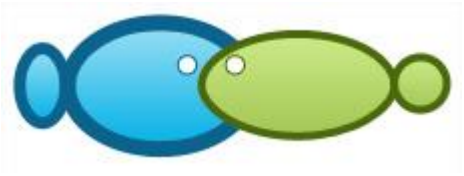

The effect of feed enriched with highly unsaturated fatty acids on the growth, fatty and amino acids of *Nereis virens*

[1]Vivi E. Herawati, [1]Seto Windarto, [2]Putut H. Riyadi, [2]YS Darmanto, [3]Novia Anggraeni

[1] Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Diponegoro, Semarang, Indonesia; [2] Department of Fisheries Product Technology, Semarang, Indonesia; [3] Department of Food Technology, National Karangturi University of Semarang, Indonesia. Corresponding author: V. E. Herawati, viviendar23@gmail.com

Abstract. The study aimed to investigate the cultivation of *Nereis virens* by feeding them with highly unsaturated fatty acids (HUFA) enriched foods in different compositions in the form of squid oil. The profile of fatty acids, amino acids, and their growth performance was determined. The study used 12 containers with 45 sea worms each, weighing in the range of 14-16 g, with 35 days of maintenance. A completely randomized experimental research method was used. In this study, the treatments presumed commercial feeding without the addition of HUFA (control - A); B - addition of 0.75 mL of HUFA; C - the addition of 1 mL of HUFA; and D - the addition of 1.25 mL of HUFA. The results showed that feeding had a significant effect ($p<0.05$) on relative growth rate (RGR), feed efficiency (FE), protein efficiency ratio (PER), and had no significant effect ($p>0.05$) on the survival rate (SR). The highest RGR, FE, PER, and SR values were found in treatment D, with the highest RGR of 5.67%, 85.2% FE, PER 2.19%, and 100% SR. The nutrient contents of *Nereis* sp. in treatment D were 52.35% protein and 24.64% lipids, with 56.96 ppm of essential amino acids and 49.14 ppm of non-essential amino acids, 11.88% of essential fatty acids (EPA), and 5.67% of non-essential fatty acids (palmitic).
Key Words: HUFA, Java, nutrition, sea worms, squid oil.

Introduction. Sea worms have a high nutritional content, namely in carbohydrates, proteins, and fatty acids, specifically in HUFA. Java Island, especially Central Java, has an abundance of *Nereis virens*. Gultom et al (2018) found an abundance of macrozoobenthic organisms, like gastropods, bivalves, and polychaetes, consisting of *Nereis* sp. and *Capitella* sp. in the mangrove area in Bedono Village, Sayung District, Demak Regency. Wibowo et al (2020) noted that *Nereis* obtained in the aquaculture area of Jeruklegi village, Cilacap, had the number of segments ranging from 80-500 segments and the average body weight ranged from 0.10 to 3.79 g. According to Wibowo et al (2020), the protein content of *N. virens* from the Jeruklegi area, Cilacap, Central Java, ranged between 42.06-51.68%, and the fat content ranged between 12.93-22%. Furthermore, according to Herawati et al (2020), *N. virens* from Jepara contained 33.19% crude protein, 19.98% fat, 15.89% crude fiber, 15.57% ash, and 16.37% of NFE (nitrogen-free extracts). Either fresh or chopped sea worms have been widely used as a substitute for fish foods. These sea worms were used to increase the growth of tiger prawns (*Penaeus monodon*) and giant prawns (*Macrobrachium rosenbergii*) (Herawati et al 2021a). *N. virens* may be developed because sea worms can be used as an alternative feed for shrimp and fish and have a fairly high price, between 1.5-5 USD.

According to Anggraeni & Handayani (2022), marine worms can be used as shrimp broodstock feed because of their nutrient content, and may improve gamete cell quality and shrimp larval viability. Currently, sea worms are not widely cultivated on a mass scale due to very limited knowledge about cultivation and methods to produce sea worms (Herawati et al 2021a). The cultivation of sea worms will contribute to preventing the

destruction of its natural habitats, namely mangroves, thereby helping the conservation of the coastal environment.

Sea worms contain essential fatty acids, especially arachidonic acid (ARA), eicosatetraenoic acid (EPA), and docosahexaenoic acid (DHA) which play a role in stimulating the maturation of the gonads of the shrimp broodstock.

