Effect of *Nereis virens* fermented meal as a fishmeal substitute in *Litopenaeus vannamei* juvenile feed on growth performance and nutritional quality

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Abstract. Pacific white shrimp (*Litopennaeus vannamei*) is an economically valuable fishery commodity. *Nereis virens* has potential as shrimp feed due to its nutritional content, especially the protein and fat content. *N. virens* requires fermentation to improve its overall dietary quality. This study aimed to determine the feed utilization efficiency, growth performance, and nutritional quality of juvenile Pacific white shrimp fed diets with different levels of fermented *N. virens* meal substituting fishmeal and to discover the best diet composition. This study 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 and three replications. The Pacific white shrimp diet was formulated with graded levels of fermented *N. virens* meal substituting fishmeal: A - 0%; B - 20%; C - 25%; D - 30%. The shrimp were fed four times daily, amounting to 20% of their biomass. The results showed that treatment diets significantly affected white shrimp's growth performance ($p<0.05$). The treatment with the best results was treatment D, resulting in a total feed consumption of 83.7 g, relative growth rate of 3.95%, feed utilization efficiency of 75.9%, a feed conversion ratio of 1.03, a protein efficiency ratio of 1.75% and a survival rate of 100%. Treatment D contained 56.33% protein, 12.12% fat, 7.98% methionine, and 8.65% EPA. Water quality during the 42 days of rearing was within the optimal range for growth.
Key Words: feed utilization, fishmeal, marine worms, production, white shrimp.

Introduction. Pacific white shrimp (*Litopenaeus vannamei*) is one of the most economically valuable fishery commodities. Some advantages of the Pacific white shrimp include being responsive to feed, resistant to infestation, fast growth, a relatively short maintenance period, a high survival rate, and it can live at high stocking densities (Herawati et al 2020c). The success of an aquaculture enterprise is influenced by several important factors, one of which is the feed. 50% of the total production costs are represented by the feed in shrimp farming activities (Yustianti et al 2013). Therefore, alternative feed ingredients with high protein content and relatively low prices are needed, one of which can be *N. virens*.

The utilization of *N. virens* in the shrimp diet is suitable because of its high nutritional content and low crude fiber. A fermentation process can further improve the nutritional quality of *N. virens* meal. The fermentation process aims to increase protein content and quality, maintain nutritional value during storage, and reduce anti-nutritional substances of feed ingredients (Abu-Elala et al 2013; Nwachi 2013; Herawati et al 2020b). Fermentation is the anaerobic dissimilation of organic compounds mediated by the activity of microorganisms or extracts from the cells of these microorganisms (Abu-Elala et al 2013). Fermentation aims to shorten the long chains of amino acids and fatty acids to facilitate feed absorption during the metabolic process. This is done by using probiotic bacteria to boost growth and add nutrients to the larval feed (Nwachi 2013). Other advantages are the production of better flavor, energy efficiency and usability.

The protein content of *Nereis* is between 42.06 to 51.68%, and the fat content is between 12.93 and 22%, which can meet the nutritional needs of shrimp (Schaum et al 2013). The dietary needs of Pacific white shrimp for protein in the diet range from 30 to 40%, carbohydrates content of 40%, and a fat range from 9 to 12% (Yustianti et al 2013; Lim et al 2011). This study aimed to determine feed utilization efficiency, growth performance, and nutritional quality of juvenile Pacific white shrimp fed diets with *N. virens* meal as a substitute for fishmeal in different ratios.

Material and Method

Preparation of N. virens meal. *N. virens* were obtained from cultivation in the Marine Science Techno Park Laboratory. The meal making process was carried out at the Marine Science Techno Park Laboratory, Jepara, Central Java. 500 g of worms are usually used to produce 100 g of flour. A higher quantity was produced for this experiment. *N. virens* meal was placed in an oven with a temperature of 40-50°C for drying. According to Julendra & Sofyan (2007), drying worms using an oven lasts 4 h at a temperature of 50°C. The following processes were to puree the dried *N. virens* with a blender into flour.

Fermentation of N. virens meal. *N. virens* meal (1 kg) was mashed, sieved, and fermented by mixing it with 1 mL of commercial bacteria containing probiotic bacteria *Lactobacillus casei* and *Saccharomyces cerevisiae* with molasses as an activator in a ratio of 1:1 in 100 mL of water (Herawati et al 2020a). The mixture was placed in a plastic bag and stored at room temperature for 7 days (Herawati et al 2015). The fermentation of *N. virens* meal is characterized by a sour smell. The next step was to open the plastic bag containing the fermented *N. virens* meal for cooling. The proximate compositions of *N. virens* fermented and fresh meals were determined (Table 1).

