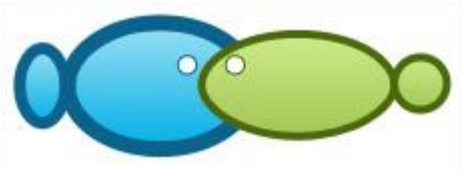

Effect of substrate media on the growth, amino acids and fatty acids profiles of the marine worm (*Nereis virens*)

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Abstract. Marine worms (*Nereis virens*) are deposit feeders that utilize organic materials as food. They are used as a natural feed for shrimp hatcheries. The type of substrate used for the growth of *N. virens* also affects their nutritional quality. This study aims to determine the best substrate media for marine worms' growth and for their amino and fatty acid profiles. The study was conducted at the Marine Science Technopark (MSTP), Undip Jepara, Central Java. Test animals were 2-3 months old marine worms, with body lengths ranging from 10-15 cm and weights ranging from 0.15-0.5 g. They were fed twice daily and had a density of 45 worms L^{-1} for 45 days. The experimental method with a completely randomized design (CRD) was used, with treatments A (clay mud media), B (sand silt media), and C (mangrove mud media). The data observed were absolute weight (Wm), specific growth rate (SGR), feed utilization efficiency (FUE), feed conversion ratio (FCR), protein efficiency ratio (PER), survival rate (SR), amino and fatty acid profiles. The conclusion is that the mangrove mud substrate had a significant effect ($p<0.05$) on Wm (6.80±0.18 g), SGR (6.4±0.01%), FUE (68.95±2.3%), FCR (1.3±0.12%), PER (1.7±0.37%), and SR (97.44±0.41%) of *N. virens*, with 48.46 ppm methionine and 8.15% EPA.
Key Words: clay mud, mangrove, nutrition, sand, seaworms.

Introduction. Marine worms (*Nereis virens*) are invertebrates belonging to the phylum Annelida. They used as natural feed for shrimp hatcheries, and contain a large number of nutrients (Asnawi & Idris 2018). The environment and food provided are the most critical factors for the growth and survival of marine worms. Appropriate care facilities promote the growth and survival of marine worms by stimulating their growth and providing optimal amounts of nutrients (Nguyen et al 2012). In the cultivation of marine worms, the substrate is one of the factors affecting growth. According to Gamis et al (2016), substrate mediator factors influence the survival of marine worms; for example, a large amount of sand in the substrate requires more energy from the worms to move. Sea worms that swim and crawl experience energy depletion, which further reduces their biomass and can lead to death.

The substrates where marine worms can be found are sand, clay, and mangrove mud. The habitat of marine worms is sandy and muddy in coastal areas near high-rise residential buildings, lagoons, and mangroves (Haryadi & Rasidi 2012; Asnawi & Idris 2018). A mangrove substrate is better suited for sea worm growth than a sandy substrate, as evidenced by the large capture of sea worms in the mangrove region. In addition to serving as a habitat for the establishment and development of aquatic species, mangrove substrates have ecological purposes such as absorbing carbon, cleaning up pollutants, avoiding abrasion and intrusion, and averting storms (Mouneyrac et al 2010; Brown et al 2011; Jayadhandhran et al 2015). Ammonia (0.09 Â mg L^{-1}) and nitrite (0.2 Â mg L^{-1}) are present in the mangrove muck in the Awur Bay region of Jepara, and organisms use these places for spawning and foraging (Jin et al 2015).

The aim of this study is to test the substrate in order to increase the growth of sea worms. This study used an experimental method to determine the suitable media used as treatments. Absolute growth, specific growth rate (SGR), feed utilization efficiency (FUE), feed conversion ratio (FCR), protein efficiency ratio (PER), survival rate (SR), and amino and fatty acids profiles were determined in this study.

Material and Method. The marine worms used in this study were in the growth phase, with an age category of 2-3 months and an initial weight of 0.15-0.5 g. PT. Matahari Cipta Sentosa, Situbondo, East Java, provided marine worms.