Because HUFA in squid oil contains attractants that can improve the palatability or capacity of the cultivar's feed, HUFA can boost feed intake and growth rate. Growth occurred because of the availability of sufficient feed where the feed consumed is sufficient for the basic needs and survival of the shrimp (Hossain et al 2013). Enrichment of nutrients in sea worms can improve their nutritional content. Efficient feeding is able to accelerate growth and produce worms rich in nutrients, which can later be utilized optimally by the shrimp broodstock. The study aimed to investigate the cultivation of *N. virens* by feeding them with highly unsaturated fatty acids (HUFA) enriched foods in different compositions in the form of squid oil.

Material and Method. The research was conducted from April to June 2022 at Marine Science Technopark, Jepara, Central Java, Indonesia. This study used 12 containers with 45 sea worms each, from the waters of Jepara, weighing in the range of 14-16 g. The experimental animals were administered commercial feed with the addition of HUFA in the form of squid oil in the following treatments: A - 0 mL; B - 0.75 mL; C - 1 mL; and D - 1.25 mL. The commercial feed used was refined flour-based commercial feed. The addition of HUFA is a development of the research done by Sulistiyo et al (2016), as an approach to adding squid oil to the artificial feed with a maximum dose of 1.25 mL. It was hoped that the addition of HUFA would create efficient sea worms feeding, with high EPA and DHA content. The animals tested had a density of 100 individuals per 30 L (per container). The substrate in this study was mangrove sand with a 10 cm thickness and water height of 3 cm (Herawati et al 2020). This study employed a completely randomized experimental design (CRD). This study used four treatments, each of which was repeated three times. In the 30 days of maintenance, feeding was *ad libitum*, 2 times a day at 7 am and 2 pm.

Relative growth rate (RGR). The RGR (relative growth rate) was calculated using the formula used by Zonneveld et al (1991), as follows:

$$RGR = (\ln W_t - \ln W_0)/t \times 100$$

Where: RGR - relative growth rate (g day^{-1}); W_t - the average weight at the end of the study (g); W_0 - average weight at the start of the study (g); t - length of maintenance (days).

Feed efficiency. Feed efficiency (FE) was calculated using the formula by Zonneveld et al (1991):

$$FE = (W_t - W_0)/F \times 100$$

Where: FE - feed efficiency (%); W_t - final biomass at the conclusion of the study (g); W_0 - biomass at the start of the study (g); F - feed consumed during the research.

Protein efficiency ratio. The PER was calculated using the following formula (Zonneveld et al 1991):

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

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

Survival rate. The survival rate was calculated using the following formula (Zonneveld et al 1991):

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

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

Water quality. As supporting data, water quality values were determined twice a day, including dissolved oxygen (DO), salinity, acidity (pH), temperature, and ammonia. The DO, temperature and pH were measured using a water quality checker (YSI environmental 550A), and salinity using a refractometer (Atago S/Mill-E).

Amino acid analysis. HPLC was used to determine the amino acid profile (Waters Corporations, USA). Thermo Scientific Acq Taclumn (3.9x150 mm) amino acid standard solution was used for calibration and was tempered at 370°C. Fluorescence detector was used, mobile phase acetonitrile 60% -AccqTag eluent A and a flow rate of 1 mL min^{-1}. Each sample received a 5 mL injection (AOAC 2005).

Fatty acid analysis. The fatty acid profile was determined using gas chromatography after the fatty acids' lipid component was converted to methyl esters (GC). Shimadzu GC-14B (Shimadzu, Japan) GC analysis was carried out using a hydrogen flame ionization detector and a capillary column. Fatty acid content was expressed as a percentage of total fatty acids (AOAC 2005).

Proximate analysis. The proximate chemical composition of the samples was determined using a standard AOAC method (AOAC 2005). The crude protein content was calculated by multiplying the total nitrogen factor. The carbohydrate content was estimated by difference.

Statistical analysis. The obtained data was analyzed using a normality test, a uniformity test, and an additivity test to ensure that the data is normal, homogeneous, and additive. ANOVA was used to determine if there are differences among the data sets from each treatment. Assuming a significant difference ($p<0.05$), Duncan's multi-region test was used to determine between which data sets the differences are significant.

Results and Discussion. Based on the investigation that had been carried out, the values of RGR, FE, PER, and SR of sea worms are presented in Table 1. Results showed that supplementing HUFA to the feed of *N. virens* had a significant effect ($p<0.05$) on RGR, FCR, FE, and PER, and no significant effect ($p>0.05$) on SR. Treatment D produced the best RGR of 5.67%, 85.2% FE, and 2.19% PER. The lowest performances were found in the control: 2.65% RGR, 61.44% FE, and 1.46% PER. SR ranged from 95-100%.

