

Survival Rate, Growth and Chemical Content of *Dendronereis pinnaticirris* (Polychaeta, Nereidae) in Maintenance with Different Feeds and Substrates

Eko Setio Wibowo^{1*}, Edy Yuwono², Purnama Sukardi³, Asrul Sahri Siregar³

¹Faculty of Biology, Jenderal Soedirman University
Jl. dr. Soeparno No.63 Purwokerto 53122, Indonesia

²Faculty of Biology, National University
Jl. Sawo Manila, Pejaten Pasar Minggu Jakarta Selatan, Indonesia

³Faculty of Fisheries and Marine, Jenderal Soedirman University
Jl. dr. Soeparno kompleks GOR Soesilo Sudarman Purwokerto, Indonesia
Email: tio_eko@yahoo.co.id

Abstract

The worm *Dendronereis pinnaticirris* is used as feed of shrimp broodstock in a hatchery, mainly because of its availability in the local market, and its nutritional content required for improving gonad maturation and post larvae production. The important economic value of the worm and the increasing demand for feed in shrimp hatcheries have led to an intense exploitation that deplete its population and the sustainability of the whole estuarine ecosystem. The study, which represents the starting point of large-scale production of the polychaete worm by culture in the artificial system, shall be undertaken. Accordingly, a production study using *D. pinnaticirris* juvenile was carried out under controlled conditions fed with two different feed (feed contains mainly plant protein and animal protein, respectively), and kept in three different substrates (substrate consists of mud and 8.78%, 37.34%, 39.17% sand, respectively). The treatments were arranged according to randomized completely block design in 8 (eight) replicates. The survival rate, body weight increment and growth, oxygen consumption, proximate body chemical, and fatty acid contents were measured. The results showed that growth and oxygen consumption was significantly influenced by a substrate and feed type ($P < 0.05$). Worms on the mud substrate with 39.17% sand, and feed containing mainly vegetable protein showed the highest oxygen consumption. Survival rate and chemical body content were not significantly influenced by the type of substrate and feed ($P < 0.05$). The protein content of the worm was 32.02-43.81%, while fat content was 2.41-9.89%. Twenty different fatty acids were identified in the worm of all treatment groups.

Keywords: *Dendronereis pinnaticirris*, fatty acids, growth, oxygen consumption, protein content

Introduction

Nereid worm *Dendronereis pinnaticirris* is an invertebrate from the Familia of Nereidae, Classis Polychaeta which live in the estuarin ecosystem as benthic organism but also actively swim in waters during reproduction (Wallace et al., 1991). Sandy soil is a common substrate inhabited by nereid worms including *Nereis diversicolor* and *N. virens*, however there are species that live in rocks like *N. pelagica* (Yuwono, 1992). In Indonesia, for example in Java and Sumatra, the worm is derived from its natural habitat in the brackish water area.

Polychaeta including the worm *D. pinnaticirris* can be used as feed of Penaeid shrimp broodstock, because its nutritional content is required to improve the quality of gamete cell and viability of shrimp larvae. *D. pinnaticirris* contains essential amino acids which are dominated by chemoattractant for shrimp and also contains unsaturated fatty acids, especially

arachidonic acid (Yuwono, 2005). *N. diversicolor* that is maintained in an integrated aquaculture with recirculation system contain high quality fatty acids especially ARA, EPA and DHA (Bischoff et al., 2009). Polychaeta also contains reproductive hormones such as prostaglandin E2 (Meunpol et al., 2005). Furthermore, Meunpol et al. (2007) showed that polychaetes *Perinereis* sp. producing progesterone (P4) and 17-alpha hydroxyprogesterone (17 α -OHP4) which have an effect on vitellogenesis and maturity of shrimp gametes.

Utilization of worm *D. pinnaticirris* as shrimp feed is not only due to its nutritional content, but also because it is easily obtained from the community around the hatchery, therefore it has an important economic value. These animals also play an important ecological role in coastal ecosystems as deposit feeders (Jumars et al., 2015). Polychaeta able to ingestion and defecation of sediment rapidly, so that the worm contributes to the regulation of the

carbon cycle, nitrogen and sulfur. Polychaeta is also able to maintain the stability of sediments that affect the sustainability of the brackish water ecosystem balance (Snelgrove, 1997).