This research was conducted at the Marine Science Techno Park (MSTP), Jepara, Central Java. The test animals were kept in containers that could hold 45 individuals each. Twelve plastic containers with a capacity of 30 L were used. Containers were washed with soap and water and dried before use to avoid contamination. The seawater came from a reservoir sterilized with 10 ppm chlorine and neutralized with 5 ppm sodium thiosulfate. Feeding was accomplished during the morning and night with 5% of the biomass weight, referring to Herawati et al (2020). Feeding was done after leaving only 1 cm of water over the substrate. The feed was an artificial feed in the form of powder with a protein content of 40%. This study used a completely randomized experimental design (CRD) with three treatments: treatment A - clay mud media; treatment B - sand silt media; treatment C - mangrove mud media.

According to Herawati et al (2020), substrate thickness for the cultivation of marine worms *Nereis* sp. was 8 cm for a survival rate (SR) of 85-95%.

The determined parameters included: absolute growth, specific growth rate (SGR), feed utilization efficiency (FUE), feed conversion rate (FCR), protein efficiency ratio (PER), survival rate (SR), amino and fatty acid profile, and water quality. All worms were weighed with a digital scale.

Absolute growth (Wm). The absolute growth is calculated as follows (Tacon 1993):

$Wm = W_t - W_0$

Where: W_t - weight at the end of the test; W_0 - the average weight at the beginning of the test (Tacon 1993).

Specific growth rate (SGR). SGR was calculated with the formula (Tacon 1993):

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

Where: W_t - final average weight; W_0 - initial average weight.

Feed utilization efficiency (FUE). The FUE formula used was the following (Tacon 1993):

$FUE = (Weight - W_0)/F \times 100\%$

Where: weight - final biomass; W_0 - initial biomass; Q - the amount of food consumed during the study.

Feed conversion ratio (FCR). The FCR was calculated using the following formula (Tacon 1993):

$FCR = F/((W_t+D)-W_0)$

Where: F - amount of feed given (g); W_t - weight of worm biomass at one time (g); W_0 - weight of worms biomass at the start of feeding (g); D - amount of dead worm biomass during weight maintenance (g).

Protein efficiency ratio (PER). PER was calculated as follows (Tacon 1993):

$PER = (W_t\text{-}W_0)/Pi \times 100\%$

Where: W_t - weight (g) of the final biomass; W_0 - initial biomass weight; Pi - protein content x feed consumed.

Survival rate (SR). The survival rate was determined with the following formula (Tacon 1993):

$SR = (N_0 - N_t)/N_0 \times 100\%$

Where: N_t - number of samples at the end of the experiment; N_0 - number of samples at the start of the experiment.

Water quality. Water quality determinations included temperature, salinity, dissolved oxygen (DO), and pH in the morning and evening. DO was measured with a DO meter, the temperature was determined with a thermometer, salinity with a refractometer, and pH with a pH meter. Table 1 shows the values of water quality parameters determined during the study. Water quality measurements for *N. virens* after 35 days of culture revealed that the DO, salinity, pH, and temperature variables were within the acceptable range.

Table 1

Water quality of the marine worms (*Nereis virens*) culture for 35 days

Variable	*Value*	*Reference*
DO (mg L^{-1})	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: DO - dissolved oxygen; * - Gamis et al (2016); ** - Asnawi & Idris (2018).

Amino acid profile. HPLC was used to determine the amino acid profile (Waters Corporations, USA). Acetonitrile 60%-AccqTag eluent was used in this test as a phase that moves. The flow rate was 1 mL min^{-1}. Each sample received a 5 mL injection volume (AOAC 2005).

Fatty acids profile. Gas chromatography (GC) was used to determine the fatty acids profile. A Shimadzu GC-14B gas chromatograph (Shimadzu Corporation, Japan) equipped with a flame ionization detector and a capillary column was used for the GC analysis (AOAC 2005).

Proximate analysis. The chemical content of the sample was determined using the standard method (AOAC 2005).

