The effect of probiotic bacteria in culture media using organic fertilizer for population density, biomass production and nutrient quality of Phronima sp. as natural feed

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The effect of probiotic bacteria in culture media using organic fertilizer for population density, biomass production and nutrient quality of *Phronima sp.* as natural feed

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Abstract

Phronima sp. is a natural feed with high nutrient content, and it can be an alternative substitute for *Artemia*. The existence of probiotic bacteria in culture media aims to improve water quality through biodegradation, maintain microbial balance and control pathogenic bacteria, so that nutrients in culture media can be utilized by *Phronima* sp. This study aimed to determine the effect of fermented organic fertilizer by probiotic bacteria in mass culture media on the population density, biomass production and nutrient content of *Phronima* sp. and to find the best culture media composition for mass culture of *Phronima* sp. The study was conducted with a completely randomized design with five treatments and three replications. The treatments were as follows: A: 0% chicken manure, 50% rice bran, 50% tofu waste; B: 25% chicken manure, 37.5% rice bran, 37.5% tofu waste; C: 50% chicken manure, 25% rice bran, 25% tofu waste; D: 75% chicken manure, 12.5% rice bran, 12.5% tofu waste and E: 100% chicken manure. The test animals were *Phronima* sp., and during cultivation, fertilization was carried out every 3 days. Population density and biomass were observed, and proximate analysis, amino acid profile analysis and fatty acid profile analysis were performed. The highest population density was at treatment B, with a peak population of 98 individuals/L that occurred on the 16th day of maintenance culture, and the highest biomass of 0.51 g was at the same treatment. The highest nutrient content was obtained at treatment B, for which the highest protein proximate analysis value was 58.90%, the proportion of the fatty acids comprised of eicosapentaenoic acid was 7.53%, and lysine amino acids were found at 44.16 ppm.

KEYWORDS
amino acid profile, *Artemia* sp., fatty acid profile, fermentation, nutrition, production

1 | INTRODUCTION

Phronima sp. is a natural feed that has many excellent qualities and the potential for economic development. The positive attributes of *Phronima* sp. include a high nutrient content, a size suitable for the mouths of fish and shrimp, and easy mass culturing requirements. *Phronima* sp. is a non-selective filter feeder so the addition of nutrients to these organisms can be achieved through the culture media (Aoki, Matsumoto-Ohsima, Hirose, and Nishikawa, 2013). The nutritional content of *Phronima* sp. depends on the culture media used. The media serve as a source of feed for *Phronima* sp. The growth performance, biomass production and nutritional content of *Phronima* sp. are influenced by various factors, one of which is the composition of nutrients in the culture media because this is related to the fulfilment of the micronutrient and macronutrient needs of these organisms (Fattah, Saenong, & Busaeri, 2014; Herawati, Nugroho, Pinandoyo, Darmanto, & Hutabarat, 2017).

Nutrient requirements can be fulfilled by the addition of organic fertilizers to the culture media. To meet the nutritional needs with natural feed during culturing, the fertilizer needs to be added to the culture media with the aim of increasing the contents of nitrogen, phosphorus and potassium to cultivate more phytoplankton that serve as a feed to meet the nutritional needs of aquacultural organisms (Dwicaksono, Suharto, & Susanawati, 2014; Herawati, Nugroho, Pinandoyo, Darmanto, & Hutabarat, 2018; Indarmawan, Mubarak, & Mahasri, 2012). Research using various fermented animal manures has been performed on *Daphnia magna* and *Tubifex* mass cultures. Based on these results, the use of chicken manure was the best treatment for the nutritional quality and growth performance of these organisms. The use of organic fertilizers, including chicken manure mixed with rice bran and tofu waste with probiotic bacteria, in mass culture media for *Phronima* sp. has not been currently investigated, as the use of organic fertilizer could impact the growth performance and nutrient content of *Phronima* sp. Nutrients supplied at the highest levels, particularly the nitrogen (N), phosphorus (P) and calcium in organic fertilizer, are feed sources for *Phronima* sp. Herawati et al. (2017), in their study, examined chicken manure containing N (4.75%); P (3.57%) and Ca (4.80%); furthermore, the analysis of dried tofu waste materials was performed by Liswahyuningsih, Khotimah, and Febriana (2011) and Herawati et al. (2017), and the materials contained crude protein (27.09%), crude fibre (22.85%), fat (7.37%), ash (35,02%) and nitrogen-free extract (6.87%). Purbowati, Sutrisno, Baliarti, Budhi, and Lestariana (2007) and Herawati et al. (2017) explained that rice bran contained crude protein (12.63%), crude fibre (0.13%), crude fat (4.63%), ash (4.19%) and nitrogen-free extract (58.42%).