The high economic value of the worm has caused intensive exploitation in its habitat. In the long term, intensive exploitation might threaten *D. pinnaticirris* population and results in the damage of overall brackish water ecosystem. This condition requires efforts to control such exploitation by conducting cultivation of worm *D. pinnaticirris* to supply aquaculture demand, thus reducing the dependence on nature. The cultivation of worm *D. pinnaticirris* can be a promising business opportunity, therefore research on the production of worm *D. pinnaticirris* that became the initial step of the development of mass cultivation has been undertaken.

The results of previous studies indicate that *D. pinnaticirris* worms cultivated on substrate enriched with finely grained bran grow better than those cultivated on substrates enriched with faeces of quail (Yuwono *et al.*, 2000). *D. pinnaticirris* maintained on a 63-250 µm diameter soil substrate showed better survival and growth than those maintained on a 250-500 µm soil substrate (Mustofa *et al.*, 2012). Feed also affects the survival and growth of marine worms, as reported by (Rasidi and Patria, 2012) that feed made from broiler chicken intestinal flour enhances the survival and growth of *Nereis* sp.

In order to complete the scientific information for the development of worm culture, a research has been conducted using juvenile of *D. pinnaticirris* obtained from mature worms spawned in the laboratory. The mature worm was collected from its natural habitat. The study aims to determine the survival, growth, metabolism and chemical content of the worm *D. Pinnaticirris* grown in sandy mud substrate and fed with animal and vegetable protein.

Materials and Methods

Juvenile worms *D. pinnaticirris* were taken from laboratory at Faculty of Fisheries and Marine Science and Technology, Unsoed, aged 5-6 months, 0.01-0.19 g in wet body weight, and number of segments was 38-131. Sandy mud substrate and seawater used for the experimental animal breeding media was obtained from Brebes and Cilacap brackish water, and the feed used was fish feed containing vegetable and animal protein.

The experimental animal was acclimated for two weeks in a plastic box sized (10x8.5x4) cm³ containing sandy mud substrate and seawater with a salinity of 15 ppt. Each container contains 3 individual juvenile *D. pinnaticirris*. Prior to use in the

experiment the substrate was sieved with a 0.02 mm strainer, dried in an oven at 90°C for 2x24 h so that the pathogen organisms perish. The experimental medium within the boxes was aerated to maintain sufficient dissolved oxygen.

The experimental worm and the feed were weighed with an analytic scale (Ohaus) with accuracy of 0.001 g. The increment of posterior segment was counted by observing under a stereo microscope at 10X magnification. The medium temperature was measured by the Celsius thermometer, while the pH was measured with a universal pH paper. Medium salinity was measured by hand refractometer (Atago) and was monitored during the experiment. The study was conducted experimentally, with 6 (six) different treatments consist of: (1) SAPN: treatment with type A substrate (8.78% sand content) and feed containing vegetable protein; (2) SAPH: treatment with type A substrate (8.78% sand content) and feed containing animal protein; (3) SBPN: treatment with type B substrate (39.17% sand content) and feed containing vegetable protein; (4) SBPH: treatment with type B substrate (39.17% sand content) and feed containing animal protein; (5) SCPN: treatment with type C substrate (37,34% sand content) and feed containing vegetable protein; (6) SCPH: treatment with type C substrate (37,34% sand content) and feed containing animal protein. The treatments were arranged in accordance with Randomized Completely Block Design in 8 replicates.

Survival rate and growth parameters that include weight gain and the number of posterior segment increment were calculated according to Hariyadi and Yuwono (1998): $SR = (Nt / No) \times 100\%$ where SR was survival rate; Nt was the number of experimental animals at the end of the study and NO was the number of experimental animals at beginning of the study. $WG = Bt - Bo$ where WG was weight gain Bt: wet weight of experimental animal at the end of the study, Bo: wet weight of experimental animal at the beginning of the study. $NSI = St - S0$ where SI was the number of segment increment; St was the number of experimental animal segment at the end of the study, S0: number of experimental animal segment at the beginning of the study. Specific growth rate was determined according to Chang *et al.* (2006): $SG = ((\ln Wt - \ln Wo) / t) \times 100\%$ where SGR: Specific Growth Rate; Wt: worm wet weight at the end of the study (g), Wo: worm wet weight at the beginning of the study (g), t: duration of the study (days). Specific growth rate were also determined by calculating the number of segment at the beginning and at the end of the study, ie: $SGR = ((\ln St - \ln So) / t) \times 100\%$; where SGR: Specific Growth Rate (%), St: number of worm segment at the end of the study, S0: number of worm segment at the beginning of the study, t: duration of study (days).

