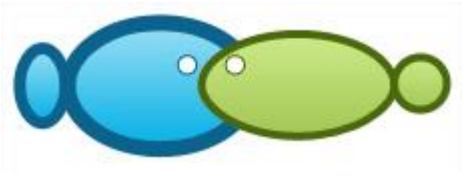

Profile of amino acids, fatty acids and growth performance of vannamei shrimp (*Litopennaeus vannamei*) fed with sea worms (*Nereis virens*) enriched with squid oil

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Abstract. Vannamei shrimp is a commodity with high economic value. The obstacle that cultivators often experience in providing natural food is the high price of *Artemia*, so it is necessary to replace *Artemia* with feed of almost the same nutritional content. One solution could be sea worms (*Nereis virens*). *N. virens* is used as natural food. Enrichment of *N. virens* to increase shrimp production can be made with squid oil, which contains high levels of EPA and DHA. The purpose of this study was to determine the profile of amino acids, fatty acids, and growth performance of vannamei shrimp (*L. vannamei*) by feeding with sea worms (*N. virens*) enriched with squid oil in different ratios. This study used an experimental method with a completely randomized design (CRD), with 4 treatments and 3 replications: treatment A (0 mL of squid oil to *Nereis* sp.), B (5 mL 200 g^{-1}), C (10 mL 200 g^{-1}), and D (15 mL 200 g^{-1}). The data observed included absolute weight growth, relative growth rate (RGR), total feed consumption (TFC), feed utilization efficiency (FUE), protein efficiency ratio (PER), survival rate (SR), proximate composition, amino acid profile, and fatty acid profile. The addition of squid oil to the feed of *Nereis* sp. had a significant effect ($p<0.05$) on the growth and survival of vannamei shrimp post-larvae. Treatment (C) was the best treatment with a RGR of 24.96 % day^{-1}, and with the best values for TFC, FUE, and PER. Treatment C had the highest nutritional content compared to other treatments, with 59.75% crude protein, 23.22% crude fat, 6.87% EPA, 7.95% DHA, and 11.95% lysine.
Key Words: DHA, EPA, feed, lysine, nutrition.

Introduction. Vannamei shrimp (*Litopenaeus vannamei*) is an aquaculture commodity with high economic value. The production of vannamei shrimp continues to increase every year. Vannamei shrimp can tolerate a wide range of salinity and temperature, are easy to cultivate at high stocking densities, easy to breed, have a fast growth, high survival, high market demand and are resistant to diseases (Amoah et al 2019). Cultured vannamei shrimp at the post-larval stage are usually given natural food in the form of *Artemia* (Riyanti et al 2020). One of the problems cultivators face in natural feeding is the high price of *Artemia*, so it is necessary to replace *Artemia* with feed of almost the same nutritional content. The proximate composition of dried *Artemia* is 56.29% crude protein, 9.28% crude fat, 13.92% ash and 2.06% crude fiber (Herawati et al 2020), while dried *Nereis* sp. meal has 56.29% crude protein. Dried *Nereis* also has 11.32% unsaturated fatty acids and a good content of essential amino acids (Yuwono 2003). Thus, *Nereis* sp. can be a natural feed substitute for *Artemia*. According to Rasidi (2012), marine worms (*Nereis* sp.) have been used as one of the natural feeds for the continuity of shrimp nauplii production in shrimp hatcheries.

Sea worms (*Nereis* sp.) are one example of marine worms commonly used as natural food for shrimp broodstock in Indonesia. The growth of vannamei shrimp at the post-larval stage requires protein in the feed ranging from 30 to 55% (Riyanti et al 2020). *Nereis* sp. has a protein content of 50.29%, and there are nine essential amino acids for shrimp that help accelerate shrimp growth (Haryadi & Rasidi 2012). Fatty acid content is related to crustacean fat needs, and usually sources such as fish oil and squid oil, and other

