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Growth performance and nutritional quality enrichment of *Phronima pacifica* by *Chlorella vulgaris* and *Chaetoceros calcitrans* as natural feed

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Abstract. *Herawati VE, Nailulmuna Z, Rismaningsih N, Hutabarat J, Pinandoyo, Elfitasari T, Riyadi PH, Radjasa OK. 2020. Growth performance and nutritional quality enrichment of* Phronima pacifica *by* Chlorella vulgaris *and* Chaetoceros calcitrans *as natural feed. Biodiversitas 21: 4253-4259. Phronima pacifica* as a natural feed has the potential to replace *Artemia* sp. because of its high nutritional content. The purpose of this study was to determine the effects of different types of feed for *P. pacifica* during its culture on its population density, growth rate, and nutritional content because of its potential use as a natural feed for fish. The test animals in this study consisted of *P. pacifica* at a stocking density of 3 ind./L, which were cultured for 18 d. Then, *Chlorella vulgaris* and *Chaetoceros calcitrans* were introduced as enrichment feed for *P. pacifica*. This study used a completely randomized design with three treatments and three replications. The treatments were as follows: A (100% *C. vulgaris*), B (100% *C. calcitrans*), and C (50% *C. vulgaris* and 50% *C. calcitrans*). The results showed that treatment increased the population density of *P. pacifica* by up to 54.67 ± 0.0038 ind./L by the 12th day. Based on the results of proximate analysis for amino acid and fatty acid profiles, the highest values for proteins and fats were in *P. pacifica* enriched with *C. vulgaris* (A) and consisted of 45.45% protein, 7.57% fat, 5.95% eicosapentaenoic acid, and 39.23% lysine. Based on an ANOVA, feeding of *P. pacifica* with *C. vulgaris* and *C. calcitrans* had a significant effect on population density, relative growth rate, biomass production, and nutrient value of proteins and fats of *P. pacifica* ($P < 0.05$). The best results, including population density, growth rate, weight of biomass, and nutrient content of *P. pacifica*, occurred after feeding with *C. vulgaris*.

Keywords: Amino acid, *Chaetoceros calcitrans*, *Chlorella vulgaris*, fatty acid, *Phronima pacifica*

INTRODUCTION

Phronima pacifica is a type of Amphipoda microcrustacean that lives at a depth from 0 to 25 m below sea level (Elder and Siebel 2015). Amphipoda has six sub-orders: Pseudingolfiellidea, Hyperiidea, Colomastigidea, Hyperiopsidea, Senticaudata, and Amphilochidea (Lowry and Myers 2017). *P. pacifica* belongs to the Hyperiidea suborder (Bishop and Geiger 2006). *P. pacifica* could be used as an alternative natural feed or a replacement for *Artemia* sp. *P. pacifica* has a high nutrient content, a size suitable for the mouth gap of fish and shrimp, and is easily mass cultured. *P. pacifica* is a nonselective filter feeder; thus, the addition of nutrients can be completed through aquaculture media (Aoki et al. 2013). The nutrient content of *P. pacifica* depends on the culture media used because it acts as its feed source (Fattah et al. 2014; Herawati et al. 2015).

Amphipoda can serve as a source of natural feed in aquaculture (Baeza-Rojano et al. 2013). *P. pacifica* has the potential to become a natural feed and serve as a substitution for *Artemia* sp. (Fattah et al. 2014), which is generally given to post-larvae shrimp. The selection of natural feed has various criteria such as the mouth gap of fish larvae and a nutritional content suitable for the needs of the cultured fish or shrimp (Herawati et al. 2015). The nutritional content of *P. pacifica* is particularly high in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); therefore, efforts are needed to support the development of *P. pacifica,* which can be a natural feed substitution, thereby reducing the use of the relatively expensive *Artemia* sp. Previous research regarding the replacement of *Artemia* sp. has focused on *Phronima* sp. as a natural feed substitute for tiger shrimp aquaculture (*Penaeus monodon*) and *Litopenaeus vannamei* post-larvae. Research conducted by Fattah et al. (2014) reported a survival rate of more than 80% and Herawati et al. (2020) using *Phronima* for vanname shrimp reported a survival rate of 95%.

