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Research Article
Evaluation of Fatty Acid and Amino Acid in Laor Worms (*Polychaetes*) from Tobelo, Indonesia Coastal Water

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Abstract

Background and Objective: Maluku water is a higher in marine biodiversity and having species richness of laor worms (*Polychaetes*). These worms used as food source by local people and they believe it's good for health. Understanding and assessment of biochemical compositions are important, thus, the purpose of this study was to determine the fatty acid and amino acid of laor worms in Tobelo coastal water and compared with others studies. **Materials and Methods:** Samples were taken using fishing net and then placed in plastic boxes. Some part of samples were fixed with 5% of formaldehyde solution then immediately observed the morphological under stereo microscope for taken photographs. Other samples were short cut then dried at 55°C in oven until constant weight. The dried samples were analyzed for total mineral, amino acid and fatty acid contents. **Results:** The result revealed that laor worms were varied in morphology. Laor worms with light green and dark green body color were dominant appeared followed by yellow-red and red-brown. Calcium and magnesium were found in samples as well as several metals. Total concentrations of Saturated Fatty Acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) were 15.42, 12.22 and 37.87%, respectively. About 15 amino acids were determined included 8 essential amino acids (41.31%) and 7 non-essential amino acids (53.34%). **Conclusion:** Overall there was a variation in composition of fatty acids and amino acids in laor worms from place to place. Totally, fatty acid and amino acid concentrations of laor worms in this study were higher than other coastal water in Indonesia.

Key words: Laor worms, morphology, marine polychaetes, fatty acids, amino acids

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Marine invertebrates such as; soft corals, sponges, tunicates and *Polychaetes* have long been recognized as the sources and diverse natural products since they provide a large proportion of bioactive compounds with different biological activities[1]. In the last several decades, research has expanded from land to ocean in order to find new leads for drug candidates. Marine ecosystem offers unlimited potential for biological and chemical diversity and comprise a continuous resource of immeasurable biological activities and natural products-based-commercial products[2,3].

Halmahera ocean is a part of indo-pacific area that is higher in marine biodiversity and having species richness of invertebrates such laor worms (*Polychaetes*). Laor is local name of Halmahera island in North Maluku and some parts of Maluku province. These worm normally used as food by local people and they believe that laor is good food for health[4,5]. The marine worms observed in Banda island has been reported since early 1900s and by native of Banda called cacing wawo or laor worms[6]. The laor is unique worms, they are benthic organisms actually, but during spawn or reproduction period they become plankton organisms and appeared in the surface water[7] and they are species richness. There are 13 different species of laor worms were found in Maluku coastal water, 2 of 13 species were identified as *Palolo viridis* and *Lysidce oele*[8]. Phenotypically identification of laor worm could be done based on the number of antennas[9]. However, in some cases it is hard to distinguish precisely because technical sample preparation failure. To date, it has developed molecular techniques to identify laor worm[10,11].

Study of biochemical compositions of laor worm in Ambon and Southeast Sulawesi coastal water have been reported[4,5,12,13]. Those studied shown that protein and fatty acid of laor worms ranged from 13.37-54.72% and 1.92-2.80% per 100 g, respectively. A literature have reviewed that biochemical compositions of marine polychaete could be varied between places due to different of ecological factors[14]. In the literatures explained that oceans are the source of a large group of structurally unique of natural products that mainly accumulated in invertebrates[15]. Understanding and assessment of biochemical compositions (fatty acid and amino acid) of laor worms are important for nutritional food and commercial uses such as; cosmetic and pharmaceutical industry[16,17]. In the marine environment, interactions of natural products mediated by marine basic life processes such as food. Food availability in sea water varies from place to place. Thus, comparing the biochemical composition of laor worms among different area are important to know their availability for natural products stock.

Tobelo city located in Halmahera Island, the appearances of laor worms in Tobelo coastal water occurred once or twice within a year, usual in May or June. During laor seasons, many people went to the shore and catch the laor worms using net. However, little is known about biochemical composition of laor worms and its potential for antibacterial (pathogen) agent. The laor worms were found also at several coastal water in Indonesia such as; Mataram, Ambon and Sulawesi coastal water. The purpose of this study was to determined the amino acids and fatty acids concentration in laor worms in Tobelo coastal water and comparing with others studies.

