The effect of using *Nereis* flour with different percentages on the growth performance and consumption of vannamei shrimp post-larvae

[1]Vivi E. Herawati, [1]Tita Elfitasari, [2]I Made B. Dirgantara, [3]Ocky K. Radjasa, [1]Seto Windarto

[1] Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Indonesia; [2] Department of Management, Faculty of Economics and Business, Diponegoro University, Semarang, Indonesia; [3] Indonesian Institute of Sciences, Jakarta, Indonesia. Corresponding author: S. Windarto, seto.windarto@live.undip.ac.id

Abstract. Vannamei shrimp (*Litopenaeus vannamei*) is a fishery commodity that has high economic value. *Nereis virens* has potential as shrimp feed due to its high protein, fat, amino acids, and unsaturated fatty acids. This study aimed to determine the growth performance and nutritional quality of fishmeal substituted with *N. virens* flour and determine the best feed composition for vannamei shrimp post-larvae. This research was conducted at the Marine Science Techno Park (MSTP), UNDIP Jepara, Central Java. The experimental research method was a completely randomized design (CRD), with four treatments and three replications. The treatments used were 0% *N. virens* flour (A); 25% *N. virens* flour (B); 30% *N. virens* flour (C); and 35% *N. virens* flour (D). The shrimp were fed four times a day, amounting to 20% of their biomass. The data observed were total feed consumption (TFC), relative growth rate (SGR), feed utilization efficiency (FUE), feed conversion ratio (FCR), protein efficiency ratio (PER), survival rate (SR), and nutritional quality of the shrimp. The results showed that the values of feed consumption level (80.5 g), relative growth rate (3.91%), feed utilization efficiency (73.93%), food consumption rate (1.35), protein efficiency ratio (1.72%), and survival rate (100%) were best in the treatment with fish meal substituted by *N. virens* flour with 30% (C). Treatment C produced shrimp with 60.23% protein, 11.44% fat, 9.10% methionine and 8.25% EPA. Feeding using 30% *N. virens* flour (C) had a significant effect ($p<0.05$) on the TFC, RGR, FUE, FCR, PER, and SR in vannamei shrimp post-larvae.

Key Words: growth, *L. vannamei, N. virens,* nutrition, post larvae.

Introduction. Vannamei shrimp (*Litopenaeus vannamei*) are a fishery commodity with high economic value. Some of the advantages of vannamei shrimp include being responsive to feed, resistant to disease, fast growth, living at high stocking densities, relatively short maintenance periods, and high survival rates (Herawati et al 2020). The success of a cultivation business is influenced by several important factors, one of which is feed. Unfortunately, shrimp farming activities spend about 50% of the total cost of production on feed (Yustianti et al 2013). This is one of the obstacles to shrimp farming activities. Therefore, alternative feeds with high protein content and relatively low prices. One alternative protein source could be *Nereis virens*, a natural shrimp feed.

N. virens is a marine worm with high economic value and which can be cultured. *N. virens* live in tidal areas, on sandy, muddy and sandy loam substrates (Asnawi et al 2018). The use of this worm as natural or artificial feed for shrimp is due to its highquality nutritional content, with high levels of protein, fat and unsaturated fatty acids, and balanced amino acids. It has high levels of essential fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Lim et al 2011). *N. virens* contains protein ranging from 42.06 to 51.68% and fat ranging from 12.93 to 22%, which can meet the nutritional needs of shrimp (Schaum et al 2013).

Protein requirements to support the growth and survival of vannamei shrimp post-larvae range from 30-40% (Yustianti et al 2013). According to Arsad et al (2017), the intensity of feeding vannamei shrimp with fish meal and pellets is at PL 1-15 2 times, PL 16-70 4 times, and PL 71-120 5 times per day. *N. virens* is a natural feed used as natural food for vannamei shrimp post-larvae. The survival rate of post-larvae shrimp and those fed with chopped marine worms can increase by 46.69% compared to those fed *Artemia* (Haryadi & Rasidi 2012). The growth of vannamei shrimp in the post-larval stage requires protein in the feed ranging from 30-55% (Brown et al 2011). Thus, shrimp need a supply of essential nutrients, including protein, fat, amino acids and fatty acids (Yustianti et al 2013).