Oxygen consumption as a metabolic rate parameter is measured at the start of exposure and the end of the study. Measurement of oxygen consumption was performed with Fry's (1971) respirometer in Brougher *et al.* (2005). The oxygen consumption apparatus consists of two tubes; tube I and tube II were 0.5 L and 10 L in volume, respectively. The tubes were aerated and equipped with electrical pump. The measurement accuracy was 0.01 ppm and 0.0001 mg⁻¹.g⁻¹.h⁻¹.

The initial dissolved oxygen was calculated by the formula according to APHA (2005). The oxygen consumption (mg⁻¹.g⁻¹.h⁻¹) of the worm was measured using the Fry (1971) method in Brougher *et al.* (2005): $KO_2 = \frac{[(DO_{ae} - DO_{tp}) - DO_{tae}] / KA}{[N \times Bi_3 / 4 \times h]}$ where KO_2 was oxygen consumption (mg⁻¹.g⁻¹.h⁻¹) DO_{ae} was dissolved oxygen of medium in aerated tube (mg.L⁻¹), DO_{tp} was dissolved oxygen of medium in treatment tube (mg.L⁻¹), DO_{tae} : dissolved oxygen of medium in tube without aeration (mg.L⁻¹); N was number of the worm; Bi: wet weight of the worm (g), h was the duration of the measurement of oxygen consumption (hr), KA was pump water flow velocity (L.h⁻¹).

The body chemical content of the experimental animals was measured by the Kjeldahl method through proximate analysis (AOAC, 1990). Measurement of fatty acid parameters was done at the beginning and at the end of the study. Measurement of fatty acid content was done by Gas Liquid Chromatography (GLC). Fatty acid levels were calculated by the formula according to McNair and Bonelli (1988): Fatty acid levels (%) = $\frac{[(L_c / L_s) / L_s] \times [(C_s \times V) / b]}{b}$; L_c : sample area, L_s : standard area, C_s : standard concentration, V : final volume, B : sample weight.

The data obtained was analyzed statistically using two-way analysis (factorial). The treatment was significantly different if $P < 0.05$. If the results of the analysis show significant differences, the analysis continues with the Tukey test for the smallest significant difference $P < 0.05$. The analysis was performed using software MINITAB 16.

Results and Discussion

The results showed that the type of substrate and feed did not affect the survival rate ($P > 0.05$). Survival rate of all treatments was 100%. These results indicate that the feed and the type of substrate used in the study are excellent for supporting the life of the worm. These results are in accordance with that of previously reported by Batista *et al.* (2003). Who achieved high survival rate in cultivating *N. diversicolor* fed with sea bream dry food

(SBDF) and dried feed of vegetable protein (tetramin). The treatments did not significantly affect ($P > 0.05$) worm survival rate. Results of the recent experiment also confirm the result of study performed by Costa *et al.* (2000) where *N. diversicolor* fed with different feed showed a high rate of survival, ie 78% -100%. The feed used in recent study perfectly supported the life of the worm *D. pinnaticirris* since the worm could consume the feed of carnivores, herbivores, suspensivores, or detritivores (Nielsen *et al.*, 1995; Riisgård, 1991 in Batista *et al.*, 2003). Another nereid worm, *Nereis diversicolor*, able to digest various food ingredients from live foods such as micro and macrozoobenthos and diatoms, organic matter including litter (Costa *et al.*, 2006) and also the feed type of deposit feeders; and the undigested sediment will be excreted in the form of faeces (Siregar, 2008). Therefore, according to Heliskov and Holmer (2001) the polychaete nereid worm plays an important role in the decomposition of organic matter and nutrient circulation in the sediments.

The high survival rate in each treatment in this study shows that the worm can live well on different types of substrate. The results of this study were in accordance with that of Mustofa *et al.* (2012), they found that the survival rate of *D. pinnaticirris* cultivated in the substrate with the size of 63-500µm and the thickness of 10 cm was 92.5-100%. According to Junardi (2001) Polychaeta live in sand-dominated substrate as well as in mud and clay substrate. The distribution of polychaetes is correlated with the substrate type and the worms generally live in soft and sandy substrates. Junardi (2001) further states that polychaeta utilizes the substrate as a living place and as foodstuff, especially those species suspension filter feeder. Filter feeder polychaetes spend most of their life in sand-dominated substrates, whereas deposit as well as subsurface deposit feeder live in mud and clay substrates.

