Effect of addition of waste vegetable fermented flour rumen fluid on the quality feed

Murni 1*, A Anwar1, Hamsah1 and E Septianingsih2

1Lecturer of Aquaculture Study Program Faculty of Agriculture, University of Muhammadiyah Makassar, Sulawesi-Indonesia
2 Researcher, brackish water aquaculture research center and fisheries extension, Maros, South Sulawesi-Indonesia

*E-mail: murni@unismuh.ac.id

Abstract. The purpose of this study was to evaluate the effect of the addition of fermented vegetable waste flour on the rumen fluid of cows and goats that could improve the quality of feed nutrition. The study used two types of feed containing starch fermented vegetable waste flour and goat flour. The treatments tested were 0 (control), 10, 20, and 30% vegetable waste flour fermented cow rumen fluid (Test feed 1) and 0 (control), 10, 20, and 30% vegetable waste flour fermented goat rumen fluid. The data obtained were analyzed descriptively by comparing various literature. The results of the proximate analysis of amino acids, and test feed fatty acids with the addition of 20% fermented vegetable waste flour in cow rumen fluid showed that the crude protein content was higher, amino acids and fatty acids were higher compared to other treatments. Likewise with the addition of 10% vegetable waste flour fermented goat rumen fluid. The results showed that the crude protein content of feed C (the addition of 20% fermented vegetable rye flour fermented cow rumen fluid) was higher than other test and control feeds. The content of amino acids and fatty acids in the test feed is more optimal based on recommendations. Feed B (addition of 10% goat rumen liquid fermented vegetable waste) obtained 38.33% higher crude protein content, and 4.81% crude fiber. The content of amino acids and fatty acids is more optimal than the control and other test feed. Based on the results of the study it can be concluded that the addition of 20% fermented vegetable waste flour rumen cow liquid and 10% fermented goat rumen liquid vegetable waste flour can improve the nutritional quality of the test feed.

1. Introduction
The feed is one component that is important and crucial in the cultivation of vannamei shrimp. Vannamei shrimp feed needs in the total cost of production range between 50-70% [1]. Therefore it takes high-quality feed, inexpensive, and sustainable.

The waste vegetable is one of the feed ingredients that contain complete nutrients, inexpensive, easy to obtain. Vegetable waste contains a high enough protein of 22.63% as a raw material shrimp feed but is constrained to the high fiber content of 30.71% [2]. Efforts made to reduce the fiber content are through the fermentation process which aims to simplify complex compounds into pure compounds. Rumen liquid is a good fermentor for reducing crude fiber of vegetable waste [2].

Rumen fluid contains cellulase, amylase, protease enzymes [3, 4, 5]; xylanase, mannanase, and phytase [6, 7] to hydrolyze feed. Rumen fluid can break down difficult material that is easily digested so that it can be utilized [2].
Previous research reported [8] that the addition of a dose of rumen liquid 10-15 mL/kg of vegetable waste can increase nutrient content and reduce the content of fermented vegetable waste fiber. [9] obtained a 4-day fermentation time with a dose of rumen liquid 15 mL/kg able to reduce the crude fiber of vegetable waste from 29.35 to 14.83%. Furthermore, [2] reported that the incubation period of 4 days with a 3% rumen fluid dose reduced the fiber content and nutritional quality of vegetable waste. This study aims to evaluate the effect of the addition of fermented vegetable waste flour to rumen fluid in cattle and goats that can improve the quality of feed nutrition.

2. Materials and methods
This research was conducted from April to 18 July 2019. Chemical analysis was carried out at the Research Center for brackish water cultivation and Maros fisheries counseling and integrated laboratories at the Faculty of Animal Science, Hasanuddin University, Makassar.

The rumen fluid of cattle and goats as a source of enzyme extracts used as a fermentor is obtained through a filtration process in 40°C temperature conditions following the method of [2] originating from slaughterhouses.