Proteins and amino acids are essential to the body because they function as ingredients involved in the buildup, regulation, and breakdown of substances. Boonyoung et al (2013) argue that, as a building block, proteins function to form new tissues for growth, replace damaged tissue, and reproduction. Valverde et al (2013) reported that protein is also a regulatory substance that plays a role in the formation of enzymes, in guarding hormones, and in the regulation of various metabolic processes in the body of the shrimp. Moreover, protein acts as a combustion agent as the carbon element contained therein can be used as an energy source when carbohydrates and fats do not meet the energy needs of the shrimp. Fat and fatty acids serve as the largest sources of energy when compared to proteins and carbohydrates. In shrimp, fatty acids play important roles as an energy source for shrimp. Fat also functions to help metabolic processes, osmoregulation, and the maintenance of the balance of organisms in water, as well as to maintain the shape and function of membranes or tissues (Fidalgo e Costa et al 2010).

This study aimed to determine the growth performance and nutritional quality of shrimp fed diets where fish meal was substituted with *N. virens* flour and determine the best feed composition for vannamei shrimp *L. vannamei* post larva.

Material and Method. The manufacture of *N. virens* flour was carried out by following the method of producing earthworm flour (*Lumbricus rubellus*) of Fidago e Costa et al (2010). The procedure for making *Nereis* flour was carried out by washing the worms with running water until they were clean and soaking them in water several times to remove adhering dirt. The clean *N. virens* were drained and then put in the oven for drying at 50^{o}C for 4-6 hours (Boonyoung et al 2013). Dried *N. virens* were mashed with a blender until flour was obtained. Proximate analysis (AOAC 2005) was conducted for the *Nereis* flour (Table 1).

Table 2 presents the formulation of the feed based on the replacement of fish meal with *Nereis* four. Proximate analysis was also conducted on the formulated feed (Table 2). Based on Boonyoung et al (2013), the process of formulating the feed begins with mixing all the raw materials from the smallest to the largest in proportion. Warm water (50-60°C) was added to the mixed material slowly, and then the feed was formed using a coconut filter. Finally, it was dried in the oven at a temperature of 30°C.

Table 1

Proximate analysis results for *Nereis* flour

	Nutritional content (%)						*Total*
	Moisture	*Protein*	*NFE*	*Fat*	*Crude Fiber*	*Ash*	*(%)*
Nereis flour	15.92	61.11	3.87	9.64	0.82	8.64	100.00

Note: NFE - nitrogen-free extract.

Vannamei shrimp post-larvae culture. Vannamei shrimp at the post-larvae (PL) stage 15 (0.029±0.001 g ind^{-1}) with a stocking density of five shrimps L^{-1} were used. Feed was administered four times a day and weighed 20% of the shrimp biomass. The salinity of seawater was 30 ppt and water has been sterilized using 60 ppm chlorine, neutralized with thiosulfate and stored in a reservoir beforehand. The water quality parameters were measured daily. In addition, the temperature was measured twice a day in the morning and evening, and the pH, salinity, and dissolved oxygen (DO) were measured once a day.

Table 2

Formulation of the feed

Feed ingredients	*Feed composition (% 100 g^{-1} feed)*			
	A	*B*	*C*	*D*
Fish meal	35.00	30.00	25.00	0.00
Nereis flour	0.00	25.00	30.00	35.00
Soybean meal flour	14.08	15.08	12.90	18.64
Bran flour	37.37	7.37	6.28	18.42
Wheat flour	4.55	13.55	16.82	18.94
Fish oil	2.00	2.00	2.00	2.00
Corn oil	2.00	2.00	2.00	2.00
Vit-Min mix	3.00	3.00	3.00	3.00
CMC	2.00	2.00	2.00	2.00
Total (%)	100.00	100.00	100.00	100.00
Protein (%)	43.47	43.46	43.47	43.48
NFE (%)	18.78	12.92	13.13	14.86
Fat (%)	7.46	9.74	9.95	9.23
En. (kkal g^{-1})	390.49	387.90	390.76	391.17
Energy-protein ratio	8.98	8.93	8.99	9.00

Note: NFE - nitrogen-free extract; En. - energy.