MATERIALS AND METHODS

Sampling site: Sampling site for laor worms collected was located in Tobelo coastal water (Fig. 1).

Samples collected and preparation: Samples collected were conducted early in the morning at 16th May, 2017 in Tobelo coastal water using fishing net. Sampled were placed in a plastic boxes. Some part of samples were fixed with 5% of formaldehyde solution and immediately observed the morphological under stereo microscope and taken photographs. Other parts were short cut then dried at 55°C in oven until constant weight. Dried sampled were used for total mineral, amino acid and fatty acid analyzed. Total minerals analyzed (included Mg, Ca, Zn, Cd and Cu) using Atomic Absorption Spectrophotometer (AAS) Shimadzu type AA 6300 flame emission refer to the laboratory procedures described by Kryazhov *et al.*[18]. While fatty acid were analyzed by using GC-MS Shimadzu QP-2010 Plus at 70 V and 320°C with auto-sampler, system equipped with Rtx-5 fused silica capillary column (30 m$\times$0.25 mm ID, film thickness 1.0 μm). Oven temperature was from 70°C for 5 min, than programmed heating from 70-220°C at the rate of 10°C for 6 min and from 280°C for 5 min, injector temperature 270°C. About 100% pure helium gas was used as a carrier gas at the constant flow rate 2.30 mL/min, split ratio 100, ion source temperature 225°C. The spectrum of the unknown components was compared with the spectrum of known components (Standard).

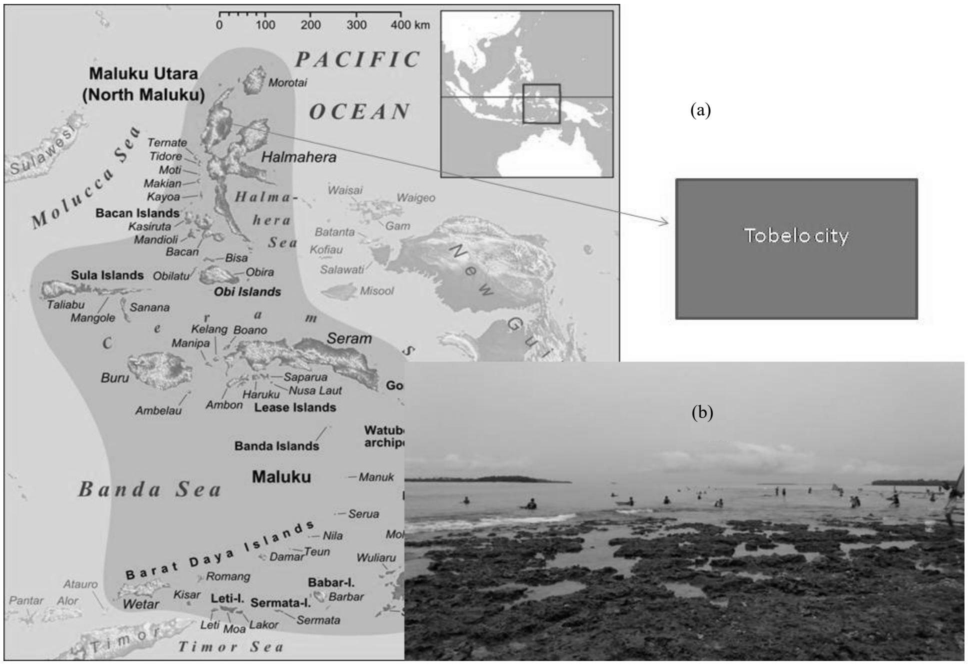

Fig. 1(a-b): Sampling site map, (a) Laor worms were collected in Tobelo coastal water and (b) Local people catching the laor worms for their food

RESULTS

Morphological aspects: Microscopical observation showed that the body of laor worms was segmented, round or flat body shape, clearly parapodia appearances. However, for some aspects such as; body color, body length and the amount of antennas were different. There were 6 different type of laor worms observed (Table 1). In addition, laor worms with light green and dark green body color were dominant appeared followed by yellow-red and red-brown. All samples varied in morphological aspects, indicating the possibility of species diversity.

Mineral and metals compounds: Selected minerals concentrations such as Ca and Mg were found in the samples. Calcium was up 1000 times higher than Mg. Also several metals such as; Zn, Cd and Cu were found. Zn concentration was higher compared Cd and Cu (Table 2). Cu concentration was lowest (<1 mg kg^{-1} dried weight).