animal oils with n-3 HUFA fatty acids are used (Pangkey 2011). The advantage of squid oil is that it contains arginine, which is part of the essential amino acids and stimulates secretion, increasing growth (Sulistyono et al 2016). Energy needs can be met if the glucose in the blood can immediately enter the cells, which depends on insulin's performance. According to Bangkit et al (2016), squid oil contains 9% EPA and DHA and 31% unsaturated fatty acid content. Addition of squid oil to *Nereis* sp. affects fat and helps the growth and survival of vannamei shrimp post-larvae (Pangkey 2011). Therefore, it is necessary to study *Nereis* sp. enriched with squid oil as feed for vannamei shrimp in different life stages. This study has a basis in the research of Yunus et al (1996), who studied the natural feed of rotifers (*Brachionus plicatilis*) added with cod liver oil and tested as a feed for mud crabs (*Scylla serrata*) larvae. This study aims to determine the best dose of adding squid oil to marine worms (*Nereis* sp.) on the growth rate and survival rate of vannamei shrimp post-larvae.

Material and Method. The test materials used in this study consisted of test animals and test feed. The test animals in this study were vannamei shrimp post-larvae 15 (PL15) from CV Riz Samudra, Marine Science Techno Park (MSTP), Jepara, Central Java, with an initial weight of 0.05-0.06 g and a stocking density of 5 ind L^{-1}. According to Herawati et al (2020[b]), PL15 vannamei shrimp are stocked with a stocking density of 5 ind L^{-1}. Fresh sea worms (*N. virens*) were obtained from the Center for Brackish Water Aquaculture Fisheries (BBPBAP) Jepara. The containers used in this study had a size of 43x30x25 cm with a volume of 15 L of water. 12 containers were used and equipped with aeration. Different setting tools were used during the rearing process, including hoses for aeration and siphons, aerators to produce oxygen, and fishing nets used as container covers.

The rearing medium used was seawater with a salinity of 29 ppt, because the salinity for rearing vannamei shrimp ranges between 29-30 ppt. Chen et al (2020) stated that water quality (temperature, dissolved oxygen and pH) should always be monitored and adapted for shrimp growth. Water temperature should be maintained in the range of 28-30^{o}C, pH between 7.4 and 8, dissolved oxygen from 6 to 7 mg L^{-1} and salinity between 31 and 32 ppt. Seawater has previously been accommodated, filtered and then sterilized using chlorine at a dose of 10 g ton^{-1}, then neutralized using sodium thiosulfate at a dose of 50% chlorine. This study used four treatments, and each treatment was repeated three times. The treatments were: Treatment A - Feed in the form of *N. virens* enriched with 0 mL of squid oil to 200 g *N. virens*; Treatment B - feed in the form of *N. virens* enriched with 5 mL of squid oil to 200 g of *N. virens*; Treatment C - feed in the form of *N. virens* enriched 10 mL of squid oil to 200 g of *N. virens*; Treatment D - feed in the form of *N. virens* enriched 15 mL of squid oil to 200 g of *N. virens*. Fresh sea worms as much as 250 g were used per treatment. 4 L of water were used per container. Each treatment was enriched with squid oil according to the dose, and 20 g of chicken egg yolk and 5 g of yeast were added. According to Yuwono (2005), shrimp larvae fed with chopped marine worms (*Nereis* sp.) showed a significantly higher survival rate. This is also confirmed by Yunus et al (1996), who noted that yeast can act as a source of vitamin B complex, and egg yolk can serve as a source of protein or amino acids. The study was conducted for 45 days of maintenance. Feeding was carried out using the fixed feeding rate method. The frequency of feeding was four times a day, at 08.00, 14.00, 18.00, and 21.00. The feeding dose was 20% of the weight of the biomass/day (Watanabe 1988). Feeding was conducted with chopped marine worms (*Nereis* sp.), enriched with squid oil. According to Asnawi et al (2018), marine worms in fresh form, chopped or flour used in feed are suitable for increasing shrimp growth.

Relative Growth Rate (RGR). The relative growth rate (RGR) of the sample was calculated using the following formula (Tacon 1993):

$$RGR = [(Wt - W_0)/(W_0 \times t)] \times 100$$

Where: RGR - relative growth rate (% day^{-1}); W_0 - 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).

Total Feed Consumption. The TFC was calculated using the following formula (Pereira 2007):

$TFC = F1 - F2$

Where: F1 - initial amount of feed (g); F2 - amount of leftover feed (g).

