# Effect of feeding with Phronima sp. on growth, survival rate and nutrient

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## Effect of feeding with *Phronima* sp. on growth, survival rate and nutrient value content of Pacific white shrimp (*Litopenaeus vannamei*) Post-larvae

Aquaculture

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#### ABSTRACT

Re amphipod Phronima sp. is used as a natural feed for aquaculture activities as it has an adequate content of polyunsaturated fatty acide namely, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)—and high protein content. It is thus used as an alternative protein source in shrimp cultures, replacing Artemia sp. In this study, we evaluated the effects of using different feed treatments including Phronima sp. on Pacific white shrimp (Litopenaeus vannamei) post-larval growth rate, survival rate, and nutritional quality. The post-larvae herein used weighed  $0.029 \pm 0.001$  g/individual 31 were maintained at a stocking density of 5 individuals/L. The experiment was carried out for 30 days. A completely randomized design was used for the experiment, with five treatments and three replicates each. The feeding treatments were: A, 100% Artemia sp.; B, 75% Artemia sp. and 25% Phronima sp.; C, 50% Artemia sp. and 50% Phronima sp.; D, 25% Artemia sp. and 75% Phronima sp.; and E, 100% Phronima sp. The shrimp were fed four times a day 20% of their biomass weight. Relative growth rate (RGR), absolute length growth, biomass weight, grazing rate, survival rate, and nutritional quality were analyzed. Treatment B produced the highest RGR (34.98  $\pm$  0.13%), absolute length growth (1.83  $\pm$  0.06 cm), biomass weight (0.0101  $\pm$  0.05), and survival rate (95  $\pm$  6.11%). Treatment A produced the highest grazing rate (21.889 ± 0.13 individuals/day). The highest nutritional quality was found in treatment B, i.e., protein and fat contents of 63.75% and 8.24%, respectively, and EPA and lysine levels of 8.25% and 48.98 ppm, respectively. In summary, we found that using a feed proportion of 75% Artemia sp. and 25% Phronima sp. (treatment B) is the best option for the aquaculture of Pacific white shrimp post-larvae. This best feed formulation should give an impact on aquaculture research and production in reducing feed production cost.

#### 14 1. Introduction

The Pacific white shrimp (*Lithopenaeus vannamei*) has the advantage of growing faster than other shrimp species and of living in the water column, which allows individuals to be stocked at high densities. Moreover, this species is more resistant to environmental conditions and diseases than other shrimp species, and it is quite popular on the international market (Rakhfid and Halida, 2018). The aquacultu 30 production business has been recently developed and is influenced by various factors, among which is the availability of high-quality natural feed. The use of natural feed can reduce feed costs during production.

Amphipoda are part of the zooplankton, and are thus a natural feed for many wild fish (Dalpadado and Bogstad, 2004) and an adequate source of natural feed for aquaculture activities (Ba 18 Rojano et al., 2013). Although Amphipoda can also be used in integrated multitrophic aquaculture (IMTA) in ponds as natural feed (9 erra-García et al., 2016; Jiménez-Prada et al., 2018), few types of Amphipoda have been used in aquaculture to date. Previous studies have shown that several caprellid, gammarid, and mysid amphipods can be used as natural feed in Common cuttlefish (*Sepia officinalis*) and Octopus (*Octopus* sp.) cultivations (Baeza-Rojano et al., 2013). Specifically, Gammarid amphigads have been shown to be of high nutritional value—as they contain polyunsaturated fatty acids, namely docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)—and to have high-level proteins. Therefas, these amphipods can be used as an alternative source of proteins for farmed fish and shrimp (Moren et al., 2006). Herawati

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et al. (2020) showed that gammarid amphipods have a protein content of 40–58%, fat content of 5–8%, and EPA and DHA contents of 1% and 7%, respectively.