There are two kinds of test feed used, and they are test feed one, which is formulated with the addition of fermented vegetable waste flour in cattle rumen fluid 0, 10, 20, and 30%. The second test feed was feed formulated with the addition of fermented vegetable waste flour, goat rumen fluid 0, 10, 20, and 30%. The fermentation process begins with cutting vegetable waste (cabbage, carrots, kale, chicory) weighing 25 grams each and placed in plastic clips that have been added to cow and goat rumen fluid at a dose of 15 mL/kg of vegetable waste, then stored in styrofoam. The fermentation process takes place anaerobically for four days, then drying and smoothing using a blender. Fermented vegetable waste flour is mixed with other feed ingredients following feed formulation, then carried out molding and drying. Test feed formulations are present in Tables 1 and 2.

Proximate analysis of test feed was analyze following the method [10]. Analysis of amino acid test feed was carried out in the Bogor IPB integrated laboratory, following the method [10]. Analysis of feed fatty acid test was carried out at the Bogor Integrated Laboratory of IPB, following the method [10].

Table 1. Test feed formulations added with fermented vegetable waste flour in cattle rumen fluid

| Raw Materials  | Treatment |
|---------------|-----------|
|               | Feed A   | Feed B  | Feed C  | Feed D  |
| Fish meal     | 0        | 10      | 20      | 30      |
| Soybean Flour | 33       | 33      | 33      | 33      |
| Fine Bran     | 22       | 18      | 15      | 11      |
| Wheat flour   | 27       | 21      | 14      | 8       |
| Cornflour     | 9        | 9       | 9       |         |
| Fish oil      | 7        | 7       | 7       |         |
| Vitamins      | 1        | 1       | 1       |         |
| Fish Oil      | 1        | 1       | 1       |         |
| Total         | **100**  | **100** | **100** | **100** |
Table 2. Formulation additional test feed waste vegetable flour fermented goat rumen fluid.

| Raw Material    | Treatment |
|-----------------|-----------|
|                 | Feed A    | Feed B    | Feed C    | Feed D    |
| Fish meal       | 0         | 10        | 20        | 30        |
| Soybean Flour   | 33        | 33        | 33        | 33        |
| Fine Bran       | 22        | 18        | 15        | 11        |
| Wheat flour     | 27        | 21        | 14        | 8         |
| Cornflour       | 9         | 9         | 9         | 9         |
| Fish oil        | 7         | 7         | 7         | 7         |
| Vitamins        | 1         | 1         | 1         | 1         |
| Fish Oil        | 1         | 1         | 1         | 1         |
| Total           | 100       | 100       | 100       | 100       |

Feed A = The addition of vegetable waste flour fermented cow’s rumen fluid 0%
Feed B = The addition of vegetable waste flour fermented cow’s rumen fluid 10%
Feed C = The addition of vegetable waste flour fermented cow’s rumen fluid 20%
Feed D = The addition of vegetable waste flour fermented cow’s rumen fluid 30%

3. Results And Discussion

3.1. Proximate analysis of test feed

Proximate analysis of test feed by the addition of different fermented vegetable waste flour from cow and goat rumen fluids during the study is presented in Tables 3 and 4.

Table 3 shows that the addition of fermented vegetable waste flour to different beef rumen fluids in feed is relatively the same, but higher than feed A (control). Although the feed with the addition of vegetable waste flour fermented rumen fluid in feed B is relatively higher than other feeds.

