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Analysis of growth and nutritional quality of sea worms (*Nereis virens*) as a mass cultured natural feed on different substrate media thicknesses

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Abstract. *Herwati VE, Elfitasari T, Rismaningsih N, Riyadi PH, Tarangkoon W, Radjasa OK, Windarto S. 2021. 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.* Sea worms (*Nereis virens*) grow slowly. The thickness of the substrate used in mass culture affects their growth, because *N. virens* is a deposit feeder that feeds on organic matter provided by other organisms; therefore, the growth rate slows down if the substrate thickness is not suitable. This study aimed to determine and analyze the growth and nutritional quality of *N. virens* as a natural feed in mass culture at different substrate media thicknesses. This study was conducted at the Marine Science Techno Park (MSTP) UNDIP, Jepara, Central Java. The test animals were two-month-old sea worms, weighing 0.15 to 0.5 g/individual. The maintenance media was the mangrove mud substrate. Feeding was carried out at a fixed feeding rate, twice a day at 07.00 and 19.00 hrs, and the animals were maintained at a density of 45 individuals/L for 35 days. The experimental research method was a completely randomized design with four treatments and three replications: treatment A (media thickness of 4 cm), B (media thickness of 6 cm), C (media thickness of 8 cm), and D (media thickness of 10 cm). The parameters studied were absolute growth, specific growth rate (SGR), efficiency of feed utilization (EFU), feed conversion ratio (FCR), protein efficiency ratio (PER), survival rate (SR), proximate analysis, profile of amino acid and fatty acids. The results showed that the highest absolute weight (6.76 g); SGR (6.34%); EFU (69.73%); FCR (1.36%); PER (1.74%); SR (97.04%); and nutritional quality (55.75% protein, 22.62% fat, 45.66 ppm methionine, and 7.7% eicosapentaenoic acid values were obtained when *N. virens* was mass-cultured in a medium with a thickness of 8 cm (Treatment C). Thus, the thickness of the media substrate had a significant effect ($P < 0.05$) on the absolute weight, SGR, EFU, FCR, PER, and SR in *N. virens*.

Keywords: Growth, medium, *Nereis virens*, nutrition, substrate thickness

INTRODUCTION

Sea worms (*Nereis virens*) are invertebrates that belong to the phylum Annelida. Their bodies have elongated segments, and they are deposit feeders that feed on organic matter (Asnawi, 2018). *N. virens* plays an important role in the maturation of gonads of broodstock and can be used as a natural shrimp feed (Asnawi, 2018). Sea worms have a high nutritional value; Asnawi et al. (2018) and Herawati et al. (2020) reported that the dry *N. virens* contains 45-55% protein, 18-23% fat, 10 -98% eicosapentaenoic acid (EPA), and 55.46 ppm methionine. The growth rate of shrimps depends upon the quality of natural feed provided. The availability of *N. virens* as a natural feed depends on the catch in nature, and therefore, mass culture of *N. virens* is essential (Asnawi et al. 2018; Herawati et al. 2020).

The most important factors for the growth and survival of sea worms are the maintenance substrate and feed provided (Nguyen et al. 2011). Media with optimal thickness along with optimal nutrition in their feed would stimulate their growth and increase their survival. Gamis et al. (2016) reported that substrate media with high sand content affects the survival of sea worms, since they must spend more energy to move through it. The substrate used for sea worm cultivation is sand and mud, since their natural habitat is typically sandy and muddy estuary and mangrove areas (Asnawi et al. 2018). The high catch of sea worms in the mangrove area indicates that a mangrove substrate is more suitable for their growth than a sandy substrate. Mangrove substrates have ecological functions, such as absorbing carbon, remediating pollutants, preventing abrasion and intrusion, and preventing storms (Brown et al. 2011; Mouneyrac et al. 2012), and can function as a habitat for the growth and development of aquatic fauna (Jayadhandhran et al. 2015). Mangrove mud in the Awur Bay area, Jepara, contains ammonia (0.09 Â mg/L) and nitrite (0.2 Â mg/L), and these areas are used as feeding and spawning grounds by organisms (Ji He et al. 2015). The energy spent by sea worms for swimming and crawling would reduce their biomass and can cause mortality. Therefore, the aim of our study was to determine the optimum substrate thickness that would facilitate increased growth and survival of *N. virens*. According to

Asnawi et al. (2018), the optimum substrate thickness for the cultivation of *N. virens* ranged from 5-15 cm that ensured a survival rate (SR) of 64-85%. Mangrove mud substrates can thus ensure and maintain the SR and specific growth rates (SGR) in sea worms.