Amphipoda belongs to zooplankton, which is a natural food for wild fish (Dalpadado and Bogstad 2004). The abundance of *P. pacifica* has been influenced by the feed in the culture media with the type of feed used being *Chlorella vulgaris* and *Chaetoceros calcitrans*. The nutrient content of *Chlorella* sp. was 45% protein, 20% fat, 20% carbohydrates, and 40% EPA (Fattah et al. 2014). Valverde et al. (2013) reported on microalgae that have high nutritional content, one of which was *Chaetoceros* sp. It had nutrient content consisting of proteins, carbohydrates, and fat with a percent dry weight of the *Chaetoceros* sp. microalgae diet consisting of 36% protein, 10% fat, 15% carbohydrate, and 15% EPA (Sihombing et al. 2013, Méndez-Martínez et al. 2018).

The type of feed affects the growth process and the nutritional quality of *Phronima* sp. The type of feed is also a factor that can support the development of *P. pacifica* as a natural feed substitute for *Artemia* sp. To date, there have been no studies that report on the effects of providing different types of natural feed on the nutritional content of *P. pacifica*. Previous research conducted by our team using various animal manures under a fermentation process was conducted using *Daphnia magna* and *Tubifex* mass culture. Based on the results, the use of chicken manure was the best treatment, providing higher nutritional quality and growth performance than did that of other treatments. The purpose of this study was to determine the growth performance, biomass production, and the quality of nutrients through proximate amino acids and fatty acids of *P. pacifica*.

MATERIALS AND METHODS

Phronima pacifica culture

Phronima pacifica, which were used as test animals, were kept at a stocking density of 3 ind./L. The culture media used was seawater with fine sand was added as a substrate. The seawater was first formulated, and then water for the culture media was prepared. After 1 d, *P. pacifica* was stocked and cultured for 18 d, and fertilizer was added every 3 d. Growth observations and water quality measurements were recorded daily. The dietary enrichment used in this study consisted of the addition of *C. vulgaris* and/or *C. calcitrans*. *P. pacifica* feeding treatments were: A (100% *C. vulgaris*), B (100% *C. calcitrans*.), C (50% *C. vulgaris* and 50% *C. calcitrans*). Feeding was conducted at 10^5 cells/individual density and occurred twice daily.

Chlorella vulgaris culture

The culture formula for *Chlorella* sp. was from a mass scale natural feed laboratory at BBBPAP Jepara. The formulation for the culture of *Chaetoceros* sp. at a bulk scale consisted of urea fertilizer 80 ppm, SP-36 40 ppm, ZA 60 ppm; NPK 1 ppm, and EDTA 5 ppm. Furthermore, the formulation was placed in 1000 L of sterile seawater and aerated continuously. To this media, 1 L of pure *Chaetoceros* sp. seeds was inserted. After 3 d, the media was ready to be used as feed for *Phronima* sp. The harvesting process was conducted by filtering with a 10 µm plankton net. The harvested contents were then used as *P. pacifica* feed.

Chaetoceros calcitrans culture

The formulation for the culture of *Chaetoceros* sp. at a bulk scale consisted of sodium dihydrogen phosphate ($NaOH_2PO_4$) 150 ppm, KNO_3 400 ppm, Na_2SiO_3 80 ppm, EDTA 50 ppm, $FeCl_3$ 10 ppm, and Vitamin B_{12} 0.01 ppm. Furthermore, the formulation was placed in 1000 L of sterile seawater and aerated continuously. To this media, 1 L of pure *Chaetoceros* sp. seeds was inserted. After 3 days, the media was ready to be used as feed for *Phronima* sp. The harvesting process was conducted by filtering with a 10 µm plankton net. The harvested contents were then used as *P. pacifica* feed.