Table 3. Proximate results of test feed by adding fermented vegetable waste flour to different cow rumen fluids.

| Composition (%) | Feed A | Feed B | Feed C | Feed D |
|-----------------|--------|--------|--------|--------|
| Crude protein   | 37.41  | 37.90  | 38.49  | 37.59  |
| Crude fat       | 10.04  | 12.36  | 13.68  | 13.67  |
| Crude fiber     | 6.62   | 5.31   | 4.35   | 3.31   |
| Ash             | 14.05  | 12.23  | 13.34  | 14.09  |
| BETN            | 31.88  | 32.20  | 30.13  | 31.34  |
Table 4. Results of the proximate test feed containing different levels of starch vegetable waste rumen fluid fermented goat.

| Composition (%) | Feed A | Feed B | Feed C | Feed D |
|-----------------|--------|--------|--------|--------|
| Crude protein   | 38.00  | 38.33  | 38.19  | 38.11  |
| Crude fat       | 14.17  | 11.43  | 10.84  | 11.60  |
| Crude fiber     | 5.69   | 4.81   | 4.53   | 4.50   |
| Ash             | 11.67  | 13.96  | 13.57  | 13.00  |
| BETN            | 30.47  | 31.47  | 32.96  | 32.79  |

The high level of test feed protein added by 20% of vegetable waste flour fermented rumen beef liquid was due to the highest crude protein C obtained (38.49%), followed by feed B (37.90%), feed D (37.59%) and the lowest in feed A (37.41%). Low levels of protein in control, influenced by the content of bran in the feed are too high (Table 3) and not through the fermentation process [11]. The results of this study are still in a decent range for vannamei shrimp juvenile cultivation, which is 30 to 43% [12, 13]. The results showed that the higher the addition of fermented vegetable waste flour to cow rumen fluid, the higher the fat content, while the lower shrub fiber content compared to the control (0%). It is due to the activity of rumen fluid cellulase, amylase, protease, and lipase enzymes [13, 14], which is secreted by bacteria in hydrolyzing vegetable waste [2]. Protein content range is 30-40% [12, 13], 5-12% fat [15, 16], fiber 4% - 4.33% [17, 18].

The results of the proximate analysis of test feed (Table 4) showed that the crude protein levels obtained in the test feed were relatively the same, but the control was low. Likewise, crude fiber is lower in feed with the addition of fermented vegetable waste flour than in controls. The levels of protein, fat, and fiber obtained (Table 4) are still in the optimal range according to [11] 30-43%, crude fat 5-12% [15], fiber 4% - 4.33% [16, 11], low fiber, and crude fat in feed with the addition of fermented vegetable waste flour showed enzyme activity in hydrolyzing vegetable waste. The enzyme activity was cellulase, lipase enzymes [3, 4, 5] used as fermenters[11, 17] reported that the bacterium Enterobacter cloacae WPL 214 isolated from rumen fluid contained cellulase enzymes.

3.2. Amino acid feed test

The composition of dietary fatty acids with the addition of different fermented vegetable rumen fluid from cow's rumen fluid is presented in Tables 5 and 6. Tables 5 and 6 show that the higher the addition of fermented vegetable waste flour to rumen fluid of cattle and goats in the test feed, the higher the amino acid content of the feed. This shows that the protein waste of vegetable waste is completely hydrolyzed from complex compounds to be simple[24] due to the activity of rumen fluid protease enzyme secreted by microbes [4]. Subsequently [8] get amylase enzyme activity of 0.250 u/mL/min, protease 0.49 u/mL/min, cellulase 0.124 u/mL/min so that it produces high amino acids and can be utilized for the growth of vannamei shrimp. [11] reports that the addition of 10 and 20% silage of fermented vegetable waste rumen can increase the amino acid content of the feed. The lowest amino acid content obtained in the 0% test feed (control) because it is affected by the bran that is too high and not fermented.
The test feed used is feed added to beef rumen liquid fermented vegetable waste obtained more optimal amino acid content in the test feed by adding 20% beef rumen liquid fermented vegetable waste flour (Table 5) compared to other treatments and controls. According to [19], the recommended leucine amino acids are 1.70%, isoleucine 1%, lysine 2.10%, and valine 1.40 [23].

According to [24, 25, 26] that the amino acids leucine, isoleucine, lysine, and valine are essential amino acids essential to support the growth of shrimp and fish. It shows that the test meal with the addition of 20% vegetable waste flour fermented rumen cattle liquid and the test food adding 10% vegetable waste flour fermented goat rumen fluid has better quality compared to other test feeds.