Phronima sp. is an amphing that has been recently mass-cultured and may be used as natural teed in shrimp aquaculture (Fattah et al., 2014; Herawati et al., 2014). However, it remains scarcely used for shrimp farming. The natural feed used in a cultivation is typically selected based on criteria such as size of the mouth gap of fish larvae and the suitability of the nutritional content to the needs of the cultured fish (Agustina and Yulisman and Mirna F., 2015). Phronima sp. has a particularly high EPA and DHA content, which is adequate for cultivating shrimp. Moreover, using Phronima sp. as natural feed is less expensive than using Artemia sp. Therefore, Phronima sp. may be used as a replacement for Artemia sp. as a food source, especially for post-larvae (Fattah et al., 2014). Fattah et al. (2014) found that using Phronima sp. as feed for Tiger shrimp (Penaeus monodon) resulted in a survival rate of over 80%. Considering this scenario, in the present study, we evaluated the effects of using Phronima sp. as a replacement for Artemia sp. as a natural feed for post-larvae of Pacific white shrimp by analyzing growth rate, survival rate, and nutritional quality. Furthermore, the most adequate feed composition using Phronima sp. is herein proposed.

#### 2. Materials and methods

#### 2.1. Materials

Pacific white shrimp (*L. vannamei*) PL15 stage were used at a stocking density of 5 shrimp/L. Feeding was performed four times a day, and the shrimp were fed 20% of their biomass weight. *Artemia* sp., at a protein content of 48.87%, and *Phronima* sp., at a protein content of 40.26%, were used as feed. According to Deslianti et al. (14.6), feed should have a protein content of 30–50% to support the growth and survival of Pacific white shrimp post-larvae. For the proximate analysis of the feed, 1 g (dry weight) of feed was used; the notice and the protein content in *Table* 1. The proximate analysis showed that the protein content in *Artemia* sp. was 8% higher than that in *Phronima* sp. (48% and 40.26%, respectively), and that the fat content was lower in the former species than in the latter (8.85% and 15.14%, respectively).

The fatty acid and amino acid profiles of feeds of both species are presented in Tables 2 and 3, respectively. Among the fatty acids, EPA had the highest content in both *Artemia* sp. (5.05%) and in *Phronima* sp. (8.19%), and among the amino acids, lysine had the highest content (45.57 ppm and 44.16 ppm, respectively).

#### 2.2. Methods

#### 2.2.1. Phronima sp. culture

A 600-L *Phronima* sp. mass culture was fed using *Chlorella* sp. and organic feed in the form of cow manure. *Phronima* sp. at approximately 2 weeks of age and 0.4 cm of size was used as feed.

| Table | 1 |
|-------|---|
|-------|---|

Results of the proximate analysis of the feed used in the present study (in %, dry weight).

| Component compositions (%) | Natural feed types |              |  |
|----------------------------|--------------------|--------------|--|
|                            | Artemia sp.        | Phronima sp. |  |
| Water content              | 10.80              | 8.43         |  |
| Protein                    | 48.87              | 40.26        |  |
| Fat                        | 8.85               | 15.14        |  |
| Ash                        | 13.25              | 17.20        |  |
| Crude fiber                | 8.32               | 8.93         |  |
| Carbohydrate               | 9.91               | 10.04        |  |

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#### Table 2

Fatty acid profiles of Artemia sp. and Phronima sp. used as feed for post-larvae shrimp.

| Fatty acids profile (%) | profile (%) Artemia sp. |                 |
|-------------------------|-------------------------|-----------------|
| Myristic                | $0.52 \pm 0.05$         | 0.48 ± 0.09     |
| Pentadecanoic           | $0.09 \pm 0.06$         | $0.15 \pm 0.08$ |
| Palmitic                | $3.14 \pm 0.09$         | $5.59 \pm 0.04$ |
| Stearic                 | $2.71 \pm 0.07$         | $2.91 \pm 0.09$ |
| Oleic/ω9                | $3.07 \pm 0.02$         | $2.61 \pm 0.01$ |
| Linoleic/ω6             | $2.83 \pm 0.09$         | $4.07 \pm 0.02$ |
| Linolenic/ω3            | $1.54 \pm 0.05$         | $3.32 \pm 0.01$ |
| Arachidic               | $2.30 \pm 0.08$         | $3.05 \pm 0.03$ |
| Arachidonic             | $0.07 \pm 0.02$         | $0.13 \pm 0.08$ |
| DHA                     | $0.03 \pm 0.05$         | $5.07 \pm 0.03$ |
| EPA                     | $5.05 \pm 0.02$         | 8.19 ± 0.08     |

#### Table 3

Amino acid profiles of Artemia sp. and Phronima sp. used as feed for Pacific white shrimp.