Table 1

Proximate composition of *Nereis virens* meal and fermented *N. virens* meal

Ingredients	*Proximate composition (%)*						*Total (%)*
	Water	*Protein*	*NFE*	*Fat*	*CF*	*Ash*	
N. virens meal	15.92	61.11	3.87	9.64	0.82	8.64	100
Fermented *N. virens* meal	8.5	63.53	5.87	11.75	4.85	5.5	100

Note: NFE - nitrogen-free extract; CF - crude fiber.

The test diet formulation using fermented *N. virens* meal as a substitute for fishmeal is presented in Table 2. Preparing the test diets started with mixing all the raw materials from the smallest to the largest. The ingredients incorporated were added slowly with warm water (50°C), then pelletized with a flour mesh sieve. The feed was placed in an oven at approximately 40°C until it dried.

Pacific white shrimp juvenile culture. Pacific white shrimp with a weight of 0.029±0.001 g were used in this study, with a stocking density of 5 shrimps L^{-1}. Feed was given four times a day, at 20% of the shrimp biomass (Herawati et al 2023). The rearing media used was seawater with a salinity of 30 ppt. The seawater was sterilized using 60 ppm chlorine and neutralized with thiosulfate, after which it was stored in a reservoir. Water change was done once a day in the morning. The water quality parameters were measured daily. The temperature was measured twice daily in the morning and evening, and the pH, salinity, and dissolved oxygen (DO) were measured once a day.

Table 2

Formulation of experimental diets using fermented *Nereis virens* meal as a fishmeal substitute

Diet ingredients	*Diet composition (% 100 g^{-1} diet)*			
	A (0%)	*B (20%)*	*C (25%)*	*D (30%)*
Fish meal	35.00	21.00	26.25	24.50
Fermented *N. virens* meal	0.00	7.00	8.75	10.50
Shrimp head meal	12.23	19.80	13.10	15.38
Soybean cake meal	31.00	30.10	27.00	25.00
Rice bran meal	12.77	13.10	15.90	15.62
Fish oil	2.00	2.00	2.00	2.00
Corn oil	2.00	2.00	2.00	2.00
Vitamin 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 (%)	39.88	39.88	39.88	39.88
NFE (%)	19.47	18.12	18.52	17.75
Fat (%)	7.45	7.64	8.01	8.16
Energy (kcal g^{-1})	373.18	369.45	374.54	372.77
E/P ratio	9.36	9.26	9.39	9.35

Note: NFE - nitrogen free extract; CMC - carboxymethyl cellulose; E/P ratio - energy per protein ratio.

Feeding of Pacific white shrimp. This study used an experimental design in the form of a completely randomized design (CRD) with four treatments and three replications. The study lasted 42 days. Containers measuring 40×50×50 cm were used for each treatment. The treatments used are: treatment A: 0% fermented *N. virens* meal; treatment B: 20% fermented *N. virens* meal; treatment C: 25% fermented *N. virens* meal; treatment D: 30% fermented *N. virens* meal.

Observed parameters. Measurement were conducted once every week to observe the parameters of growth performance, which consist of: total feed consumption (TFC), relative growth rate (RGR), the efficiency of feed utilization (EFU), the protein efficiency ratio (PER), feed conversion ratio (FCR), and survival rate (SR).

Total feed consumption (TFC). TFC represents the total quantity of feed consumed by the shrimp. TFC can be calculated using the formula (Zonneveld et al 1991):

F= C- S

Where: F - feed consumption (g); C - administered feed (g); S - remaining feed (g).

Relative growth rate (RGR). The RGR of shrimp was calculated using the formula (Steffens 1989):

$$RGR = \frac{Wt - Wo}{Wo \times t} \times 100\%$$

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).

The efficiency of feed utilization (EFU). EFU was calculated using the formula (Tacon 1993):

EFU=[(Wt-Wo)/F]x100

Where: Wt - final biomass at the end of the study (g); Wo - baseline biomass at the start of the study (g); F - the amount of feed consumed during the study.

Protein efficiency ratio (PER). The following formula was used to calculate PER (Tacon 1993):

PER=[(Wt-Wo)/Pi]x100

Where: Wt - weight of test animals at the end of maintenance (g); Wo - weight of test animals at the beginning of maintenance (g); P - protein content x amount of feed consumed.