Table 5. Composition of essential amino acids and non-essential feed containing different levels of vegetable waste flour from fermented rumen beef

| Amino Acid     | Feed A | Feed B | Feed C | Feed D | recommended |
|----------------|--------|--------|--------|--------|-------------|
|                |        |        |        |        |             |
| **Essential**  |        |        |        |        |             |
| Threonin       | 1.14   | 1.29   | 1.32   | 1.35   | 1.40 [19]   |
| Histidin       | 0.65   | 0.71   | 0.73   | 0.79   | 0.80 [20]   |
| Methionin      | 0.64   | 0.68   | 0.71   | 0.76   | 0.90 [21]   |
| Arginin        | 1.28   | 1.43   | 1.46   | 1.51   | 1.90 [21]   |
| Phenylalanin   | 0.77   | 0.91   | 0.95   | 1.02   | 1.40 [22]   |
| Valin          | 1.89   | 1.21   | 1.24   | 1.30   | 1.40 [23]   |
| Leusin         | 1.51   | 1.75   | 1.78   | 1.81   | 1.70 [22]   |
| Isoleusin      | 1.09   | 1.14   | 1.19   | 1.24   | 1.00 [22]   |
| Lysine         | 1.65   | 2.06   | 2.12   | 2.14   | 2.10 [21]   |
| Tryptophan     | -      | -      | -      | -      | 1.3         |
| **Non-Essential** | | | | | |
| Asam Aspartat  | 2.48   | 2.68   | 2.80   | 2.83   | -           |
| Tirosin        | 1.21   | 1.59   | 1.62   | 1.66   | -           |
| Prolin         | 2.26   | 2.71   | 2.74   | 2.78   | -           |
| Alanin         | 0.97   | 1.01   | 1.09   | 1.13   | -           |
| Asam Glutamat  | 6.11   | 6.14   | 6.53   | 6.72   | -           |
| Serin          | 1.14   | 0.98   | 1.18   | 1.21   | -           |
Table 6. Composition of essential amino acids and non-essential feed containing different levels of vegetable waste flour from goat rumen liquid fermentation

| Amino Acid | Feed A | Feed B | Feed C | Feed D | Recommend |
|------------|--------|--------|--------|--------|-----------|
| Histidin   | 0,95   | 1,00   | 1,02   | 1,16   | 0,80[20] |
| Threonin   | 1,46   | 1,47   | 1,51   | 1,54   | 1,40[19] |
| Arginin    | 1,66   | 1,70   | 1,76   | 1,78   | 1,90[21] |
| Methionin  | 0,98   | 1,09   | 1,17   | 1,22   | 0,90[21] |
| Valin      | 1,39   | 1,31   | 1,36   | 1,37   | 1,40[23] |
| Phenilalanin | 1,22   | 1,24   | 1,26   | 1,27   | 1,40[22] |
| Isoleusin  | 1,39   | 1,41   | 1,44   | 1,45   | 1,00[22] |
| Leusin     | 2,00   | 2,09   | 2,12   | 2,30   | 1,70[21] |
| Lysine     | 2,28   | 2,31   | 2,38   | 2,41   | 2,10[21] |
| Tryptophan | -      | -      | -      | -      | 1,3       |

| Amino Acid Non-Esential | Feed A | Feed B | Feed C | Feed D | Recommend |
|-------------------------|--------|--------|--------|--------|-----------|
| Prolin                  | 3,10   | 3,18   | 3,27   | 3,27   | -         |
| Tirosin                 | 1,67   | 1,65   | 1,70   | 1,79   | -         |
| Asam Aspartat           | 3,15   | 3,21   | 3,26   | 3,20   | -         |
| Alanin                  | 1,27   | 1,31   | 1,38   | 1,40   | -         |
| Asam Glutamat           | 7,18   | 7,21   | 7,30   | 7,36   | -         |
| Serin                   | 1,25   | 1,29   | 1,30   | 1,32   | -         |

3.3. Feed the fatty acid test
The composition of dietary fatty acids containing different fermented vegetable waste starch rumen fluid is present in Tables 7 and 8.