This study aimed to determine the optimum substrate media thickness for *N. virens* in terms of parameters, such as absolute growth, SGR, efficiency of feed utilization (EFU), feed conversion ratio (FCR), protein efficiency ratio (PER), SR, and nutritional quality amino acids and fatty acids used as natural feed sources through proximate analysis.

MATERIALS AND METHODS

Sea worms (*N.virens*) were obtained from the culture stock of PT. Matahari Cipta Sentosa, Situbondo, East Java, Indonesia and were used as test animals. Twelve containers with a total capacity of 30 liters were used, and 45 test animals were reared per container. Feeding was carried out in the morning and evening at 07.00 WIB and 19.00 WIB, respectively, with a fixed feeding rate of 5% of the weight of biomass, according to the protocol reported by Herawati et al. (2020). Sea worms were fed by commercial feed (MS Feng-Li feed for shrimp and fish larvae) from Matahari Cipta Sentosa with a protein content of 40% that was easily digested by the worms. Feeding was carried out by removing the water approximately 1 cm from the surface of the substrate. The experimental design used in this study was a completely randomized design. The results obtained by Asnawi et al. (2018), indicated that mangrove mud substrate could optimize the SR and SGR in the maintenance of sea worms leading to an SR of±83%. But, in his research didn't mention the thickness of the substrate.

Four treatments were used in this study, and each treatment was repeated three times. The treatments were as follows: (i) Treatment A, mangrove mud media with a thickness of 4 cm; (ii) Treatment B, mangrove mud media with a thickness of 6 cm; (iii) Treatment C, mangrove mud media with a thickness of 8 cm; and (iv) Treatment D, mangrove mud media with a thickness of 10 cm. The mangrove mud media was obtained from the mangrove area and then filtered before being used.

Variables

Calculation of absolute growth and SGR used live *N. virens*, which were harvested in total by separating *N. virens* from the substrate, while for proximate analysis, amino acid profile, and fatty acid profile, *N. virens* was dried at a temperature below 60°C.

Absolute growth (Wm)

Absolute growth (Wm), known based on the average biomass of marine worms (*N. virens*), is calculated using the following formula (Tacon, 1993). $Wm = W_t - W_0$, which Wm: Average absolute growth of test animals (g); Wt: average weight of test animals at the end of the study (g); and W_0: average weight of test animals at the start of the study (g).

Specific growth rate (SGR)

The formula (Tacon, 1993) calculated the specific growth rate: SGR: $(\ln W_t - \ln W_0)/t \times 100\%$. SGR: Specific growth rate (%); W_t: Mean weight of worms at the end of the study (g); W_0: Mean weight of worms at the beginning of the study (g).

The efficiency of feed utilization (EFU)

Efficiency of Feed Utilization (EFU)was calculated using Tacon's formula (1993). EFU: $(W_t - W_0)/F \times 100\%$. EFU: Efficiency of Feed Utilization (%); W_t: final biomass at the end of the study (g); W_0: baseline biomass at the start of the study (g); F: the amount of feed consumed during the study

Feed conversion ratio (FCR)

The calculation of the feed conversion ratio is based on Tacon's formula (1993), FCR: $F/((W_t + D) - W_0)$. FCR: Feed conversion ratio; F: the amount of feed given (g); W_t: Weight of worms biomass at a time (g); W_0: weight of worms biomass at the beginning of maintenance (g); D = weight of dead worms biomass during maintenance (g).

Protein efficiency ratio (PER)

The protein efficiency ratio can be calculated using the Tacon (1993) formula: $PER = (W_t - W_0)/Pi \times 100\%$. PER: Protein efficiency ratio; W_t: Weight of test animals' biomass at the end of maintenance (g); W_0: 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: $(N_0 - N_t)/N_0 \times 100\%$. SR: Survival Rate; N_t: Number of individuals at the end of the study (individual); N_0: number of individuals at the beginning of the study (individual).

Water quality

Measurement of water quality includes temperature, salinity, DO (dissolved oxygen), and pH, carried out in every morning and evening. DO measurements used a DO meter, 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.