Feed Utilization Efficiency. The FUE was calculated using the following formula (Watanabe 1988):

$FUE = ((W_t + D - W_0)/F) \times 100$
Where: W_t - tested fish biomass weight at the end of the observation (g); W0 - tested fish biomass weight at the beginning of the observation (g); D - dead fish weight (g); F - the amount of feed administered during observation (g).

Protein Efficiency Ratio. The PER was calculated using the following formula (Tacon 1993):

$PER = [(W_t - W_0)/Pi] \times 100$

Where: W_t - tested fish weight at the end of the observation (g); W_0 - tested fish weight at the beginning of the observation (g); Pi - the weight of feed consumed × % of feed protein.

Survival rate. The survival rate of Pacific white shrimp was calculated using the formula (Tacon 1993):

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

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 sample's protein, fat, ash, carbohydrates, fiber, and water content. The protein analysis was conducted by the Kjeldahl method, while the fat content was determined using the Soxhlet method. Analysis of water and ash content employed gravimetric principles. Content of carbohydrates was calculated manually based on the proximate analysis results.

Amino acid analysis. The amino acid profile was determined using HPLC type 1100 apparatus with Eurosphere 100-5 C18, 250×4.6 mm column, with the initial column P/N: 1115Y535. The wastes 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 the samples were placed into a closed glass, and 15 mL of 6M HCl were added. The mixture was vortexed for homogeneity and hydrolyzed in an autoclave at 110ºC for 12 h before being cooled to room temperature and neutralized with 6M NaOH. After adding 2.5 mL of 40% lead acetate and 1 mL of 15% oxalic acid, 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 plus 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 into the HPLC system (AOAC 2005).

Fatty acid analysis. The fatty acid profile was determined using the QP-2010 Gas Chromatograph - Mass Spectrophotometer (GCMS) (Shimadzu), which has a length of 50

m, a diameter of 0.22 mm Wall Coat Open Tubular CP-SIL-88 column (Agilent, Santa Clara, CA, USA). Analyses were carried out over a column temperature range of 120-200°C. The method used was *in-situ* trans-esterification. 100 mg of the sample were homogenized using 4 mL of water. The homogenate (104 mg) obtained was transferred into the test tube. 100 µL of methylene chloride were added, along with 1 mL of 0.5M NaOH in methanol. After adding nitrogen, the tubes were tightly closed. They were heated to 90ºC for 10 min. The test tubes were then cooled, and 1 mL of 14% BF3 in methanol was added. After adding nitrogen, it was heated at the same temperature for 10 min. The test tubes were 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 GC analysis (AOAC 2005).

Water quality. Water quality measurements included temperature, pH, salinity and dissolved oxygen (DO), which were measured using a Water Quality Checker (WQC). Ammonia was tested at the Environmental Quality Laboratory of the Center for Brackish Water Aquaculture (BBPBAP) Jepara, Indonesia. Measurements of temperature, salinity and pH were carried out twice a day, oxygen was measured once a day, and ammonia was measured once a week.

Data analysis. The data obtained were analyzed using the analysis of variance (ANOVA). A normality test, homogeneity test, and additivity test were carried out to determine whether the data was normal, homogeneous, and additive. If significant differences were determined ($p<0.05$), Duncan's Multiple Area Test was used to determine the pairs of sets with significant differences. Water quality data were analyzed descriptively.

Results and Discussion. The parameter values for the growth of shrimp are presented in Table 1.

Table 1

The average values of growth parameters of *Litopenaeus vannamei* during the study

Parameter	*Treatment*			
	A	*B*	*C*	*D*
Relative Growth Rate (% day^{-1})	$19.74^{a}\pm1.98$	$22.18^{bc}\pm0.96$	$24.96^{bc}\pm2.50$	$21.48^{ab}\pm0.45$
Total Feed Consumption (g)	$472.06^{a}\pm0.32$	$472.82^{bc}\pm0.06$	$472.59^{c}\pm0.10$	$472.29^{ab}\pm0.10$
Feed Utilization Efficiency (%)	$2.28^{a}\pm0.03$	$2.37^{bc}\pm0.07$	$2.45^{c}\pm0.04$	$2.34^{ab}\pm0.11$
Protein Efficiency Ratio (%)	$3.59^{a}\pm0.06$	$4.13^{bc}\pm0.13$	$4.55^{c}\pm0.06$	$4.08^{ab}\pm0.20$
Survival rate (%)	$95.56^{ab}\pm1.93$	$98.89^{c}\pm1.92$	$98.89^{ab}\pm1.92$	$94.44^{a}\pm1.93$

Note: different superscripts show significant differences ($p<0.05$).