The fermentation of fertilizer has been proven to be effective in increasing the nutrient content of culture media. Herawati, Hutabarat, and Radjasa (2015) explained that the aims of fermentation are to produce a feed material that contains higher nutrient contents, a longer storage time and better organoleptic characteristics and nutritional qualities. Probiotic bacteria are supportive of the health of organisms (Nwachi, 2013). These bacteria also serve to decompose and ferment organic materials (Yuniwati, Iskarima, & Padulemba, 2012). Decomposition is a biological process by which most bacteria are able to produce growth substances, hormones, vitamins and other enzymes (Gunawan & Subhan, 2012).

The purpose of this study was to examine the population density and biomass production as growth performance parameters, and quality of nutrients through proximate, amino acid and fatty acid analysis of *Phronima* sp. mass cultured using fermented organic fertilizer including chicken manure, rice bran and tofu waste. The fertilizer was fermented with probiotic bacteria (*Lactobacillus casei* and *Saccharomyces cerevisiae*).

2 | MATERIALS AND METHODS

2.1 | Fermentation of organic materials

The fermentation of the organic matter began with activating the probiotic bacteria (*L. casei* and *S. cerevisiae*) by mixing probiotic bacteria of *Lactobacillus* sp. with 1 ml dosage and molasses with 100 L dosage then allowing the mixture to stand for ±30 min. After that, the organic materials including rice bran, tofu waste and chicken manure were weighed according to the dose of each treatment and blended until smooth and homogeneous. The blended organic material was then added to the activated probiotic bacteria (*L. casei* and *S. cerevisiae*) and then stirred until blended. The probiotic bacteria were bought commercially at a density of 1.5 × 106 CFU/ml. The organic material mixture was then placed into a tightly sealed container and allowed to stand for 28 days (Herawati et al., 2018). After the fermentation was completed, the organic materials were added to the maintenance media of *Phronima* sp. periodically (once every 3 days).

2.2 | *Phronima* sp. culture

Phronima sp. were used as test animals and maintained at a stocking density of 3 individuals/L. The initial weight of Phronima sp. was 0.05 g for each individual. The culture media were seawater with a volume of 500 L. The seawater was prepared first; afterwards, the culture media were formulated with 200 g/L dosage. Culture media were amended with fertilizer made of organic matter according to each treatment and were applied for 1 day. After 1 day, *Phronima* sp. was stocked and maintained for 24 days, and fertilizer was added every 3 days; water quality measurements are carried out daily. The treatments with various combinations of organic fertilizers of different compositions were based on the research of Herawati et al. (2017) and Herawati et al. (2018) and were as follows:

- A Treatment: 0% chicken manure, 50% rice bran and 50% tofu waste.
- B Treatment: 25% chicken manure, 37.5% rice bran and 37.5% tofu waste.
- C Treatment: 50% chicken manure, 25% rice bran and 25% tofu waste.
- D Treatment: 75% chicken manure, 12.5% rice bran and 12.5% tofu waste.
- E Treatment: 100% chicken manure.

2.3 | Population density

The population density of *Phronima* sp. was calculated once every 2 days during the 36 days of mass culture. Population density calculations were performed by taking 1 L of culture media at six sampling points and then calculating the number of *Phronima* sp.

presents. The six sampling points included two points at the right and left ends of the front of the tub, two points at the right and left ends of the back of the tub and two points at the right and left ends of the centre of the tub. At the time of sampling, the culture media were stirred in advance so that the *Phronima* sp. was spread evenly. The calculations were performed with three repetitions (Darmawan, 2014).

2.4 | Biomass production of *Phronima* sp

Measurement of biomass (wet weight) production of *Phronima* sp. aimed to find out the number of the production of *Phronima* sp. in a practical and simple method (Wardhana, 2003). The calculation of *Phronima* sp. biomass production used the following formula:

$$W = W_t - W_0$$

Description:

W = Biomass of *Phronima* sp. (g).
W_0 = Initial weight of *Phronima* sp. (g).
W_t = Final weight of *Phronima* sp. (g).

