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Dietary enrichment with Highly Unsaturated Fatty Acid (HUFA) during rearing phase of *Nereis virens*: growth performance, fatty and amino acid

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Abstract. *Herawati VE, Windarto S, Arfan M. 2023. Dietary enrichment with Highly Unsaturated Fatty Acid (HUFA) during the rearing phase of marine worms (*Nereis virens*) in growth performance, fatty acid profiles, and amino acid profiles. Biodiversitas 24: 3828-3834.* Nereis virens is a species of marine worm that belongs to Polychaeta. *Nereis virens* is often utilized as a natural feed for shrimp broodstock related to its main content of fatty acids to accelerate gonad maturity. The enrichment of Highly Unsaturated Fatty Acid (HUFA) is thought to stimulate diet protein and support growth, survival, and nutritional content. This study aimed to discover and examine the growth performance, amino acid profile, and fatty acid profile of *N. virens* in the rearing stage with a HUFA-enriched diet. The research was conducted from May to September 2022 at MSTP (Marine Science Techno Park) Jepara. The experimental animals used were 45 marine worms per rearing container, weighing 14-16g/container. The research method is experimental, with a completely randomized design (CRD) of 4 treatments and 3 replicates. The treatments in this study consisted of a commercial diet without the HUFA (A), with the enrichment of 0.50 ml HUFA (B), 1 ml HUFA (C), and 1.50 ml HUFA (D). The results showed that diets with the enrichment with HUFA had a significant effect ($P<0.05$) on Absolute Growth (Ag); Relative Growth Rate (RGR); Efficiency of Feed Utilization (EFU), Protein Efficiency Ratio (PER) and no significant effect ($P>0.05$) on SR. The highest RGR, EFU, PER, and SR values were in the treatment with the enrichment with 1.50 ml HUFA (D) with the following values of 5.77 ± 0.20g/day, 85.20 ± 2.75g, 2.05 ± 0.08%, and 100 ± 0.00%. Nutritional quality based on proximate protein and fatty acid fat results were highest in the enrichment treatment with 1.50 ml HUFA (D), 53.80% protein and 19.67% fat. The highest amino acid and fatty acid profiles in the same treatment were 41.15 ppm and 6.60% EPA.

Keywords: Feed, *Nereis virens*, nutrition, production

INTRODUCTION

Marine worms have a high nutritional content, especially in carbohydrates, protein, and fatty acid: EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) (Monoroig and Kabeya 2018). Marine worms have been widely utilized as feed by Aqua-culturists, both fresh in the form of chopped or flour mixed as a fish feed ingredient. These marine worms accelerate the growth of tiger shrimp (*Penaeus monodon*) and king prawns (*Litopenaeus vannamei*) (Hermawan et al. 2015; Rohmanawati et al. 2022). The problem currently occurs is the requirement for the nutritional content of marine worms as a natural diet has yet to be adequately fulfilled, and its availability is limited to be utilized by shrimp farmers. According to Wibowo et al. (2018), marine worms can be used as feed for shrimp broodstock, as they contain nutrients that can improve the quality of gametes and the viability of shrimp larvae. According to Hartanti and Suyono (2015), marine worms still need to be mass-produced due to a lack of knowledge about cultivation and optimal methods to produce high-quality marine worms. The cultivation of marine worms will contribute to preventing damage to natural habitats, specifically mangroves, thus helping coastal environmental conservation efforts.

According to Gamis et al. (2016), the carbohydrate value of marine worms is higher than the value of protein and fat and ash content. According to research by Herawati et al. (2000), the nutritional value of marine worm protein is 56.29%, fat is 11.32%, and ash is 14.34%. Marine worms contain essential fatty acids, especially Arachidonic acid (ARA), Eicosapentaenoic acid (EPA), and Docosahexaenoic acid (DHA), which play a role in stimulating the maturation of shrimp broodstock gonads.