Fatty acids: The fatty acid compositions of laor worms are given in Table 3 and grouped as Saturated Fatty Acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA).

There were 3 types of SFA (Nonadecanoic acid, palmitic acid and heptadecanoic acid) were determined. The content of nonadecanoic acid a bit higher than palmitic and heptadecanoic acids. For MUFA group, only 10.13 eicosadienoic acid C20:2 was detected. While in PUFA group, docosahexaenoic acid was higher followed by arachidonic and

Table 1: Morphological aspect of collected samples

Samples	Body color	Body length (cm)	Number of antennas
1	Light green	4.0	3
2	Dark green	7.0	3
3	Yellow-green	4.4	3
4	Yellow-red	5.5	5
5	Red-brown	6.0	3
6	Orange	6.4	2

Table 2: Selected minerals of laor worms

Selected minerals	Concentrations (mg kg^{-1} of dry weight)	References
Zn	29.540	Present study
	14.01	Latumahina[4]
Ca	2,740	Present study
	1,340	Nurhikma *et al.*[13]
	636.86	Latumahina[4]
Mg	149.73	Present study
	7,000	Nurhikma *et al.*[13]
Cu	0.85	Present study
	13.0	Latumahina[4]
Cd	10.15	Present study
	<0.24	Latumahina[4]

Table 3: Fatty acids content of laor worms

Groups	Fatty acid types	Concentrations (% w/w)
SFAs	Heptadecanoic acid C17:0	4.76
	Palmitic acid C16:0	4.36
	Nonadecanoic acid C19:0	6.30
	Total SFAs	15.42
MUFA	10,13 eicosadienoic acid C20:2	12.22
	Total MUFA	12.22
PUFAs	Docosahexaenoic acid C22:6	16.78
	Tetracosapentaenoic acid C24:5	6.32
	Arachidonic acid C20:4	14.77
	Total PUFAs	37.87

Table 4: Amino acids content of laor worm

Amino acids	Concentrations (% w/w)
Essential	
Threonine	4.63
Methionine	2.19
Valine	10.34
Phenylalanine	4.42
Isoleucine	7.55
Leucine	8.27
Lysine	0.98
Arginine	2.94
Total essential	41.31
Non-essential	
Aspartic acid	4.35
Glutamic acid	6.65
Asparagine	2.09
Serine	3.34
Glycine	12.84
Tyrosine	12.03
Alanine	12.04
Total non-essential	53.34

Table 5: Total fatty acids compared to other studies

Total fatty acids group	Concentration (% w/w)	References
SFA	15.42	Present study
	6.40	Nurhikma *et al.*[13]
	3.10	Asghari *et al.*[26]
MUFA	12.22	Present study
	0.48	Nurhikma *et al.*[13]
	3.00	Rettob *et al.*[25]
	3.85	Asghari *et al.*[26]
PUFA	37.87	Present study
	6.26	Nurhikma *et al.*[13]
	33.40	Rettob *et al.*[25]
	10.27	Asghari *et al.*[26]

tetracosapentaeonic, respectively. Overall total PUFAs two time higher than total SFAs and MUFA, indicating fatty acids composition in laor worms were majority consists of PUFA (Docosahexaenoic acid and Arachidonic acid, respectively).

Amino acids profile: About 15 of amino acids were found in laor worms, encompassed 8 of essential and 7 of non-essential are given in Table 4.

Essential amino acids: Among 8 essential amino acids were found in laor worms. Lysine, methionine and arginine were the lowest value, ranged from 0.98-2.19%. While the higher value was found for isoleucine, leucine and valine in the range of 7.55-10.34 (Table 4).