The addition of squid oil had a significant effect ($p<0.05$) on the growth and survival of vannamei shrimp post-larvae, with the best results being in treatment C (10 mL of added squid oil), with a RGR of 24.96% day^{-1}; 472.59 g total feed consumption; FUE of 2.45%, a PER of 4.55% and a high SR of 98.89%. Based on the results of the calculation of the data in the table above, it can be seen that the RGR (% day^{-1}) of treatment C (10 mL of addition of squid oil) had a significant effect compared to the control (0 mL of addition of squid oil), but no significant effect compared to treatments B and D. The calculation of total feed consumption (g) showed that treatment C (10 mL of addition of squid oil) had a significant effect compared to treatments A and D, but no significant effect compared to treatment B. The results of the calculation of FUE (%) showed that treatment C (10 mL of addition of squid oil) had a significant effect compared to treatments A and D, but had no significant effect compared to treatment B. The calculation of PER (%) showed that treatment C (10 mL of addition of squid oil) had a significant effect compared to treatments

A and D, but had no significant effect compared to treatment B. The results of the SR (%) showed that treatment C (10 mL of squid oil) had no significant effect compared to treatments A and D, but had significant effect compared to treatment B.

Proximate analysis results for *N. virens* enriched with squid oil at different doses are presented in Table 2.

Table 2

Proximate analysis results for *Nereis virens* enriched with squid oil at different doses

Proximate	*Dry weight content percentage*			
	A (0 mL)	*B (5 mL)*	*C (10 mL)*	*D (15 mL)*
Protein	45.55±0.04^{b}	51.32±0.06^{b}	55.75±0.07^{b}	48.16±0.07^{b}
Fat	21.14±0.07^{a}	21.32±0.05^{a}	22.62±0.03^{b}	21.59±0.03^{a}
Crude fiber	18.25±0.09^{b}	11.72±0.03^{b}	10.19±0.07^{b}	12.82±0.02^{b}
Ash	8.18±0.03^{a}	8.51±0.03^{a}	5.19±0.01^{b}	8.83±0.01^{a}
Carbohydrate	6.88±0.02^{a}	6.83±0.05^{a}	6.25±0.02^{a}	8.6±0.05^{b}

Note: different superscripts show significant differences ($p<0.05$).

N. virens enriched with squid oil at different doses gave good results, treatment C producing a 55.75% protein content, 22.62% fat content, 5.19% ash content and 0.19% crude fiber. The proximate analysis of Pacific white shrimp post-larvae under different treatments is presented in Table 3.

Table 3

Proximate analysis of pacific white shrimp (*Litopenaeus vannamei*) under different treatments

Proximate	*Dry weight content percentage*			
	A (0 mL)	*B (5 mL)*	*C (10 mL)*	*D (15 mL)*
Protein	51.25±0.04^{b}	54.64±0.06^{b}	59.75±0.07^{b}	52.56±0.07^{b}
Fat	21.34±0.07^{a}	21.30±0.05^{a}	23.22±0.03^{b}	21.59±0.03^{a}
Crude fiber	11.25±0.09^{b}	8.72±0.03^{b}	6.19±0.07^{b}	8.42±0.02^{b}
Ash	8.68±0.03^{a}	8.51±0.03^{a}	5.19±0.01^{b}	8.60±0.01^{a}
Carbohydrate	7.38±0.02^{a}	6.33±0.05^{a}	5.65±0.02^{a}	8.83±0.05^{b}

Note: different superscripts show significant differences ($p<0.05$).

According to Table 3, pacific white shrimp in treatment C had the best composition, with a protein content of 59.75%, 23.22% fat, and 5.19% ash. The amino acid profile of *N. virens* enriched with squid oil at different doses is presented in Table 4.