Data analysis. The variance analysis (ANOVA) was used to analyze the data in this study. The normality, homogeneity, and additivity were determined in the first step. If significant differences were observed ($p<0.05$), the Duncan Multiple Area Test was used to determine differences between treatments.

Results. The results of growth performance and SR of *N. virens* are presented in Table 2.

Table 2

Marine worms (*Nereis virens*) cultured in different substrate media for 35 days

Variables	*Treatment*		
	A (clay mud)	*B (sand silt)*	*C (mangrove mud)*
Wm (g)	4 ± 0.5^{a}	5.3 ± 0.52^{ab}	6.8 ± 0.18^{bc}
SGR (%)	4.65 ± 0.05^{a}	5.58 ± 0.04^{ab}	6.4 ± 0.01^{bc}
FUE (%)	40.85 ± 5.24^{b}	54.2 ± 5.12^{ab}	68.95 ± 2.3^{a}
FCR (%)	2.24 ± 0.15^{a}	1.71 ± 0.18^{b}	1.3 ± 0.12^{b}
PER (%)	1.01 ± 0.11^{b}	1.36 ± 0.12^{ab}	1.7 ± 0.37^{a}
SR (%)	84.44 ± 0.52^{a}	91.11 ± 0.18^{bc}	$97.44\ \pm0.41^{ab}$

Note: Wm - absolute growth; SGR - specific growth rate; FUE - feed utilization efficiency; FCR - feed conversion ratio; PER - protein efficiency ratio; SR - survival rate; different superscripts in the same row show significant differences.

N. virens cultured in treatment C had the highest Wm value of 6.8 g, SGR of 6.4%, FUE of 68.95%, FCR of 1.30%, PER of 1.7% and SR of 97.44%. *N. virens* cultured in treatment A had the lowest Wm (4 g), SGR (4.65%), FUE (40.85%), FCR (2.24%), PER 1.01%), and SR (84.44%).

Proximate analysis. The proximate analysis of marine worms cultured on different substrate media for 35 days is presented in Table 3. The highest protein and fat contents of *N. virens* were in treatment C, 56.95% and 22.47% from dry matter, respectively. In contrast, sea worms from treatment A had 51.85% crude protein and 21.29% fat, and from treatment B had 54.62% crude protein and 21.54% fat, respectively.

Table 3

Proximate analysis of marine worms (*Nereis virens*) cultured in different substrate media for 35 days

Proximate composition	Treatment		
	A (Clay Mud)	B (Sand Silt)	C (Mangrove Mud)
Protein	51.85 ± 0.02^{b}	54.62 ± 0.05^{b}	$56.95\pm\ 0.02^{b}$
Fat	21.29 ± 0.01^{a}	21.54 ± 0.02^{a}	22.47 ± 0.07^{b}
Crude fiber	15.1 ± 0.08^{b}	10.5 ± 0.01^{b}	9.19 ± 0.08^{b}
Ash	5.18 ± 0.02^{a}	6.51 ± 0.07^{a}	5.19 ± 0.02^{b}
Carbohydrate	6.58 ± 0.01^{a}	6.53 ± 0.09^{a}	6.2 ± 0.03^{a}

Amino acid profile. The amino acid profile analysis was used to determine the contents of amino acid-, peptide- and protein-containing samples. The results of the amino acid analysis are presented in Table 4. Methionine had the highest content, in treatment C, 48.46 ppm of methionine.

Fatty acid profile. The essential fatty acid with the highest content was EPA, in treatment C, with 8.15%. The fatty acid profile of marine worms cultured on different substrate media for 35 days is presented in Table 5.