Table 1. Water quality in marine worms (*N. virens*) maintenance for 35 days

Variable	Value	Reference
DO (mg/L)	5-6.5	0.8-9.3**
pH	7-8.4	7-8.5**
Temperature (°C)	28-31	25-31*
Salinity	29-31	25-40**

Note: Gamis et al. (2016)*, Asnawi et al. (2018)**

The results of measuring water quality parameters in *N. virens* maintenance for 35 days, the values of the variables DO, salinity, pH, temperature are still in the appropriate range to be used as a medium for cultivation of marine worms (*N. virens*).

Amino acid profile

Amino acid profiles were determined by HPLC (waters corporations, USA). The amino acid standard solution used for calibration from Thermo Scientific, Acq Taclumn (3.9 mm x 150 mm), at 37^0C temperate; mobile phase acetonitrile 60%-AccqTag Eluent A. Flow rate 1.0ml. min^{-1} with fluorescence detector. The volume injected for each sample was 5µL (A0AC., 2005).

Fatty acids profile

The Fatty acid profile was determined by gas chromatography (GC) after converting the fatty acid component of 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 (A0AC 2005).

Proximate analysis

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

Data analysis

Analysis of variance (ANOVA) was used to determine the differences in the growth parameters among the treatments, and the significance level was set at $P < 0.05$, 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 ($P < 0.05$) or ($P < 0.01$), 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 absolute weight and specific growth rate of marine worms (*N. virens*) were presented in Figure 1.

The highest Wm of 6.76 g was observed in the *N. virens* cultured on the substrate with a thickness of 8 cm (C), whereas the lowest growth rate 4.00 g was observed in the *N. virens* cultured on the substrate with a thickness of 4 cm (A). Treatment A was not significantly different from Treatment B, and D. Treatment B was not significantly different from Treatment C and D, and Treatment C was not significantly different from Treatment D. Duncan's significance value was 0.063. There was a significant difference in SGR between the treatments ($P < 0.05$) with P value 0.02. The result of the specific growth rate of marine worms (*N. virens*) During 35 days of maintenance was presented in Figure 2.

The highest SGR of 6.34% was observed in the *N. virens* cultured on the substrate with a thickness of 8 cm (C), whereas the lowest growth rate (4.6%) was observed in the *N. virens* cultured on the substrate with a thickness of 4 cm (A). Treatment A was significantly different from Treatment B, C, and D. Treatment B was not significantly different from Treatment C and D, and Treatment C was not significantly different from Treatment D. Duncan's significance value was 0.076. There was a significant difference in SGR between the treatments ($P < 0.05$) with P value 0.01. The efficiency of feed utilization (EFU), of marine worms (*N. virens*) during 35 days of maintenance was presented in Figure 3.

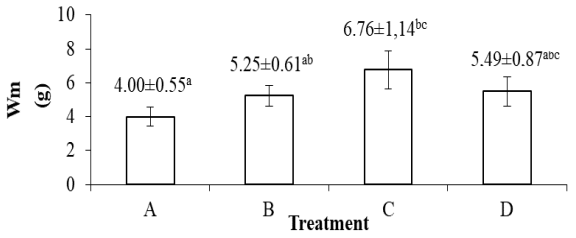

Figure 1. Absolute weight (Wm) of N. virens during research 35 days. *Different superscript letters indicate statistical significance.

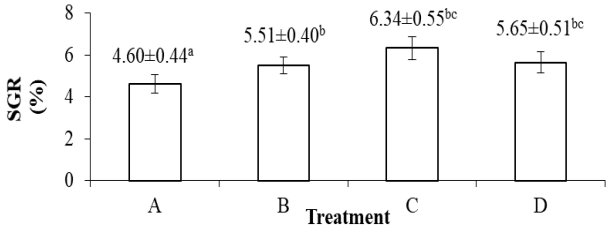

Figure 2. The specific growth rate of *N. virens* during research 35 days. *Different superscript letters indicate statistical significance.