Table 4

The amino acid profiles of *Nereis virens* enriched with squid oil at different doses

Amino acid (%)	*Treatment*			
	A (0 mL)	*B (5 mL)*	*C (10 mL)*	*D (15 mL)*
L-aspartic acid	3.89±0.05^{a}	2.06±0.06^{d}	3.19±0.09^{b}	2.98±0.04^{c}
L-proline	3.55±0.09^{a}	1.10±0.01^{d}	3.03±0.03^{b}	1.23±0.07^{c}
L-serine	4.56±0.02^{a}	2.26±0.05^{c}	4.19±0.03^{b}	2.20±0.01^{d}
L-glutamic acid	3.66±0.02^{a}	4.26±0.07^{c}	5.03±0.06^{b}	3.90±0.09^{d}
Glycine	4.93±0.06^{a}	2.80±0.05^{c}	3.90±0.09^{b}	2.75±0.08^{d}
L-histidine	5.78±0.02^{a}	2.67±0.07^{c}	3.36±0.08^{b}	2.56±0.02^{d}
L-arginine	4.05±0.01^{a}	1.89±0.09^{c}	1.78±0.06^{d}	1.96±0.08^{b}
L-threonine	3.02±0.09^{b}	2.95±0.08^{c}	3.20±0.09^{a}	2.23±0.09^{d}
L-alanine	5.22±0.03^{a}	3.75±0.09^{c}	4.87±0.07^{b}	2.92±0.01^{d}
L-valine	3.24±0.09^{d}	4.90±0.04^{a}	4.83±0.03^{b}	3.99±0.08^{c}
L-methionine	3.11±0.02^{d}	4.99±0.03^{b}	6.50±0.05^{a}	3.98±0.03^{c}
L-Lysine HCl	3.57±0.07^{d}	5.20±0.04^{b}	6.98±0.03^{a}	4.98±0.05^{c}
L-isoleucine	3.99±0.01^{b}	3.09±0.02^{d}	4.23±0.02^{a}	3.99±0.03^{bc}
L-leucine	4.44±0.05^{a}	1.38±0.01^{d}	3.25±0.03^{b}	2.57±0.01^{c}
L-phenylalanine	4.99±0.07^{b}	2.98±0.09^{c}	5.98±0.01^{a}	1.96±0.06^{d}

Note: different superscripts show significant differences ($p<0.05$).

Lysine was the most common amino acid found after adding 10 mL of squid oil to *N. virens*, with 6.98%. It was lowest in treatment A, without squid oil, with 3.57±0.07%. Amino acid profiles of post-larval Pacific white shrimp under different treatments are presented in Table 5.

Table 5

Amino acid profiles of post-larval Pacific white shrimp (*Litopenaeus vannamei*) under different treatments