2.5 | Water quality

During the study, the water was maintained at a temperature of 28–30°C, 3.5–4.5 ppm of dissolved oxygen (DO) and 8.1–8.2 pH, which is in the ideal range. This is in line with the statement of Fattah et al. (2014) that the proper conditions for *Phronima* sp. culture are a temperature of 25–30°C, DO of 3.5–4.5 ppm and pH of 6.5–9. Excellent water quality helps grow phytoplankton and algae to advance the growth of *Phronima* sp.

2.6 | Statistical analysis

This research employed a completely randomized design of five treatments with three replications. The biomass was analysed using analysis of variance (ANOVA) to determine differences among treatments. The parameters analysed were the growth, biomass production and nutritional content of *Phronima* sp.

2.7 | Biochemical analysis

2.7.1 | Proximate analysis

The proximate chemical composition of the samples was determined using a standard procedure (AOAC, 2005 and Herawati et al., 2017). The crude protein content was calculated by multiplying the total nitrogen factor. The carbohydrate content was estimated by the difference.

2.7.2 | Essential amino acid profile

The amino acid composition of the samples was determined using high-performance liquid chromatography (HPLC) (Shimadzu LC-6A) (AOAC, 2005 and Herawati et al., 2017).

2.7.3 | Fatty acid profile

The fatty acid composition of the samples was determined using a gas chromatograph (Shimadzu) (AOAC, 2005 and Herawati et al., 2017).

3 | RESULTS

The results organic fertilizer nutrient analysis from all treatments for *Phronima* sp. mass culture which was used in the study is presented in Table 1. Based on the results of the analysis of NPK contents from fermented culture media for 28 days, the highest value was on the B treatment (25% of chicken manure, 37.5% of rice bran and 37.5% of tofu waste); they were 6.73% of nitrate, 4.82% of Potassium and 3.25% of Phosphor.

3.1 | Population density

Phronima sp. were cultured for 36 days using fermented organic fertilizer (chicken manure, rice bran, tofu waste) as a culture medium. During the 36 days of the maintenance period of the *Phronima* sp. mass culture process, growth of *Phronima* sp. was calculated 18 times, once every 2 days. The growth of the *Phronima*

TABLE 1 Analysis of organic fertilizer nutrient in all treatments for *Phronima* sp. mass culture which was used in this research

	Results (%)					
Parameters	A	B	C	D	E	Methods
Nitrogen (N)	4.73	6.73	2.49	3.94	3.14	Kjeldhal
Potassium (K)	3.12	4.82	2.35	2.47	2.85	AOAC 983.02.2000
Phosphor (P)	2.26	3.25	2.06	1.60	2.73	AOAC 983.02.2000

Note: Description: The treatments were A: 0% chicken manure, 50% rice bran, 50% tofu waste; B: 25% chicken manure, 37.5% rice bran, 37.5% tofu waste; C: 50% chicken manure, 25% rice bran, 25% of tofu waste; D: 75% chicken manure, 12.5% rice bran, 12.5% tofu waste; E: 100% chicken manure.

sp. mass cultured using fermented organic fertilizer is presented in Figure 1.

The growth pattern had the same pattern for each treatment. The highest growth was realized with B treatment (25% chicken manure, 37.5% rice bran and 37.5% tofu waste) on the 16th day of maintenance, which was as many as 98 individuals/L. The lowest population density was observed with the E treatment (100% chicken manure, 0% rice bran and 0% tofu waste) on the 16th day of maintenance, which was as many as 73 individuals/L.

3.2 | Biomass production of *Phronima* sp

Biomass production was obtained by weighing *Phronima* sp. twice—before dispersion stocking (W_0) and on the last day of maintenance or on the 36th day (W_t). The *Phronima* sp. biomass production achieved with mass culture using fermented organic fertilizer is presented in Figure 2.

The highest biomass production was realized with B treatment (25% chicken manure, 37.5% rice bran and 37.5% tofu waste), which was as much as 0.51 grams, while the lowest biomass production was observed for the E treatment (100% chicken manure, 0% tofu waste and 0% rice bran) and was as much as 0.07 grams.