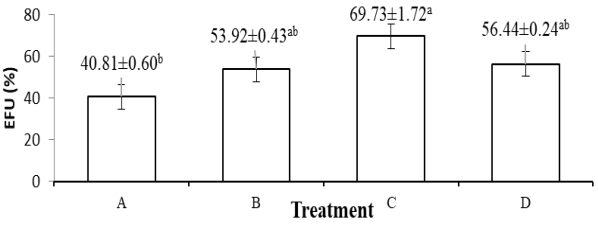

Figure 3. The efficiency feed utilization of *N. virens* during research 35 days. *Different superscript letters indicate statistical significance.

The highest EFU 69.73% was observed in the *N. virens* cultured on the substrate with a thickness of 8 cm (C), whereas the lowest (40.81%) was observed in the *N. virens* cultured on the substrate with a thickness of 4 cm (A). Treatment A was not significantly different from Treatment B, and D. Treatment B was not significantly different from Treatment C, and D. Treatment C was not significantly different from Treatment D. Duncan's significance value was 0.063. A significant difference was observed between the groups ($P < 0.05$) with P-value 0.02. The result of the feed conversion ratio of sea worms (*N. virens*) during 35 days of maintenance was presented in Figure 4.

The highest FCR (1.36%) was observed in the *N. virens* cultured on the substrate with a thickness of 8 cm (C), whereas the lowest (2.25%) was observed in the *N. virens* cultured on the substrate with a thickness of 4 cm (A). Treatment A was significantly different from Treatment B, C, and D. Meanwhile, Treatment B was not significantly different from Treatment C, and D. Duncan's significance value was 1.00. A significant difference was observed between the groups ($P < 0.05$) with P-value 0.007. The result of the protein efficiency ratio of sea worms (*N. virens*) during 35 days of maintenance is presented in Figure 5.

The highest PER (1.74%) was observed in the *N. virens* cultured on the substrate with a thickness of 8 cm (C), whereas the lowest (1.02%) was observed in the *N. virens* cultured on the substrate with a thickness of 4 cm (A). Treatment A was not significantly different from Treatment B, and D. Treatment B was not significantly different from Treatment C, and D. Treatment C was not significantly different from Treatment D. Duncan's significance value was 0.061. A significant difference was observed between the groups ($P < 0.05$) with P-value 0.02. The survival rate of sea worms (*N. virens*) during 35 days of maintenance is presented in Figure 6.

The highest SR (97.04%) was observed in *N. virens* cultured on the substrate with a thickness of 8 cm (C), whereas the lowest value (84.44%) was observed in the *N. virens* cultured on the substrate with a thickness of 4 cm (A). Treatment A was significantly different from Treatment B and C, but not significantly different from Treatment D. Treatment B was not significantly different from Treatment C and D, and treatment C was very significantly different from Treatment D. Duncan's significance value was 0.080. A significant difference was observed between the groups ($P < 0.05$) with P-value 0.01. The proximate analysis of the nutritional quality of protein and fat content of the mass cultures of *N. virens* showed that *N. virens* grown on a substrate with a thickness of 8 cm (C) had the highest protein (55.75%) and fat (22.62%) content, whereas the lowest values (45.55% protein and 21.14% fat) were observed in the *N. virens* cultured on the substrate with a thickness of 4 cm (A). Proximate analysis of Sea Worms (*N. virens*) For 35 Maintenance Days is presented in Table 2. Duncan's significance value is 0.080.

The results of the amino acid profiles showed that *N. virens* cultured on the substrate with a thickness of 8 cm (C) had the highest methionine content (45.46 ppm), whereas the lowest value (28.26 ppm) was observed in the *N. virens* cultured on the substrate with a thickness of 4 cm (A). Results of amino acid analysis of Sea Worms (*N. virens*) For 35 maintenance days are presented in Table 3.

The analysis of the essential fatty acid profiles revealed that *N. virens* cultured on the substrate with a thickness of 8 cm (C) had the highest EPA content of 7.75%, whereas the lowest value (1.65%) was observed in the *N. virens* cultured on the substrate with a thickness of 4 cm (A). Results of analysis of fatty acids in sea worms (*N. virens*) For 35 maintenance days are presented in Table 4.

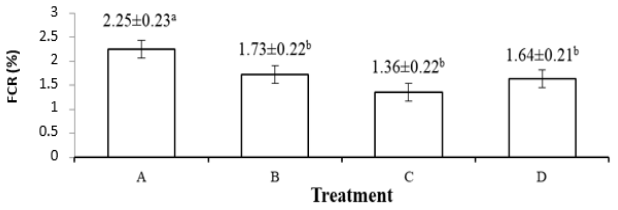

Figure 4. The feed conversion ration of *N. virens* during research 35 days. *Different superscript letters indicate statistical significance.