Highly Unsaturated Fatty Acid (HUFA) has engaging content that can improve the palatability or ability of feed to be consumed by cultivators so that HUFA can increase the growth and feed consumption rate. Growth occurs due to the availability of a diet in sufficient quantities where the feed consumed is adequate for the basic needs and survival of the cultivators (Asnawi et al. 2018). Nutritional enrichment can be an option for the utilization of marine worms so that the cultivar can optimize the nutrient content (Mustari 2016); the enrichment of HUFA is expected to optimize the diet of marine worms in achieving high EPA and DHA content in marine worms. Efficient feeding can accelerate growth and generate nutrient-rich worms that can be optimally utilized by shrimp brood stock. This study aimed to discover and examine the growth performance,

amino acid profile, and fatty acid profile of *N. virens* in the rearing stage with HUFA-enriched feed.

MATERIALS AND METHODS

This study used experimental animals, which were marine worms weighing 14-16 g/container, with a total of 45 animals per container. The experimental animals were given commercial feed with the enrichment of HUFA in the form of squid oil by 0 ml, 0.50 ml, 1 ml, and 1.50 ml on 10g diet/day. The rearing tanks in this study used containers with a volume of 15 L and as many as 12 pieces. The equipment used as a rearing container is cleaned and soaked in a chlorine solution of 10 ppm. Hermawan et al. (2015); the preliminary step to use the tools was first sterilized in seawater using 10 ppm chlorine and neutralized with 5 ppm sodium thiosulfate. The media used in this study was a mangrove sand substrate with a thickness of 8 cm.

The experimental design used in this study was a completely randomized design (CRD). This study used 4 treatments, and each treatment was repeated three times. The treatments in the study using the enrichment with HUFA in the diet are as follows: (i) Treatment A: Diet without the enrichment of HUFA, (ii) Treatment B: enrichment with HUFA by 0.50 ml on 10 g of diet, (iii) Treatment C: enrichment with HUFA by 1 ml on 10 g diet, (iv) Treatment D: enrichment with HUFA of 1.50 ml on 10 g diet. This study was conducted for 42 days with feeding as much as 5% of biomass weight ad satiation (Gamis et al. 2016). After 42 days of rearing, the worms were harvested, and the final weight was weighed. Furthermore, the worms were dried, and then after drying, they were analyzed for proximate, fatty, and amino acids.

Variables

Absolute Growth (Ag)

Absolute growth (Ag), known based on the average biomass of marine worms (*N. virens*), is calculated using the following formula (Tacon 1993). Ag = Wt-W0, in which Ag: Average Initial Weight of worm (g); Wt: The average weight of test animals at the end of the study (g); and W0: The average weight of test animals at the start of the study (g).

Relative Growth Rate (RGR)

The formula (Tacon 1993) calculated the specific growth rate: RGR= (Wt-W0)/ (W0 × t) x 100 %. RGR: Relative growth rate (%); Wt: Mean weight of worms at the end of the study (g); W0: Mean weight of worms at the beginning of the study (g); t: time of the study (days).

Efficiency of Feed Utilization (EFU)

Feed utilization efficiency was calculated using Tacon's formula (1993). EFU= (Wt-W0)/F × 100%. EFU: Efficiency of Feed Utilization (%); Wt: Final biomass at the end of the study (g); W0: Baseline biomass at the start of the study (g); F= The amount of feed consumed during the study.

Protein Efficiency Ratio (PER)

The protein efficiency ratio can be calculated using the Tacon (1993) formula: PER = (Wt-W0)/Pi x 100%. PER: Protein efficiency ratio; Wt: Weight of test animals' biomass at the end of maintenance (g); W0: weight of test animals at the beginning of maintenance (g); Pi: protein content x amount of feed consumed.

Survival Rate (SR)

The survival rate formula, according to Tacon (1993). SR= (N0-Nt)/N0 × 100%. SR: Survival Rate; Nt: Number of individuals at the end of the study (individual); N0: number of individuals at the beginning of the study (individual).

Water quality

Water quality measurement includes temperature, salinity, DO (dissolved oxygen), and pH, carried out in the morning and evening. DO measurements used a DO meter, and temperature measurements used a thermometer, salinity measurements used a refractometer, and pH measurements used a pH meter. The result of water quality can be seen in Table 1.

The results of measuring water quality parameters in *N. virens* maintenance for 42 days, the values of the variables DO, salinity, pH, and temperature are still in the appropriate range to be used as a medium for the cultivation of marine worms (*N. virens*).