Feed conversion ratio (FCR). The following formula was used to calculate the FCR (Tacon 1993):

FCR=F/(Wt+D-Wo)

Where: F - amount of feed given (g); Wt - weight of test animals at the end of maintenance (g); Wo - weight of test animals at the beginning of maintenance (g); D - weight of dead shrimp during maintenance (g).

Survival rate (SR). The SR of Pacific white shrimp was calculated using the following formula (Steffens 1989):

SR=(Nt/N0)x100

Where: SR - survival rate of Pacific white shrimp (%); Nt - the number of shrimp at the end of the study; N_0 - the number of shrimp at the beginning of the study.

Proximate analysis. Proximate analysis (AOAC 2005) was used to determine the protein, fat, ash, carbohydrate, and water content of shrimp. The protein determination was carried out using the Kjeldahl method, while the fat content was determined using the Soxhlet method. Analyses of water and ash content were done using gravimetric principles, and the analysis of carbohydrates was done through calculations based on the other proximate analysis results.

Amino acid analysis. The amino acid profile for shrimp and diets was determined using high-performance liquid chromatography (HPLC), with a type 1100 apparatus with a Eurosphere 100-5 C18, 250×4.6 mm column, and an initial column P/N: 1115Y535. The buffer was: 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 were placed in a glass chamber, and 15 mL of 6 M HCl were added. Furthermore, the mixture was vortexed for homogeneity, 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 were placed in the HPLC system (AOAC 2005).

Fatty acid analysis. The fatty acid profile for shrimp and diets was 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. 100 mg of sample were homogenized using 4 mL of water. The 100 µL homogenate obtained was transferred into a test tube. 100 µL of methylene chloride

were added, along with 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 cooled, and 1 mL of 14% BF3 in methanol was added. After adding nitrogen, the mixture was heated at the same temperature for 10 min. Afterwards, the test tube was cooled to ambient temperature, and 1 mL of water and 200-500 µL of hexane were added. The mixture was stirred for 1 min to extract the methyl esters 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, weight, SR, proximate composition, amino acid results, and fatty acid results were statistically analyzed using the normality, homogeneity, and additivity tests before being compared using the analysis of variance (ANOVA). Further tests used Duncan's multiple range test, with significance at $p<0.05$. The water quality parameters were analyzed descriptively and compared with references.

Results. The study's results on growth performance and feed utilization of juvenile Pacific white shrimp during the 42 days of maintenance are presented in Table 3.

Table 3

Growth performance and feed utilization efficiency in juvenile *Litopenaeus vannamei* for 42 days of maintenance

Variable	*Treatments*			
	A (0%)	*B (20%)*	*C (25%)*	*D (30%)*
TFC (g)	74.84±1.7^{a}	78.9±1.7^{bc}	81.1±0.95^{c}	83.7±1.03^{c}
RGR (%)	2.85±0.1^{a}	3.05±0.18^{ab}	3.36±0.37^{bc}	3.95±0.88^{d}
EFU (%)	55.98±1.1^{a}	60.17±1.1^{b}	68.65±1.86^{bc}	75.9±2.42^{d}
FCR	1.76±0.15^{a}	1.5±0.15^{b}	1.18±0.27^{c}	1.03±0.25^{d}
PER (%)	1.32±0.02^{a}	1.32±0.02^{a}	1.55±0.02^{b}	1.75±0.01^{c}
SR (%)	92.22±1.92^{a}	92.22±1.92^{a}	98.89±1.92^{b}	100^{b}

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

Fishmeal substitution using fermented *N. virens* meal in juvenile Pacific white shrimp had a significant effect ($p<0.05$) on TFC, RGR, EFU, FCR, PER, and SR. The best values were obtained in treatment D, with a TFC of 83.7 g, a RGR of 3.95%, an EFU of 75.9%, a FCR of 1.03, a PER of 1.75% and a SR of 100%. Treatment A had the poorest results.

Proximate composition. The results of the proximate analysis of juvenile Pacific white shrimp after 42 days of maintenance are presented in Table 4.