Table 1

The average value of relative growth rate (RGR), feed efficiency (FE), the protein efficiency ratio (PER), and survival rate (SR) of *Nereis* sp. during the study

Variable	*Treatment*			
	A	*B*	*C*	*D*
RGR	2.65 ± 0.79^{a}	2.94 ± 1.60^{ab}	3.58 ± 1.26^{b}	5.67 ± 0.33^{c}
FE	61.44 ± 5.23^{a}	65.74 ± 4.99^{ab}	67.57 ± 3.32^{b}	85.20 ± 2.66^{c}
PER	1.46 ± 0.12^{a}	1.67 ± 0.26^{b}	1.63 ± 0.20^{b}	2.19 ± 0.05^{c}
SR	95.56 ± 2.23^{a}	99.26 ± 1.28^{b}	97.78 ± 2.22^{b}	100 ± 0.00^{b}

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

From the analysis, it was found that the highest values regarding the chemical composition of *Nereis* for protein, fat, and carbohydrates were in treatment D, with 52.35% protein,

24.64% fat, and 7.73% carbohydrates respectively. Table 2 shows a proximate analysis of sea worms (*N. virens*) after 35 days of maintenance.

Table 2

Proximate analysis of sea worms (*N. virens*) for 35 maintenance days

Proximate	*Nereis sp. after treatments (%)*			
	A	*B*	*C*	*D*
Protein	48.16±0.06^{a}	50.12±0.03^{b}	49.65±0.03^{ab}	52.35±0.05 ^{c}
Fat	18.59±0.04^{a}	22.22±0.02^{b}	21.04±0.09^{b}	24.64±0.02 ^{c}
Crude fiber	15.82±0.02^{a}	10.92±0.07^{b}	14.65±0.07^{a}	9.19±0.05 ^{c}
Ash	8.83±0.08^{a}	8.91±0.01^{a}	7.18±0.09^{b}	6.09±0.02 ^{c}
Carbohydrate	7.60±0.01^{a}	7.53±0.02 ^{a}	7.48±0.01^{b}	7.73±0.01^{a}

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

The highest amino acid profile was found in *N. virens* from treatment D, with a methionine content of 56.96 ppm and a glutamic acid content of 49.14 ppm. The lowest content of amino acids was found in *Nereis* sp. from control, with 30.26 ppm methionine and 30.15 ppm glutamic acid. Table 3 shows the analysis results of the total amino acid profile found in sea worms (*N. virens*) after 35 maintenance days.

Table 3

Amino acids content of sea worms (*Nereis virens*) after treatments

Amino acid (ppm)	*A*	*B*	*C*	*D*
L Histidine	13.96±0.04^{a}	16.28±0.02 ^{b}	15.32±0.08^{b}	21.07±0.04^{c}
L-Threonin	20.3±0.03^{a}	26.75±0.04 ^{b}	17.3±0.05^{c}	28.48±0.06^{d}
L-Proline	20.79±0.01^{a}	25.38±0.02 ^{b}	20.59±0.03^{a}	27.49±0.04^{c}
L-Tyrosine	20.63±0.03^{a}	25.2±0.03^{b}	19.23±0.02^{c}	25.79±0.03^{b}
L-Leucine	32.93±0.01^{a}	34.47±0.02^{b}	30.93±0.05^{c}	35.12±0.02^{b}
L-Aspartate	31.04±0.04^{a}	32.25±0.02^{a}	30.04±0.06^{b}	43.68±0.03^{c}
L-Lysine	22.99±0.06^{a}	19.19±0.03^{b}	20.99±0.03^{b}	22.3±0.02^{a}
Glycine	36.99±0.01^{a}	41.5±0.04^{b}	34.99±0.02^{c}	45.61±0.03^{d}
L-Arginine	9.41±0.03^{a}	7.749±0.03^{b}	7.41±0.03^{b}	9.71±0.01^{c}
L-Alanine	36.49±0.03^{a}	34.9±0.04^{b}	30.49±0.09^{c}	35.88±0.03^{ab}
L-Valin	21.65±0.03^{a}	23.34±0.03^{b}	19.65±0.06^{c}	28.37±0.01^{d}
L-Isoleucine	19.81±0.02^{a}	21.18±0.05^{b}	15.81±0.01^{b}	26.34±0.01^{c}
L-Phenylalanine	26.33±0.04 ^{a}	29.46±0.04 ^{b}	22.93±0.04 ^{c}	35.15±0.05 ^{d}
L-Glutamic Acid	30.15±0.04 ^{a}	32.78±0.04^{b}	32.75±0.04^{b}	49.14±0.04^{c}
L-Serin	21.18±0.01 ^{a}	23.21±0.02^{a}	18.98±0.01^{b}	28.53±0.04^{c}
L-Tryptophan	4.72±0.04 ^{ac}	5.53±0.04^{b}	3.92±0.04^{ac}	8.98±0.06^{d}
L-Methionine	30.26±0.03 ^{a}	48.56±0.02^{b}	39.26±0.03^{c}	56.96±0.04^{d}
L-cystine	16.34±0.04 ^{a}	15.62±0.04^{a}	15.32±0.04^{a}	20.1±0.04^{b}