Amino acid profile

Amino acid profiles were determined by HPLC (waters Corporation, USA). The amino acid standard solution used for calibration from Thermo Scientific, Acq Taclumn (3.9 mm x 150 mm), at 37ºC ; mobile phase acetonitrile 60%-AccqTag Eluent A. Flow rate 1.0ml. min^{-1} with fluorescence detector. The volume injected for each sample was 5µL (AOAC 2005).

Fatty acids profile

The Fatty acid profile was determined by gas chromatography (GC) after converting the fatty acid component to the lipid to their methyl esters. The GC analysis was performed on a Shimadzu GC-14B (Shimadzu Seisakusho, Japan) equipped with a flame ionization detector and a capillary column. Fatty acid content was expressed as the relative weight percentage of total fatty acids (AOAC 2005).

Table 1. Water quality in marine worms (*Nereis virens*) maintenance for 42 days

Variable	Units	Result	Optimized standard
Temperature	°C	27-31	28-30[b]
DO	mg/L	5-6.3	4.20-5.40[b]
PH	-	7.1-8.2	6.5-8.0[c]
Salinity	Ppt	28-31	5-35[a]
Ammonia	Mg/L	0.00009	0,006-0,008[a]

Note: [a]Wibowo et al. (2018); [b]Herawati et al. (2020); [c]Subaidah et al. (2017)

Proximate analysis

The proximate chemical composition of the samples was determined using a standard (AOAC 2005). Proximate analysis was used to describe the protein, fat, ash, carbohydrates, and water content in the sample. The protein analysis sample was calculated using the Kjeldahl method, while the sample fat content was calculated using the Soxhlet method. Analysis of water and ash content using gravimetric principles. Analysis of carbohydrates is calculated manually based on the results of the proximate analysis.

Data analysis

The data obtained were analyzed using variance (ANOVA), which first carried out the normality test, homogeneity test, and additivity test to determine whether the data was normal, homogeneous, and additive. Suppose it is known that there is a significant ($P < 0.05$), then proceed with the Duncan Multiple Area Test to determine the difference in the mean between treatments and determine the best treatment. Water quality data were analyzed descriptively.

RESULTS AND DISCUSSION

Results

Based on the research, the growth performance of marine worms during 42 days of rearing is presented in Table 2.

The results showed that diets with the enrichment of HUFA had a significant effect ($P<0.05$) on RGR, AG, TFC, EFU, and PER and no significant effect ($P>0.05$) on SR. RGR (P: 0.025, $P < 0.05$); TFC (P: 0.038, $P < 0.05$); AG (P: 0.026, $P < 0.05$); EFU (P: 0.001, $P < 0.05$); and PER (P: 0.019, $P < 0.05$). The highest RGR, Ag, EFU, PER, and SR values were in the treatment with the enrichment of HUFA of 1.50 ml/10 g feed (D) with the following values: 5.77g/day; 6.75g; 85.20g; 2.05g; and 100%. Based on the results of the lowest RGR, AG, EFU, and PER values in the treatment with the enrichment of HUFA at 0 ml/10 g feed (A) with the following values 3.55g/day; 4.00g; 61.45g; 1.63% and 95.56% survival. The results of the proximate analysis of worms during 42 days of rearing are presented in Table 3.

The results of nutritional quality analysis (proximate analysis) of protein and fat of *N. virens* were highest in the treatment of enrichment with HUFA at 1.50 ml/10 g diet at 53.80% protein and 19.67% fat, while the lowest protein and fat of *N. virens* in the treatment of enrichment with HUFA at 0 ml/10 g feed at 40.40% protein, and 16.29% fat. The results of amino acid analysis of marine worms (*N. virens*) for 42 days of rearing are presented in Table 3.

The results of the highest amino acid profile of *N. virens* in the treatment of the enrichment with HUFA at 1.50 ml/10 g diet was 41.15 ppm of methionine. In comparison, the lowest amino acid profile was found of *N. virens* in the treatment of the enrichment of HUFA at 0 ml/10 g diet, which was 27.98 ppm. The results of the fatty acid analysis in marine worms (*N. virens*) for 35 days of maintenance are presented in Table 4.