| Amino acids (ppm)  | Artemia sp.      | Phronima sp.     |
|--------------------|------------------|------------------|
| 4<br>Aspartic acid | $38.92 \pm 0.08$ | 43.94 ± 0.01     |
| Serine             | $15.61 \pm 0.03$ | $17.62 \pm 0.01$ |
| Glutamic acid      | $36.61 \pm 0.04$ | $32.37 \pm 0.07$ |
| Glycine            | $19.36 \pm 0.04$ | $19.19 \pm 0.01$ |
| Histidine          | 9.78 ± 0.03      | $9.70 \pm 0.01$  |
| Arginine           | $20.51 \pm 0.04$ | $27.28 \pm 0.01$ |
| Threonine          | $19.02 \pm 0.09$ | $20.37 \pm 0.01$ |
| Alanine            | $40.65 \pm 0.05$ | $32.51 \pm 0.09$ |
| Proline            | $20.25 \pm 0.05$ | $19.00 \pm 0.06$ |
| Valine             | $30.24 \pm 0.05$ | $28.87 \pm 0.04$ |
| Methionine         | $21.10 \pm 0.08$ | $39.40 \pm 0.04$ |
| Lysine             | $45.57 \pm 0.04$ | $44.16 \pm 0.01$ |
| Isoleucine         | $19.97 \pm 0.03$ | $22.79 \pm 0.04$ |
| Leucine            | $32.44 \pm 0.05$ | $36.88 \pm 0.05$ |
| Phenylalanine      | $15.49 \pm 0.07$ | $16.98 \pm 0.10$ |

#### 2.2.2. Artemia sp. culture

A 600-L Artemia sp. mass culture was fed using rice bran. Artemia sp. at approximately 2 weeks of age and 0.4 cm of size was used as feed.

#### 2.3. Feeding of Pacific white shrimp post-larvae

Pacific white shrimp PL15 stage were used at a stocking density of 5 shrimp/L. Three 30-L containers were used, one for each treatment. Approximately 450 shrimp were included in our analysis. The shrimp were fed four times a day approximately 20% of their biomass (approximately 80 individuals per shrimp). The study period was of 30 days. Initial biomass was measured before the treatment, and 50 post-larvae were randomly selected for length measurement. The proximate analysis of fatty acids and amino acids was performed using shrimp after the treatment.

In this research, a completely randomized design (CRD) with five treatments and three replications was used. *Artemia* sp. was replaced with *Phronima* sp. as feed at different dosages: Treatment A, 100% *Artemia* sp.; Treatment B, 75% *Artemia* sp. and 25% *Phronima* sp.; Treatment C, 50% *Artemia* sp. and 50% *Phronima* sp.; Treatment D, 25% *Artemia* sp. and 75% *Phronima* sp.; and Treatment E, 100% *Phronima* sp.

2.4. Relative growth rate





There RGR is relative growth rate (%/day),  $W_0$  is shrimp biomass weight at the beginning of the study (g),  $W_t$  is shrimp biomass weight at

 $= L_t - L_0$ 

the end of the study (g), and t is length of the study (days).

#### 2.5. Absolute length growth

According to Zonneveld et al. (1991), absolute length growth can be alculated using the following formula:

where L is absolute length growth (cm),  $L_0$  is shrimp body length at the beginning of the study (cm), and  $L_t$  is shrimp body length at the end of the study (cm).

#### 2.6. Biomass weight of Pacific white shrimp

Biomass weight was determined using the following formula (Steffens, 1989):

 $BW = \frac{W_t - W_0}{W_t - W_0}$ 

Where BW is the biomass weight (g),  $W_t$  is final shrimp larval weight (g),  $W_0$  is initial larval weight (g), and t is length of study (days).

#### 2.7. Survival rate

According 19 teffens (1989), the survival rate of Pacific white shrimp can be calculated using the following formula:

 $SR = \frac{N_t}{N_0} \times 100\%$ 

where SR is survival rate of Pacific white shrimp (%),  $N_t$  is the number of shrimp at the end of the study, and  $N_0$  is the number of shrimp at the beginning of the study.

#### 2.8. Water quality

All water quality parameters of the shrimp cultures during the study are presented in Table 4.

#### 2.9. Grazing rate

The amount of feed consumed during the aquaculture period was determined by comparing the amount of feed initially provided with the amount of leftover feed in the aquaculture media. The consumption of natural feed was calculated by dividing the amount of feed consumed during the aquaculture period by the number of Pacific white shrimp in the cultures, following Widiastuti et al. (2012).