The weight gain of *D. pinnaticirris* significantly affected by substrate and feed type ($P < 0.05$), but it did not affected by the interaction between substrate and feed type (Table 1.). Analysis variance showed that experimental animals kept in substrate A (sand 8.78%) and fed with feed contain animal protein had the highest body weight gain. This result was in accordance with that of Costa (1999) in which *N. diversicolor* maintained in sandy substrate (500-250 µm in diameter) showed higher body weight than maintained on mud substrate (12.5-63 µm in diameter). Mustofa *et al.* (2012) also reported that sand substrate of 63-250 µm in diameter with a thickness of 10 cm provided a better growth in *D. pinnaticirris* than those maintained in substrates of 250 to 500 µm in diameter. This phenomenon showed that deposit-eating biota in general likes fine-

grained substrates, because it can utilize the organic material contained in the substrate as food (Nybakken, 1988).

D. pinnaticirris fed with feed with its protein component mainly animal protein showed the highest body weight gain. The feed containing mainly animal protein proved to be more advantageous for the worm growth than feed containing mainly vegetable protein. According to Millamena *et al.* (2002) animal raw materials are superior to vegetable raw materials because they contain amino acids methionin and lysin, which are indispensable for growth. The efficiency of animal feed absorption is generally higher than vegetable feed (Yuwono, 2008) and has been proven by Yuwono *et al.* (2000) that compost wick contain animal protein as a culture medium resulted in better growth rates compared to vegetable protein composts. Batista *et al.* (2003) also reported a similar phenomenon, in *N. diversicolor* fed fish feed contains animal protein resulted in a relatively higher weight gain compared to those that contain vegetable protein.

The type of substrate and feed showed a significant effect on the increment of number of *D. pinnaticirris* segments (Table 2.). The increment of the number of segment was significantly affected by substrate type and feeding with different protein content ($P < 0.05$), but it did not significantly affected by the interaction between substrate and feed type ($P > 0.05$). The worms maintained in the substrate containing 8.78% sand showed the highest increment of the number of segments. This result is in accordance with that of Costa (1999) which shows that the addition of setae on *N. diversicolor* maintained on sand substrate is relatively higher than that maintained on mud substrate. *D. pinnaticirris* may be better preserved on substrate with low sand proportions, as Barnes (1987) suggests that sandy mud substrate is more suitable for animals living in burrows and only out at certain times for feeding usually during night.

The worms *D. pinnaticirris* fed on animal protein have the highest increment of number of segments. This phenomenon confirms the results of Yuwono *et al.* (2002) suggesting that feed with animal protein content (*Brachionus*) fed to young worms produces higher number of segment compared to that fed vegetable protein (*Spirulina* and *Chlorella*). Animal protein is more complete in its essential amino acid content, whereas vegetable protein lacks one or more essential amino acids that inhibit protein synthesis and restricts the use of other amino acids and consequently affects growth (Campbell *et al.*, 2004).

The specific growth rates of *D. pinnaticirris* calculated by weight gain parameters (Table 3.) and

posterior segment growth parameters (Table 5.) were significantly different between treatments. Substrate type and feed type had significant effect ($P < 0.05$) on specific growth rate, but not so with interaction between substrate type and feed type. The substrate type A (sand 8.78%) yielded the highest specific growth rate compared to substrate C (sand 37.34%) and substrate B (sand 39.17%). This phenomenon is corresponding with the results of Costa (1999) study on *N. diversicolor* maintained with sand substrate (500-250 μm) and sludge (125-63 μm) which showed length growth rates for sand and sludge, 0.97 and 0.84 $\text{mm}\cdot\text{day}^{-1}$, respectively, and the worms achieved commercial size in the market within 72 days with length reached 7 cm (70% of initial size).