The highest fatty acid profile result of *N. virens* in the treatment of enrichment with HUFA at 1.50 ml/10 g diet is 6.60% EPA. In comparison, the lowest fatty acid profile efficiency value is found in *N. virens* treatment of 0 ml/10 g diet with HUFA at 2.15% EPA.

Table 2. Growth performance of marine worms (*Nereis virens*) for 42 maintenance days

Variable	Treatment			
	A (0)	B (0.50)	C (1)	D (1.50)
RGR (%)	3.55±0.79abc	2.90±1.60ab	2.60±1.26a	5.77±0.20c
TFC (g)	22.41±0.62 a	23.99±6.00c	24.59±5.25bc	24.85±1.60a
Absolute weight (Ag) (g)	4.00±0.62a	5.50±0.55ab	5.75±0.87abc	6.75±1.20bc
EFU (%)	61.45±3.32a	65.90±4.99ab	68.55±5.23abc	85.20±2.75bc
PER (%)	1.63±0.12ab	1.57±0.26abc	1.46 ±0.20abc	2.05±0.08a
SR (%)	95.56±2.23bc	99.26±1.28bc	97.78±2.22ab	100±0.00a

Description: different superscripts values indicate that the treatment had a significant effect ($P < 0.05$).

Table 3. Proximate analysis of marine worms (*Nereis virens*) for 42 maintenance days

Proximate (%)	Treatment			
	A (0)	B (0.50)	C (1)	D (1.50)
Protein	40.40 ± 0.03^{b}	45.39 ± 0.05^{b}	50.63 ± 0.03^{b}	53.80 ± 0.06^{b}
Fat	16.29 ± 0.02^{a}	17.72 ± 0.03^{a}	18.34 ± 0.07^{a}	19.67 ± 0.04^{b}
Crude fiber	10.10 ± 0.07^{b}	10.50 ± 0.01^{b}	10.70 ± 0.04^{b}	8.14 ± 0.05^{b}
Ash	16.33 ± 0.03^{a}	11.60 ± 0.05^{a}	9.50 ± 0.04^{a}	7.19 ± 0.03^{b}
Carbohydrate	16.88 ± 0.08^{a}	13.25 ± 0.06^{b}	10.53 ± 0.02^{a}	10.20 ± 0.01^{a}

Table 3. Results of amino acid analysis of sea worms (*Nereis virens*) for 42 maintenance days

Amino acid (ppm)	A (0)	B (0.50)	C (1)	D (1.50)
L Histidine	17.30± 0.04[b]	18.10 ± 0.03[b]	19.50 ± 0.03[b]	21.15 ± 0.03[b]
L-Threonin	10.12± 0.05[b]	15.60 ± 0.05[b]	19.56 ± 0.05[b]	25.03± 0.05[b]
L-Proline	11.48 ± 0.02[b]	16.80 ± 0.03[b]	20.50 ± 0.09[b]	26.66 ± 0.07[b]
L-Tyrosine	15.23 ± 0.08[b]	18.88 ± 0.02[a]	20.16 ± 0.02[a]	25.38 ± 0.02[a]
L-Leucine	25.93 ± 0.02[b]	27.77 ± 0.09[b]	30.25 ± 0.08[b]	33.49 ± 0.07[b]
L-Aspartate	24.04 ± 0.09[b]	28.05 ± 0.01[b]	30.50 ± 0.01[b]	32.68 ± 0.08[b]
L-Lycine	21.99 ± 0.04[b]	26.66 ± 0.06[b]	27.55 ± 0.08[b]	32.67 ± 0.04[b]
Glycine	10.99 ± 0.02[b]	12.75 ± 0.02[b]	15,70 ± 0.05[b]	20.61 ± 0.08[b]
L-Arginine	7.41 ± 0.05[a]	8.05 ± 0.07[a]	8.50 ± 0.06[a]	9.05 ± 0.01[9]
L-Alanine	19.55 ± 0.07[b]	22.23 ± 0.07[b]	24.55 ± 0.07[b]	27.65 ± 0.03[b]
L-Valin	18.70 ± 0.02[b]	20.14 ± 0.08[b]	23.20 ± 0.04[b]	25.37 ± 0.09[b]
L-Isoleucine	16.90 ± 0.06[b]	19.03 ± 0.08[b]	21.23 ± 0.06[b]	25.88 ± 0.03[b]
L-Phenylalanine	19.80 ± 0.09[b]	22.46 ± 0.03[b]	20.35 ± 0.04[b]	25.85 ± 0.06[b]
L-Glutamic Acid	29.87 ± 0.01[b]	31.50± 0.05[b]	32.25 ± 0.02[b]	35.70 ± 0.09[b]
L-Serin	17.75 ± 0.06[b]	23.70 ± 0.09[b]	24.95 ± 0.03[b]	28.95 ± 0.05[b]
L-Tryptophan	4.45 ± 0.05[b]	5.53 ± 0.03[b]	7.15 ± 0.02[b]	8.98 ± 0.03[b]
L-Methionine	27.98 ± 0.02[b]	35.77 ± 0.01[b]	38.54 ± 0.05[b]	41.15 ± 0.07[b]
L-cystine	14.70 ± 0.01[a]	15.50 ± 0.05[a]	17.35 ± 0.08[b]	21.90 ± 0.05[b]