3.3 | Nutrient content of *Phronima* sp

The highest values of protein and fat found, which were 58.90% and 8.24%, respectively, in the nutritional quality analysis of *Phronima* sp. was observed for the fermented culture media containing 25% chicken manure, 37.5% tofu waste and 37.5% rice bran (B). The lowest protein and fat contents, which were 41.26% and 5.04%, respectively, were found for the fermented culture media containing 100% chicken manure, 0% tofu waste and 0% rice bran (E). The proximate analysis results for *Phronima* sp. mass cultured using fermented organic fertilizer as culture media are presented in Table 2.

Based on the results of the study, the highest fatty acid profile was observed for *Phronima* sp. mass cultured using fermented culture media containing 25% chicken manure, 37.5% tofu waste and 37.5% rice bran (B) as well as the highest EPA fatty acid contents of as much as 7.53%. The lowest fatty acid profile was observed in *Phronima* sp. mass cultured using fermented culture media containing 100% chicken manure, 0% tofu waste and 0% rice bran (E), with EPA fatty acids levels of as much as 2.64%. The results of the analysis of the total fatty acid profiles of *Phronima* sp. mass cultured using fermented organic fertilizer during the study are presented in Table 3.

The highest amino acid contents were observed in *Phronima* sp. mass cultured using fermented culture media contai7ning 25% chicken manure, 37.5% tofu waste and 37.5% rice bran (B), with the highest lysine amino acid level of 44.16 ppm. The lowest amino acid contents were observed in *Phronima* sp. mass cultured using fermented culture media containing 100% chicken manure, 0% tofu waste and 0% rice bran (E), with lysine amino acid contents of as much as 20.59 ppm. The highest and lowest yield difference in lysine essential amino acids was 23.57 ppm. The results of the analysis of the total amino acid profile of *Phronima* sp. mass cultured using fermented organic fertilizer during the study are presented in Table 4.

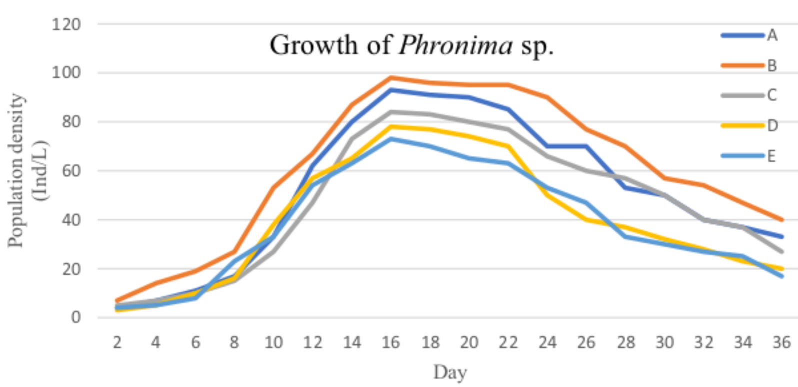

FIGURE 1 The growth of *Phronima* sp. mass cultured using fermented organic fertilizer

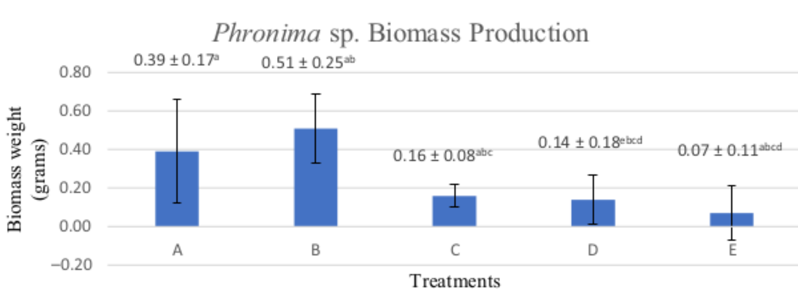

FIGURE 2 The biomass production of *Phronima* sp. mass cultured using fermented organic fertilizer during research

TABLE 2 The results of proximate analysis of *Phronima* sp. mass cultured in fermented organic fertilizer medium during the study