The results of this study also showed that the *D. pinnaticirris* fed with feed mainly contain animal protein resulted in the highest specific growth rate (Table 3.). This is presumably due to feed containing animal protein has more advantage for the growth than that contain mainly vegetable protein. According to Hariyadi *et al.* (2000) vegetable food is more difficult to digest than animal feed because it contains cellulose. Difficulty in digestion decreases energy allocation for growth. This result is in accordance with that of Costa *et al.* (2000) where *N. diversicolor* fed with animal protein granule for post larvae shrimp showed higher specific growth rates than those fed with vegetable protein. Batista *et al.* (2003) reported that *N. diversicolor* fed with dried animal-protein dried foods for *S. auratus* showed higher specific growth rates than those fed vegetable-protein containing feed for ornamental fish, i.e. 7.88%.d⁻¹ and 7.78 %.d⁻¹. Nielsen *et al.* (1995) reported that *N. diversicolor* fed shrimp feed showed a specific growth rate twice as high as that fed algae.

Oxygen consumption

The parameter for metabolism in this study is the rate of oxygen consumption. The experimental results showed that the type of substrate and feed type significantly affected ($P < 0.05$) the rate of oxygen consumption of *D. pinnaticirris* (Table 4.); but the interaction between substrate type and feed type ($P > 0.05$) did not so. Worms maintained in a substrate with a sand content of 39.17% and fed vegetable protein feed showed the highest oxygen consumption.

The worm is influenced by environmental conditions, i.e. those kept in substrate with higher sand content shows higher oxygen consumption, whereas those kept in substrate with lower sand content show lower oxygen consumption. This phenomenon suggests that the worm *D. pinnaticirris* is an oxyconformer organism, i.e. the animal whose oxygen consumption rate adjusts to the availability of

Table 1. Body weight gain (g) of worms *D. pinaticirris* maintained with different substrate and feed. The figures followed by different letters in the same column are significantly different ($P < 0.05$).

Treatment	Initial Weight	Final Weight	Weight Growth Range	Average+ SD
SAPN	0,0621	0,2925	0.1233 - 0.3233	0.2304±0.0636 ^{ab}
SAPH	0,0429	0,3033	0.2033 - 0.3500	0.2604±0.0443 ^a
SBPN	0,0558	0,1254	0.0033 - 0.1200	0.0696±0.0393 ^d
SBPH	0,0495	0,1883	0.1000 - 0.2000	0.1388±0.0377 ^c
SCPN	0,0683	0,2788	0.1400 - 0.2600	0.2021±0.0470 ^b
SCPH	0,0550	0,3108	0.1867 - 0.2933	0.2558±0.0430 ^{ab}

Table 2. Increment of the number of worm segments of *D. pinaticirris* maintained with different substrate and feed. The figures followed by different letters on the same column are significantly different ($P < 0.05$).

Treatment	Initial Segment Count	Final Segment Count	Range Number of Segment	Average + SD
SAPN	94,5000	133,9583	19.0000 - 52.6667	39.4583±12.5153 ^{abc}
SAPH	80,5833	138,0000	46.6667 - 72.6667	57.4167±7.5441 ^a
SBPN	96,2083	118,2500	7.6667 - 46.0000	22.0417±12.3795 ^c
SBPH	84,4583	126,6250	26.6667 - 58.3333	42.1667±15.0248 ^{ab}
SCPN	95,7083	125,0417	13.6667 - 44.6667	29.3333±12.6215 ^{bc}
SCPH	87,7917	130,9583	31.3333 - 66.0000	43.1667±11.7527 ^{ab}

Table 3. Specific growth rates based on body weight parameters (%) of worm *D. pinaticirris* maintained with different substrate and feed. The numbers followed by different letters on the same column are significantly different ($P < 0.05$).

Treatment	Range	Average + SD
SAPN	0.0162 - 0.0348	0.0266±0.0057 ^{ab}
SAPH	0.0206 - 0.0541	0.0362±0.0115 ^a
SBPN	0.0004 - 0.0290	0.0159±0.0089 ^b
SBPH	0.0134 - 0.0400	0.0250±0.0100 ^{ab}
SCPN	0.0202 - 0.0330	0.0241±0.0047 ^{ab}
SCPH	0.0215 - 0.0475	0.0313±0.0070 ^a