Table 4. Results of analysis of fatty acids in sea worms (*Nereis virens*) for 42 maintenance days

Fatty acids (%)	A	B	C	D
C 6:0	0.30 ± 0.04[a]	0.35 ± 0.04[a]	0.42 ± 0.03[b]	0.45± 0.06[a]
C 8:0	0.53 ± 0.03[b]	0.80 ± 0.06[b]	0.95 ± 0.05[b]	1.55 ± 0.09[b]
C 10:0	0.15 ± 0.02[b]	0.20 ± 0.07[b]	0.56 ± 0.08[b]	1.35 ± 0.06[a]
C 11:0	0.37 ± 0.06[a]	0.45 ± 0.03[a]	0.55 ± 0.02[a]	0.95± 0.02[a]
C12:0	2.05 ± 0.07[b]	3.79 ± 0.09[b]	4.45 ± 0.07[b]	2.79 ± 0.09[a]
C 13:0	0.15 ± 0.01[a]	1.20 ± 0.08[a]	1.90 ± 0.09[b]	2.45 ± 0.03[b]
C 14:0	1.35 ± 0.08[a]	1.95 ± 0.06[a]	2.05 ± 0.02[b]	2.75 ± 0.06[a]
C 14:1	0.17 ± 0.02[a]	0.30 ± 0.02[a]	0.90 ± 0.05[b]	1.80 ± 0.04[a]
C 15:0	0.30 ± 0.06[a]	0.51 ± 0.09[a]	0.75 ± 0.09[a]	1.05 ± 0.09[a]
C 16:0	3.55 ± 0.09[b]	4.30 ± 0.05[b]	4.80 ± 0.02[b]	5.67 ± 0.02[b]
C 16:1	0.39 ± 0.02[a]	0.30 ± 0.03[a]	0.95 ± 0.08[b]	1.50 ± 0.01[a]
C 17:0	0.12 ± 0.01[a]	0.57 ± 0.02[a]	3.52 ± 0.07[b]	0.83 ± 0.08[a]
C 18:0	1.09 ± 0.05[a]	1.05 ± 0.08[b]	1.23 ± 0.05[a]	1.50 ± 0.08[a]
C 18:1	1.98 ± 0.06[b]	2.05 ± 0.01[b]	2.75 ± 0.03[b]	0.98 ± 0.04[b]
C 18:2	1.13 ± 0.02[a]	1.53 ± 0.08[b]	2.10 ± 0.02[b]	3.20 ± 0.09[a]
C 18:3	1.35 ± 0.08[a]	2.7 ± 0.05[b]	3.50 ± 0.09[b]	4.20 ± 0.02[b]
C 20:0	0.37 ± 0.03[a]	0.40 ± 0.08[a]	0.44 ± 0.05[a]	0.80 ± 0.09[a]
C 20:1	0.55 ± 0.02[b]	0.50 ± 0.02[b]	0.66 ± 0.02[b]	0.98 ± 0.02[b]
C 20:2	0.75 ± 0.08[a]	0.98 ± 0.09[a]	1.07 ± 0.09[b]	1.55 ± 0.09[a]
C 20:4	0.69 ± 0.09[a]	0.64 ± 0.07[a]	0.98 ± 0.01[a]	1.02 ± 0.01[a]
EPA	2.15 ± 0.02[a]	3.75 ± 0.09[b]	4.50 ± 0.09[b]	6.60 ± 0.05[a]
DHA	1.30 ± 0.09[b]	3.40 ± 0.09[b]	4.15 ± 0.02[b]	5.25 ± 0.01[b]