Amino acid (%)	*Treatments*			
	A (0 mL)	*B (5 mL)*	*C (10 mL)*	*D (15 mL)*
Aspartic acid	4.63±0.05^{a}	5.18±0.09^{b}	5.19 ± 0.09^{bc}	6.06 ± 0.06^{d}
Proline	5.08±0.09^{a}	3.84 ± 0.03^{b}	4.87 ± 0.07^{c}	5.75 ± 0.09^{d}
Serine	6.76 ± 0.02^{a}	6.46 ± 0.09^{b}	6.31 ± 0.03^{b}	6.10 ± 0.01^{d}
Glutamic acid	6.36 ± 0.07^{a}	6.16 ± 0.08^{b}	5.30 ± 0.03^{c}	5.35 ± 0.05^{d}
Glycine	6.33 ± 0.02^{a}	5.98 ± 0.05^{c}	5.75 ± 0.06^{d}	6.26 ± 0.07^{b}
Histidine	4.65 ± 0.03^{a}	4.79 ± 0.11^{b}	5.90 ± 0.09^{c}	5.80 ± 0.05^{d}
Arginine	5.85 ± 0.07^{a}	6.27 ± 0.02^{b}	6.36 ± 0.08^{b}	6.28± 0.07^{b}
Threonine	6.47 ± 0.07^{a}	6.67 ± 0.04^{c}	7.78 ± 0.06^{d}	5.89 ± 0.09^{b}
Alanine	6.51 ± 0.01^{a}	5.17 ± 0.03^{b}	5.20 ± 0.09^{c}	4.95 ± 0.08^{a}
Valine	4.72 ± 0.03^{a}	6.98 ± 0.08^{c}	7.10 ± 0.03^{d}	4.90 ± 0.04^{b}
Methionine	4.89 ± 0.06^{a}	5.88 ± 0.03^{c}	8.33± 0.05^{d}	5.09 ± 0.03^{b}
Lysine	6.70 ± 0.06^{a}	8.80 ± 0.03^{c}	11.95 ± 0.03^{d}	7.75 ± 0.04^{b}
Isoleucine	5.17 ± 0.02^{a}	4.95 ± 0.08^{b}	5.23 ± 0.02^{c}	4.09 ± 0.02^{a}
Phenylalanine	4.41 ± 0.07^{a}	4.80 ± 0.07^{b}	5.48 ± 0.01^{c}	4.98 ± 0.09^{b}
Leucine	5.47 ± 0.05^{a}	5.07 ± 0.02^{c}	4.75 ± 0.03^{b}	3.38 ± 0.01^{a}
L-Triphtophan	6.23 ± 0.06^{a}	7.78 ± 0.07^{c}	4.19 ± 0.01^{a}	7.93 ± 0.01^{c}

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

The amino acid profile showed that the amino acid with the highest concentration was lysine in treatment (C), 11.95%. The lowest concentration of lysine was in the control group, 6.7%.

The fatty acid profile of *N. virens* enriched with squid oil at different doses is presented in Table 6.

Table 6

Total fatty acid in *N. virens* enriched with squid oil at different dose

Fatty acid profile (%)	*Treatment*			
	A (0 mL)	*B (5 mL)*	*C (10 mL)*	*D (15 mL)*
C14:0 (Myristic)	9.52±0.09^{a}	5.41±0.02^{b}	5.48±0.09^{c}	10.49±0.06^{d}
C15:0 (Pentadecanoic)	6.76±0.08^{a}	6.17±0.04^{c}	6.15±0.08^{d}	7.18±0.03^{b}
C16:0 (Palmitic)	5.14±0.07^{a}	7.97±0.08^{c}	8.59±0.04^{b}	6.29±0.09^{d}
C18:0 (Stearic)	5.71±0.05^{a}	5.52±0.03^{c}	4.91±0.09^{d}	6.65±0.01^{b}
C18:1 n-9 (Oleic/ω9)	8.07±0.03^{a}	4.89±0.08^{c}	6.61±0.01^{d}	4.95±0.03^{b}
C18:2 n-6 (Linoleic/ω6)	4.83±0.09^{a}	6.49±0.07^{c}	8.07±0.02^{b}	5.46±0.07^{d}
C18:3 n-3 (Linolenic/ω3)	3.54±0.02^{a}	4.89±0.03^{c}	7.32±0.01^{b}	4.76±0.08^{d}
C20:0 (Arachidic)	3.30±0.04^{a}	6.02±0.04^{b}	3.05±0.03^{c}	2.83±0.02^{d}
C20:4 n-6 (Arachidonic)	4.71±0.03^{a}	6.15±0.09^{c}	8.23±0.07^{b}	4.15±0.03^{d}
C20:5 n-3 (EPA)	6.19±0.08^{a}	10.78±0.02^{c}	13.23±0.08^{b}	9.64±0.07^{d}
C22:6 n-3 (DHA)	4.23±0.05^{a}	5.17±0.04^{c}	6.07±0.01^{b}	3.08±0.03^{d}

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

EPA was the most commonly found among the fatty acids, with the highest level in treatment C (13.23±0.08%), while the lowest EPA level was observed in the control (6.19±0.08%). DHA (C22:6n-3) levels in treatment C were the highest among other treatments (6.07±0.03%), as well as arachidonic acid content (8.23±0.07%). Fatty acid profiles of Pacific white shrimp under different treatments are presented in Table 7.

The highest EPA level was in treatment C, 6.87%, while the lowest level was in treatment A, 4.23%.