dissolved oxygen in its external environment (Moyes and Schulte, 2008). Substrate with higher sand content has higher dissolved oxygen content than substrate with lower sand content. According to Junardi (2001) the finer the substrate grain, the lower the substrate oxygen because there is more limited space between the substrate grains. The results of this study confirm that of Hardewig *et al.* (1991); at *Sipudusus nudus* living in intertidal areas that are regularly exposed to low oxygen conditions capable of responding to hypoxia by decreasing oxygen consumption. Suadicaní *et al.* (1991) also reported that Polychaeta *Cirinformia tentaculata* able to adapt and survive on temporary hypoxia. Under hypoxia, these animals slowly change from aerobic metabolism to anaerobic metabolism, with the accumulation of alanine and succinate, resulting in low levels of oxygen consumption. The ability to perform anaerobic and aerobic metabolism for energy production enabling this animal to adapt to extremely low oxygen supply conditions (hypoxia). According to Connell and Miller (1995) to compensate for the low dissolved oxygen, some

benthic organisms form a high concentration of pigment respiration in body fluids. These respiratory pigments consist of proteins and non-protein components, for example those in polycheta in the form of hemeythrin and chlorocruoin contained in body fluids (Yuwono, 2008).

High consumption of oxygen is one of the high figures of the body's metabolism process in digesting vegetable protein, because vegetable protein has more fiber than animal protein feed. According to Darmadi *et al.* (2003) the activity of animal metabolism can not be separated from the food consumed that acts as a source of energy. Furthermore, according to Ranjhan (1993) fibrous foods will cause the increase of energy needed in the digestive process, so that energy that can be used to increase body tissue, is expended for the process of digesting fibrous feed.

The higher metabolism in an animal's cell leads to an increased need for oxygen followed by increased oxygen consumption by the animal (Hochachka, 1991). According to Cook *et al.* (2000); Zimmermann and Kunzmann (2001) oxygen consumption is influenced by changes in the ability of digestibility to food. Oxygen consumption is also influenced by internal factors; namely type, size, reproduction status and daily activities; and external factors such as temperature, salinity and dissolved oxygen content in the environment (Suadicaní *et al.*, 1991). Oxyconformer animal such as *D. pinaticirris* is apparently able to decrease the rate of metabolism when exposed to an environment with low soluble oxygen content such as on a substrate with a low sand proportion.

Chemical content of the body

The result of the proximate analysis of the *D. pinaticirris* worm body at the end of the study obtained the highest protein content to the lowest in the treatment of SBPN, SAPH, SCPH, SBPH, SAPN and SCPN, while the fat content of the highest in treatment of SCPH, SAPN, SAPH, SBPH, SBPN and The lowest SCPN (Table 5.). The type of substrate and feed does not affect the protein content and body fat ($P>0.05$). This result is in consistent with Mustofa *et al.* (2012) who reported that *D. pinnaticirris* maintained with different substrate particle sizes yielded no different protein retention.

D. pinnaticirris protein content ranged from 32.02 to 43.81%, while the fat content ranged from 2.41 to 9.89%, lower than the protein and fat content of *Nereis* sp. from nature, that obtained by Rachmad and Yuwono (2000), namely 52.26% protein and 29.83% fat. The content of *D. pinnaticirris* protein is also slightly lower than that of *N. virens*, which is 62.64% (Yuwono, 2005); polychaeta *Perinereis* sp. 64,87%; *Marphysa* sp. 50,90% (Meunpol *et al.*, 2005). The highest fat content in *D. pinnaticirris* is higher than the fat content of *N. diversicolor*, which is only 19.3% (Luis & Pasos, 1985). This fat content is also higher compared to that of *N. diversicolor* fed different feeds, which ranges from 7.9-20% (Costa *et al.*, 2000).

The body protein content in the treatment with vegetable diet was lower than feed protein (46,99%), while that with animal protein was higher than feed protein (34,17%). The content of protein and body fat was strongly influenced by nutritional composition of the feed, but in the treatments have not demonstrated differences in body protein levels. According Yuwono (2008) animal protein generally have better quality than vegetable protein. Protein in the feed will be absorbed and used to build or repair damaged body cells, and very efficient for energy (Lovell, 1989).

Table 4. The rate of oxygen consumption ($\text{mg}^{-1}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) of *D. pinaticirris* maintained with different substrate and feed. The figures followed by different letters on the same column are significantly different ($P<0.05$).