Discussion

The results showed that feeding with the enrichment with HUFA had a significant effect ($P<0.05$) on the relative growth rate, total biomass weight, feed consumption rate, feed utilization efficiency, and protein efficiency ratio, but no significant effect ($P<0.05$) on SR. The highest values of absolute growth (AG); relative growth rate (RGR); efficiency of feed utilization (EFU), protein efficiency ratio (PER) and survival rate (SR) were in the treatment with the enrichment with 1.50 ml HUFA (D) with consecutive values of 5.77±0.20g/day, 85.20±2.75g, 2.05±0.08%, and 100±0.00%. The enrichment with HUFA in the diet is thought to improve the growth performance of *N. virens*, as found through relative growth rate, total biomass weight, feed consumption rate, feed utilization efficiency, and protein efficiency ratio. Unsaturated fatty acids (HUFA) cannot be produced in the body for this reason, it is necessary to add HUFA to the diet. The HUFA in the diet is converted with the help of enzymes into long hydrocarbon chains, forming double bonds to generate HUFA, EPA, and DHA are essential for metabolic functions. This statement follows the results of research by Watanabe (1988), unsaturated fatty acids (HUFA) cannot be in the manufacture of the body and must be obtained

from feed, then converted into long hydrocarbon chains to further form double bonds and generate the HUFA, EPA, and DHA which are very important for metabolic functions and components in cell membranes. Ibeas et al. (2020) also claimed that a marine cultivar does not have an enzyme system like the one found in freshwater cultivars, so marine cultivars need long-chain n-3 and n-6 HUFA from feed for optimal growth.

Based on the study's results, growth performance was observed to be different in each treatment, and the different levels of HUFA enrichment influenced this in the diet. Watanabe (1988) stated that squid oil contains 13.4%-17.4% EPA and 12.8%-15.6% DHA. Animal fat contains many fatty acids from the n-3 Highly Unsaturated Fatty Acid (HUFA) group, such as 20:5n-3 (EPA/Eicosa Pentaenoic Acid) and 22:6n-3 (DHA/Docosa Hexaenoic Acid) (Marzuqi et al. 2006). This factor is supported by Herawati et al. (2022), which stated that the difference in growth rate could be affected by the diet provided. The content of HUFA in squid oil added to the diet may increase the feed consumption rate of marine worms. HUFA can be an attractant that can increase the palatability or ability of feed to be consumed.

The relative growth rate (RGR) results showed that the treatment with the enrichment of 1.50 ml of HUFA in the diet (D) was the best result of this study and had a significant effect on the growth performance of marine worms. This is because the content of HUFA in squid oil added to the diet can increase the total feed consumption, resulting in the growth of *N. virens*. However, the use of HUFA enrichment has only partially affected each treatment. Enrichment 1.50 ml HUFA in treatment D showed an increase in growth performance compared to the non-enrichment control treatment. In contrast to the treatment of the enrichment with 0 ml HUFA in diet (A), the enrichment with 0.50 ml HUFA in diet (B), and the enrichment with 1 ml HUFA in diet (C). This can be expected because the worms in treatments A, B, and C perform a more active movement compared to treatments A and D, where worms have a habit of staying in the form of the letter U with the tip of the head on the surface of the substrate. This is also reinforced by Gamis et al. (2016), marine worms that actively move will spend more energy where nutrients from the diet that should be fat and worm weight will be used to fuel their movement on the substrate. In addition, HUFA in squid oil is also an attractant, a stimulant compound given to a diet to stimulate the fish's sense of smell so that the fish can respond quickly to the food provided.