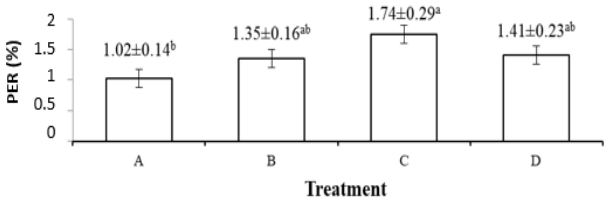

Figure 5. Protein efficiency ratio of Nereis sp during research 35 days. *Different superscript letters indicate statistical significance.

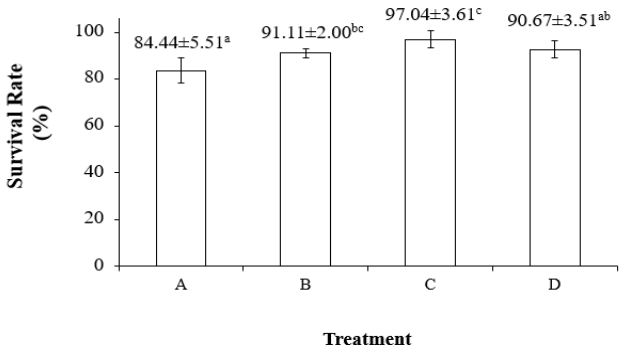

Figure 6. The survival rate of sea worms (N. virens) during 35 days of maintenance. Note: *Different superscript letters indicate statistical significance.

Table 2. Proximate analysis of sea worms (*N. virens*) for 35 maintenance days

Proximate	Treatment			
	A (4 cm)	B (6 cm)	C (8 cm)	D (10 cm)
Protein	45.55±0.04b	51.32±0.06[a]	55.75±0.07[a]	48.16±0.07[a]
Fat	21.14±0.07a	21.32±0.05[b]	22.62±0.03[a]	21.59±0.03[a]
Crude fiber	18.25±0.09b	11.72±0.03[a]	10.19±0.07[a]	12.82±0.02[a]
Ash	8.18±0.03a	8.51±0.03[b]	5.19±0.01[a]	8.83±0.01[a]
Carbohydrate	6.88±0.02a	6.83±0.05[b]	6.25±0.02[a]	8.6±0.05[a]

Table 3. Results of amino acid analysis of sea worms (*N. virens*) for 35 maintenance days

Amino acid (ppm)	A (4 cm)	B (6 cm)	C (8 cm)	D (10 cm)
L Histidine	10.12±0.08[b]	16.28±0.02[b]	20.07±0.04[b]	18.07±0.04[b]
L-Threonine	17.30±0.05[b]	26.75±0.04[b]	27.48±0.06[b]	24.48±0.04[b]
L-Proline	10.59±0.03[b]	25.38±0.02[b]	28.49±0.04[b]	24.49±0.03[b]
L-Tyrosine	15.23±0.02[b]	25.20±0.03[a]	26.19±0.03[a]	25.76±0.05[a]
L-Leucine	27.93±0.05[b]	30.47±0.02[b]	34.12±0.02[b]	32.12±0.03[b]
L-Aspartate	27.04±0.06[b]	29.25±0.02[b]	32.68±0.03[b]	30.68±0.01[b]
L-Lycine	20.99±0.03[b]	29.19±0.03[b]	35.30±0.02[b]	27.30±0.01[b]
Glycine	14.99±0.02[b]	11.50±0.04[b]	20.61±0.03[b]	18.61±0.03[b]
L-Arginine	7.41±0.03[a]	7.749±0.03[a]	8.71±0.01[a]	8.50±0.03[a]
L-Alanine	20.49±0.09[b]	24.90±0.04[b]	24.88±0.03[b]	28.81±0.01[b]
L-Valin	19.65±0.06[b]	23.34±0.03[b]	28.37±0.01[b]	23.37±0.01[b]
L-Isoleucine	15.81±0.01[b]	21.18±0.05[b]	26.34±0.01[b]	21.34±0.02[b]
L-Phenylalanine	17.93±0.04[b]	22.46±0.04[b]	25.15±0.05[b]	20.15±0.05[b]
L-Glutamic acid	30.75±0.04[b]	32.78±0.04[b]	44.14±0.04[b]	34.14±0.04[b]
L-Serin	18.98±0.01[b]	23.21±0.02[b]	28.53±0.04[b]	24.53±0.03[b]
L-Tryptophan	3.92±0.04[b]	5.53±0.04[b]	8.98±0.06[b]	6.98±0.04[b]
L-Methionine	28.26±0.03[b]	35.56±0.02[b]	45.46±0.04[b]	39.37±0.03[b]
L-cystine	15.32±0.04[a]	15.62±0.04[a]	21.60±0.06[b]	18.60±0 .04[b]