The values of the water quality parameters during the study are presented in Table 8. Water quality during the study was in a suitable condition for vannamei shrimp post-larvae cultivation.

Table 7

Fatty acid profiles of pacific white shrimp (*Litopenaeus vannamei*) under different treatments

Fatty acid profile (%)	*Treatments*			
	A (0 mL)	*B (5 mL)*	*C (10 mL)*	*D (15 mL)*
C14:0 (Myristic)	6.57±0.04[a]	3.50±0.02[a]	4.68±0.09[b]	4.75±0.05[b]
C15:0 (Pentadecanoic)	2.38±0.06[a]	2.27±0.04[b]	2.45±0.08[b]	1.72±0.06 [a]
C16:0 (Palmitic)	4.77±0.08[a]	4.97±0.08[a]	8.09±0.04[c]	5.14±0.09 [b]
C18:0 (Stearic)	5.75±0.02[a]	5.52±0.03[c]	3.11±0.09[b]	2.71±0.07 [a]
C18:1 n-9 (Oleic/ω9)	1.90±0.03[a]	3.89±0.08[b]	5.85±0.01[c]	3.37±0.02[b]
C18:2 n-6 (Linoleic/ω6)	2.30±0.07[a]	4.49±0.07[b]	5.37±0.02[c]	4.60±0.09 [b]
C18:3 n-3 (Linolenic/ω3)	2.30±0.09[a]	3.39±0.03[c]	4.32±0.01[d]	2.54±0.05[b]
C20:0 (Arachidic)	2.70±0.02[a]	2.02±0.04[a]	4.05±0.03[d]	2.30±0.08[b]
C20:4 n-6 (Arachidonic)	4.19±0.05[a]	4.15±0.02[a]	5.13±0.08 [b]	4.07±0.02 [a]
C20:5 n-3 (EPA)	3.08±0.04[a]	4.07±0.01[c]	6.87±0.03[d]	3.83±0.05[b]
C22:6 n-3 (DHA)	4.23±0.07[a]	5.68±0.02[c]	7.95±0.08	5.23±0.02[b]

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

Table 8

Results of water quality measurements on post-larvae of vannamei shrimp in the aquaculture media during the study

Variable	*Measurement unit*	*Interval*	*Reference*
Temperature	^{o}C	27.9-29.9	26-32[b]
Dissolved oxygen	mg L^{-1}	4.09-5.82	>3[a]
pH	-	7.43-8.1	7-8.5[a]
Salinity	Ppt	30-33	5-35[c]
Ammonia	mg L^{-1}	0.023-0.09	≤0.1[a]

Note: [a]Arsad et al (2017); [b]Rakhfid et al (2018); [c]Umami et al (2018).

Treatment C with *N. virens* enriched with 10 mL of squid oil produced the better results of RGR, TFC, FUE, PER, and SR of vannamei shrimp. The nutritional content of the feed and the level of feed consumption of the post-larvae of vannamei shrimp affect its growth. Feed efficiency can be achieved by paying attention to feeding management and feeding frequency. Saopiadi et al (2012), in their research, stated that the main factor that determines the high and low value of FUE is the nutritional value of the feed. According to Marzuqi et al (2012), feed efficiency shows how much feed can be utilized. Low feed efficiency values indicate that shrimp need more feed to gain weight because only a small part of the energy from the feed is used for growth. The addition of an appropriate dose of squid oil can increase FUE in vannamei shrimp.

Hepher (1988), in his research, stated that a higher PER value shows the higher quality of the feed, so that protein is utilized optimally. A greater feed efficiency value shows the more efficient feed use in the fish body (Riyanti et al 2020). The level of utilization efficiency is influenced by the nutrient content of the feed (Herawati et al 2020[a]).

The nutrient content of *N. virens* feed enriched with 10 mL of squid oil was the highest among treatments, with 55.75% protein and 22.62% fat. The high protein level in the feed accelerated the growth of vannamei shrimp post-larvae. *N. virens* feed enriched with squid oil has a protein content that can meet the nutritional needs of post-larvae vannamei shrimp, which is 35-40%. The growth of vannamei shrimp was different in each treatment due to the feed administered. Marine worms (*Nereis* sp.) have 55.75% protein, and there are nine essential amino acids for shrimp that help accelerate growth. This is

also confirmed by Bangkit et al (2016), who observed that squid oil has the potential to be a good source of fatty acids (HUFA), with high levels of arachidonic acid, EPA, and DHA. Feeds with high DHA levels will provide better growth rates (Kim et al 2017).