HUFA contains attractants that increase feed's palatability or ability to be consumed. HUFA in the diet as an enrichment ingredient also functions as an attractant to increase the palatability or ability of feed to be consumed so that HUFA can increase the total value of feed consumption and growth. This statement follows the results of the study by Khasani (2013), which states that attractants are ingredients mixed in the diet in small amounts to enhance the food intake, growth, and consumption of cultivars to feed. This is also reinforced by Gamis et al. (2016), which state that one factor determining the success of cultivation depends on the quality of the feed provided.

The highest total feed consumption during the study in the same treatment was with the enrichment with 1.50 ml of HUFA in the diet (D). This is because *N. virens* has physiology and feeding habits actively, which means that the cultivar's response to the feed given is fast so this marine worm has the highest feed consumption rate. This is reinforced by Subaidah et al. (2017), stating that factors that affect the diet on growth include physiological activity, metabolic processes, and digestibility which are different in each fish. High feed consumption will grow the fish body if the diet can be digested properly. The diet will affect growth, and an animal protein-based diet has a higher body weight growth than vegetable protein-based diets. This statement was reinforced by Wibowo et al. (2018). *N. virens* with animal protein diets experienced higher body weight growth than vegetable protein diets. The amount of feed consumed (TFC) by *N. virens* needs to be considered as its efficiency. The highest efficiency of feed utilization (EFU) in this study was found in the treatment with the enrichment with 1.50 ml HUFA in the diet (D), which was 85.20%; the feed utilization efficiency shows the optimization of *N. virens* in consuming the feed given. Asnawi et al. (2018), in their research, stated that the provision of HUFA at the appropriate dose can increase feed efficiency utilization because feed can be appropriately utilized and digested by the body.

The method used in the research is ad satiation, which is gradually feeding according to the biomass weight in the treatment. The attractants are contained, and the structure of the diet is good so that the feed's level of feed utilization and protein absorption in the feed is good. The diet consumed by *N. virens* will be optimally utilized. The weight gain can find this to body weight, and this occurs through the process of protein absorption by *N. virens* using its energy. The large number of diets consumed by *N. virens* will be broken down into nutrients and body weight, thus increasing growth performance. Each treatment has a different absorption efficiency following the level of the HUFA enrichment to the diet. Therefore, feed efficiency can be found through the weight of feed consumed in proportion to the resulting body weight. This is reinforced by Mustari (2016), which states that the more efficient feed can be found through the amount of feed consumed in proportion to the body weight produced.

The cultured *N. virens* also requires adequate nutritional requirements to support its growth and survival. Optimal feeding will result in better growth. Therefore the feed must contain a high-energy source. The energy possessed by marine worms can break down carbohydrates and feed protein so that the protein is deposited into the body of marine worms. Increased EPA and DHA indicate optimization in absorbing the given diet. This is reinforced by Monoroig and Kabeya (2018), which state that marine worms can biosynthesize from the feed given, especially on EPA and DHA contained in the diet.

Nutritional content through proximate analysis (Table 2) in sea worms (*N. virens*). Research results for the range of protein of 40-53% and 16-19% fat, as for the treatment

of *N. virens* that the treatment with the enrichment with 1.50 ml HUFA in the diet (D) the highest results on protein and fat, 53.80% and 19.67%. This study's protein and fat contents were higher than the research results by Herawati et al. (2020), 55.75% protein and 22.62% fat in *N. virens* that the treatment with the enrichment with 0 ml HUFA in diet (A). High nutrient content in *N. virens* treatment with the enrichment with 1.50 ml HUFA in the diet (D), quality diet fulfills the nutrients required by cultivated organisms. The nutritional requirements of organisms generally include protein, fat, carbohydrates, and other minerals. Cultured marine worms also need sufficient nutrients to support their growth and survival. Asnawi et al. (2018) in their research, explained that development could occur due to the availability of a diet in sufficient quantities where the feed consumed is adequate for basic needs and survival. HUFA in squid oil is also an attractant. An attractant is a stimulant compound given to the diet to stimulate the fish's sense of smell to respond quickly to the feed. HUFA contains attractants that can increase the palatability or ability of feed to be consumed so that HUFA can increase the value of feed consumption and growth rates. This statement is reinforced by the results of research by He et al. (2019) and Herawati et al. (2020), that a reasonable growth rate indicates that the quality of the diet provided is following the nutritional needs of *N. virens* so that it will be able to increase its nutritional content as natural feed. The high fatty acids contained in it are essential for ovarian growth and development. Tocher (2015) stated that the fat content required by shrimp is 10%, and fat is an important nutritional component needed for the development of the ovary of shrimp.