Table 4

Amino acids profile of marine worms (*Nereis virens*) cultured in different substrate media for 35 days

Amino acid (ppm)	*A (clay mud)*	*B (sand silt)*	*C (mangrove mud)*
L Histidine	15.12±0.03[b]	18.28±0.04[b]	20.07 ± 0.02[b]
L-Threonine	17.30±0.06[b]	25.75±0.06[b]	26.48± 0.04[b]
L-Proline	13.59±0.01[b]	25.38±0.03[b]	25.49 ± 0.02[b]
L-Tyrosine	15.23±0.06[b]	24.20±0.02[a]	26.19 ± 0.05[a]
L-Leucine	28.93±0.02[b]	30.47±0.05[b]	32.12 ± 0.03[b]
L-Aspartate	27.04 ± 0.09[b]	30.25±0.01[b]	32.68 ± 0.02[b]
L-Lysine	22.75±0.04[b]	29.19±0.06[b]	36.30 ± 0.06[b]
Glycine	15.99±0.01[b]	15.50±0.02[b]	20.61 ± 0.07[b]
L-Arginine	6.41±0.04[a]	7.749±0.01[a]	8.71±0.04[a]
L-Alanine	20.49±0.05[b]	24.90±0.07[b]	24.88±0.06[b]
L-Valine	19.65±0.02[b]	23.34±0.08[b]	28.37±0.07[b]
L-Isoleucine	15.81±0.09[b]	21.18±0.06[b]	26.34±0.09[b]
L-Phenylalanine	17.93±0.02[b]	22.46±0.03[b]	25.15±0.02[b]
L-Glutamic Acid	30.75±0.03[b]	32.78±0.02[b]	44.14±0.09[b]
L-Serine	18.98±0.09[b]	23.21±0.08[b]	28.53±0.03[b]
L-Tryptophan	3.92±0.05[b]	5.53±0.02[b]	8.98±0.07[b]
L-Methionine	32.26±0.08[b]	39.56±0.06 [b]	48.46±0.08[b]
L-Cysteine	15.32±0.03[a]	15.62±0.07[a]	15.60±0.02[b]

Note: different superscripts in the same row show significant differences ($p<0.05$).

Table 5

Fatty acids profile of marine worms (*Nereis virens*) cultured for 35 days in different substrates

Fatty acids (%)	*A (clay mud)*	*B (sandy mud)*	*C (mangrove mud)*
C 6:0	0.30±0.04[a]	0.35±0.03[a]	0.48±0.05[b]
C 8:0	0.85±0.05[b]	0.54±0.09[b]	1.78±0.03[b]
C 10:0	0.18±0.02[b]	0.16±0.05[b]	1.36±0.08[b]
C 11:0	0.15±0.07[a]	0.36±0.06[a]	0.38±0.09[a]
C12:0	2.15±0.03[b]	3.79±0.07[b]	4.45±0.05[b]
C 13:0	0.17±0.05[a]	0.12±0.02[a]	2.42±0.09[b]
C 14:0	1.88±0.03[a]	1.98±0.05[a]	2.68±0.04[b]
C 14:1	0.17±0.08[a]	0.27±0.02[a]	1.79±0.03[b]
C 15:0	0.45±0.09[a]	0.63±0.04[a]	0.98±0.06[a]
C 16:0	3.55±0.03[b]	4.53±0.02[b]	5.67±0.09[b]
C 16:1	0.39±0.02[a]	0.38±0.09[a]	1.65±0.02[b]
C 17:0	0.12±0.07[a]	0.57±0.05[a]	3.52±0.04[b]
C 18:0	0.99±0.03[a]	1.86±0.08[b]	0.93±0.09[a]
C 18:1	1.78±0.09[b]	2.58±0.07[b]	2.98±0.05[b]
C 18:2	1.33±0.08[a]	1.53±0.09[b]	3.53±0.08[b]
C 18:3	1.35±0.09[a]	2.70±0.08[b]	4.45±0.09 [b]
C 20:0	0.37±0.05[a]	0.40±0.02[a]	0.44±0.08[a]
C 20:1	0.55±0.01[b]	0.48±0.05[b]	0.76±0.05[b]
C 20:2	0.98±0.08[a]	0.98±0.09[a]	1.70±0.03[b]
C 20:4	0.99±0.09[a]	0.64±0.06[a]	0.98±0.02[a]
EPA	1.35±0.02[a]	4.65±0.03[b]	8.15±0.07[b]
DHA	0.35±0.09[b]	3.50±0.04[b]	5.78±0.02[b]

Note: different superscripts in the same row show significant differences ($p<0.05$).