#### Table 4

Results of the measurement of water quality parameters for pacific white shrimp (L. vannamei) in the aquaculture media during the study.

| Treatments | Water quality parameter value range |                      |           |                    |  |
|------------|-------------------------------------|----------------------|-----------|--------------------|--|
|            | Temperature (°C)                    | рН                   | DO (mg/L) | Salinity (ppt)     |  |
| A          | 29-31.5                             | 8                    | 4.54-5.10 | 25-28              |  |
| В          | 28-30.5                             | 8                    | 4.34-4.12 | 25-28              |  |
| С          | 29.8-31.5                           | 8                    | 4.25-5.00 | 25-28              |  |
| D          | 29.2-30.4                           | 8                    | 4.78-5.05 | 25-28              |  |
| E          | 29.5-31.6                           | 8                    | 4.21-4.98 | 25-28              |  |
| References | 26-32 °C                            | 7.7–8.7 <sup>b</sup> | 3-8°      | 15-35 <sup>d</sup> |  |

<sup>a</sup> Tahe and Hidayat (2011).

<sup>b</sup> Purba (2012).

Syukri and Ilham (2016).

d Wyban and Sweeney (1991).

#### 2.10. Data analysis

RGR, absolute length growth, biomass weight, grazing rate, and SR were statistically analyzed using normality tests, homogeneity tests, and additivity tests followed by analysis of variance (ANOVA). When the values were found to be statistically different in 21 ANOVA (p < .05), a Duncan's multiple range test was performed. Water quality data were analyzed descriptively and compared with reference values. For the proximate analysis and amino acid and fatty acid p 2 ile analyses, 60 g (dry weight) of shrimp were used (20 g for each analysis).

The proximate chemical composition of the samples was determined using a standard procedure (AOAC, 2005; Herawati et al., 2018). The crude protein content was calculated by multiplying the total nitrogen factor. The carbohydrate content was estimated by the difference.

Amino acid content was determined using High Performance Liquid Chromatography (HPLC) (Shima u LC-6A) (AOAC, 2005; Herawati et al., 2018). Fatty acid content was determined using a gas chromatograph (Shimadzu) (AOAC, 2005; Herawati et al., 2018).

#### 3. Results

#### 3.1. Relative growth rate

Our result howed that all treatments replacing Artemia sp. with Phronima sp. had a significant effect (p > .05) on the growth rate of Pacific white shrimp. The RGR values for each treatment are shown in Fig. 1.

Duncan's test showed that treation is a significantly different from treatments A, C, D, and E, and that treatment C was significantly different from treatments A and but not from treatment D (p > .05). Furthermore, treatment D was significantly different from treatments A and E, whereas treatment A was significantly different from E (p > .05). The highest (34.98%/day) and lowest (32.49%/day; 2.49%/day lower than the former value) RGRs were observed for treatments B and E, respectively.

Our results als 5 howed that all treatments replacing Artemia sp. with  $P_{5}$  nima sp. had a significant effect (p > .05) on the absolute length growth of Pacific white shrimp. The absolute length growth values of each treatment are shown in Fig. 2.

Duncan's test showed that treatment B was significantly different from treatments A and E, but not from C and D. Treatment C was significantly different from treatment E, but not from treatments D and A. Treatment A was not significantly different from treatment E. This shows that treatments D, A, and E do not have any effect on the absolute growth rate of Pacific white shrimp. The highest (1.83 cm) and lowest (1.60 cm; 0.23 cm lower than the former value) absolute length growth values were found in treatments B and E, respectively.

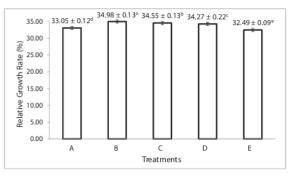


Fig. 1. Relative growth rates of Pacific white shrimp (L. vannamei) under different treatments.

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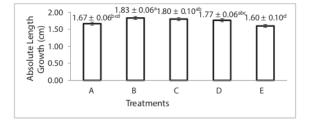


Fig. 2. Absolute length growth of Pacific white shrimp (L. vannamei) under different treatments.

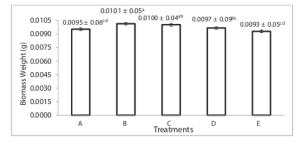


Fig. 3. Biomass weight values of Pacific white shrimp (L. vannamei) under different treatments.