Specific growth rate (r)

The specific growth rate (ind/day) was calculated using the Krebs formula (1972), as follows:

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

Where: r: growth rate (ind/day); t: days needed to achieve maximum growth (days); N_t: density of *Phronima* sp. on day t; N_0: initial density of *P. pacifica*.

Population density

The population density of *P. pacifica* was calculated daily during the 18 d of aquaculture. Population density calculations were performed by taking 1 L of culture media from six sampling points and calculating the number of *P. pacifica*. At the time of sampling, the culture media was stirred in advance such that *P. pacifica* were evenly spread. The calculations were performed with three repetitions (Aoki et al. 2013).

Biomass

Measurement of biomass production to determine the number of plankton produced was conducted with a practical and simple method (Krebs 1972). Calculation of *P. pacifica*. biomass production was calculated using the following formula:

$$W = W_t - W_0$$

Where: W: biomass (g); W_0: initial weight (g); W_t: final weight (g).

Nutrient content

Nutrient content was obtained from proximate analysis tests, which included proteins, carbohydrates, fats, crude fiber, and water content.

Proximate analysis

The proximate chemical composition of the samples was determined using a standard procedure (AOAC 2005; Herawati et al. 2018). The carbohydrate content was estimated based on the difference.

Essential amino acid profiles

The amino acid composition of the sample was determined using high-performance liquid chromatography (HPLC) (Shimadzu LC-6A) (AOAC 2005; Herawati et al. 2018). The essential amino acid profile of *P. pacifica* was determined by examining its essential amino acid content. The essential amino acid analysis was conducted using an HPLC type 1100 with a Eurospher 100-5 C18, 250 × 4.6 mm column with a P/N 1115Y535 pre-column. The effluents were A) 0.01 M acetate buffer at pH 5.9 and B) 0.01 M MeOH acetate buffer at pH 5.9. Conditions were THF > 80, 15:5 Λ fluorescence, Ext 340 nm, and Em 450 nm. Approximately 2.5 g of the sample was placed into a sealed glass. Then, 15 mL of HCl 6N was added. The mixture was then *vortexed* for homogeneity and underwent hydrolysis using an autoclave at 110°C for 12 h, before being cooled to room temperature and neutralized with NaOH 6 N. After the addition of 2.5 mL of 40% Pb acetate and 1 mL of 15% oxalate acid, approximately 3 mL of the mixture was filtered with 0.45 μm millex. For the injection for HPLC, 25 μL of the filtered mixture plus 475 μL of OPAA solution was *vortexed* and incubated for 3 min. Finally, 30 μL of the final mixture was added for the HPLC.

Fatty acid profile

The fatty acid composition of the samples was determined using gas chromatograph (Shimadzu) (AOAC 2005; Herawati et al. 2018). The fatty acid profile of *P. pacifica* could be determined by analyzing its total fatty acid content. The equipment used for this purpose was a gas chromatograph (GCMS) and a QP-2010 Mass Spectrophotometer with a W Cot fused Silica Coating CP-SIL-88 column with 50 m length, 0.22 mm diameter, and a column temperature of 120-200°C. The method employed was in-situ transcertification. A 100 mg sample of *P. pacifica* was homogenized using 4 mL of water. The resulting 100 μL homogenate was then transferred into a reaction tube. One hundred microliters of methylene chloride were then added, along with 1 mL of 0.5 N NaOH in methanol. Once nitrogen was added and the tube was sealed, it was heated to 90°C for 10 min. The reaction tube was then cooled, and 1 mL of 14% BF_3 in methanol was added. After the addition of nitrogen, heating continued at the same temperature for the next 10 min. Next, the reaction tube was cooled to ambient temperature, and 1 mL of water and 200-500 μL of hexane were added. The mixture was then *vortexed* for 1 min to extract the fatty acid methyl ester. After centrifugation, the upper layer of the sample was ready for GC analysis.