Feeding PL vannamei shrimp. This study used an experimental design in the form of a completely randomized design (CRD) with 4 treatments and 3 replications, and the length of the study was 30 days. Four containers, measuring 40×50×50 cm with 30 L of water were used for each treatment. The following is the arrangement of treatments and different dosage compositions for the use of worm flour (*N. virens*): 0% *N. virens* flour (A); 25% *N. virens* flour (B); 30% *N. virens* flour (C); 35% *N. virens* flour (D).

Total feed consumption (TFC). The TFC was calculated according to Tacon (1993):

$$TFC=C-S$$

Where: C - administered feed (g); S - leftover feed (g).

Relative growth rate (RGR). The RGR was calculated using the following formula (Tacon 1993):

$$RGR = [(Wt-Wo)/(Wo \times t)] \times 100\%$$

Where: RGR - relative growth rate (% day^{-1}); Wo - shrimp biomass weight at the beginning of the study (g); Wt - shrimp biomass weight at the end of the study (g); t - time of the study (days).

Feed utilization efficiency (FUE). The FUE was calculated based on Tacon (1993):

$$FUE=[(Wt-W0)/TFC]x100$$

Where: Wt - biomass at the end of the study (g); W0 - initial biomass at the beginning of the study (g); TFC - total feed consumed during the study.

Feed conversion ratio. The feed conversion ratio (FCR) was calculated using the formula used by Tacon (1993):

$$FCR=TFC/[(Bt+Bd)-Bo]$$

Where: TFC - total feed consumption (g); Bt - final fish biomass (g); Bd - dead fish biomass (g); Bo - initial fish biomass (g).

Protein efficiency ratio (PER). The protein efficiency ratio (PER) was calculated using the formula of Tacon (1993):

$$PER=[(Wt–W0)/Pi]x100$$

Where: Wt - total weight of fish at the end of research (g); W0 - total weight of fish at the start of research (g); Pi - total feed consumed multiplied by fish protein content.

Survival rate. The survival rate (SR) of vannamei shrimp shrimp was calculated using the following formula (Tacon 1993):

$$SR = (Nt/N_0) \times 100\%$$

Where: Nt - the number of shrimps at the end of the study; N_0 - the number of shrimps at the beginning of the study.

Amino acid analysis. Amino acids were analyzed using high-performance liquid chromatography (HPLC), with a 1100 Apparatus with a Eurosphere 100-5 C18, 250×4.6 mm column, and an initial column P/N: 1115Y535. The buffers used were: A) 0.01 M acetate buffer at pH 5.9; and B) 0.01 M MeOH acetate buffer at pH 5.9; THF >80: 15: 5 Λ Fluorescence: Extra: 340 mm; Em: 450 nm. Approximately 2.5 g of each sample was placed in a glass chamber, and 15 mL of 6 M HCl was added. Furthermore, the mixture was vortexed for homogeneity and hydrolyzed in an autoclave at 110°C for 12 h, cooled to room temperature, and neutralized with 6 M NaOH. After adding 2.5 mL of 40% lead acetate and 1 mL of 15% oxalic acid, approximately 3 mL of the mixture was filtered with a 0.45 µm Millex-HV filter (Merck KGaA, Darmstadt, Germany). 25 µL of the filtered mixture and 475 µL of the OPA anhydrase solution were stirred and incubated for 3 min for injection into the HPLC system. Finally, 30 µL of the final mixture was placed in an HPLC system (AOAC 2005).

Fatty acid analysis. *L. vannamei* fatty acids were analyzed using the QP-2010 Gas Chromatograph Mass Spectrophotometer (GCMS) (Shimadzu) and a mass spectrophotometer with a length of 50 m and a diameter of 0.22 mm wall coat open tubular CP-SIL-88 column (Agilent, Santa Clara, CA, USA), with analyses carried out over a column temperature range of 120-200°C. The method used was *in situ* transesterification. First, 100 mg of the sample were homogenized using 4 mL of water. The 100 µL homogenate obtained was then transferred into a test tube. 100 µL of methylene chloride were then added and 1 mL of 0.5 M NaOH in methanol. After nitrogen was added and the tubes were tightly closed, they were heated to 90°C for 10 min. The test tube was then cooled, and 1 mL of 14% BF_3 in methanol was added. After adding nitrogen, the mixture was heated at the same temperature for 10 min. After that, the test tube was cooled to ambient temperature, and 1 mL of water and 200-500 µL of hexane were added. The mixture was then stirred for 1 min to extract the methyl ester from the fatty acids. After centrifugation, the top layer of the sample was ready for gas chromatography (GC) analysis (AOAC 2005).