Table 4. Results of analysis of fatty acids in sea worms (*N. virens*) for 35 maintenance days

Fatty acids (%)	A	B	C	D
C 6: 0	0.35±0.01[a]	0.37±0.01[a]	0.47±0.09[b]	0.30±0.05[a]
C 8: 0	0.80±0.01[b]	0.52±0.08[b]	1.79±0.01[b]	0.65±0.01[b]
C 10: 0	0.18±0.01[b]	0.19±0.01[b]	1.36±0.04[b]	0.23±0.03[a]
C 11: 0	0.25±0.01[a]	0.33±0.01[a]	0.38±0.02[a]	0.30±0.04[a]
C12: 0	2.05±0.04[b]	3.79±0.02[b]	4.45±0.01[b]	2.79±0.01[a]
C 13: 0	0.17±0.02[a]	0.12±0.01[a]	2.42±0.04[b]	0.95±0.03[b]
C 14: 0	1.88±0.02[a]	1.98±0.01[a]	2.68±0.04[b]	1.33±0.03[a]
C 14: 1	0.17±0.01[a]	0.27±0.01[a]	1.79±0.01[b]	0.89±0.01[a]
C 15: 0	0.45±0.05[a]	0.63±0.03[a]	0.98±0.03[a]	0.51±0.03[a]
C 16: 0	3.55±0.04[b]	4.53±0.03[b]	5.67±0.03[b]	2.89±0.01[b]
C 16: 1	0.39±0.04[a]	0.38±0.01[a]	1.65±0.03[b]	0.17±0.05[a]
C 17: 0	0.12±0.01[a]	0.57±0.04[a]	3.52±0.04[b]	0.83±0.04[a]
C 18: 0	0.99±0.01[a]	1.86±0.01[b]	0.93±0.02[a]	0.67±0.04[a]
C 18: 1	1.98±0.01[b]	2.58±0.04[b]	2.98±0.03[b]	0.98±0.04[b]
C 18: 2	1.13±0.03[a]	1.53±0.04[b]	3.53±0.05[b]	1.35±0.01[a]
C 18: 3	1.35±0.03[a]	2.7±0.02[b]	4.95±0.04 [b]	1.45±0.03[b]
C 20: 0	0.37±0.02[a]	0.40±0.04[a]	0.44±0.06[a]	0.19±0.05[a]
C 20: 1	0.55±0.01[b]	0.48±0.03[b]	0.96±0.04[b]	0.47±0.04 [b]
C 20: 2	0.98±0.03[a]	0.98±0.02[a]	1.78±0.04[b]	0.69±0.02[a]
C 20: 4	0.69±0.01[a]	0.64±0.05[a]	0.98±0.04[a]	0.55±0.04[a]
EPA	1.65±0.01[a]	4.75±0.02[b]	7.75±0.01[b]	3.09±0.05[a]
DHA	0.35±0.03[b]	3.40±0.03[b]	5.20±0.03[b]	4.68±0.02[b]