Water quality parameters

Measurement of water quality parameters, including temperature (°C), pH, salinity (ppt), and dissolved oxygen (mg/L), was performed daily in-situ. Dissolved oxygen (DO) was measured using a DO meter, the temperature was measured using a thermometer, and pH was measured using a pH meter. The results of the measurements of water quality parameters in *P. pacifica* are presented in Table 1.

Data analysis

Data analyzed included growth rate (r) and biomass weight. If the test results showed that the data were distributed normally and were homogeneous and additive, then the analysis was continued with a one-way analysis of variance (ANOVA) to determine the influence of the observed variables. If there was a significant effect ($P < 0.05$), then it was followed by Duncan's multiple range test to determine the pairwise difference in mean values between treatments, and consequently, the best treatment.

RESULTS AND DISCUSSION

Population density

Based on population density data during the study, a graph can be made which is presented in Figure 1. The growth pattern on each treatment had the same pattern. The highest growth was on the A treatment (fed by 100% *C. vulgaris*) on the 12th day of cultivation, which was as many as 58 individuals/L. The lowest population density was on C treatment (fed by 50% *C. vulgaris* and 50% *C. calcitrans*) on the 12th day of cultivation which was as many as 20 individuals/L.

Growth rate (r)

Based on data from the Growth Rate (r) of *P. pacifica* during the study, a histogram can be made and presented in Figure 2. Based on the ANOVA analysis of growth rates of *P. pacifica*, it showed that different treatment of sea grapes stocking density had a significant effect on the growth of *P. pacifica* ($P<0.05$). The growth rate histogram shows the highest growth rate of 0.2418 ind/day in the treatment of *P. pacifica* fed by 100% *C. vulgaris* (A), while the lowest growth rate is in the treatment of *P. pacifica* fed by 50% *C. vulgaris* and 50% *C. calcitrans* (C) about 0.1443 Ind/day.

Biomass production

Biomass production was obtained by weighing *Phronima* sp. twice, those were before dispersion stocking (W_0), and on the last day of cultivation or on the 18th day (W_t). *Phronima* sp. biomass production from enrichment with *C. vulgaris* and *C. calcitrans* is presented in Figure 3. The highest biomass production was 0.35 grams in the treatment of feeding 100% *C. vulgaris* (A), while the lowest biomass production was in the feeding treatment in the form of a combination of 50% *C. vulgaris* and 50% *C. calcitrans* which was 0.09 grams. Based on the ANOVA analysis of growth rates of *P. pacifica*, it showed that different treatment of sea grapes stocking density had a significant effect on the growth of *P. pacifica* ($P<0.05$).

Table 1. Results of water quality parameter measurement of *Phronima pacifica* aquaculture

Treatments	Water quality parameter range			
	Temp. (°C)	pH	DO (mg/L)	Salinities (ppt)
A	29.2-30.8	8.2	4.69-5.38	30
B	28.7-30.5	8.09	4.53-4.98	30
C	27.2-29.1	8.1	4.78-5.05	30
References	27-31	8.0-9.0	4.7-5.6	30

Note: The references were based on Fattah et al. (2014)

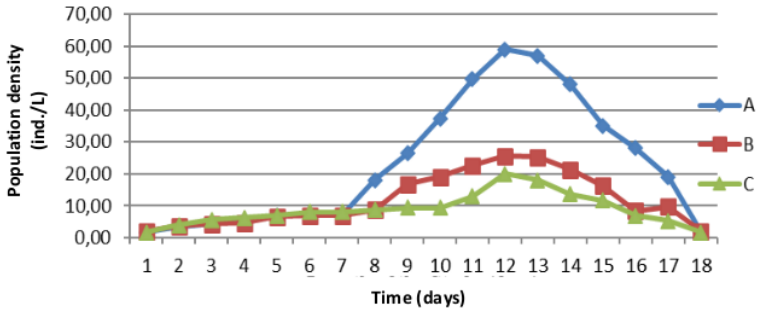

Figure 1. Population density of *Phronima pacifica* during the study (ind./L)