Table 7. Composition of dietary fatty acids with the addition of different fermented vegetable waste flour in cattle rumen fluid.

| Types of fatty acid | Feed A | Feed B | Feed C | feed D |
|---------------------|--------|--------|--------|--------|
| Laurat              | 0,13   | 0,20   | 0,14   | 0,28   |
| Miristat            | 1,10   | 1,69   | 1,29   | 1,73   |
| Palmitat            | 22,61  | 21,87  | 28,85  | 24,82  |
| Stearat             | 1,38   | 1,01   | 0,96   | 0,94   |
| Oleat               | 20,25  | 26,02  | 22,37  | 24,45  |
| Linoleat            | 51,41  | 39,39  | 49,30  | 39,98  |
| Linolenat           | 2,66   | 5,55   | 2,24   | 6,49   |
Table 8. Composition of dietary fatty acids containing different fermented vegetable waste starch

| Types of fatty acid | Feed A | Feed B | Feed C | Feed D |
|---------------------|--------|--------|--------|--------|
| Laurat              | 0.17   | 0.21   | 0.11   | 0.20   |
| Miristat            | 1.49   | 1.68   | 1.68   | 1.95   |
| Palmitat            | 26.64  | 28.16  | 30.57  | 28.20  |
| Stearat             | 0.86   | 0.73   | 0.75   | 0.84   |
| Oleat               | 21.95  | 28.89  | 20.74  | 28.58  |
| Linoleat            | 46.83  | 34.88  | 44.80  | 33.71  |
| Linolenat           | 1.35   | 3.77   | 2.70   | 5.18   |

Omega-3 and omega-6 essential fatty acids are unable to be synthesized by shrimp and fish, so they must be added through the feed. According to[27] that there are four types of essential fatty acids needed for all types of shrimp, namely linoleic acid (18: 2n-6), alpha-linolenic acid (18: 3n-3), eicosapentaenoic acid (20: 5n-3), and docosahexaenoic acid (22: 6n-3). [28]. The ideal Linoleic acid for Penaeus aztecus is 1-2%; [32] 2.5%.

Based on Tables 7 and 8, the analysis of test feed fatty acids by adding fermented vegetable waste flour to rumen fluid in cattle and goats showed that oleic fatty acids were higher than other test and control feeds. The content of linolenic acid obtained in feed C (addition of 20% rumen liquid fermented vegetable waste flour) was 2.24%, and feed B (addition of 10% goat rumen liquid fermented vegetable waste flour) was 3.77. [29] provide feed with the addition of fatty acids above 5% whereas [30] recommend for Penaeus monodon omega-3 31.2 mg/day. The ideal concentration of linolenic acid for Penaeus indicus is 2%. [31] report that the presence of FUPA must reach a proportion of feed fatty acids as a condition for predicting weights. The linolenic fatty acid is an essential fatty acid that functions to stimulate shrimp growth [32].

4. Conclusion
Based on the results of research on the effect of the addition of fermented vegetable waste flour rumen fluid of cattle and goats to the nutritional quality of feed, it can be concluded that the addition of 20% fermented vegetable waste flour of cow rumen fluid and 10% fermented vegetable waste flour of goat rumen fluid can improve the nutritional quality of feed.

Acknowledgment
Thanks to DRPM DIKTI This research was funded by The Applied Research of University Priority (PTUPT) in the year 2020, therefore the authors gratefully acknowledge the Indonesia Higher Education especially DP2M for funding this research through PTUPT scheme for the year 2020.