Discussion

Mangrove mud substrate media thickness had a significant effect ($P < 0.05$) on the absolute weight, Specific Growth Rate (SGR), Efficiency of Feed Utilization (EFU), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Survival Rate (SR). Mangrove mud was used as the media substrate because it contains soft clay that has a texture suitable for sea worms. Mangrove mud at the research location, the Teluk Awur area, Jepara, contains ammonia 0.09 mg/L and nitrite 0.2 mg/L, and this area is used for feeding and breeding purposes by worms (Mouneyrac et al. 2018). Higher sand content in the substrate would require *N. virens* to spend more energy to move through and carry out activities to accelerate its growth (Herawati et al. 2020). This statement is evidenced by the results of the study, the thicker the substrate, the lower the growth. The media substrate thicknesses used in the study were 4 cm, 6 cm, 8 cm, and 10 cm. The results of our present study showed that the highest SGR value of 6.34% was observed in *N. virens* cultured on the substrate thickness of 8 cm (C). *N. virens* could grow optimally in this medium and perform activities without spending much energy looking for food. In addition, the substrate with a thickness of 8 cm had sufficient organic matter to balance the feed. The SGR tended to be higher, allowing worms to carry out tube formation, decomposition of organic matter, and gaseous exchange with a relatively ideal oxygen supply. This is reinforced by Schaum et al. (2013), who reported that the content of organic matter affects the SGR because it provides the energy required for worms to carry out their activities. The lowest SGR of 4.6% was observed in *N. virens* cultured on the substrate with a thickness of 4 cm (A); this was thought to be due to the low consumption of feed by *N. virens.* It is suspected that the low amount of substrate used in the maintenance media resulted in increased competition for space between the *N. virens* individuals, resulting in difficulty in procuring oxygen.

The thickness of the media affected the maintenance of the sea worms; the thicker the media used, the greater the energy available for the sea worms to carry out their activities. The substrate with a thickness of 10 cm (D) resulted in poor growth compared to the substrate with a thickness of 8 cm (C). This is probably because the high amount of energy provided by the substrate with a thickness of 10 cm increased the activity levels of the worms, resulting in reduced biomass and greater mortality. This was reinforced by the study conducted by Muruganatam et al. (2015), which reported that energy depletion occurs in sea worms that are more active, leading to reduced biomass and increased mortality. Moreover, Jayadhamdran et al. (2015) showed that sea worms that move less save more energy, leading to increased biomass. Asnawi et al. (2018) stated that a sandy substrate plays a role in the oxygen exchange process within the substrate and acts as a medium for the entry of nutrient particles (organic matter), while a clay substrate acts as storage for organic matter.

Feed is another factor that affects the growth rate of *N. virens*. Muruganatam et al. (2015) reported that the SGR reflects the quality of the feed. Our results support this statement. Treatment C supported the highest biomass weight (6.76 g) in *N. virens*; the more significant the biomass weight, the higher the feed percentage. The feed used in this study was a commercial feed with a protein content of 40% that was easily digested by the worms. Feeding was carried out in the morning at 07.00 WIB and at night at 19.00 WIB, with a fixed feeding rate of 5% of the biomass weight. The results showed that the EFU and PER were the highest (69.73%, and 1.74%, respectively) in treatment C (substrate thickness of 8 cm). These results

indicate that the feed is digested and absorbed efficiently by the worms. The highest FCR (1.36%) was also observed in treatment (C). The FCR is the ratio between the amount of food given and weight gain; the lower the FCR value, the more efficiently the animal converts its food into an energy source. This efficiency depends on several factors, including protein quality, protein content in feed, other nutritional components, such as fat and carbohydrates, and feeding frequency (Costa et al. 2000).

Table 2 shows the results of the proximate analysis of the nutritional content of the mass cultures of *N. virens*. The protein content ranged from 45-55%, whereas the fat content ranged from 21-22%. *N. virens* cultured on the substrate with a thickness of 8 cm (C) yielded the highest amounts of protein and fat (55.75% and 22.62%, respectively). The protein and fat contents reported in this study were higher than those reported by Herawati et al. (2020), where the *N. virens* maggots that were fed on flour and coconut cake contained 54.05% protein and 22.54% fat. The excellent growth rate and high nutritional content observed in treatment (C) were probably because the thickness of the substrate and quality of the feed was suitable and could meet the nutritional needs of *N. virens.* Feed that is derived from animal protein is easily digested by sea worms. The source of fat in the feed can also be used as an energy source for growth. This is reinforced by the study conducted by He et al. (2019) and Herawati et al. (2020), who found that a reasonable growth rate indicates that the quality of the feed given follows the nutritional needs of *N. virens,* such that it can be used as a highly nutritious natural food. *N. virens* is the best live food for shrimps, and its high fatty acid content is important for ovarian growth and development in shrimps. Nguyen et al. (2011) showed that shrimps require a fat content of 10% in their feed, and fat is an essential nutritional component needed for ovarian development in shrimps (Tocher, 2015).