	Dry weight content percentage				
Treatments	Protein (%)	Carbohydrate (%)	Crude fat (%)	Ash (%)	Crude fibre (%)
A	45.45 ± 0.02	15.07[a] ± 0.05	7.57[a] ± 0.02	26.13[a] ± 0.03	5.78[a] ± 0.03
B	58.90[b] ± 0.04	14.22[a] ± 0.03	8.24[a] ± 0.03	16.19[b] ± 0.03	2.45[b] ± 0.08
C	40.44[a] ± 0.06	14.87[a] ± 0.05	5.89[b] ± 0.02	30.61[b] ± 0.02	5.19[b] ± 0.05
D	42.07[a] ± 0.02	13.25[a] ± 0.02	5.40[b] ± 0.02	33.68[b] ± 0.06	5.60[b] ± 0.02
E	41.26[a] ± 0.06	14.40[a] ± 0.02	5.04[b] ± 0.04	33.67[b] ± 0.07	5.63[b] ± 0.06

[a]The value was not significantly different.

[b]The value was significantly different.

TABLE 3 Fatty acid profile of *Phronima* sp. mass cultured in fermented organic fertilizer medium during the study

Fatty acids profile	A (%)	B (%)	C (%)	D (%)	E (%)
Myristic	0.52[a] ± 0.05	0.48[a] ± 0.09	0.41[a] ± 0.02	0.50[a] ± 0.05	0.49[a] ± 0.04
Pentadecanoic	0.09[b] ± 0.06	0.15[a] ± 0.08	0.17[a] ± 0.304	0.08[a] ± 0.02	0.18[a] ± 0.06
Palmitic	3.14[b] ± 0.09	5.59[b] ± 0.04	1.97[a] ± 0.08	2.12[b] ± 0.01	2.29[b] ± 0.08
Stearic	2.71[b] ± 0.07	2.91[b] ± 0.09	0.52[a] ± 0.03	2.08[b] ± 0.05	1.65[b] ± 0.02
Oleic/ω9	3.07[b] ± 0.02	2.61[b] ± 0.01	0.89[a] ± 0.08	2.55[b] ± 0.03	0.95[a] ± 0.03
Linoleic/ω6	4.83[b] ± 0.09	5.37[b] ± 0.02	2.49[b] ± 0.07	3.75[b] ± 0.02	2.46[b] ± 0.07
Linolenic/ω3	3.54[b] ± 0.05	3.32[b] ± 0.01	2.39[b] ± 0.03	2.56[b] ± 0.07	2.38[b] ± 0.09
Arachidic	2.30[b] ± 0.08	3.05[b] ± 0.03	1.02[a] ± 0.04	1.25[a] ± 0.05	2.83[b] ± 0.02
Arachidonic	0.07[a] ± 0.02	0.13[a] ± 0.08	0.15[a] ± 0.02	0.06[a] ± 0.08	0.15[a] ± 0.05
Eicosapentaenoic	0.06[a] ± 0.07	3.52[b] ± 0.06	0.50[a] ± 0.04	0.05[a] ± 0.02	2.53[a] ± 0.09
AA	2.71[b] ± 0.03	0.13[a] ± 0.07	0.15[a] ± 0.09	2.18[b] ± 0.03	0.15[a] ± 0.04
DHA	0.83[a] ± 0.05	1.07[a] ± 0.03	0.07[a] ± 0.01	0.39[a] ± 0.08	0.08[a] ± 0.04
EPA	6.05[b] ± 0.02	7.53[b] ± 0.08	3.68[b] ± 0.02	4.03[b] ± 0.05	2.64[b] ± 0.07

Note: Description: The treatments were A: 0% chicken manure, 50% rice bran, 50% tofu waste; B: 25% chicken manure, 37.5% rice bran, 37.5% tofu waste; C: 50% chicken manure, 25% rice bran, 25% of tofu waste; D: 75% chicken manure, 12.5% rice bran, 12.5% tofu waste; E: 100% chicken manure.

[a]The value was not significantly different.

[b]The value was significantly different.

4 | DISCUSSION

Organic fertilizer in the form of chicken manure mixed with rice bran and tofu waste fermented for 28 days was used in *Phronima* sp. mass culture media to produce different growth results. The results showed that *Phronima* sp. mass cultured using fermented culture media containing 25% chicken manure + 37.5% rice bran + 37.5% tofu waste produced the highest growth of 98 individuals/L (Figure 1), and the highest biomass was 0.51 gram (Figure 2). This was due to differences in the nutrient content of the culture media under each treatment. *Lactobacillus* sp. and *Saccharomyces cerevisiae* were added as probiotic bacteria in the fermentation process to increase the protein content in the organic fertilizer. Probiotic bacteria degrade complex compounds into simpler compounds and synthesize proteins so that they will be easier to digest. This statement is in accordance with Hersoelistyorini, Sumanto, and Najih (2010), who stated that the fermentation process can increase energy, protein and crude fibre contents. The fermentation process can produce enzymes because these bacteria degrade complex compounds into simpler forms and synthesize proteins so that they will be more easily digested and decomposed into energy to support the growth of cultured organisms.