#### 3.2. Biomass weight

Our result showed that all treatments replacing Artemia sp. with Phronima sp. had a significant effect (p < .05) on the biomass weight of Pacific white shrimp. The biomass weights of each treatment are shown in Fig. 3.

Duncan's test showed that treatment B was 17 ificantly different from C, D, A, and E, and that treatment C was significantly different from A and E but not from D (p < .05). Treatment D was significantly different from A and E, whereas A was significantly different from E (p < .05). The highest (0.0101 g/day) and lowest (0.0093 g/day; 0.0008 g/day lower than the former value) biomass weights were found for treatments B and E.

#### 3.3. Survival rate

The survival rates of Pacific white shrimp under different treatments are shown in Fig. 4. The data from all containers of each treatment were summed and then divided by 3. Our results sh 23 d that replacing *Artemia* sp. with *Phronima* sp. in the feeding used had no significant effect on the survival rate of PL15 Pacific white shrimp during the 30-day rearing period. Total survival rate was of 85–95%, whereas those of

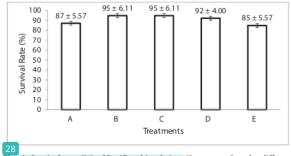


Fig. 4. Survival rate (%) of Pacific white shrimp (L. vannamei) under different treatments.

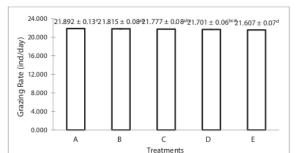


Fig. 5. Grazing rate in the culture of Pacific white shrimp (L. vannamei) under different treatments.

treatments A, B, C, D, and E were of 81–100%, 88–96%, 85–100%; 85–91%, and 80–90%, respectively. Treatments B and C had the highest survival rates, whereas treatment E had the lowest survival rate.

#### 3.4. Grazing rate

The grazing rates of Pacific white shrimp under different treatments are shown in Fig. 5. Our results showed that all treatments had a significant effect (p < .05) on the grazing rate. The highest (21.889  $\pm$  0.13 individuals/day) and lowest (21.607  $\pm$  0.07 individuals/day) values were found for treatments A and E, figectively. Moreover, Duncan's test short it that treatment A was significantly different from B, C, D, and E; troment B was not significantly different from treatment C, but it was significantly different from treatments D and E; treatment C was significantly different from D and E; and treatment D was significantly different from treatment E.

As shown in Table 5, the proximate analysis showed that the highest and lowest protein and fat contents were obtained in treatments B (63.75% protein and 8.24% fat) and E (50.26% protein and 5.04% fat), respectively. The fatty acid profiles are shown in Table 6; the highest fatty acid content was of EPA in treatment B (8.25%), whereas the lowest 3 then the treatment E (4.94%). The amino acid profiles are shown in Table 7; the highest amino acid content is that of lysine in treatment B (48.98 ppm), whereas the lowest is that of treatment E (30.59 ppm).

#### 4. Discussion

In the present study, the growth rate of Pacific white shrimp under different feed treatments was evaluated by calculating RGR, absolute length growth, biomass weight, and grazing rate. The nutritional contents of natural feed using *Artemia* sp. and *Phronima* sp. were found to be suitable for the nutritional needs of Pacific white shrimp post-larvae. The protein contents of *Artemia* sp. and *Phronima* sp. natural feed were 48.87% and 40.26%, respectively; these values are both suitable for this shrimp species' post-larval growth according to Deslianti et al. (2016), who previously stated that a protein content of 30–50% is required for this species to grow and survive. Although *Artemia* sp. has a higher protein content than *Phronima* sp., it is also more expensive. A feed formulation that brings good growth rate results and is cost-effective shopped have a positive impact on aquaculture businesses worldwide.

Artemia sp. is one of the most common natural feeds used in shrimp larval cultures because of its high protein (52.19%), fat (14.75%), ash (11.2%), and crude fiber (1.73%) contents. However, the market price of 400 g Artemia sp. reaches Rp 850,000.00 (Herawati et al., 2014), which is quite expensive. In addition, Artemia sp. has to be imported from various countries to meet the demand in Indonesia. Finally, Artemia sp. is marketed in the form of preserved eggs or in cans, which results in higher prices and had limited supply on the market.