Statistical analysis. The RGR, absolute length growth, biomass weight, grazing rate, SR, proximate analysis, amino acid analysis, and fatty acid analysis were statistically analyzed using the normality, homogeneity, and additivity tests before being compared using the analysis of variance (ANOVA). Further tests were performed using Duncan's multiple range test, with significance set at $p<0.05$. Finally, the water quality parameters were analyzed descriptively and compared with the references.

Results and Discussion. The results of TFC, RGR, FUE, FCR, PER and SR of *L. vannamei* are presented in Table 3.

Table 3

The average value of total feed consumption (TFC), relative growth rate (RGR), feed utilization efficiency (FUE), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR) of *Litopenaeus vannamei* during the study

Parameters	*Treatment*			
	A	*B*	*C*	*D*
TFC (g)	73.95±0.80^{a}	77.64±0.48^{bc}	80.05±0.25^{d}	76.68±0.99^{b}
RGR (%)	2.84±0.06^{a}	3.33±0.06^{c}	3.91±0.03^{d}	3.08±0.05^{b}
FUE (%)	58.07±1.00^{a}	64.84±0.86^{c}	73.93±0.42^{d}	60.75±1.14^{b}
FCR	1.79±0.04^{d}	1.54±0.02^{b}	1.35±0.01^{a}	1.65±0.03^{c}
PER (%)	1.35±0.02^{a}	1.51±0.02^{c}	1.72±0.01^{d}	1.41±0.03^{b}
SR (%)	94.44±1.92^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

Note: TFC - total feed consumption; RGR - relative growth rate; FUE - feed utilization efficiency; FCR - feed conversion ratio; PER - protein efficiency ratio; SR - survival rate; different superscripts indicate significant differences (p<0.05).

Based on Table 3, the results showed the better values of TFC, RGR, FUE, FCR, PER, and highest SR were in treatment C (30% *N. virens*), with the values of 80.05. g, 3.91%, 73.93%, 1.35, 1.72%, and 100%, respectively.

Proximate feed analysis for *L. vannamei* post-larvae was highest in treatment C, with 50.23% protein and 10.24% fat. The proximate analysis of vannamei shrimp post-larvae is presented in Table 4.

Table 4

Proximate analysis of vannamei shrimp (*Litopenaues vannamei*) post-larvae

Treatments	*Dry weight content*				
	Protein (%)	*Carbohydrate (%)*	*Crude fat (%)*	*Ash (%)*	*Crude fiber (%)*
A	45.26±0.03^{a}	18.80±0.02	6.24±0.04^{a}	24.07±0.07	5.33±0.06
B	55.45±0.02^{b}	17.57±0.05	8.77±0.02^{b}	15.51±0.03	3.70±0.03
C	60.43±0.01^{c}	15.02±0.03	11.44±0.03^{c}	10.26±0.03	2.85±0.08
D	52.90±0.04^{b}	15.72±0.03	8.34±0.03^{a}	17.55±0.03	5.49±0.08

The proximate analysis results of vannamei shrimp post-larvae were best in treatment C, with 60.43% protein and 11.44% fat. The amino acid profile of the feeds is presented in Table 5.