Discussion. The difference of substrate media has a significant effect on Wm, SGR, FUE, FCR, PER, and SR of marine worms. The research results showed that the highest absolute values for SGR, FUE, FCR, PER, and SR were obtained in treatment C (mangrove mud media): 6.8 g, 68.95%, 1.3, 1.7, and 97.44%, respectively. Sea worms prefer the texture of mangrove mud because it contains a soft clay. Mangrove mud in the Awur Bay area of Jepara contains 0.09 mg L^{-1} ammonia and 0.2 mg L^{-1} nitrites (Herawati et al 2021). The mangrove forest ecosystem has a high productivity compared to other ecosystems. Organic matter resulting from the decomposition of materials from mangrove forests is the primary ecological linkage connecting it with the surrounding waters. The abundance of organic matter makes the mangrove forest a food source and a place to nurture various biota (Junardi & Wardoyo 2008). The results of this study follow the results of Mouneyrac et al (2010), who noted that mangrove mud contains a soft clay, marine worms being very fond of this texture. Djunaedi et al (2020) also stated that mangrove mud is a living medium for marine worms as a place for feeding and breeding. For substrates with more sand, a higher amount of energy is required to perform the activities that accelerate growth and to find food within the substrate. According to Lewerissa et al (2018), from an ecological perspective, mangrove ecosystem substrates serve as life support systems for various aquatic and terrestrial organisms, as feeding grounds, nurseries, and spawning grounds. The study used 8 cm thick substrate medium, which promotes the growth and development of sea worms (Herawati et al 2020).

The results in treatment C were in contrast with the growth of *N. virens* cultured on clay and sandy mud substrates in treatments A and B. A more concentrated substrate used for rearing media made it difficult for the test animals to have sufficient space to move, affecting their growth. Marine worms in the substrate containing clay mud grew less than then in sand and mangrove mud substrate media. This is considered a significant energy loss for marine worms. Food search and oxygen procurement are increasingly complex, requiring enormous energy to perform the activities. Hermawan et al (2015) stated that energy loss occurs in marine worms on dense substrates such as clay. This reduces its biomass and can cause death. This is also reinforced by the opinion of Mustofa (2012), that marine worms stay in one pore of an unsuitable substrate medium to conserve energy and increase biomass. Oxygen is known to play an essential role in initiating biological growth. Asnawi & Idris (2018) stated that sand structures play a role in oxygen exchange processes within the matrix and act as media for nutrient particles (organic matter), and clay structures act as reservoirs for organic matter particles within the matrix. This statement was supported by Schaum et al (2013), who state that sufficient organic matter and forage levels influence specific growth rates.

This study's SGR values showed that treatment C had significantly higher values than treatments A and B. The SGR of treatment C was 6.4%. The lowest growth rate was 4.65% (treatment A). SGR indicates excellent forage quality. The lower SGR may be due to the lower feed consumption in treatment A. Growth rate can be used to determine feed quality. According to Haryadi & Rasidi (2012), the evaluation of SGR values indicates good forage quality. This is due to the type of feed used in the research, which used animal ingredients, making it easier for nematodes to digest. Treatments A, B, and C had marine worms with a minimum biomass weight of 4 g and maximum biomass of 6.8 g. The trend toward higher SGRs provides ample opportunity for worms to perform a tube-forming activity, organic matter decomposition, and exchange processes.

The feed sample administered during the 35-day maintenance period was an artificial feed in the form of powder with a protein content of 40%. According to the research findings, the highest FCR and PER were in the treatment C. These results indicate that the marine worm's body effectively and efficiently absorbs the food. FCR is affected by the amount of each component in the food and feed nutrient sources. The efficiency is determined by several factors, including the protein content, the quality of the food, and the frequency of meals (Tacon 1987).