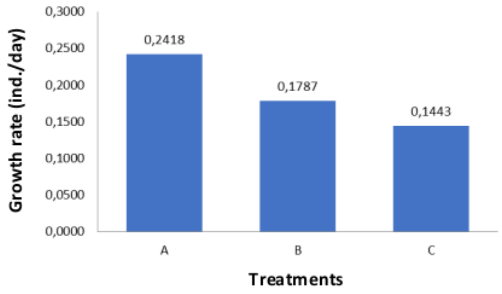

Figure 2. *Phronima pacifica* growth rate (r) during research (ind./day)

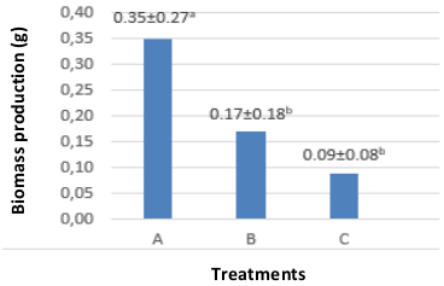

Figure 3. *Phronima pacifica* biomass production from enrichment with *Chlorella vulgaris* and *Chaetoceros calcitrans*

Nutritional quality

The highest results of the analysis of the nutritional quality of protein and fat of *P. pacifica* could be determined based on the results. The highest value was 45.45% protein and 7.57% fat in the treatment of *P. pacifica* fed by 100% *C. vulgaris* (A), while the lowest protein and fat was in the feeding treatment in the form of a combination of 50% *C. vulgaris* and 50% *C. calcitrans* which was 40.44% and 5.89%. Proximate analysis results of *P. pacifica* are presented in Table 2.

The ANOVA analysis of protein and fat of *P. pacifica*, it showed that different treatment of enrichment feed of *P. pacifica* had a significant effect on the protein and fat of *P. pacifica* (P<0.05). Based on the results of the analysis of fatty acid profiles of *P. pacifica*, the highest value was in the EPA for about 5.45 in the treatment of *P. pacifica* fed by 100% *C. vulgaris* (A), whereas the lowest value was in the treatment of 50% *C. vulgaris* and 50% *C. calcitrans*, with EPA of 0.68%. The results of analysis of the total fatty acid profile of *P. pacifica* with the enrichment of *C. vulgaris* and *C. calcitrans* during the study are presented in Table 3.

Based on the ANOVA fatty acid profile of *P. pacifica*, it showed that different treatment of enrichment feed of *P. pacifica* had a significant effect on the fatty acid profile of *P. pacifica* (P<0.05). The results of the analysis of amino acid profiles of *P. pacifica* the highest value was in the amino acid lysine 39.23 ppm in the treatment of feeding 100% *C. vulgaris* (A), while the lowest value of the feeding treatment was at a combination of 50% *C. vulgaris* and 50% *C. calcitrans* with EPA of 18.19 ppm. The results of analysis of amino acid profile of *P. pacifica* enriched by *C. vulgaris* and *C. calcitrans* during the study are presented in Table 4. Based on the ANOVA analysis of growth rates of *P. pacifica*, it showed that different treatment of enrichment feed of *P. pacifica* had a significant effect on amino acid profile of *P. pacifica* (P<0.05).

Discussion

The results of each treatment included a fairly high population increase phase before a dramatic decline. Each *P. pacifica* treatment experienced the highest phase on the 12th d of maintenance, before eventually declining in number until the 18th d. Growth patterns in this study exhibited faster maintenance time for the growth of *Phronima* sp. compared to that of Fattah et al. (2014), who obtained results that increased until the 17th d and decreased until the 24th d, as well as that of Herawati et al. (2020), who obtained populations that increased until the 16th d and decreased until the 36th d.