Nutrients from culture media are very influential for the supply of plankton and bacteria to increase the population growth and biomass of *Phronima* sp. The results of this study were confirmed by Siswati & Theodorus (2009), who stated that probiotic bacteria such as *L. casei* and *S. cerevisiae* have unique properties as fermenters because they can neutralize acidic or basic organic matter. Proteolytic microbes are able to produce protease enzymes that will breakdown proteins, and proteins that have been overhauled are converted into polypeptides and subsequently become simple peptides; then, these peptides are overhauled into amino acids. These amino acids are used by microbes to multiply themselves. The number of microbial colonies that are sources of single-cell protein can increase during the fermentation process.

TABLE 4 Amino acid profile of *Phronima* sp. mass cultured in fermented organic fertilizer medium during the study

	Phronima sp.				
Amino acid	A	B	C	D	E
L-aspartic acid	48.92 ± 0.08[a]	53.94[a] ± 0.01	45.52[a] ± 0.05	36.14[a] ± 0.05	37.85[a] ± 0.05
L-serine	15.61 ± 0.03[b]	17.62[b] ± 0.01	17.63[b] ± 0.07	13.40[b] ± 0.05	14.76[b] ± 0.02
L-glutamic acid	76.61 ± 0.04[b]	72.37[b] ± 0.07	64.35[b] ± 0.03	54.39[b] ± 0.03	57.36[b] ± 0.07
Glycine	19.36 ± 0.04[b]	19.19[b] ± 0.01	18.78[b] ± 0.06	16.48[b] ± 0.04	17.33[b] ± 0.02
L-histidine	9.78 ± 0.03[b]	9.70[b] ± 0.01	9.50[b] ± 0.05	8.48[b] ± 0.04	8.65[b] ± 0.03
L-arginine	20.51 ± 0.04[b]	27.28[b] ± 0.01	27.89[b] ± 0.08	18.56[b] ± 0.05	20.85[b] ± 0.07
L-threonine	19.02 ± 0.09[b]	20.37[b] ± 0.01	20.56[b] ± 0.03	16.17[b] ± 0.02	18.47[b] ± 0.07
L-alanine	40.65 ± 0.05[a]	32.51[b] ± 0.09	32.98[a] ± 0.05	29.21[a] ± 0.05	32.51[a] ± 0.01
L-proline	20.25 ± 0.05[b]	19.00[b] ± 0.06	17.44[b] ± 0.04	18.98[b] ± 0.07	18.08[b] ± 0.09
L-valine	30.24 ± 0.05[a]	28.87[b] ± 0.04	28.13[b] ± 0.02	23.74[a] ± 0.04	25.72[b] ± 0.03
L-methionine	11.10 ± 0.08[b]	10.40[b] ± 0.04	13.40[b] ± 0.05	7.82[b] ± 0.04	10.89[b] ± 0.06
L-Lysine HCl	35.57 ± 0.04[a]	44.16[a] ± 0.01	25.99[b] ± 0.03	31.56[a] ± 0.06	20.59[b] ± 0.06
L-isoleucine	19.97 ± 0.03[b]	22.79[b] ± 0.04	21.20[b] ± 0.07	16.95[b] ± 0.05	18.87[b] ± 0.02
L-leucine	32.44 ± 0.05[a]	36.88[b] ± 0.05	35.46[a] ± 0.07	30.67[a] ± 0.04	31.47[a] ± 0.05
L-phenylalanine	15.49 ± 0.07[b]	16.98[b] ± 0.10	15.97[b] ± 0.05	14.62[b] ± 0.03	14.41[b] ± 0.07

Note: The treatments were A: 0% chicken manure, 50% rice bran, 50% tofu waste; B: 25% chicken manure, 37.5% rice bran, 37.5% tofu waste; C: 50% chicken manure, 25% rice bran, 25% of tofu waste; D: 75% chicken manure, 12.5% rice bran, 12.5% tofu waste; E: 100% chicken manure.