Treatment	Range	Average + SD
SAPN	0.0025 - 0.0084	0.0060±0.0022 ^{abc}
SAPH	0.0067 - 0.0110	0.0090±0.0014 ^a
SBPN	0.0011 - 0.0070	0.0035±0.0019 ^c
SBPH	0.0038 - 0.0106	0.0070±0.0030 ^{ab}
SCPN	0.0021 - 0.0077	0.0046±0.0024 ^{bc}
SCPH	0.0044 - 0.0114	0.0068±0.0023 ^{abc}

D. pinnaticirris, fed with vegetable protein, had higher body fat content than feed fat content (3.47%) except in SCPN treatment, whereas in *D. pinaticirris* worms fed with animal protein the body fat content is higher of feed fat content (4.5%). Body fat content is strongly influenced by nutritional composition of feed. This result was consistent with that of Luis and Passos (1995) which indicate that food composition is the critical factor in determining the *N. diversicolor* fatty acid composition.

Fatty acid content composition

The results of qualitative analysis indicate that the worms *D. pinaticirris* in all treatments contained 20 types of fatty acids. The fatty acids consist of 9 types of saturated fatty acids (SAFAs), namely lauric acid (C12: 0), myristic acid (C14: 0), pentadecanoic acid (C 15: 0), palmitate (C16: 0), heptadecanoic acid C17: 0), stearic acid (C18: 0), heneicosanoic acid (C21: 0), behenat acid (C22: 0), trichosanoic acid (C23: 0). Monointoleic acid (16: 1), elaidic acid (C18: 1, n-9t) acidic acid (C18: 1, n-9c). Polyric acid (C18: 3n6), linoleic acid (C18: 3n6), eicosadienic acid (C20: 2), eicosatrienaat acid), docosadienoic acid (C22: 2), eicosapentanoate / EPA (C20: 5n3), and docosahexaenoic acid / DHA (C22: 6n3) (Table 6).

The fatty acid content of SAPN treatment consists of 20 species, SAPH 17 types, SBPN 19 species, 16 species SBPH, 18 species SCPN and 17 species SCPH. These fatty acids are divided into three types: SAFA, MUFA and PUFA (Table 7.). SAFA content in each treatment is relatively the same, this condition according to Sukarsa (2004) caused of the fatty acid group SAFA is the basic component of the fat forming system in living things. While the high content of unsaturated fatty acids might be caused of these fatty acids are more easily metabolized compared with saturated fatty acids.

The composition of fatty acid content of each treatment was relatively similar, ie palmitic (C 16: 0), EPA (C 20: 5n-3), stearic (C 18:0), elaidic (C18:1n9t), oleic (C18:1n9c), Linoleic (C18:3n3) and small amounts of myristic acid (C14:0), palmitoleic acid (C16:1), docosahexaenoic acid (DHA) (C22: 6n3). This result was similar to that of Costa *et al.* (2000) which derives the majority of *N. diversicolor* fatty acids in all treatments of C 16: 0, C 18: 1n-9, C 18: 1n-7, C 18: 2n-6 and C 20: 5n-3. A small number of C 16: 1n-7, C 18: 0, C 18: 3n-3, C20: 2n-6 and C 20: 4n-6. The fatty acid composition obtained in accordance with Yuwono's research (2005) in the form of myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and EPA. The high content of palmitic acid and stearic acid is also suitable with the research of Dorgham *et al.* (2015) in *P. cultrifera* worms from the

Table 5. Proksimat body (%) worm *D. pinaticirris* maintained with different substrate and feed

Sample	Water %	Dry Weight %	% Dry Weight				
			Protein	Fat	Fiber	Ash	Ingredients Without Nitrogen Extract (NFE)
SAPN	17.31	82.69	36.18	7.51	0.73	5.11	50.49
SAPH	15.06	84.95	39.40	5.92	0.28	8.73	45.68
SBPN	12.01	87.99	42.91	3.89	4.76	1.75	46.70
SBPH	12.03	87.98	39.14	5.79	9.94	6.09	39.04
SCPN	31.17	68.83	32.85	2.63	7.08	8.46	48.68
SCPH	8.33	91.67	39.67	9.66	7.68	1.28	40.88

Table 6. The composition of fatty acid body worm *D. pinaticirris* maintained with different substrate and feed