Table 2. The results of proximate analysis of *Phronima pacifica* enriched by *Chlorella vulgaris* and *Chaetoceros calcitrans* during the study

Treatments	Dry weight content percentage				
	Protein (%)	Carbohydrate (%)	Crude fat (%)	Ash (%)	Crude fiber (%)
A	45.45 ± 0.02^{c}	15.07 ± 0.05	7.57^{b} ± 0.02	26.13 ± 0.03	5.78 ± 0.03
B	42.90 ± 0.04^{b}	16.22 ± 0.03	6.24^{b} ± 0.03	29.19 ± 0.03	5.45 ± 0.08
C	40.44 ± 0.06^{a}	14.87 ± 0.05	5.89^{b}± 0.02	30.61 ± 0.02	5.19 ± 0.05

Note: A: 100% *C. vulgaris*, B: 100% *C. calcitrans*, C: 50% *C. vulgaris* and 50% *C. calcitrans*

Table 3. The results of analysis of the total fatty acid profile of *Phronima pacifica* enriched by *Chlorella vulgaris* and *Chaetoceros calcitrans* during the study

Fatty acids profile (%)	A	B	C
Myristic	0.52 ± 0.05^{a}	0.48 ± 0.09^{a}	0.41 ± 0.02^{a}
Pentadecanoic	0.09 ± 0.06^{a}	0.15 ± 0.08^{a}	0.17 ± 0.04^{a}
Palmitic	3.14 ± 0.09^{b}	5.59 ± 0.04^{c}	1.97 ± 0.08a
Stearic	2.71 ± 0.07^{b}	2.91 ± 0.09^{b}	0.52 ± 0.03^{a}
Oleic/ω9	3.07 ± 0.02^{b}	2.61 ± 0.01^{b}	0.89 ± 0.08^{a}
Linoleic/ω6	4.83 ± 0.09^{b}	5.37 ± 0.02^{c}	2.49 ± 0.07^{a}
Linolenic/ω3	3.54 ± 0.05^{b}	3.32 ± 0.01^{b}	2.39 ± 0.03^{b}
AA	2.71 ± 0.03^{b}	0.13 ± 0.07^{b}	0.15 ± 0.09^{b}
DHA	2.83 ± 0.05^{b}	1.17 ± 0.03^{b}	0.97 ± 0.01^{a}
EPA	5.95 ± 0.02^{c}	3.88 ± 0.08^{b}	0.68 ± 0.02a

Note: A: 100% *C. vulgaris*, B: 100% *C. calcitrans*, C: 50% *C. vulgaris*. and 50% *C. calcitrans*. Different superscript letter indicates significant differences between treatments ($p < 0.05$)

Table 4. *Phronima pacifica* enriched by *Chlorella vulgaris* and *Chaetoceros calcitrans* during the study

Amino acid (ppm)	*P. pacifica*		
	A	B	C
L-aspartic acid	18.92±0.08^{c}	13.94±0.01^{b}	5.52±0.05^{b}
L-serine	17.62± 0.01^{a}	17.62± 0.01^{a}	14.76±0.02^{a}
L-glutamic acid	32.37±0.07^{c}	12.37±0.07^{b}	17.36±0.07^{b}
Glycine	19.19±0.01^{a}	19.19±0.01^{a}	17.33±0.02^{a}
L-histidine	9.70±0.01^{a}	9.70±0.01^{a}	8.65±0.03^{a}
L-arginine	27.28±0.01^{c}	20.28±0.01^{c}	10.85±0.07^{c}
L-threonine	20.37±0.01^{c}	10.37±0.01^{c}	18.47±0.07^{c}
L-alanine	21.51±0.09^{c}	12.51±0.09^{c}	10.51±0.01^{c}
L-proline	19.00±0.06^{c}	15.00±0.06^{c}	9.08±0.09^{c}
L-valine	28.87±0.04^{c}	17.87±0.04^{c}	5.72±0.03^{c}
L-methionine	15.40±0.04^{c}	8.57±0.04^{c}	5.89±0.06^{c}
L-Lysine	39.23±0.01^{c}	25.73±0.01^{c}	18.19±0.06^{c}
L-isoleucine	22.79±0.04^{c}	17.53±0.04^{c}	8.87±0.02^{c}
L-leucine	16.88±0.05^{c}	8.88±0.05^{c}	11.47±0.05^{c}
L-phenylalanine	15.49±0.07^{b}	16.98±0.10^{b}	14.41±0.07^{b}