[a]The value was not significantly different.

[b]The value was significantly different.

The poorest growth results were obtained with *Phronima* sp. mass cultured using 100% chicken manure, 0% rice bran and 0% tofu waste and were as many as 73 individuals/L. The growth of *Phronima* sp. showed the same pattern on maintenance media. Although the amount of *Phronima* sp. in each medium was different, the phase of increasing and decreasing the amount that was produced was relatively similar. The difference in the number of *Phronima* sp. in each different maintenance medium showed that the nutrient content in each medium was different, and the nutrients affected the growth of *Phronima* sp. The difference in the population densities was due to the ability of cells to utilize nutrients for growth.

The role of probiotic bacteria as biological control in culture media is to suppress the growth of pathogenic bacteria, accelerate the degradation of organic matter and waste, increase the availability of essential nutrients and do nitrogen fixation. Probiotic bacteria also affect the speed of organic fertilizer fermentation which is used as a culture medium so that it can be used directly by phytoplankton that grows in culture media as food for zooplankton so as to increase population density and biomass production of *Phronima* sp. The fermentation process that uses the probiotic bacteria *Lactobacillus* sp. able to decompose complex compounds to be simple ones so that it can be utilized by fish and microorganisms which are able to synthesize vitamins and amino acids needed. This statement is in line with the results of previous study conducted by Fuller (1992) that the fermentation process is able to break down complex compounds to be simple one so that it can be used by fish and a number of microorganisms which able to synthesize vitamins and amino acids needed by aquatic animal larvae.

The role of *Lactobacillus* sp. is able to balance the digestive tract microbes so that it can improve the digestibility of fish by converting carbohydrates to lactic acid which can reduce pH, thereby stimulating enzyme production. This statement is in accordance with Arsyad and Muharam (2015) that the *Lactobacillus* sp. able to balance the digestive tract microbes so as to increase the digestibility of fish by converting carbohydrates to lactic acid which can reduce pH, thereby stimulating the production of endogenous enzymes to increase nutrient absorption, feed consumption, growth and inhibit pathogenic organisms. Crab, Avnimelech, Defoirdt, Bossier, and Verstraete (2007) stated that growth is defined as population growth and weight over certain time. The excess energy needed for maintenance and bodily activities is used for growth. *Lactobacillus* sp. and *S. cerevisiae* will move when they enter digestion by growing and colonizing. Lactobacillus will convert carbohydrates into lactic acid; then, lactic acid can create a lower pH atmosphere. In acidic conditions, *Lactobacillus* has the ability to inhibit pathogenic bacteria and spoilage bacteria so that the nutrients present in the culture media can be maximally utilized by phytoplankton.

The nutrients in culture media affect the amount of phytoplankton contained in the media, and the nitrate levels determine the amount of phytoplankton as it functions as a natural feed source for *Phronima* sp. in addition to the bacteria and detritus contained in the media. Based on the results of the study, the abundance of plankton that grew and dominated the culture media was *Chlorella* sp. Darmawan (2014), in his research, explained that the greater the abundance of phytoplankton and organic matter contained in the media is the faster the growth rate.

In general, each treatment was showed a phase of very high population increases before finally undergoing a drastic decrease. In each treatment, *Phronima* sp. showed the highest phase from the 16th day to the 36th day during maintenance, before finally showing a decrease in the population on the 36th day. The growth pattern in this study indicates that a longer maintenance time is required for *Phronima* sp. compared with that used in previous research conducted by Fattah et al. (2014), in which results were obtained on the 17th day. *Phronima* sp. experienced an increase in numbers and then a decrease on the 24th day.

There was a significant population decrease after *Phronima* sp. experienced an increase in population density. This may have been caused by *Phronima* sp. entering the death phase. This death phase can be caused by plankton deaths that occur much faster than plankton production. This phase is caused by the accumulation of organic matter, which ultimately inhibits growth. This statement is in accordance with the results of research by Aoki et al. (2013), who stated that the decrease in the number of *Phronima* sp. because of the ineffective use of excess nutrients produces a mass of organic material that is toxic and can ultimately inhibit growth. If nutrients are added to the culture media in excessive amounts, they become toxic, which can inhibit growth, because the effectiveness of cell metabolism is directly disrupted.