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#### Table 5

| Proximate analysis of post-larval Pacific | c shrimp (L. vannamei) | under different treatments. |
|---|------------------------|-----------------------------|
|---|------------------------|-----------------------------|

| Treatments Dry weight content |                  |                  |                 |                  | Crude fiber (%) |
|-------------------------------|------------------|------------------|-----------------|------------------|-----------------|
|                               | Protein (%)      | Carbohydrate (%) | Crude fat (%)   | Ash (%)          |                 |
| A                             | $58.65 \pm 0.09$ | $13.07 \pm 0.04$ | $6.57 \pm 0.02$ | $15.93 \pm 0.02$ | $5.78 \pm 0.03$ |
| В                             | $63.75 \pm 0.03$ | $12.28 \pm 0.03$ | $8.24 \pm 0.03$ | $11.28 \pm 0.03$ | 4.45 ± 0.08     |
| С                             | $54.74 \pm 0.02$ | $11.87 \pm 0.05$ | $6.89 \pm 0.02$ | $21.31 \pm 0.01$ | $5.19 \pm 0.05$ |
| D                             | $53.67 \pm 0.01$ | $10.25 \pm 0.02$ | $6.40 \pm 0.02$ | $24.08 \pm 0.06$ | $5.60 \pm 0.02$ |
| E                             | $50.26 \pm 0.05$ | $12.55 \pm 0.02$ | $5.04 \pm 0.04$ | $26.52 \pm 0.07$ | $5.63 \pm 0.06$ |

Previous studies have focused on amphipods other than *Phronima* sp. that can be used an ged, such as caprellids, gammarids, and mysids in the statistical sp. ltivation of Cuttlefish (*Sepia officinalis*) and Octopus (*Octopus* sp.) (Baeza-Rojano et al., 2010; Baeza-Rojano et al., 2013).

Besides being economically advantageous for shrimp cultures, *jornima* sp. also has an adequate nutritional content, as it contains 136 unsaturated fatty acids (DHA and EPA) and high levels of protein (Moren et al., 2006; Herawati et al., 2020). Moreover, according to Fattah et al. (2014), Phronimidae increased Tiger shrimp production and generated a survival rate of approximately 70%, which shows that this group could be a health indicator.

The highest RGR (34.98%), absolute length growth (1.83 cm), and biomass weight (0.0101 g) were found in treatment B; this may be explained by this treatment having more than one type of feed source, which means that the shrimp received a larger variety of nutrients. Shrimp 35 with a natural combination of *Artemia* sp. and *Phronima* sp. had a high growth rate, which is because of the similar but complementary nutritional composition of *Artemia* sp. and *Phronima* sp., as previously described by Nofiyanti et al. (2014). A variety of natural feed types (including phytoplankton and zooplankton) regarding size and nutritional content should meet the shrimp larvae's nutritional requirements. Panjaitan et al. (2014), for instance, reported satisfactory results for Pacific white shrimp fed with mixed plankton.

Riani et al. (2012) defined growth as the increase in size, length, or weight over time. Growth results from the mitotic division of cells with input from energy and proteins 25 jived from feed. Feeding using *Artemia* sp. and *Phronima* sp. had a significant effect on the growth and biomass of the shrimp, especially when a combination of both amphipods was used. Previous studies have suggested that EPA, DHA, fatty acid, and amino acid defficiencies affect growth and biomass weight. Santoso, l. (2006), for instance, argued that EPA and DHA assist in the maintenance of shrimp survival and accelerate growth. Nuhman (2008) also suggested that shrimp grow when they can obtain the required energy.

RGR, absolute length growth, and biomass weight were lowest in treatment E; this treatment consisted exclusively of *Phronima* sp., which has a protein content lower than that of *Artemia* sp. and may have not reached the shrimp' nutritional requirements. Moreover, *Phronima* sp.

typically attaches to aeration stones, which makes it difficult for the shrimp to eat it. However, it has higher amino acid content and EPA and DHA levels compared to *Artemia* sp. In addition, *Phronima* sp. is easier to maintain and its size is adequate for the shrimp' mouth gap. After the shrimp eats, the ingested feed is digested, metabolized, and the nutrients are used for development and movement. Prawira (2017) stated that proteins are needed by fish to produce energy and for growth. Proteins are the primary energy source that provides the amino acids required by fish.

In the present study, treatment B had better results than those of treatments C and D. Nuhman (2008) stated that the dose of components of the feed is a factor that must be considered in feed management as it plays an important role in the effectiveness of feed usage. The daily grazing rate shows the number of *Artemia* sp. and *Phronima* sp. that were 34 leed consumed by the shrimp, along with the increasing size of the Pacific where 32 shrimp. The use of natural feed is believed to indirectly affect the growth rate of Pacific white shrimp.