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

The best fatty acid profile was found in *N. virens* from treatment D, with the highest EPA (11.88%) and 5.67% palmitate. The fatty acid profile of *N. virens* from control had 3.88% EPA and 3.63% palmitate. Table 4 shows the results of fatty acid analysis on sea worms (*N. virens*) after 35 days of maintenance.

The results showed that feeding HUFA in the form of squid oil had a significant effect ($p<0.05$) on PER, FE, and RGR, and had no significant effect ($p>0.05$) on SR. The addition of HUFA in the form of squid oil in feed can increase the growth of sea worms because HUFA cannot be produced by the body and must be obtained from food. The addition of HUFA in sea worm feed will affect the formation of double bonds, producing HUFA, EPA, and DHA, which function in metabolic processes. The results of this study are supported by the results of Rasidi (2012), who noted that marine cultivars, including *N. virens*, lack an enzyme system like freshwater cultivars, requiring HUFA in feed for optimal growth.

Table 4

Fatty acids of sea worms (*Nereis virens*) in the treatments

Fatty acids (%)	*A*	*B*	*C*	*D*
C 6:0	0.27±0.01^{a}	0.37±0.01^{b}	0.32±0.05^{ab}	0.47±0.09^{c}
C 8:0	0.79±0.01^{a}	0.52±0.08^{b}	0.35±0.01^{c}	1.59±0.01^{d}
C 10:0	0.18±0.01^{a}	0.19±0.01^{a}	0.17±0.03^{a}	1.36±0.04^{b}
C 11:0	0.28±0.01^{a}	0.33±0.01^{b}	0.29±0.04^{a}	0.38±0.02^{b}
C12:0	2.01±0.04^{a}	3.79±0.02^{b}	3.19±0.01^{c}	4.45±0.01^{d}
C 13:0	0.12±0.02^{a}	0.52±0.01^{b}	0.75±0.03^{b}	2.42±0.04^{c}
C 14:0	1.48±0.02^{a}	1.98±0.0^{b}	1.57±0.03^{a}	2.68±0.04^{c}
C 14:1	0.17±0.01^{a}	0.27±0.01^{a}	0.77±0.01^{c}	1.79±0.01^{d}
C 15:0	0.49±0.05^{a}	0.63±0.03^{b}	0.51±0.03^{c}	0.98±0.03^{d}
C 16:0	3.63±0.04^{a}	4.53±0.03^{b}	3.79±0.01^{a}	5.67±0.03^{c}
C 16:1	0.29±0.04^{a}	0.38±0.01^{b}	0.37±0.05^{b}	0.65±0.03^{c}
C 17:0	0.12±0.01^{a}	0.57±0.04^{b}	1.13±0.04^{c}	3.52±0.04^{d}
C 18:0	0.99±0.01^{a}	1.86±0.01^{b}	1.67±0.04^{c}	0.93±0.02^{a}
C 18:1	1.88±0.01^{a}	2.58±0.04^{b}	1.58±0.04^{c}	2.98±0.03^{d}
C 18:2	1.13±0.03^{a}	2.43±0.04^{b}	1.18±0.01^{a}	4.53±0.05^{c}
C 18:3	1.35±0.03^{a}	4.37±0.02^{b}	0.45±0.03^{c}	6.55±0.04^{d}
C 20:0	0.37±0.02^{a}	0.48±0.04^{b}	0.39±0.05^{a}	0.44±0.06^{ab}
C 20:1	0.55±0.01^{a}	0.58±0.03^{a}	0.67±0.04^{ab}	0.96±0.04^{c}
C 20:2	0.98±0.03^{a}	0.98±0.02^{a}	0.79±0.02^{b}	1.78±0.04^{c}
C 20:4	0.69±0.01^{a}	0.64±0.05^{ab}	0.55±0.04^{b}	0.98±0.04^{c}
EPA	3.88±0.01^{a}	7.68±0.02^{b}	6.09±0.05^{c}	11.88±0.01^{d}
DHA	2.35±0.03^{a}	5.58±0.03^{b}	4.68±0.02^{c}	7.36±0.03^{d}