References
[1] Prakash C B, Reddy C P K, Ghosh T K, Ramalingaiah D and Kanudan S C 2016 Effect of different dietary protein sources of growth, survival and carcass composition of Litopenaeus vannamei (Boone, 1931). Journal of Experimental Zoology, 19(1): 205-13.
[2] Murni, Aslamyah S and Sonjaya H 2018 The Nutrition Waste Vegetables with Invitro Using Rumen Liquids for Feed. Journal of Food and Nutrition Sciences 6(2): 58.
[3] Martin C, Devillard E and Michalet-Doreau B 1999 Influence of sampling site on concentrations and carbohydrate-degrading enzyme activities of protozoa and bacteria in the rumen. *Journal of animal science, 77*(4): 979-87.

[4] Lee S S, Kim C H, Ha J K, Moon Y H, Choi N J and Cheng K J 2002 Distribution and activities of hydrolytic enzymes in the rumen compartments of Hereford bulls fed alfalfa-based diet. *Asian-Australasian journal of animal sciences 15*(12): 1725-31.

[5] Kamra D 2005 Rumen microbial ecosystem. *Current Science 89*(1): 124-35.

[6] Budiansyah A, Resmi, Nahrowi K G, Wiryawan M T, Suhartono and Widjastuti Y 2011 Hidrolisis Zat Makanan Pakan oleh Enzim Cairan Rumen Sapi Asal Rumah Potong. *Jurnal Agrinirka 1*(1)

[7] Andriani Y 2015 Assessment on cow rumen fluid celluloseamylase enzyme activity as an alternative source of crude fiber degrading enzyme in fish feed materials. *Lucrări Științifice-Universitatea de Științe Agricole și Medicină Veterinară, Seria Zootehnie 63:* 242-45.

[8] Murni M, Darmawati D and Amri M I 2017 Optimasi lama waktu fermentasi limbah sayur dengan cairan rumen terhadap peningkatan kandungan nutrisi pakan ikan nila (*Oreochromis Niloticus*). *Octopus: Jurnal Ilmu Perikanan, 6*(1): 541-45.

[9] Murni M, Darmawati D and Amri M I 2017 Optimasi lama waktu fermentasi limbah sayur dengan cairan rumen terhadap peningkatan kandungan nutrisi pakan ikan nila (*Oreochromis Niloticus*). *Octopus: Jurnal Ilmu Perikanan, 6*(1): 541-45.

[10] AOAC (Association of Official Analytical Chemists) 1990 *Official Methods of Analysis, 12th edition.* (Washington, D.C: Association of Official Analytical Chemists) p 1141

[11] Murni 2018 *Cairan Rumen sebagai Biodegradator Limbah Sayur dalam Pakan terhadap Kinerja Pertumbuhan Udang Vannamei.* Disertasi. Pascasarjana UNHAS.

[12] Ayyappan S and Ali S A 2007 Analysis of feeds and fertilizers for sustainable aquaculture development in India. *FAO Fisheries Technical Paper, 497* (FAO: Rome) p 191.

[13] Zainuddin Z, Haryati H, Aslamsyah S and Surianti S 2015 Pengaruh level karbohidrat dan frekuensi pakan terhadap rasio konversi pakan dan sintasan juvenile *Litopenaeus vannamei*. *Jurnal Perikanan Universitas Gadjah Mada, 16*(1): 29-34.

[14] McVey J P 1993. *CRC Handbook of Mariculture*, Vol I. Crustacean Aquaculture, 2nd Edn. (London: CRC Press)

[15] Shivaram C M and Raj R P 1997 Dietary Lipid requirements of the juveniles of indian white prawn *Penaeus indicus* H. Milne Edwards. *Journal of Aquaculture in the Tropics, 12:* 165-80.

[16] Hertrampf J 2006 A quick method for crude fiber estimation. *Feed Technology 10*(2): 29–31

[17] Lokapirnasari W P, Nazar D S, Nurhajati T, Supranianondo K and Yulianto A B 2015 Production and assay of cellulolytic enzyme activity of Enterobacter cloacae WPL 214 isolated from bovine rumen fluid waste of Surabaya Abbatio, Indonesia. *Veterinary World, 8*(3): 367.