Parameter (% w/w)	Treatment					
	SAPN	SAPH	SBPN	SBPH	SCPN	SCPH
Fat Content	5.54	4.54	4.17	6.65	5.91	7.14
<i>Fatty Acid</i>						
Lauric Acid, C12:0	0.01	0	0.02	0	0	0
Myristic acid C14:0	1.38	1.67	0.98	0.36	0.86	0.7
Pentadecanoic Acid C15:0	0.37	0.47	0.3	0.2	0.33	0.2
Palmitic acid C16:0	10.09	12.38	8.27	4.65	8.14	6.3
Palmitoleic Acid, C16:1	1.1	1.32	0.91	0.43	0.9	0.59
Heptadecanoic Acid, C17:0	2.59	0	2.37	1.63	2.4	1.55
Stearic Acid, C18:0	3.78	4.48	3.22	2.36	3.49	2.35
Elaidic Acid, C18:1n9t	2.45	2.98	1.74	0.86	1.67	1.36
Oleic Acid, C18:1n9c	3.77	4.35	3.36	0.86	1.67	1.36
Linoleic Acid, C18:3n3	3.28	3.33	2.59	1.92	2.84	2.01
g-Linolenic Acid, C18:3n6	0.04	0	0	0	0	0
Heneicosanoic Acid, C21:0	0.57	0.06	0.05	0.04	0.05	0.03
Cis-11,14-Eicosadienoic Acid, C20:2	1.36	1.39	0.92	0.5	0.87	0.65
Behenic Acid, C22:0	0.75	0.85	0.55	0.33	0.61	0.37
Cis-11,14,17-Eicosatrienoic Acid, C20:3n3	0.32	0.37	0.25	0	0.26	0.25
Arachidonic Acid, C20:4n6 (AA)	2.96	3.4	1.84	2.07	2.67	2.18
Tricosanoic Acid, C23:0	0.22	0.27	0.16	0.13	0.24	0.13
Cis-13,16-Docosadienoic Acid, C22:2	0.05	0.04	0.02	0	0.05	0
Cis-5,8,11,14,17-Eicosapentaenoic Acid, C20:5n3 (EPA)	6.56	6.73	3.48	4.36	5.22	3.27
Cis-4,7,10,13,16,19-Docosahexaenoic Acid, C22:6n3 (DHA)	1.84	2.14	0.96	1.1	1.39	1.31

Table 7. Group of fatty acids body worm *D. pinaticirris* maintained with different substrate and feed

Group of Fatty Acid	Treatment					
	SAPN	SAPH	SBPN	SBPH	SCPN	SCPH
SAFA	9	7	9	8	8	8
MUFA	3	3	3	3	3	3
PUFA	8	7	7	5	7	6

Mediterranean Sea. Likewise, according to Palmer *et al.* (2014), which states that palmitate and stearate acids are commonly found in many types of Polychaeta.

Fatty acid composition obtained in recent study is in accordance with that revealed by Yuwono (2005) in the form of myristic acid, palmitic acid,

palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and EPA. The study also showed a DHA content ranging from 0.96-2.14% w/w. According to Costa *et al.* (2000) fatty acid composition in *N. diversicolor* are unsaturated fats that reflect the fatty acid composition of the feed and demonstrate the ability to biosynthesize some fatty acids such as C 20: 5n-3 (EPA) and C 22: 6n-3 (DHA). Furthermore Costa

et al. (2000) showed that polychaeta *N. diversicolor* fed low EPA and DHA content contained a higher amount of DHA than those contained in their diet. When worms are fed high DHA (dry feed for sea bream or shrimp larvae), its DHA profile is lower than that contained in the feed. The results also showed that *D. Pinnaticirris*, which was maintained on substrates with smaller sand content, had relatively higher AA, EPA, and DHA contents. These results are in accordance with that reported by Meunpol et al. (2005), they shows that Polychaeta *Perinereis* sp. living in the sand have a lower proportion of AA: EPA: DHA, which is 6.40%: 3.94%: 0.54% compared to Polychaeta *Marphysa* sp who lives in mud: 7.78%: 7.52%: 1, 34%.

Conclusion

D. pinaticirris could live on different substrate type with vegetable and animal feed type, with 100% survival. Animal feed and substrate type with lower sand content (8.78%) could provide higher growth of worm *D. pinaticirris* than vegetable and substrate types with high sand content (37.34% n 39.17%). Metabolic rate of worm *D. pinaticirris* was higher when maintained with vegetable feed and substrate with high sand content. Worm is oxiconformer organisms. Body composition (protein, fat, fiber, ash and NFE) worms were relatively same that on maintenance in different substrates. The composition of the fatty acid content of the worm *D. pinaticirris* body fed with vegetable was more complete than that fed the animal feed kept with the same substrate type. Worms fed on animals and kept on substrate types with low sand content indicate higher EPA and DHA content.

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