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

Treatment D had the best RGR (5.67%), FE (85.2%), and PER (2.19%) among treatments. Ramdhani et al (2018) found that feed supplemented with nutrients needed by the cultivars will boost their growth. Environmental conditions, quality, and quantity of feed itself affect the growth of cultivars and have a relationship with both the high and low resulting conversion of the feed. This is also validated by Rasidi & Patria (2012). FE showed a different level for each treatment. The HUFA in the feed consumed was utilized optimally, so that there was an increase in growth. According to Herawati et al (2021), factors that influence growth include physiological activity, metabolic processes, and digestibility. The addition of HUFA in the appropriate dose can increase FE because the feed can be consumed and digested properly. The extractants contained in squid oil and a good feed structure will increase feed consumption and protein absorption in the feed. According to Khasani (2013), attractants are a small amount of ingredients are mixed in the food to increase food intake, growth, and consumption of feed.

HUFA added to feed was in the form of squid oil. According to Watanabe (1988), squid oil contains 13.4-17.4% EPA and 12.8-15.6% DHA. Meanwhile, according to Wibowo et al (2020), squid meat contains useful polyunsaturated fatty acids, namely n-3 fatty acids. Animal fats contain many fatty acids from the n-3 group of HUFA, such as 20:5n-3 (EPA) and 22:6n-3 (DHA) (Watanabe 1988). The content of HUFA in squid oil added to the feed could increase the level of food consumption of sea worms. HUFA could be an attractant that could to increase the palatability of feed.

Our findings reveal that there were differences between each treatment. *N. virens* in treatment D had the highest protein and fat content, at 54.05% and 22.54%, respectively, corresponding to 20.86% and 2.56% increases. The control treatment had the lowest level of nutrition, with 48.16% protein and 18.59% fat. The findings of this study are also validated by the results of Rasidi & Patria (2012). The growth rate indicates the quality of the feed supplied (Herawati et al 2015). The fat in the feed can also be used as an energy source or for growth. *N. virens* is the best live shrimp food (Brown et al 2011). Its high fatty acid content is good for ovarian growth and development (Costa et al 2010). Shrimp must have a minimum fat content of 10% in their composition (Nguyen et al 2011; Tocher 2015). Fat is an essential nutrient for shrimp ovulation development (Tocher 2015).

Treatment D had the highest amino acid content (56.96 ppm methionine and 49.14 ppm glutamic acid). The control had the lowest amino acid content, with 30.26 ppm methionine and 30.15 glutamic acid. The essential amino acid methionine works to improve the balance and utilization of other amino acids (Boonyoung et al 2013; Herawati et al 2018). It is necessary for protein synthesis and other physiological functions. In animals, the main sources of sulphur amino acids are methionine and cysteine. The body needs methionine for nucleic acid formation and tissue and protein synthesis. It also forms vitamins (choline) with other amino acids (cysteine). Methionine produces S-adenosylmethionine (SAM) catalyzed by methionine-adenosyl transferase (MAT) (Zhou et al 2021). Methionine, in conjunction with vitamin B12 and folic acid, assist the body in regulating excess protein in a high-protein diet. 2.3% methionine is required in fish feed (Tacon 1987; Soares et al 2015; Herawati et al 2021b).

The integrity and quantity of amino acids that enter or are transported to tissue cells are the primary determinants of tissue protein synthesis. The ribosome's synthetic process is highly dependent on the presence of amino acids, which are taken up by DNA and translated into tissues (Rolland et al 2015). The completeness and balance of circulating and tissue-translated amino acids have a strong influence on the efficiency and degree of protein synthesis in histiocytes. Methionine is required for fish to begin protein synthesis and has the potential to affect muscle growth (Belghit et al 2014). The addition of methionine to the diet has been shown to enhance growth and immune response (Yuan et al 2011; Kuang et al 2012; Boonyoung et al 2013; Ma et al 2013; Rolland et al 2015). Methionine deficiency can impair turbot (*Psetta maxima*) and cobia (*Rachycentron canadum*) growth and survival (Ma et al 2013; Boonyoung et al 2013).

Treatment D had the best total fatty acid profile, with 11.88% EPA and 5.67% palmitic acid, while treatment A was the poorer treatment, with 3.88% EPA and 2.35% palmitic acid (Table 4). Monounsaturated and polyunsaturated fats, including omega 3 fatty acids (EPA and DHA), help to lower triacylglycerol levels and boost excretion. They also make cell membranes more fluid and produce eicosanoids. They reduce platelets and are necessary for the development of the brain and retina (Costa et al 2010). Fatty acids contribute to energy production and storage, membrane structure, gene expression, and hormone responsiveness. Fatty acids of different lengths and degrees of unsaturation are notorious for their various effects on human health (Bajramova & Spegel 2022). Fatty acids also play an important role in the gonad maturation process for the production of high-quality eggs (Costa et al 2010). These are important factors to consider when feeding shrimp during gonad maturation.