Table 4

Proximate analysis of juvenile Pacific white shrimp (*Litopenaeus vannamei*) during the 42 days of maintenance

Dry weight content	*Treatments*			
	A (0%)	*B (20%)*	*C (25%)*	*D (30%)*
Protein (%)	48.35±0.04^{a}	50.5±0.03^{a}	52.4±0.08^{b}	56.33±0.02^{b}
Carbohydrate (%)	16.15±0.05^{b}	14.4±0.04^{b}	14.32±0.03^{b}	12.12±0.08^{a}
Crude fat (%)	10.5±0.08^{b}	12.34±0.07^{a}	11.3±0.05^{b}	13.12±0.09^{a}
Ash (%)	16.4±0.05^{a}	16.55±0.03^{a}	14.93±0.02^{b}	12.2±0.09^{b}
Crude fiber (%)	8.5±0.05^{b}	6.21±0.02^{a}	7.05±0.09^{b}	6.25±0.01^{a}

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

The best results were found in treatment D, with 56.33% protein and 13.12% fat. The lowest protein and fat contents were found in treatment A.

Amino acid profile. The results of the amino acid profile analysis of the experimental diets are presented in Table 5.

Table 5

The amino acid profile of experimental diets

Amino acid (%)	*Treatments*			
	A (0%)	*B (20%)*	*C (25%)*	*D (30%)*
Aspartic acid	2±0.05^{a}	2.95±0.03^{a}	3.17±0.05^{b}	3.98±0.02^{b}
Proline	1.04±0.03^{a}	2.5±0.02^{a}	2.9±0.03^{b}	3.15±0.04^{b}
Serine	0.82±0.04^{a}	1.75±0.07^{b}	2.25±0.02^{b}	2.88±0.06^{b}
Glutamic acid	2.19±0.05^{a}	3.29±0.02^{b}	3.85±0.07^{b}	4.35±0.04^{b}
Glycine	1.09±0.02^{a}	2.35±0.04^{a}	2.88±0.04^{b}	3.2±0.05^{b}
Histidine	0.86±0.01^{a}	1.45±0.02^{b}	1.9±0.02^{b}	2.2±0.02^{b}
Arginine	2.61±0.05^{a}	2.75±0.06^{a}	3.25±0.01^{b}	3.98±0.09^{b}
Threonine	2.05±0.08^{a}	2.3±0.02^{a}	2.7±0.08^{b}	3.2±0.02^{b}
Alanine	3.23±0.09^{b}	3.5±0.09^{a}	3.68±0.06^{a}	4.05±0.04^{a}
Valine	0.9±0.02^{a}	1.60±0.07^{a}	2.37±0.02^{b}	2.85±0.05^{b}
Methionine	5.4±0.04^{a}	6.05±0.04^{b}	6.4±0.05^{b}	6.95±0.03^{b}
Lysine	3.6±0.03^{b}	4.25±0.02^{b}	5.7±0.06^{a}	6.55±0.02^{b}
Isoleucine	3.24±0.03^{a}	3.8±0.01^{a}	4.15±0.03^{b}	4.55±0.01^{b}
Phenylalanine	1.04±0.09^{a}	1.8±0.09^{a}	2.05±0.01^{b}	2.44±0.09^{b}

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

The best results regarding the amino acid profile were found in diet treatment D, with 6.95% methionine and 4.35% glutamic acid. The amino acid profiles of juvenile Pacific white shrimp after 42 days of maintenance are presented in Table 6.

Table 6

Amino acid profile of juvenile Pacific white shrimp (*Litopenaeus vannamei*) for 42 days of maintenance