The RGR, TFC, FUE, PER and SR were lowest in treatment A. In this treatment, the nutritional content was lower than in other treatments. The low nutrient content in the feed greatly affects the growth performance of post-larvae. The protein requirement of post-larval shrimp based on research by Moren et al (2006) is 35-40%, while Lovell (1988) stated that it is 45% protein, 12- 15% fat, and 2-5% EPA. The nutrient content in the feed and the availability of natural feed affect the growth of vannamei shrimp. Protein and fat are the main sources of energy. Low protein and fat in the diet are only sufficient for tissue repair; there is no storage of nutrients for growth and a source of energy. This statement is reinforced by Prawira (2017), who states that post-larval shrimp need protein both for energy generation and growth.

The lysine content in treatment C was 6.98%. Lovell (1988) stated that the requirement for lysine at the post-larval stage is 6.97%. The role of lysine is to form carnitine, which functions as a growth promoter, protects against ammonia poisoning, increases the body's defenses and protects against changes in temperature (Ovie & Eze 2013). In addition, lysine also has an important role in blood antibodies, strengthens the circulatory system and maintains average cell growth (Baeza-Rojano et al 2012). Lysine can increase the digestibility of other amino acids, one of which is the non-essential amino acid tyrosine, which regulates appetite and stress response (Ovie & Eze 2013).

One of the good providers of essential fatty acids is squid oil. Squid contains attractant ingredients such as glycine and betaine, important in stimulating appetite (Khasani 2013). *Nereis* sp. enriched with squid oil and used as post-larval feed for vannamei shrimp is expected to meet the nutritional needs of vannamei shrimp. According to Herawati et al (2020[a]), *Nereis* sp. has a high content of amino acids and fatty acids needed in shrimp's metabolism. Saturated fatty acids can be metabolized and formed into energy for shrimp growth. The essential amino acid lysine, added to commercial feed as a precursor of carnitine, helps in fatty acid metabolism (Meyer 2004).

The shrimp's highest EPA and DHA contents were 6.87% and 7.95%, respectively, in treatment C. The content of EPA and DHA can also affect the growth rate of vannamei shrimp. This is confirmed by Sinclair (1993), who observed that the high content of fatty acids in the feed can provide the possibility to accelerate growth and increase the survival rate of shrimp. Sinclair (1993) explained that HUFA group fatty acids (EPA and DHA) contribute to cell metabolism and that HUFAs are more active than linoleic and linolenic acids. The ability of shrimp to survive in different environments is strongly influenced by the nutritional quality of the feed consumed, especially the content of essential n-3 HUFAs, such as EPA and DHA (Rudtanatip et al 2019). In addition to supporting the survival of shrimp, n-3 essential HUFA is very important to increase the ability of shrimp to respond to stress (Pangkey 2011).

The treatment of vannamei shrimp by feeding *N. virens* enriched with squid oil did not significantly affect the survival rate. Shrimp death occurred because the shrimp experienced cannibalism. Healthy vannamei shrimp will attack weaker shrimp, especially when sick. Rakhfid et al (2018) stated that the moulting process is a complicated process where mortality is difficult to avoid. The highest survival rate reached 98.89%. The high survival rate is because of water quality and the quality of feed. Water quality during maintenance was in optimal conditions for post-larvae of vannamei shrimp. Herawati et al (2021) argue that feeding with nutritional content following the needs and following the larval mouth opening will increase the growth and survival of the larvae.

Conclusions. The addition of squid oil to *N. virens* for feeding vannamei shrimp post larvae had a significant effect ($p<0.05$) on the growth and survival of vannamei shrimp. Feeding treatment C was the best treatment, with a higher RGR (24.96%/day), TFC (472.59 g), FUE (2.45 %), PER (4.55%), and SR (98.89%). Treatment C also had the highest nutritional content compared to other treatments.

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