Nereis with the addition of HUFA as vannamei shrimp feed with a maintenance period of 35 days resulted in 95-100% SR, with the highest concentrations discovered in treatment D and A. The results were higher than those of Rasidi & Patria (2012), who fed *N. virens* with chicken intestinal meal, blood meal, and shrimp head meal, obtaining a SR of 80.56-92.22%. The SR is also higher than the SR obtained by Herawati et al (2020). The SR is affected by multiple factors, such as water quality, grooming media and feed. The obtained measurement results indicated optimal water quality. Water quality is a critical factor to aquaculture success, so its management must adhere to optimal standards to support the growth and survival of organisms (Rasidi et al 2012; Hamid et al 2022). Table 5 summarizes the water quality findings.

Table 5

Results of water quality measurements during the study

No	*Variable*	*Measurement*	*Results*	*Appropriateness*
1	Temperature	^{o}C	27-31	28-30[b]
2	DO	mg L^{-1}	5–6.3	4.2-9.4[b]
3	pH	–	7.1–8.2	6.5-9[c]
4	Salinity	Ppt	28–31	5-35[a]
5	Ammonia	mg L^{-1}	0.00009	0.006-0.008[c]

Note: a - Arsad et al (2017); b - Rakhfid & Mauga (2018); c - Umami et al (2018).

Conclusions. Cultivation of *Nereis virens* by feeding them with highly unsaturated fatty acids (HUFA) enriched foods in different compositions in the form of squid oil had a significant effect ($p<0.05$) on relative growth rate (RGR), feed efficiency (FE), protein efficiency ratio (PER), and had no significant effect ($p>0.05$) on survival rate (SR). The highest RGR, FE, PER, and SR values were found in treatment D, with the highest RGR of 5.67%, 85.2% FE, PER 2.19%, and 100% SR. The nutrient contents of *Nereis* sp. in treatment D were 52.35% protein and 24.64% lipids, with 56.96 ppm of essential amino acids and 49.14 ppm of non-essential amino acids, 11.88% of essential fatty acids (EPA), and 5.67% of non-essential fatty acids (palmitic).

Acknowledgements. This research was funded by the Directorate of Research, Technology and Community Service, Directorate General of Higher Education, Research and Technology, Ministry of Education, Culture, Research, and Technology under a grant in 2022, with the contract number 187-16/UN.7.6.1/PP/2022.

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

References

Anggraeni N., Hatmiyarni T. H., 2022 [Consumer acceptance and nutritional value of catfish cendol (*Clarias batrachus*) with the addition of coffee powder]. Agromix 13(1):1-8. [In Indonesian].

Arsad S., Afandy A., Purwadhi A. P., Maya B. V., Saputra D. K., Buwono N. R., 2017 [Study of vannamei shrimp (*Litopenaeus vannamei*) enlargement cultivation activities with the application of different maintenance systems]. Jurnal Ilmiah Perikanan dan Kelautan 9(1):1-14. [In Indonesian].

Bajramova A., Spegel P., 2022 A comparative study of the fatty acid profile of common fruits and fruits claimed to confer health benefits. Journal of Food Composition and Analysis 112:104657.

Belghit I., Skiba-Cassy S., Guerden I., Das K., Surget A., Kaushik S., Panserat S., Seiliez I., 2014 Dietary methionine availability affects the main factors involved in muscle protein turnover in rainbow trout (*Oncorhynchus mykiss*). J The British Journal of Nutrition 112(4):493-503.

Boonyoung S., Haga Y., Satoh S., 2013 Preliminary study on effects of methionine hydroxy analogue and taurine supplementation in a soy protein concentrate-based diet on the biological performance and amino acid composition of rainbow trout [*Oncorhynchus mykiss* (Walbaum)]. Aquaculture Research 44(9):1339-1347.

Brown N., Eddy S., Plaud S., 2011 Utilization of waste from a marine recirculating fish culture system as a feed source for the polychaete worm, *Nereis virens*. Aquaculture 322–323:177-183.

Costa P. F., Narciso L., Cancela L., Fonseca, 2010 Growth, survival, and fatty acid profile of *Nereis diversicolor* (O. F. Müller, 1776) fed on six different diets. Bulletin of Marine Science 67(1):337-343.