The highest grazing rate was found for treatment A (21.892 individuals/day), because most of the food in this treatment was ingested by the shrimp. However, the highest growth rates were obtained using a combination of *Phronima* sp. and *Artemia* sp. This is because the EPA (7.53%) and DHA (1.09%) levels in *Phronima* sp. were higher than those in *Artemia* sp. Santoso, l. (2006) stated that the high content of fatty acids in the feed accelerates the growth of shrimp during the aquaculture. Herawati et al. (2020) also stated that the protein contained in feed will affect shrimp growth. Feed with optimal protein content will produce maximum growth.

The highest protein (63.75%) and fat (8.24%) contents were found in treatment B, whereas the lowest ones (50.26% and 5.04%, respectively) were in E. Furthermore, the highest EPA level (8.25%) was found in B, whereas the set fatty acid profile was found in E (4.94%). Monounsati and fatty acids and polyunsaturated fatty acids, including the omega-3 fatty acids EPA and DHA, play a role in reducing triacylglycerol levels and increasing the excretion process, besides increasing cell membrane fluidity, forming eicosanoids that reduce platelets, and playing an important role in brain and retinal development (Sinclair, 1993)

Lysine was dominant in the amino acid profile of treatment B

#### Table 6

Fatty acid profiles of post-larval Pacific shrimp (L. vannamei) under different treatments.

| Fatty acid profile (%) | Treatments      |                 |                  |                 |                        |  |  |
|------------------------|-----------------|-----------------|------------------|-----------------|------------------------|--|--|
|                        | A               | В               | С                | D               | Е                      |  |  |
| Myristic               | $2.81 \pm 0.05$ | $3.68 \pm 0.09$ | $2.51 \pm 0.02$  | $1.50 \pm 0.05$ | 1.49 ± 0.04            |  |  |
| Pentadecanoic          | $1.19 \pm 0.06$ | $2.25 \pm 0.08$ | $0.17 \pm 0.304$ | $1.08 \pm 0.02$ | $2.18 \pm 0.0$         |  |  |
| Palmitic               | $6.14 \pm 0.09$ | $8.09 \pm 0.04$ | $4.97 \pm 0.08$  | $4.12 \pm 0.01$ | $4.09 \pm 0.03$        |  |  |
| Stearic                | $2.71 \pm 0.07$ | $3.11 \pm 0.09$ | $1.52 \pm 0.03$  | $2.08 \pm 0.05$ | $1.65 \pm 0.0$         |  |  |
| Oleic/ω9               | $3.07 \pm 0.02$ | $5.91 \pm 0.01$ | $3.89 \pm 0.08$  | $2.55 \pm 0.03$ | $1.95 \pm 0.0$         |  |  |
| Linoleic/ω6            | $4.83 \pm 0.09$ | $5.37 \pm 0.02$ | $3.49 \pm 0.07$  | $3.75 \pm 0.02$ | $2.46 \pm 0.0$         |  |  |
| Linolenic/ω3           | $3.54 \pm 0.05$ | $4.32 \pm 0.01$ | $2.39 \pm 0.03$  | $2.56 \pm 0.07$ | $2.38 \pm 0.0$         |  |  |
| Arachidic              | $2.30 \pm 0.08$ | $4.05 \pm 0.03$ | $2.02 \pm 0.04$  | $2.25 \pm 0.05$ | $2.83 \pm 0.0$         |  |  |
| Arachidonic            | $2.07 \pm 0.02$ | $2.13 \pm 0.08$ | $0.15 \pm 0.02$  | $0.06 \pm 0.08$ | $0.15 \pm 0.0$         |  |  |
| DHA                    | $1.83 \pm 0.05$ | $5.07 \pm 0.03$ | $1.07 \pm 0.01$  | $1.39 \pm 0.08$ | $1.08 \pm 0.0$         |  |  |
| EPA                    | $6.05 \pm 0.02$ | $8.25 \pm 0.08$ | $5.68 \pm 0.02$  | $5.03 \pm 0.05$ | $4.94 \pm 0.0^{\circ}$ |  |  |

#### Table 7

Amino acid profiles of post-larval Pacific shrimp (L. vannamei) under different treatments.