[18] Palupi R and Imsya A 2011 Pemanfaatan kapang Trichoderma viridae dalam proses fermentasi untuk meningkatkan kualitas dan daya cerna protein limbah udang sebagai pakan ternak unggas. In Seminar Nasional Teknologi Peternakan dan Veteriner. Pusat Penelitian dan Pengembangan Peternakan, Badan Penelitian dan Pengembangan Pertanian (Bogor: Departemen Pertanian) pp 672-77

[19] Alam M S Teshima S I, Koshio S, Ishikawa M, Uyan O, Hernandez L H H and Michael F R 2005 Supplemental effects of coated methionine and lysine to soy protein isolate diet for juvenile shrimp, Marsupenaeus japonicus. *Aquaculture, 248* (1-4): 13-9.

[20] Millamena O M, Bautista M N, Reyes O S and Kanazawa A 1997 Threonine requirement of juvenile marine shrimp *Penaeus monodon*. *Aquaculture, 151*(1-4): 9-14.

[21] Richard L, Blanc P P, Rigolet V, Kaushik S J and Geurden I 2010 Maintenance and growth requirements for nitrogen, lysine and methionine and their utilisation efficiencies in juvenile black
tiger shrimp, *Penaeus monodon*, using a factorial approach. *British Journal of Nutrition*, **103**(7): 984-95.

[22] Millamena O M, Teruel M B, Kanazawa A and Teshima S 1999 Quantitative dietary requirements of postlarval tiger shrimp, *Penaeus monodon*, for histidine, isoleucine, leucine, phenylalanine and tryptophan. *Aquaculture*, **179**(1-4): 169-79.

[23] Millamena O M, Bautista-Teruel M N, Reyes O S and Kanazawa A 1998 Requirements of juvenile marine shrimp, *Penaeus monodon* (Fabricius) for lysine and arginine. *Aquaculture*, **164**(1-4): 95-104.

[24] Brown M 2002 Preparation and assessment of microalga concentrates as feeds for larva and juvenile pacific oyster crassostrea. *Journal of the World Aquaculture Society*. 7: 189-199.

[25] Brown M and Robert R 2002 Preparation and assessment of microalgal concentrates as feeds for larval and juvenile Pacific oyster (*Crassostrea gigas*). *Aquaculture*, **207**(3-4): 289-309.

[26] Herawati V E 2014 Transfer nutrisi dan energi larva udang Vanname (Litopenaeus vannamei) dengan pemberian pakan Artemia sp. produk lokal dan impor. *AQUASAINS*, **2**(2): 177-86.

[27] Lima R B and Figueiredo-Lima D F 2016 Critical review: essential fatty acids on shrimp feeding. *Scientia Agraria Paranaensis*, **15**(3): 236-43.

[28] Shewbart K L and Mies W L 1973 Studies on nutritional requirements of brown shrimp - the effect of linolenic acid on the growth of Penaeus aztecus. In *Proceedings of the annual workshop - World Mariculture Society* (Oxford, UK: Blackwell Publishing Ltd) **4**(1-4) pp. 277-87

[29] Merican Z O and Shim K F 1997 Quantitative requirements of linolenic and docosahexaenoic acid for juvenile Penaeus monodon. *Aquaculture*, **157**(3-4): 277-95.

[30] Glencross B D and Smith D M 2001 A study of the arachidonic acid requirements of the giant tiger prawn, *Penaeus monodon*. *Aquaculture Nutrition*, **7**(1): 59-69.

[31] Rees J F, Curé K, Piyatiratitivorakul S, Sorgeloos P and Menasveta P 1994 Highly unsaturated fatty acid requirements of *Penaeus monodon* postlarvae: an experimental approach based on Artemia enrichment. *Aquaculture*, **122**(2-3): 193-207.

[32] Juwana S 1985 Pellet udang. jurnal. *Oseana*. **10**(4): 150-59.