Gultom C. R., Muskananfola M. R., Purnomo P. W., 2018 [Relationship of macrozoobenthos abundance with organic materials and sediment textures in mangrove areas in Bedono Village, Sayung District, Demak Regency]. Jurnal Maquares 7:172-179. [In Indonesian].

Hamid S. H. A., Lananan F., Noor N. A. M., Endut A., 2022 Physical filtration of nutrients utilizing gravel-based and lightweight expanded clay aggregate (LECA) as growing media in aquaponic recirculation system (ARS). Aquacultural Engineering 98:102261.

Herawati V. E., Hutabarat J., Pinandoyo, Ocky K. R., 2015 Growth and survival rate of tilapia (*Oreochromis niloticus*) larvae fed by *Daphnia magna* cultured with organic fertilizer resulted from probiotic bacteria fermentation. HAYATI Journal of Biosciences 22(4):169-173.

Herawati V. E., Nugroho R. A., Pinandoyo, Hutabarat J., Prayitno B., Radjasa O. K., 2018 The growth performance and nutrient quality of Asian swamp eel *Monopterus albus*

in Central Java Indonesia in a freshwater aquaculture system with different feeds. Journal of Aquatic Food Product Technology 27(6):658-666.
Herawati V. E., Pinandoyo, Nurmanita R., Putut H. R., Tita E., Woraporn T., Ocky K. R., Seto W., 2021a Analysis of growth and nutritional quality of sea worms (*Nereis virens*) as a mass cultured natural feed on different substrate media thicknesses. Biodiversitas 22:3299-3305.
Herawati V. E., Pinandoyo, Seto W., Nurmanita R., Putut H. R., Darmanto Y. S., Ocky K. R., 2020 Nutritional value and growth performance of sea worms (*Nereis* sp.) fed with *Hermetia illucens* maggot flour and grated coconut (*Cocos nucifera*) as natural feed. Biodiversitas 2:5431-5437.
Herawati V. E., Windarto S., Prasetyo D. Y. B., Prayitno S. B., Radjasa O. K., 2021b The effect of mass cultured *Phronima pacifica* feed using *Caulerpa lentillifera* substrate on growth performance and nutritional quality of Pacific white shrimp (*Litopenaeus vannamei*). Journal of Applied Aquaculture, DOI: 10.1080/10454438.2021.2018378
Hossain M. I., Kamal M. M., Mannan M. A., Bhuyain M. A. B., Hossain M. A., 2013 Effects of Probiotics on Growth and Survival of Shrimp (*Penaeus monodon*) in Coastal Pond at Khulna, Bangladesh. Journal of Scientific Research 5(2):363-370.
Khasani I., 2013 [Attractants in fish feed: Types, functions, and responses of fish]. Jurnal Media Akuakultur 8:127-133. [In Indonesian].
Kuang S. Y., Xiao W. W., Feng L., Liu Y., Jing J., Jiang W. D., Hu K., Li S. H., Tang L., Zhou X. Q., 2012 Effects of graded levels of dietary methionine hydroxy analogue on immune response and antioxidant status of immune organs in juvenile Jian carp (*Cyprinus carpio* var. Jian). Fish and Shellfish Immunology 32(2):629-636.
Ma R., Hou H., Mai K., Bharadwaj A. S., Cao H., Ji F., Zhang W., 2013 Comparative study on the effects of L-methionine or 2-hydroxy-4-(methylthio) butanoic acid as dietary methionine source on growth performance and anti-oxidative responses of turbot (*Psetta maxima*). Aquaculture 412-413:136-143.
Nguyen B. T., Koshio S., Sayikama K., Ishikawa M., Yokoyama S., Kader M. A., 2011 Effects of polychaete extracts on reproductive performance of kuruma shrimp, *Marsupenaeus japonicus* Bate. - Part II. Ovarian maturation and tissue lipid compositions. Aquaculture 334-337:65-72.
Rakhfid A., Mauga U., 2018 [Growth and survival of vannamei shrimp (*Litopenaeus vannamei*) at various doses of fertilizer and stocking solids]. Jurnal Akuakultur, Pesisir dan Pulau-Pulau Kecil 2(2):53-60. [In Indonesian].
Ramdhani S., Setyowati D. N., Astriana B. H., 2018 [Addition of different prebiotics to feed to increase the growth of vannamei shrimp (*Litopenaeus vannamei*)]. Jurnal Perikanan 8(2):50-57. [In Indonesian].
Rasidi, 2012 [Sea worm hatchery *Dendronereis pinnaticirris* - an initial effort to provide sea worms for aquaculture]. Media Akuakultur 7(2):88-91. [In Indonesian].
Rasidi, Patria M. P., 2012 [Growth and survival of sea worms (*Nereis* sp.) (Polychaeta, Annelida) given different types of feed]. Jurnal Riset Akuakultur 7:447-464. [In Indonesian].
Rolland M., Dalsgaard J., Holm J., Gómez-Requeni P., Skov P. V., 2015 Dietary methionine level affects growth performance and hepatic gene expression of GH– IGF system and protein turnover regulators in rainbow trout (*Oncorhynchus mykiss*) fed plant protein-based diets. Comparative Biochemistry and Physiology. Part B 181:33-41.
Soares M., Fracalossi D. M., Lima de Freitas L. E., Rodrigues M. S., Redig J. C., Mouriño J. L. P., Seiffert W. Q., do Nascimento Vieira F., 2015 Replacement of fish meal by protein soybean concentrate in practical diets for Pacific white shrimp. Revista Brasileira de Zootecnia 44(10):343-349.
Sulistyono B., Isriansyah, Sumoharjo, 2016 [Feeding *Artemia* sp. enriched with squid oil against the survival and growth of snakehead (*Channa striata*) larvae]. Jurnal Sains dan Teknologi Akuakultur 2(1):11-18. [In Indonesian].
Tacon A. G. J., 1987 The nutrition and feeding of farmed fish and shrimp - A training manual. A report prepared for the FAO Trust Fund GCP/RLA/075/ITA Project Support to the Regional Aquaculture Activities for Latin America and the Caribbean, pp. 106-109.