Table 5

Amino acid profile of the feed in the four treatments

Amino acid (%)	*Treatments*			
	A	*B*	*C*	*D*
Aspartic acid	1.98±0.09^{a}	2.06±0.06^{a}	3.19±0.09^{c}	2.98±0.04^{b}
Serine	1.46±0.09^{b}	1.10±0.01^{a}	3.03±0.03^{c}	1.23±0.07^{a}
Glutamic acid	2.16±0.08^{a}	2.26±0.05^{a}	4.19±0.03^{b}	2.20±0.01^{a}
Glycine	3.98±0.05^{a}	4.26±0.07^{b}	5.03±0.06^{c}	3.90±0.09^{a}
Histidine	4.79±0.11^{c}	2.80±0.05^{a}	3.90±0.09^{b}	2.75±0.08^{a}
Arginine	3.27±0.02^{b}	2.67±0.07^{a}	3.36±0.08^{b}	2.56±0.02^{a}
Threonine	3.43±0.04^{b}	1.89±0.09^{a}	1.78±0.06^{a}	1.96±0.08^{a}
Alanine	2.17±0.03^{a}	2.95±0.08^{b}	3.20±0.09^{c}	2.23±0.09^{a}
Proline	2.84±0.03^{a}	3.75±0.09^{b}	4.87±0.07^{c}	2.92±0.01^{a}
Valine	2.73±0.08^{a}	4.90±0.04^{c}	4.83±0.03^{c}	3.99±0.08^{b}
Methionine	1.88±0.03^{a}	4.99±0.03^{c}	6.98±0.05^{d}	3.98±0.03^{b}
Lysine	3.09±0.03^{a}	5.20±0.04^{c}	6.50±0.03^{d}	4.98±0.05^{b}
Isoleucine	1.16±0.08^{a}	3.09±0.02^{b}	4.23±0.02^{d}	3.99±0.03^{c}
Leucine	3.23±0.02^{c}	1.38±0.01^{a}	3.25±0.03^{c}	2.57±0.01^{b}
Phenylalanine	3.06±0.07^{c}	2.98±0.09^{b}	5.98±0.01^{d}	1.96±0.06^{a}

Note: different superscripts indicate significant differences between treatments (p<0.05).

Based on the amino acid profile of feed for *L. vannamei* post-larvae, the highest value was for methionine (6.98%), in treatment C. The amino acid profile of vannamei shrimp post-larvae is presented in Table 6.

Table 6

Amino acid profile of vannamei shrimp (*Litopenaeus vannamei*) post-larvae

Amino acid (%)	*Treatments*			
	A	*B*	*C*	*D*
Aspartic acid	3.98±0.09[b]	3.26±0.06[a]	5.23±0.09[c]	3.78±0.04[b]
Serine	1.46±0.09[a]	3.10±0.01[c]	3.19±0.03[c]	2.23±0.07[b]
Glutamic acid	3.16±0.08[a]	4.26±0.05[b]	5.89±0.03[d]	4.80±0.01[c]
Glycine	3.98±0.05[a]	6.25±0.07[c]	6.93±0.06[d]	5.99±0.09[b]
Histidine	3.79±0.11[a]	3.80±0.05[a]	4.98±0.09[b]	4.85±0.08[b]
Arginine	3.27±0.02[c]	2.67±0.07[a]	4.86±0.08[d]	2.96±0.02[b]
Threonine	3.43±0.04[c]	1.19±0.09[a]	1.78±0.06[b]	1.09±0.08[a]
Alanine	2.17±0.03[a]	2.95±0.08[b]	3.20±0.09[c]	2.23±0.09[a]
Proline	2.84±0.03[a]	3.75±0.09[b]	5.87±0.07[d]	4.92±0.01[c]
Valine	2.73±0.08[a]	4.90±0.04[c]	4.83±0.03[c]	3.99±0.08[b]
Methionine	1.88±0.03[a]	6.99±0.03[c]	9.10±0.05[d]	5.18±0.03[b]
Lysine	3.09±0.03[a]	5.70±0.04[c]	7.98±0.03[d]	4.85±0.05[b]
Isoleucine	1.16±0.08[a]	3.59±0.02[b]	5.23±0.02[c]	3.68±0.03[b]
Leucine	3.23±0.02[c]	1.78±0.01[a]	3.25±0.03[c]	2.55±0.01[b]
Phenylalanine	5.06±0.07[c]	2.98±0.09[b]	5.08±0.01[c]	1.96±0.06[a]
Tryptophan	1.03±0.02[a]	1.78±0.01[c]	2.25±0.03[d]	1.55±0.01[b]

Note: different superscript letters indicate significant differences between treatments ($p<0.05$).