Table 3 shows the amino acid profiles for all the treatments. The highest methionine content of 45.46 ppm was found in *N. virens* cultured on the substrate with a thickness of 8 cm. These results were lower than those of Herawati et al. (2020), where the methionine content in *N. virens* maggots that were fed on flour and coconut cake was 55.46 ppm. Methionine is an essential amino acid that is required for the formation of nucleic acids and tissues. Moreover, it is required for protein synthesis and for the formation of other amino acids such as cysteine and vitamins such as choline. Methionine works along with vitamin B12 and folic acid to regulate excessive protein in the body. Fish and shrimp need 2.3% methionine in their feed for adequate growth. Methionine can improve the balance and utilization of other amino acids to promote growth. It plays an essential role in protein synthesis and other physiological functions. Boonyoung et al. (2013) stated that methionine and cysteine are the primary sources of amino sulfate in animals. However, cysteine is not essential because it can be synthesized from methionine. Rolland et al. (2015) explained how ribosomal protein synthesis depends on the presence of amino acids and their uptake into the cells of tissues. Tissue protein synthesis is primarily determined by the amounts of different amino acids that are transported into tissue cells, and the efficiency and amount of protein synthesis in tissue cells are strongly influenced by the balance of amino acids circulating in the tissue. In addition, methionine is needed to initiate protein synthesis in fish and can affect muscle growth (Belghit et al. 2014). Addition of methionine to fish feed has been proven to increase growth and enhance immune responses (Yuan et al. 2011; Kuang et al. 2012; Boonyoung et al. 2013; Ma et al. 2013; Rolland et al. 2015).

The highest EPA content (7.75%) was found in *N. virens* cultured on the substrate with a thickness of 8 cm (C), and the lowest value (3.88%) was observed in *N. virens* cultured on the substrate with a thickness of 8 cm (A). Fatty acids play an essential role in the maturation of gonads and production of good quality eggs. Costa et al. (2010) confirmed the results of this study by showing that the essential fatty acid EPA, 20: 5n-3, especially plays a role in the growth and survival of shrimps. EPA is an important component of membrane phospholipids and nervous tissue. Shrimp larvae have a very high neurosomatic index during the onset of feeding, and hence, they need a high n-3 HUFA content to prevent abnormalities in nerve formation. Tocher (2015) found that fatty acids are an essential factor that must be considered when providing feed for shrimps during gonad maturation.

The highest SR of *N. virens* after 35 days was 97.04%. This indicates that the thickness and quality of the substrate and the quantity of feed provided were sufficient to meet the basic nutritional needs of *N. virens* and facilitate their growth. According to Asnawi et al. (2018), the substrate thickness and texture, as well as the habit patterns of sea worms, affect their growth, movement ability, and other distinctive properties. Sea worms commonly inhabit estuary areas with sandy mud substrate conditions. Water quality generally shows DO values in the range of 5-6.5 mg/L, pH values in the range of 7-8.4, temperatures in the range of 28-31 °C, and salinity values in the range of 29-31 ppt during regular maintenance. According to Asnawi et al. (2018), water temperatures ranging from 23-32 °C and salinity values ranging from 14-31 ppt are suitable for sea worms. The water quality in this study was suitable for sea worm cultivation as seen from its effect on growth and survival. The SR of organisms is influenced by good cultivation management, including stocking density, feed quality, water quality, and parasites or diseases. According to Gamis et al. (2016), the abundance of *N.virens* is greatly influenced by the substrate as well as the water conditions.

The results showed that the highest absolute weight values, SGR, EFU, FCR, PER, and SR, were obtained in the treatment of *N. virens* in mass culture with a thickness of 8 cm (C), 6.76 g; 6.34%; 69.73%; 1.36%; 1.74%; 97.04%; protein 55.75%; fat 22.62%; Methionine 45.66 ppm and EPA 7.7%. The thickness of the media substrate had a significant effect ($P < 0.05$) on the absolute weight, SGR; EFU; FCR; PER, and SR in marine worms. *N. virens* is the best natural feed for shrimp farming. By finding a suitable substrate thickness to increase the growth and nutritional quality of *N. virens* in this research, it can meet the feed requirements for shrimp culture.

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