Note: A: 100% *C. vulgaris*, B: 100% *C. calcitrans*, C: 50% *C. vulgaris* and 50% *C. calcitrans*

Phronima pacifica experienced an increase in population density and then decreased significantly. This could be caused by individuals that had entered the phase of death. This death phase could be caused by the death of plankton that was the food source for *P. pacifica*. This is in accordance with the results of Aoki et al. (2013), who stated that the decrease in the number of *Phronima* sp. occurred because excess nutrients were not utilized effectively such they produced a large amount of toxic organic material and the amount of plankton as a natural feed source for *Phronima* sp. was not sufficient for its needs.

Enrichment of the *P. pacifica* mass culture with *C. vulgaris* and *C. calcitrans* yielded different growth results. The results showed the highest growth and biomass in *P. pacifica* mass cultures were yielded by feeding 100% *C. vulgaris* (treatment A), with the highest growth of 59 ind./L (Figure 1), the highest biomass was 0.35 g (Figure 3), and the growth rate was 0.24% (Figure 2). This was because the difference in size and nutrient content of *C. vulgaris* was 2-10 µm (with 45% protein and 20% fat) and that of *C. calcitrans* was 10-50 µm (with 36% protein and 10% fat). The higher nutrient content and smaller size of *C. vulgaris* resulted in a density of 59 ind./L, biomass production of 0.35 g, and 0.24% growth rate of *P. pacifica,* which was higher than that of those that feed on *C. calcitrans*, which resulted in a yield of 20 ind./L, biomass production of 0.09 g, and 0.14% growth rate of *P. pacifica* because *C. vulgaris* is more easily digested by *P. pacifica* as feed. This statement was reinforced by previous studies conducted by Ball (1977) and Diebel (1988), wherein a nonselective filter feeder received an addition of nutrients through the media. Thus, growth is highly dependent on the feed and culture media of *P. pacifica*.

The results of this study were lower than those of Herawati et al. (2020), wherein the mass culture of *Phronima* sp. used a culture media in the form of fermented organic waste, which resulted in a density of 98 ind./L and biomass production of 0.51 g over 35 d of rearing. Enrichment using *C. vulgaris* and *C. calcitrans* was not sufficient to provide better results because the culture media nutrient was highly influenced by the supply of plankton and bacteria, by which the population and biomass growth increased. Growth and production of *P. pacifica* biomass are influenced not only by the nutrient content in the culture media but also by environmental conditions. According to Agung et al. (2104), plankton growth patterns are influenced by several factors, including the physical condition of the water, type of feed, and concentration of the feed. When these three factors are optimal, the growth rate of plankton will increase and produce a higher population peak. One of the influential environmental conditions was water quality in the culture media of *P. pacifica*. During the culture period of the *P.*

pacifica culture media, water quality was controlled by measuring water parameters daily. Water quality was measured *in situ*.

Growth of *P. pacifica* showed the same pattern as maintenance media. Although the amount in each media was different, the phases of increase and decrease and relative to the amount received were relatively the same. Differences in the number of *P. pacifica* in different maintenance media showed that the nutrient content in each medium was different and nutrients affected the growth of *P. pacifica*. The difference in population density was caused by the ability of cells to utilize nutrients for growth. Nutrients in culture media will affect the amount of phytoplankton contained in the media, and nitrate determines the amount of phytoplankton that functions as a natural feed source for *P. pacifica,* as well as bacteria and detritus contained in the media. Based on the results, the highest abundance of plankton that grew and dominated the culture media was *Chlorella* sp. Agung et al. (2104) in their research explained that the greater the abundance of phytoplankton and organic matter contained in the media, the greater the growth rate.

