with *Chaetoceros calcitrans* resulted in the best growth performance (18,06±0.24 ind.mL⁻¹ total density, 0,145±0.001.day⁻¹ population growth rate, and 26,75±0.96 eggs.ind.⁻¹ egg production) in *O. similis* culture with optimum salinity (19.4 ppt). Based on the population growth rate and egg production of *O. Similis* produced, the optimum point of protein content in *O. Similis* organic feed was on 30% protein content.

4. Conclusions

The difference of protein content in organic feed has a significant influence on the growth and egg production of *O. similis* in which the organic feed containing 30% protein content showed the best growth performance and egg production (18.06±0.24 ind.mL⁻¹ total density, 0.145±0.001.day⁻¹ population growth rate, and 26.75±0.96 eggs.ind⁻¹ egg production).

Furthermore, based on population-specific growth rate and egg production, the optimum protein content of organic feed for *O. similis* was 30%.

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