High SR suggests that food quality and quantity were adequate to meet basic needs and promoted growth. According to Asnawi & Idris (2018), the importance of the substrate type in the lives of *N. virens* is closely related to the function and type of each texture, as well as the marine worms' habitual patterns of motility. Marine worms performed best when

treated with a mangrove mud substrate, allowing them to absorb the maximum amount of food.

Marine worms eat residual organic matter. *N. virens* generally live in bays with mangrove mud substrate conditions. This study used medium-sized mangrove mud as a substrate in treatment C. Marine worms can use the organic matter of the substrate as natural feed, resulting in improved growth rates during culture. In addition, the water quality during cultivation showed DO values of 5-6.5 mg L^{-1}, pH values in the range of 7-8, a temperature in the range of 28-31°C, and salinity of 29-31 ppt. Water quality suitable for marine worms is between 23 and 32°C and salinity between 1 and 31 ppt. The water quality during the culture of *N. virens* was suitable due to their growth and survival. Good management influences organism survival, including stocking density, forage quality, water quality, parasites, and diseases. The status of *N. virens* is strongly influenced by environmental conditions, substrate, and water (Gamis et al 2016).

The protein results ranged from 51-56.95% and from 21-22% for fat. The treatment of *N. virens* in mass culture on a media substrate with mangrove mud gave the highest yields on protein and fat, 56.95% and 22.47%, respectively. This study's protein and fat content were higher than the research results of Herawati et al (2020), with 54.05% protein and 22.54% fat. The high nutrient content in *N. virens* is presumably due to the excellent growth rate in treatment C. Animal protein-based foods are more manageable for marine worms to digest. Dietary fat sources can also be used as an energy source and for growth. He et al (2019) and Herawati et al (2020) showed that food that meets the nutritional needs of *N. virens* can increase its nutrient content based on reasonable growth rates. *N. virens* is one of the best natural feeds for shrimp due to its high content of fatty acids, which are essential for ovarian growth and development (Gamis et al 2016). According to Nguyen et al (2012), the required oil content for shrimp is 10%, and fat is an important food component for shrimp ovaries' development (Tocher 2015). The bulk culture of *N. virens* using clay as a substrate had the least amount of nutrients, with 51.85% protein and 21.29% fat.

The amino acid profile of *N. virens* cultured in mangrove mud was the best, especially the methionine content, which was 46.46 ppm. The lowest methionine content was in treatment A, 32.26 ppm. An essential amino acid for the body, methionine is required for nucleic acid and tissue formation, protein synthesis, and for the formation of other amino acids (cysteine), and vitamins (choline). According to Boonyoung et al (2013), methionine and cysteine are animals' most important sources of amino sulfate. Cysteine is not always necessary, because it can be synthesized from methionine. Rolland et al (2015) showed that the ribosome's synthetic process depends on the amino acids needed. The composition of amino acids that enter or are released from tissue cells primarily determines tissue protein synthesis. The integrity and balance of circulating amino acids in the tissue greatly influence the efficiency and quantity of protein synthesis in tissue cells. Methionine is required by the fish body to initiate protein synthesis and has the potential to affect muscle growth (Belghit et al 2014).

The essential eicosapentaenoic fatty acid (EPA; 20:5n-3) plays a vital role in shrimp survival, particularly growth. EPA essential fatty acids are phospholipids found in membranes and nerve tissue. Larvae have a very high neurosome index when they first feed and require much n-3 HUFA to avoid neurodysplasia (Costa et al 2010). According to Tocher (2015), fatty acids are essential when feeding shrimp during gonadal maturation. So, as shrimp feed, marine worms must have a high content of fatty acids, especially EPA, according to shrimp needs. The highest EPA was found in *N. virens* mass cultured in mangrove mud (8.15%), and the lowest was in *N. virens* in clay mud (1.35%).

Conclusions. Mass-cultured marine worms in mangrove mud substrate had the highest absolute weight, SGR, FUE, FCR, PER, and SR values of 6.8 g, 6.4%, 68.95%, 1.3, 1.7 and 97.44%, respectively, with 48.46 ppm of methionine and 8.15% of EPA.

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

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