Nutritional quality based on proximate analysis, as shown in Table 2, showed that the highest values of protein and fat were observed in *P. pacifica* fed *C. vulgaris* being 45.45% and 7.57%, respectively. Additionally, the lowest values occurred in the treatment wherein *P. pacifica* was fed 50% *C. vulgaris* and 50% *C. calcitrans*. High protein content and low fat resulted in high nutrients in the culture media of *P. pacifica,* and higher nitrate and phosphate levels resulted from the higher protein level produced. Tocher and Glencross (2015) stated that the higher the N and P content, the higher the protein content in the culture. The fat content was inversely proportional to protein content. The results of this study were supported by the results of Lim et al. (2011), who stated that higher protein content was always proportional to fat because the fat in the body works twice as much as protein.

Based on the total fatty acid profile (Table 3), the highest fatty acid profile for EPA occurred for the *P. pacifica* fed *C. vulgaris*, whereas the lowest value was observed in *P. pacifica* fed 50% *C. vulgaris* and 50% *C. calcitrans,* which was 2.64%. EPA fatty acids function as a basic substrate in the formation of long-chain polyunsaturated fatty acids (PUFAs) and help to avoid blood clotting. The results of this study were higher than those of Herawati et al. (2020), wherein a *P. pacifica* mass culture using fermented organic waste from probiotic bacteria as feed had an EPA content of 5.95%. PUFAs are an important nutrient for the formation of LC-PUFAs, such that it could form or be able to produce EPA and DHA and help avoid blood clotting. The results of this study were reinforced by the findings of Monroig et al. (2013), and Tocher and Glencross (2015), in which PUFAs were a very important nutrient for the formation of LC PUFAs, from which EPA and DHA are formed, depending on the species.

Based on the results of the amino acid profile of *P. pacifica,* the highest value was observed in *P. pacifica* fed *C. vulgaris*, whereas the lowest value was observed in *P. pacifica* fed 50% *C. vulgaris* and 50% *C. calcitrans,* which was 2.64%. The function of the amino acid lysine, as stated in the research of Ovie and Ovie (2006), Valverde et al. (2013), and Herawati et al. (2017) is to serve as a structural framework for vitamin B1 and anti-virals, increasing the absorption of calcium, stimulating appetite, and aiding in the production of carnitine to convert fatty acids into energy. The high content of lysine in *P. pacifica* with a natural feed could increase growth, and it is also a basic ingredient of blood antibodies, strengthens the circulatory system, and improves cells. Lysine deficiency can cause fin erosion and fish death. This is in line with the results of Nafisi Bahabadi et al. (2018), who stated that the function of lysine in fish growth was very important, among others reasons, for the basic ingredients of blood antibodies, strengthening of the circulation system and metabolism, and the repair of cells. Based on an ANOVA, feeding *P. pacifica* with *C. vulgaris* and *C. calcitrans* had a significant effect on population density, relative growth rate, biomass production, and nutrient values for protein and fat in *P. pacifica* ($P < 0.05$). The best research results were for *P. pacifica* fed *C. vulgaris,* which resulted in better growth, including population density, growth rate, biomass weight, and nutrient content.

In conclusion, the utilization of *C. vulgaris* and *C. calcitrans* as enrichment for *P. pacifica* culture had been reported. Enrichment of the *P. pacifica* mass-cultured with *C. vulgaris* (treatment A) resulted in the highest growth of 59 ind./L (Figure 1), the highest biomass was 0.35 g (Figure 3), and the growth rate was 0.24% (Figure 2). The highest values for proteins and fats were also in (treatment A) and consisted of 45.45% protein, 7.57% fat, 5.95% eicosapentaenoic acid, and 39.23% lysine. Since there have been no studies that report on the effects of providing different types of natural feed on the nutritional content of *P. pacifica*, the results of this study should have quite impact on improving quality of *P. pacifica* as natural feed.

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