Amino acid (%)	*Treatments*			
	A (0%)	*B (20%)*	*C (25%)*	*D (30%)*
Aspartic acid	1.78±0.03^{a}	3.08±0.02^{b}	3.56±0.04^{b}	4.1±0.04^{b}
Serine	1.45±0.04^{a}	2.77±0.01^{a}	2.35±0.02^{b}	2.7±0.06^{b}
Glutamic acid	1.17±0.02^{a}	2.18±0.05^{b}	2.75±0.01^{b}	4.5±0.07^{b}
Glycine	2.2±0.01^{a}	3.08±0.06^{b}	3.17±0.06^{b}	3.35±0.05^{b}
Histidine	1.2±0.07^{a}	2.4±0.08^{a}	2.9±0.08^{b}	3.15±0.03^{b}
Arginine	1.29±0.08^{a}	1.76±0.02^{a}	2.06±0.03^{b}	2.4±0.06^{b}
Threonine	2.8±0.03^{a}	2.99±0.04^{a}	3.4±0.02^{b}	4.1±0.03^{b}
Alanine	2.3±0.09^{b}	2.87±0.01^{a}	2.95±0.05^{a}	3.35±0.07^{a}
Proline	3.3±0.02^{a}	3.75±0.06^{b}	3.9±0.08^{b}	4.18±0.09^{b}
Valine	1.2±0.06^{b}	1.95±0.05^{b}	2.4±0.02^{a}	3.07±0.02^{a}
Methionine	3.75±0.02^{a}	5.6±0.03^{a}	6.95±0.01^{a}	7.98±0.01^{a}
Lysine	5.4±0.09^{b}	6.26±0.06^{a}	6.83±0.08^{a}	7.6±0.04^{a}
Isoleucine	3.3±0.05^{b}	4.18±0.03^{a}	4.56±0.02^{a}	4.98±0.02^{a}
Leucine	1.04±0.02^{a}	1.8±0.08^{a}	2.05±0.04^{b}	2.44±0.08^{b}
Phenylalanine	2.2±0.06^{a}	3.07±0.03^{b}	3.47±0.08^{b}	4.05±0.03^{b}
Tryptophan	1.35±0.01^{a}	2.88±0.01^{b}	3.15±0.02^{b}	3.88±0.02^{b}

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

Based on the amino acid profile of juvenile pacific white shrimp after 42 days of maintenance, treatment D produced the best profile, with 7.98% methionine and 4.5% glutamic acid.

Fatty acid profile. The results of the fatty acid profile of the experimental diets are presented in Table 7.

Table 7

Fatty acid profile of the experimental diet

Fatty acids profile (%)	*Treatments*			
	A (0%)	*B (20%)*	*C (25%)*	*D (30%)*
C14:0 (Myristic)	2.52±0.09^{b}	3.29±0.06^{b}	3.41±0.02^{a}	4.3±0.05^{a}
C15:0 (Pentadecanoic)	1.76±0.08^{a}	2.18±0.03^{b}	3.17±0.04^{a}	3.15±0.07^{a}
C16:0 (Palmitic)	3.14±0.07^{a}	4.29±0.09^{b}	4.67±0.08^{b}	5.45±0.05^{b}
C18:0 (Stearic)	1.71±0.05^{a}	2.65±0.01^{b}	3.52±0.03^{b}	3.91±0.01^{b}
C18:1 n-9 (Oleic/ω9)	3.07±0.03^{a}	3.95±0.03^{a}	4.09±0.08^{b}	5.20±0.02^{b}
C18:2 n-6 (Linoleic/ω6)	3.83±0.09^{a}	4.46±0.07^{b}	4.49±0.07^{b}	5.27±0.08^{b}
C18:3 n-3 (Linolenic/ω3)	3.54±0.02^{a}	4.76±0.08^{b}	4.89±0.03^{b}	5.32±0.03^{b}
C20:0 (Arachidic)	2.3±0.04^{b}	3.23±0.02^{a}	3.53±0.04^{a}	3.95±0.09^{a}
C20:4 n-6 (Arachidonic)	2.71±0.03^{a}	3.15±0.03^{b}	3.75±0.09^{b}	4.37±0.02^{b}
C20:5 n-3 (EPA)	3.19±0.08^{a}	4.24±0.07^{b}	4.88±0.02^{b}	5.9±0.04^{b}
C22:6 n-3 (DHA)	1.23±0.05^{a}	2.27±0.04^{b}	3.16±0.01^{b}	3.97±0.02^{b}

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

Based on the fatty acid profile of the experimental diet, the best results were found in diet treatment D, with 5.9% EPA and 5.45% palmitic non-essential fatty acid. The results of the fatty acid profile of Pacific white shrimp juveniles after 42 days of maintenance are presented in Table 8.

Table 8

Fatty acid profiles of Pacific white shrimp (*Litopenaeus vannamei*) juveniles after 42 days of maintenance