The amino acid with the highest concentration in *L. vannamei* post-larvae was methionine (9.10%) in treatment C. The fatty acid profiles of the feeds are presented in Table 7.

Table 7

Fatty acid profile of feed

Fatty acids profile (%)	*Treatments*			
	A	*B*	*C*	*D*
Myristic	0.81±0.05[a]	2.51±0.02[c]	3.68±0.09[d]	1.50±0.05[b]
Pentadecanoic	1.09±0.06[b]	0.17±0.304[a]	2.25±0.08[c]	1.08±0.02[b]
Palmitic	2.14±0.09[a]	3.97±0.08[b]	4.79±0.04[d]	4.12±0.01[c]
Stearic	1.71±0.07[b]	1.52±0.03[a]	3.11±0.09[d]	2.08±0.05[c]
Oleic/ω9	1.07±0.02[a]	2.89±0.08[c]	3.91±0.01[d]	2.55±0.03[b]
Linoleic/ω6	0.83±0.09[a]	3.49±0.07[b]	3.37±0.02[b]	3.75±0.02[c]
Linolenic/ω3	0.54±0.05[a]	2.39±0.03[b]	3.32±0.01[d]	2.56±0.07[c]
Arachidic	1.30±0.08[a]	2.02±0.04[b]	3.05±0.03[d]	2.25±0.05[c]
Arachidonic	2.07±0.02[c]	1.15±0.02[b]	2.13±0.08[c]	0.06±0.08[a]
DHA	1.83±0.05[b]	2.87±0.01[c]	3.50±0.03[d]	1.39±0.08[a]
EPA	1.05±0.02[a]	2.68±0.02[c]	4.97±0.08[d]	2.33±0.05[b]

Note: different superscript letters indicate significant differences between treatments ($p<0.05$).

Based on the fatty acid profile of feed, the fatty acid with the highest concentration was EPA (4.97%), in treatment C. Fatty acid profiles of vannamei shrimp post-larvae are presented in Table 8.

Table 8

Fatty acid profile of vannamei shrimp (*Litopenaeus vannamei*) post-larvae

Fatty acids profile (%)	*Treatments*			
	A	*B*	*C*	*D*
Myristic	2.81±0.05^{c}	2.51±0.02^{b}	3.68±0.09^{d}	1.50±0.05^{a}
Pentadecanoic	1.19±0.06^{b}	0.17±0.30^{a}	2.25±0.08^{c}	1.08±0.02^{b}
Palmitic	6.14±0.09^{c}	4.97±0.08^{b}	8.09±0.04^{d}	4.12±0.01^{a}
Stearic	2.71±0.07^{c}	1.52±0.03^{a}	3.11±0.09^{d}	2.08±0.05^{b}
Oleic/ω9	3.07±0.02^{b}	3.89±0.08^{c}	5.91±0.01^{d}	2.55±0.03^{a}
Linoleic/ω6	4.83±0.09^{c}	3.49±0.07^{a}	5.37±0.02^{d}	3.75±0.02^{b}
Linolenic/ω3	3.54±0.05^{b}	2.39±0.03^{a}	4.32±0.01^{c}	2.56±0.07^{a}
Arachidic	2.30±0.08^{b}	2.02±0.04^{a}	4.05±0.03^{c}	2.25±0.05^{b}
Arachidonic	2.07±0.02^{b}	0.15±0.02^{a}	2.13±0.08^{b}	0.06±0.08^{a}
DHA	1.83±0.05^{c}	1.07±0.01^{a}	5.07±0.03^{d}	1.39±0.08^{b}
EPA	6.05±0.02^{c}	5.68±0.02^{b}	8.25±0.08^{d}	5.03±0.05^{a}

Note: different superscript letters indicate significant differences between treatments ($p<0.05$).

The highest fatty acid of *L. vannamei* post-larvae was EPA (8.25%) in treatment C.

The values of the water quality parameters for white shrimp during the study are presented in Table 9.