| Amino Acid (ppm)   | Treatments       |                  |                  |                  |                  |
|--------------------|------------------|------------------|------------------|------------------|------------------|
|                    | Α                | в                | С                | D                | E                |
| 4<br>Aspartic acid | $14.18 \pm 0.09$ | $15.19 \pm 0.09$ | $13.06 \pm 0.06$ | $13.98 \pm 0.04$ | $11.85 \pm 0.05$ |
| Serine             | $11.46 \pm 0.09$ | $19.03 \pm 0.03$ | $17.10 \pm 0.01$ | $17.23 \pm 0.07$ | $14.76 \pm 0.02$ |
| lutamic acid       | $4.16 \pm 0.08$  | $18.19 \pm 0.03$ | $12.26 \pm 0.05$ | $14.20 \pm 0.01$ | 57.36 ± 0.0      |
| lycine             | $12.98 \pm 0.05$ | $19.03 \pm 0.06$ | $16.26 \pm 0.07$ | $17.90 \pm 0.09$ | $17.33 \pm 0.0$  |
| listidine          | $13.79 \pm 0.11$ | $18.90 \pm 0.09$ | $13.80 \pm 0.05$ | $14.75 \pm 0.08$ | $8.65 \pm 0.03$  |
| rginine            | $16.27 \pm 0.02$ | $24.36 \pm 0.08$ | $21.67 \pm 0.07$ | $22.56 \pm 0.02$ | $20.85 \pm 0.0$  |
| Threonine          | $14.43 \pm 0.04$ | $21.78 \pm 0.06$ | $17.89 \pm 0.09$ | $18.96 \pm 0.08$ | 18.47 ± 0.0      |
| lanine             | $12.17 \pm 0.03$ | $23.20 \pm 0.09$ | $18.95 \pm 0.08$ | $20.23 \pm 0.09$ | $32.51 \pm 0.0$  |
| Proline            | $12.84 \pm 0.03$ | $25.87 \pm 0.07$ | $23.75 \pm 0.09$ | $24.92 \pm 0.01$ | $18.08 \pm 0.0$  |
| aline              | $25.73 \pm 0.08$ | $28.83 \pm 0.03$ | $22.90 \pm 0.04$ | $25.99 \pm 0.08$ | $20.72 \pm 0.0$  |
| Aethionine         | $32.88 \pm 0.03$ | $37.10 \pm 0.05$ | $33.09 \pm 0.03$ | $29.98 \pm 0.03$ | $30.89 \pm 0.0$  |
| ysine              | $44.09 \pm 0.03$ | 48.98 ± 0.03     | $35.20 \pm 0.04$ | $34.98 \pm 0.05$ | $30.59 \pm 0.0$  |
| soleucine          | $11.16 \pm 0.08$ | $15.23 \pm 0.02$ | $13.09 \pm 0.02$ | $13.99 \pm 0.03$ | $18.87 \pm 0.0$  |
| eucine             | $9.23 \pm 0.02$  | $13.25 \pm 0.03$ | $11.38 \pm 0.01$ | $12.57 \pm 0.01$ | $31.47 \pm 0.0$  |
| henylalanine       | $10.06 \pm 0.07$ | $15.98 \pm 0.01$ | $12.98 \pm 0.09$ | $14.96 \pm 0.06$ | $14.41 \pm 0.0$  |

(48.98 ppm), whereas treatment E had the amino acid profile with the lowest value (30.59 ppm). Ovie and Ovie (2006), Valverde et al. (2013), and Herawati and Hutabarat (2015) stated that lysine participates in th 2 formation of vitamin B1 and antibodies, assists the absorption of calcium, stimulates appetite, and helps in the production of camitine to convert fatty acids into energy.

The 95% survival rate is believed to result from the high protein feed provided to the shrimp. Other factors of the culture medium may also support survival and reduce stress. Pr 5 ira (2017), for instance, stated that the factors that most influence the survival rate of Pacific white shrimp larvae are water quality and feed quality. Feed availability during the maintenance period can also affect survival rate. Herawati and Hutabarat (2015) stated that, if the feed is chosen according to nutrient content and size (i.e., considering the larval mouth gap), it can increase shrimp growth and survival.

#### Declaration of Competing Interest

We declare that there is no potential conflict of interest among authors, and as the corresponding author, I agree all the terms mentioned on the journal.

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