Fatty acids profile (%)	*Treatments*			
	A (0%)	*B (20%)*	*C (25%)*	*D (30%)*
C14:0 (Myristic)	3.05±0.08^{a}	3.79±0.05^{a}	3.4±0.03^{b}	4.85±0.03^{b}
C15:0 (Pentadecanoic)	2.25±0.05^{a}	2.3±0.02^{a}	2.98±0.06^{b}	3.3±0.07^{b}
C16:0 (Palmitic)	3.4±0.03^{a}	4.6±0.01^{b}	4.88±0.02^{b}	5.95±0.04^{b}
C18:0 (Stearic)	2.08±0.02^{a}	2.9±0.06^{a}	3.6±0.08^{b}	4.2±0.03^{b}
C18:1 n-9 (Oleic/ω9)	3.35±0.04^{a}	4.1±0.02^{b}	4.25±0.09^{b}	5.2±0.07^{b}
C18:2 n-6 (Linoleic/ω6)	3.9±0.01^{a}	4.65±0.08^{b}	4.9±0.03^{b}	5.4±0.09^{b}
C18:3 n-3 (Linolenic/ω3)	3.54±0.03^{a}	4.76±0.09^{b}	4.89±0.08^{b}	5.32±0.05^{b}
C20:0 (Arachidic)	2.45±0.02^{a}	3.5±0.03^{b}	3.98±0.09^{b}	4.2±0.03^{b}
C20:4 n-6 (Arachidonic)	2.95±0.06^{b}	3.23±0.02^{b}	3.9±0.04^{a}	3.6±0.08^{a}
C20:5 n-3 (EPA)	4.98±0.07^{a}	5.77±0.08^{b}	7.2±0.08^{b}	8.65±0.09^{b}
C22:6 n-3 (DHA)	1.6±0.02^{a}	3.85±0.09^{b}	4.74±0.04^{b}	5.98±0.08^{b}

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

Based on the fatty acid profile of Pacific white shrimp juveniles after 42 days of maintenance, treatment D produced the best results, with of 8.65% EPA and 5.95% palmitic acid.

Water quality. The water quality parameters during the study are presented in Table 9.

Table 9
Water quality parameters for juvenile Pacific white shrimp (*Litopenaeus vannamei*) in the aquaculture media during the study

Treatments	*Value*			
	Temperature (°C)	*pH*	*DO (mg L^{-1})*	*Salinity (ppt)*
A	29±0.07	8±0.07	4.54±0.05	25±0.02
B	28±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
References	26-32°C[a]	7.7-8.7[b]	3-8[c]	15-35[b]

Note: DO - dissolved oxygen; a - Tahe & Hidayat (2011); b - Herawati et al (2020c); c - Syukri & Ilham (2016).

Discussion. This study aimed to determine feed utilization efficiency, growth performance, and nutritional quality of juvenile Pacific white shrimp fed diets with N. virens meal through fermentation as a substitute for fishmeal in different ratios. Probiotics used to ferment *N. virens* meal can generate a distinctive odor (attractant) and flavor in the diet, thus stimulating to approach and consumption of the feed. According to Pamungkas (2013), and Abidin et al (2015), differences in feed consumption rates could be influenced by the content of the diet and feed palatability. This study determined that the highest feed consumption rate was in treatment D, and the lowest in treatment A. Diets treated with probiotics have a fresher aroma than untreated diets (Noviana et al 2014).

The protein requirement for the growth of Pacific white shrimp post-larval stage PL 15 ranges from 30 to 45% (Yustianti et al 2013). The fermentation process improves the quality of nutrients in *N. virens* meal. The fermentation will convert the complex compounds into simple compounds, allowing the feed to be easily utilized by the shrimp efficiently and effectively. Probiotics contain a variety of beneficial microorganisms. *Lactobacillus* is beneficial for fermenting organic matter into a lactic acid compound. Photosynthetic bacteria absorb toxic gases and heat from the fermentation process. Yeast has roles in fermenting organic matter into alcohol compounds. Sugars, amino acids, and actinomycetes serve to produce antibiotic compounds which are toxic to pathogenic bacteria and able to dissolve phosphate ions and other micro ions (Fadri et al 2016). The protein content in the diets with fermented *N. virens* meal can meet the needs and support the growth of juvenile Pacific white shrimp. The effect of protein value on growth can be measured through PER (Ma et al 2013). PER shows the amount of shrimp weight produced from each unit of protein weight in the diet, assuming that all protein is used for growth. The best PER in this study was obtained in treatment D. High EFU will result in better growth, being associated with a low FCR. The FCR value is this study was classified as excellent and optimum for the Pacific white shrimp. This is supported by Bahri et al (2020), who noted that an excellent FCR for Pacific white shrimp is 1.3-1.4. Using efficient feed produces a high EFU, so that the energy requirements of the shrimp can be fulfilled and the rest is used for growth (Belghit et al 2014).