Table 9

Water quality parameters for vannamei shrimp (*Litopenaues vannamei*) in the aquaculture media during the study

Treatments	*Parameter*			
	Temperature (°C)	*pH*	*DO (mg L^{-1})*	*Salinity (ppt)*
A	29.0±0.07	8±0.07	4.54±0.05	25±0.02
B	28.0±0.03	8±0.04	4.34±0.03	25±0.03
C	29.8±0.02	8±0.05	4.25±0.06	25±0.06
D	29.2±0.04	8±0.08	4.78±0.07	25±0.08
Optimal values	26-32°C^{a}	7.7–8.7^{b}	3–8^{c}	15–35^{b}

Note: references: a - Fendjalan et al (2016); b - Herawati et al (2020); c - Gamis et al (2016).

Based on the study results, the experimental feeds had a significant effect on the growth of vannamei shrimp post-larvae reared for 30 days. Treatment C resulted in the best TFC, RGR, FUE, FCR, and PER values. The high protein in *N. virens* flour was 61.11% (Table 1), higher than in fishmeal or maggot meal (Fendjalang et al 2016; Herawati et al 2019).

High protein can support the growth of vannamei shrimp. The effect of protein value on growth can be measured through PER (Ma et al 2013). This statement is proven by the results of this study, where the best PER was in treatment C, 1.72, and the lowest in treatment A, 1.35. Protein and fat are essential nutrients for shrimp. The protein requirements needed for the growth of vannamei shrimp post-larvae ranges from 30 to 45% (Yustianti et al 2013). According to Jayadhandran et al (2015), the protein and fat content of *Nereis* sp. can meet the needs of various shrimp species. The nutritional content of feed following the nutritional needs of the species will accelerate its growth (Lim et al 2011; Nwaichi 2013).

The use of *N. virens* flour as a substitute for fish meal was able to provide good growth for shrimp. A high level of feed consumption resulted in high growth, being evidenced by the best RGR in the same treatment. Belghit et al (2014) stated that high levels of feed consumption influence high growth rates. Factors that influence growth include feed, cultivar heredity, and water quality (Valverde et al 2013).

Based on the research results, high FUE results in better growth and a low feed conversion ratio (FCR). FUE and food consumption rate in treatment C have the best values in this study. The FCR value from the results of the study was classified as optimum for vannamei shrimp. Bahri et al (2020) stated that the value of a good FCR for vannamei shrimp is 1.3-1.4. Treatment A gave the lowest value among the treatments because the feed had nutritional qualities that were less able to meet the nutritional needs of shrimp. According to Ma et al (2013), the value of the FCR is inversely proportional to weight gain, so a lower the value means that the shrimp is more efficient in utilizing the feed. The growth of vannamei shrimp post-larvae in this study was best in treatment C, and the lowest growth was in the control. Fendjalang et al (2016) stated that the commercial feed for vannamei shrimp is known to have a FCR ranging from 1.6-2.49%.

Nereis flour contains amino acids that meet the nutritional needs of vannamei shrimp. Based on the statement of Kuang et al (2012), the protein must have a high level of digestibility and the amino acids should be similar to those of the species being cultivated. The amino acids in *N. virens* flour are more suitable for shrimp needs, where there are high levels of methionine, phenylalanine, and lysine (Mouneyrac et al 2012). The amino acid methionine can act as a chemoattractant to increase appetite and support shrimp growth. The essential amino acid with high concentration in the feed from treatment C was methionine (6.98%). The need for the methionine required by vannamei shrimp post larvae is 3.2% (Brown et al 2011). The function of methionine for shrimp, among others, is to improve the balance and utilization of other amino acids to increase shrimp growth. In addition, methionine has an essential part in protein synthesis and physiological functions. Bhagavan (1992) stated that the body of fish or shrimp needs methionine to form nucleic acids and tissues, as well as for protein synthesis. Methionine cooperates with vitamin B12 and folic acid in helping the body regulate excessive protein supply in a high-protein diet. It has been proven that feeds with high methionine content increase growth and immune responses (Yuan et al 2011; Kuang et al 2012; Boonyoung et al 2013; Ma et al 2013; Rolland et al 2015).

Some of the fatty acids in *N. virens* flour (EPA and DHA) are needed by shrimp. The results of the total fatty acid profile (Table 8) showed the highest in EPA in vannamei shrimp post-larvae from treatment C was 8.25%, and the lowest was in treatment A, 1.05%. The EPA and DHA requirements for post-larva vannamei shrimp are 2.6% and 1.5%, respectively (Tocher 2015). EPA is an essential component of phospholipids in membranes and nervous tissues and plays a role in survival and growth (Lim et al 2011). Limsuwatthanathamrong et al (2012) stated that fatty acids are an essential factor that must be considered when providing feed for shrimp during the gonad maturation process.