Growth and biomass production of *Phronima* sp., in addition to being influenced by the nutrient content in the culture media, are also influenced by environmental conditions. According to Darmawan (2014), this pattern of plankton growth is influenced by several factors, including the physical condition of the waters, the type of feed and the concentration of the feed. When all three factors are ideal, the growth rate of plankton will increase, and more population peaks will occur. One of the influential environmental conditions is the water quality of the maintenance media of *Phronima* sp. *Phronima* sp. culture media during the maintenance period is controlled by measuring the water quality every day. Water quality measurements are carried out by means of in situ measurements.

Nutritional quality based on proximate analysis, as shown in Table 2, showed that the highest protein and fat contents of *Phronima* sp. were realized with (B) treatment and were as much as 58.90% and 8.24%, while the lowest protein and fat contents observed with (E) treatment and were as much as 41.26% and 5.04% respectively. The high protein content and low fat obtained in this study were caused by the high nutrient content in the *Phronima* sp. culture media; the higher the nitrate and phosphate contents are, the higher the amount of protein produced. Widianingsih, Ridho, Hartati, and Harmoko (2008) stated that the higher the N and P contents are, the higher the amount protein in the culture media. The fat content is inversely proportional to the protein content. The results of this study were supported by Lim, Aksoy, and Klesius (2011), which stated that the higher protein content is always inversely proportional to the fat content because fat in the body works twice as hard compared with protein.

The total fatty acid profile (Table 3) showed that the highest content of EPA fatty acids in *Phronima* sp. was obtained with (B) treatment and was much as 7.53%, while the lowest fatty acid content was obtained with (E) treatment and was 2.64%. EPA fatty acids function as a basic substrate in the formation of long chains PUFAs and help avoid blood clotting. The results of this study are reinforced by the studies of Pratiwi, Hardjito, and Goreti (2009), Zengin, Vural, and Çelik (2013) and Herawati et al. (2015), which confirm that EPA fatty acids serve as a basic substrate to form long chains of DHA.

The results showed that the amino acid content of *Phronima* sp. mass cultured using organic fertilizer with a composition of 25% chicken manure + 37.5% rice bran + 37.5% tofu waste (B) was 44.16%. The lowest amino acid content was observed in the treatment of *Phronima* sp. mass cultured using fermented organic fertilizer with a composition of 100% chicken manure + 0% rice bran + 0% tofu waste (E and was 20.59%. The function of the amino acid lysine in the research of Ovie and Ovie (2006), Valverde et al. (2013) and Herawati et al. (2015) was as a structural framework of vitamin B1 and an anti-virus compound, helping in the absorption of calcium, stimulating appetite and aiding the production of carnitine to convert fatty acids into energy.

5 | CONCLUSION

Based on the results of this study, *Phronima* sp. mass cultured in media with fermented organic fertilizer including 25% chicken manure + 37.5% rice bran + 37.5% tofu waste produced the highest growth and biomass production. The highest nutritional quality based on proximate analysis, and the fatty acid and amino acid profiles obtained with the same media. Therefore, we can conclude that the existence of probiotic bacteria in organic fertilizer affect the quality of culture medium besides the medium's formulated compositions. Thus, *Phronima* sp. could be considered an alternative natural feed substitute for *Artemia* because the nutritional content of *Phronima* sp. specifically, the EPA and DHA contents, is higher than *Artemia*. Therefore, *Phronima* sp. can be used as a substitute for *Artemia* in shrimp feed in particular.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interests.

AUTHOR CONTRIBUTIONS

Vivi Endar Herawati conceived the study design, performed the experiments, wrote the manuscript and edited the manuscript. Pinandoyo conceived the study design, performed the experiments and did the data analysis. Nurmanita Rismaningsih did the data analysis, wrote the manuscript and edited the manuscript. YS. Darmanto conceived the study design, performed the experiments and did the data analysis. Johannes Hutabarat performed the experiments and did the data analysis. Ocky Karna Radjasa performed the experiments and did the data analysis.

ETHICAL APPROVAL

The research did not need any ethical approval to be conducted.

DATA AVAILABILITY STATEMENT

The authors confirm that all data generated or analysed during this study, and the data supporting the findings of this study are available within the article.

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