The amino acid profile (Table 3) of *N. virens* during 42 days of maintenance gave the highest results in the treatment of *N. virens* with the enrichment of 1.50 ml HUFA in the diet (D), which was 41.15 ppm. The results of this study were lower than those of Herawati et al. (2020), which was 45.46 ppm in *N. virens* by feeding maggot flour and coconut meal. In comparison, the lowest was found in the treatment of *N. virens* by the enrichment with 0 ml of HUFA in the diet (A), which reached 27.98 ppm. The body's essential amino acid methionine is needed to form nucleic acids and tissues, protein synthesis, and other amino acids (cysteine) and vitamins (choline). Methionine works with vitamin B12 and folic acid to help the body regulate excessive protein supply- *N. virens*, as a natural food, fish or shrimp requires 2.30% methionine in the feed for growth. The essential amino acid methionine function can improve the balance and utilization of other amino acids to promote growth. Methionine has a necessary part in protein synthesis and other physiological processes. Boonyoung et al. (2013) state that methionine and cysteine are the primary sources of amino sulfate for animals, but cysteine is not essential because it can be synthesized from methionine. Rolland et al. (2015) explained that the ribosome's synthetic process depends on the amino acids needed and comes picked up by DNA into the tissue. Tissue protein synthesis is primarily determined by the completeness and level of amino acids entered or transported into tissue cells; the efficiency and amount of protein synthesis in tissue cells are strongly influenced by the completeness and balance of amino acids circulating in the tissue. Besides, methionine is needed by the fish body to start protein synthesis and can affect muscle growth (Belghit et al. 2014). It has been proven that feed with the addition of methionine to feed affects increasing growth and immune response (Boonyoung et al. 2013; Ma et al. 2013; Rolland et al. 2015).

The results of the total fatty acid profile study (Table 4) found the highest EPA of *N. virens* treatment with the enrichment with 1.50 ml HUFA in the diet (D), 6.60%, while the lowest was in the *N. virens* treatment with the enrichment with 0 ml HUFA in the diet (A), 2.15%. Fatty acids also play an essential role in the maturation process of the parent gonads to produce quality eggs. Watanabe (1988) confirmed this study's results that the essential fatty acid eicosapentaenoic acid (EPA; 20: 5n-3) plays a role in survival, especially for shrimp growth. EPA essential fatty acid is a necessary component of phospholipids in membranes and nervous tissue. When they first eat, larvae have a very high neurosomatic index, so they need a high (n-3 HUFA) not to experience nerve formation abnormalities. Tocher (2015), fatty acids are an essential factor that must be considered when providing feed for shrimp during the gonad maturation process.

The survival rate of sea worms (*N. virens*) for 42 days, the highest survival rate, was found in the treatment with the enrichment of 1.50 ml HUFA into the diet (D), which was 100%. Several factors, including water quality, rearing media, and diet, can influence the survival rate in this study. The diet given during the study fulfilled the nutritional requirements of *N. virens*. It can be seen from the diet provided that the enrichment with HUFA has an effect on the growth of marine worms.

In conclusion, the results showed that diets with the enrichment with HUFA had a significant effect ($P<0.05$) on RGR, TFC, EFU, and PER and no significant effect ($P>0.05$) on SR. The highest RGR, EFU, PER, and SR values were in the treatment with the enrichment with 1.50 ml HUFA (D) with the following values of 5.77 ± 0.20g/day, 85.20 ± 2.75g, 2.05 ± 0.08%, and 100 ± 0.00%. Nutritional quality based on proximate protein and fatty acid fat results were highest in the enrichment treatment with 1.50 ml HUFA (D), namely 53.80% protein and 19.67% fat. The highest amino acid and fatty acid profiles in the same treatment were 41.15 ppm and 6.60% EPA.

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