Tocher D. R., 2015 Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aquaculture 449:94-107.
Umami I. R., Hariyati R., Utami S., 2018 [Diversity of phytoplankton in vannamei shrimp pond (*Litopenaeus vannamei*) in Tireman, Rembang Regency, Central Java]. Jurnal Biologi 7(3):27-32. [In Indonesian].
Watanabe I., 1988 Nutrition and growth. In: Intensive fish farming. Shepherd C. J., Promage N. R. (eds), BSP Profesional Books, pp. 154-197.
Wibowo E. S., Puspitasari I. G. A. R., Palupi E. S., Atang, 2020 The potential of *Nereis* sp. (Polychaete) as prawn feed at the coastal farming of Jeruklegi, Cilacap. IOP Conference Series: Earth and Environmental Science 550:012030.
Yuan Y. C., Gong S. Y., Yang H. J., Lin Y. C., Yu D. H., Luo Z., 2011 Effects of supplementation of crystalline or coated lysine and/or methionine on growth performance and feed utilization of the Chinese sucker (*Myxocyprinus asiaticus*). Aquaculture 316(1-4):31-36.
Zhoua Y., He J., Su N., Masagounder K., Xu M., Chen L., Liu Q., Ye H., Sun Z., Ye C., 2021 Effects of DL-methionine and a methionine hydroxy analogue (MHA-Ca) on growth, amino acid profiles and the expression of genes related to taurine and protein synthesis in common carp (*Cyprinus carpio*). Aquaculture 532:735962.
Zonneveld N., Huisman E. A, Boon J. H., 1991 [Fish cultivation principles]. Gramedia Pustaka Utama, Jakarta, 318 p. [In Indonesian].
*** AOAC, 2005 Official methods of analysis. 18th Edition. Association of Official Analytical Chemists, Benjamin Franklin Station, Washington.

Received: 23 August 2022. Accepted: 22 September 2022. Published online: 10 November 2022.
Authors:
Vivi Endar Herawati, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University. Jl. Prof. Jacub Rais, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: viviendar23@gmail.com
Seto Windarto, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Jacub Rais, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: seto.windarto@live.undip.ac.id
Putut Har Riyadi, Department of Fisheries Product Technology, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Jacub Rais, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: putut.riyadi@live.undip.ac.id
YS Darmanto, Department of Fisheries Product Technology, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Jacub Rais, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: ysdarmanto@lecturer.undip.ac.id
Novia Anggraeni, Department of Food Technology, National Karangturi University of Semarang, Rejomulyo, Semarang Timur, Semarang, 50227 Central Java, Indonesia, e-mail: novia.anggraeni@unkartur.ac.id

How to cite this article:
Herawati V. E., Windarto S., Riyadi P. H., Darmanto Y. S., Anggraeni N., 2022 The effect of feed enriched with highly unsaturated fatty acids on the growth, fatty and amino acids of *Nereis virens*. AACL Bioflux 15(6):2813-2821.