Survival rate (SR) is one of the essential parameters and measures the success of a cultivation activity. Based on the results of the study, it can be seen that the SR of vannamei shrimp post-larvae reared for 30 days was the highest treatments C, D, and B (100%), and lowest in treatment A (94.44%). Several factors affect the survival rate of vannamei shrimp post-larvae, one of which is water quality in the rearing media. Based on the measurement results, it can be said that the water quality of the vannamei shrimp post-larval rearing media during 30 days of rearing was within reasonable limits, supporting the growth and survival of the vannamei shrimp post larvae. This follows Mouneyrac et al (2012) and Nwaichi (2013), who state that good metabolic processes and physiological processes are supported by good water quality.

The temperature of the maintenance medium during the study ranged from 28.2 to 31.8^{o}C, being optimal for the growth of vannamei shrimp post-larvae. According to Kuang et al (2012), the optimal temperature required for vannamei shrimp ranges from 28 to 32^{o}C. The pH was 7.41-8.3. This value is optimal for the growth and survival of vannamei shrimp, which ranges from 7 to 8.5. Shrimp can tolerate pH values in the range of 6.5-9. A pH value below the tolerance range can disrupt the molting process, so that the carapace becomes soft and can decrease survival (Gamis et al 2016).

DO values ranged from 3.89 to 5.67 mg L^{-1}. These values are suitable for supporting the growth and survival of vannamei shrimp. Schaum et al (2013) noted that the DO value to support the life of vannamei shrimp is >3 mg L^{-1}. Salinity in rearing water ranged from 29-31 ppt, within the limits of 28-32 ppt that support shrimp life (Tocher 2015). Vannamei shrimp have euryhaline properties, and can live at 0.5-40 ppt (Yustianti et al 2013).

Ammonia obtained from the measurement results at the beginning of maintenance was 0.023 mg L^{-1} and at the end of maintenance was 0.19 mg L^{-1}. At the beginning of the rearing, the ammonia value was good for vannamei shrimp, but at the end of the experiment, the ammonia value was relatively high. Nevertheless, the ammonia value was still within the tolerance limit of vannamei shrimp, so it can still be said that it did not negatively affect the growth and survival of vannamei shrimp post-larvae. This is reinforced by Herawati et al (2020), who noted that the ammonia value for shrimp must be lower than 1 ppm. This statement is also supported by Yustianti et al (2013). High ammonia level are usually caused by the accumulation of feed residues and feces at the bottom of the water, increasing the content of toxic nitrite, and ultimately causing stunted growth and death (Schaum et al 2013). The mortalities in this study were due to cannibalism.

Conclusions. Substituting fishmeal with 30% *N. virens* flour showed the best results on total feed consumption, relative growth rate, feed utilization efficiency, feed conversion rate, protein efficiency ratio, and survival rate with values of 80.05 g, 3.91%, 73.93%, 1.35, 1.72%, and 100%, respectively. The best nutrient values of vannamei shrimp in this research were 60.23% crude protein, 11.44% fat, 9.1% methionine and 8.25% EPA. In addition, feeding using 30% *N. virens* flour had a significant effect ($p<0.05$) on total feed consumption, relative growth rate, feed utilization efficiency, feed conversion ratio, protein efficiency ratio and survival rate in vannamei shrimp post-larvae.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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Authors:
Vivi Endar Herawati, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: viviendar23@gmail.com
Tita Elfitasari, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: t.elfitasari@gmail.com
I Made Bayu Dirgantara, Department of Management, Faculty of Economics and Business, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: imadebdirgantara@lecturer.undip.ac.id
Ocky Karna Radjasa, Indonesian Institute of Sciences, 10 Jend. Gatot Subroto St., 12710 Jakarta, Indonesia, e-mail: ocky_radjasa@yahoo.com
Seto Windarto, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Soedarto, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: seto.windarto@live.undip.ac.id

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