Diets using 0% fermented *N. virens* meal are difficult to digest and utilize by the body of juvenile Pacific white shrimp (*L. vannamei*). Arief et al (2014) stated that low feed efficiency is influenced by digestive activity that is not assisted by the presence of probiotic bacteria. According to Greiner & Konietzny (2011), phytic acid can reduce enzyme activity in the digestive tract, reducing protein solubility and digestibility. The absence of a fermentation process in the feed may result in phytic acid in the diet that cannot be removed.

Fermented *N. virens* meal has amino acid content suited to the nutritional requirements of juvenile Pacific white shrimp. In their research, Kuang et al (2012) stated that quality protein must have a high digestibility rate and amount of amino acids similar to the cultured species. Essential amino acids lysine and methionine needed by juvenile Pacific white shrimp are 3.2% and 4.6%, respectively (Mouneyrac et al 2010; Brown et al 2011). Glutamic acid is a non-essential amino acid that is found in blood plasma and muscles. Glutamine is a substrate for several aminotransferases involved in

synthesizing purines, glucosamine, pyrimidine, and asparagine (Jusadi et al 2015). Glutamine is an energy source and plays a role in the biosynthesis of glucose, sugar amines, and glutathione. Glutamine can improve intestinal structure and function, which is essential for shrimp, considering the undeveloped digestive tract. Adding glutamine is expected to strengthen intestinal performance in digesting the diet (Jusadi et al 2015). Methionine can act as a chemoattractant, increasing appetite and supporting shrimp growth. The essential amino acid methionine improves the balance and utilization of other amino acids with roles in shrimp growth. Moreover, methionine has an essential part in protein synthesis and physiological functions. The body of shrimp needs methionine to form nucleic acids and tissues, for protein synthesis; methionine also works with vitamin B12 and folic acid to help the body regulate excessive protein supply in a high-protein diet (Bhagavan 1992). It has been shown that diets with high methionine content increase growth and immune response (Yuan et al 2011; Kuang et al 2012; Boonyoung et al 2013; Ma et al 2013; Rolland et al 2015). However, methionine deficiency could cause decreased growth and survival (Takagi et al 2001).

The requirement of EPA and DHA in post-larval Pacific white shrimp is 2.6% EPA and 1.5% (Tocher 2015). The content of EPA and DHA found in the present experimental diet was higher than the EPA content (4.97%) found in Pacific white shrimp fed a diet with 30% *N. virens* meal substituting fish meal without fermentation (Herawati et al 2022). EPA in the diet serves as an essential component of phospholipids in membranes and nervous tissue. Moreover, EPA plays a role in the growth and survival of shrimp (Lim et al 2011). EPA is an important component of phospholipids in membranes and nervous tissue. Larvae have a very high neurosomatic index at first feeding, and they need a high n-3 HUFA content in the feed to avoid nerve formation abnormalities. Fatty acids are essential in providing feed for shrimp during the gonad maturation process (Limsuwatthanathamrong et al 2012).

SR is one of the critical indicators and a measure of farming success. The SR of juvenile Pacific white shrimp fed treatment diet D was 100%. The lowest SR was in the control. This is because several factors affect the survival rate of Pacific white shrimp larvae, one of which is water quality in the maintenance media. White shrimp will have difficulty molting, and can undergo death at low salinity (Anita et al 2017). According to Rakhfid & Mauga (2018), the optimum salinity value for Pacific white shrimp farming is between 20-35 ppt. Salinity during the maintenance period is suitable for the requirements of shrimp. Constant salinity levels can minimize stress and increase survival. In addition to salinity, temperature changes can also impact the levels of stress of Pacific white shrimp. Temperatures that tend to be stable can enhance the immune system of Pacific white shrimp and increase endurance. The temperature during maintenance ranged from between the optimum interval of 25.8-30.4^{o}C (Putra & Manan 2014). The suitability of water quality and diet for Pacific white shrimp can maintain the its body condition (Herawati et al 2020d). Proper metabolic and physiological processes are supported by water quality (Mouneyrac et al 2010; Nwachi 2013).

Conclusions. Treatment diets significantly affected Pacific white shrimp's growth performance ($p<0.05$). The treatment with the best results was treatment D (30% *N. virens* meal), resulting in a TFC of 83.7 g, RGR of 3.95%, EFU of 75.9%, a FCR of 1.03, a PER of 1.75% and a SR of 100%. Treatment D contained 56.33% protein, 12.12% fat, 7.98% methionine, and 8.65% EPA.

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

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