Table 6: Composition of amino acids in this study compared to other studies

Amino acids	Composition (%) Nurhikma *et al.*[13]	Rettob *et al.*[25]	Latumahina[4]	Present study
Essential				
Arginine	0.37	-	1.28	2.94
Histidine	3.04	0.34	0.31	nd
Isoleucine	1.1	0.62	0.68	7.55
Leucine	2.49	0.81	0.89	8.27
Lysine	2.19	0.77	1.08	0.98
Methionine	0.75	0.23	0.27	2.19
Phenylalanine	0.84	0.53	0.47	4.42
Threonine	1.78	0.57	0.69	4.63
Tryptophan	-	-	-	nd
Valine	1.08	0.68	0.68	10.34
Total	13.54	4.55	6.35	41.32
Non-essential				
Alanine	2.38	-	1.22	12.04
Asparagine	-	-	-	2.09
Aspartate	3.08	-	1.26	4.35
Cysteine	nd	-	1.63	nd
Glutamate	6.53	-	-	6.65
Glutamine	-	-	-	nd
Glycine	3.29	-	1.67	12.84
Serine	1.26	-	0.38	3.34
Proline	-	-	-	nd
Tyrosine	0.97	0.34	0.36	12.03
Total	16.54	0.34	6.52	53.34

Nd: Not determined

Non-essential amino acids: Among 7 non-essential amino acids were found in laor worms. Asparagine, serine and aspartic acid were the lowest value in the range of 2.09-4.35%. While tyrosine, alanine and glycine were highest value ranging from 12.03-12.84% (Table 4).

Comparison of amino acid and fatty acid concentration: The types of SFA, MUFA and PUFA in this study were less varied then other studies. However, a comparison of total concentrations of SFA, MSFA and with other studies were summarized in Table 5. Other researchers have found that, there was a variations in type and total amount of laor worm amino acid from place to place (Table 6).

DISCUSSION

Laor worms surrounding Maluku coastal water are higher in diversity. There were 5 family of laor worms from Ambon island have been identified based on body parts containing sex cells including Eunicidae, Euphrosinidae, Lumbrineridae, Nereidae and Scalibregmatidae[11]. The number of antennas could be done to indentify the type of laor worms. *Neridae* genus usually have 2 antennas, while *Lysidice* or *Palola* genus have 3 or 5 antennas[9]. In this study, the number of antennas were observed ranging from 2-5. Based on the number of antennas and its similar with the number were found in Ambon.

Previous researchers have reported that taxonomy of polychaetes are very complex[19,20] and its due to the large number of species and the diversity of forms. Even, it's still unsolved with a dilemma in terms of what taxa to include and what leave out[21].

A selected mineral and heavy metals were determined from the samples and it varied from place to place. The Ca concentration observed and was two times higher than Ca concentration in laor worms from Southeast Sulawesi and Ambon[4,13]. The presence of minerals and metals in laor worms attributed to their metabolisms and physiological processes. Cadmium, zinc and calcium influencing sexual maturation, egg laying, fertilization and zygote hatching[22-25].

There have many studies documenting fatty acids composition of marine invertebrates included polychaetes worms,[13,25-28]. Those studied shown the various fatty acid profiles were recorded from laor (*Polychaetes*) worms. In these study total concentrations of SFA, MUFA and PUFA were higher compared with other studies (Table 5).

The palmitic acid member of SFA was the most prevalent saturated in many *Polychaete* species[16]. However, in this study, palmitic acid was found bit lower than nonadecanoic acids (margaric acid). omega-3 polyunsaturated fatty acids (arachidonic acid and docosahexaenoic acid) were observed. It's very important for the brain, the eye the cardiovascular system and general human growth[29].

Moreover, a few recent studies have been reported that fatty acids could be used as antimicrobial agent. For example, arachidonic acid and docosahexaenoic acid could be used to induce the death of the *Plasmodium* species, inhibit viral replication, exert anti-hepatitis C virus activity and exhibit growth of the Gram (-) marine pathogen *Listonella anguillarum*, *multiresistant-Staphylococcus aureus* and fungicidal effects, suggested that omega-3 PUFAs function as endogenous antimicrobial molecule[30,31].

Amino acid compositions from laor worms have been documented by several researchers and varied in compositions, number and places (Table 6).

This study showed total 15 of amino acids was found. About 8 of 10 Essential Amino Acids (EAA) were determined, while 2 leaf EAA (histidine and tryptophan) was not detected. In contrast with other studied reported[13] that histidine was found in laor worms. Almost 7 of 10 non-essential amino acids were determined in this study, while cysteine, glutamine and praline were not detected. In contrast with asparagines, it was not recorded from other studies (Table 6).

Overall, total amino acid concentrations (both essential and non-essential) were higher compared to other studies reported[4,5,12,13]. The variation in compositions and concentrations of amino acids of laor worms might be related with their metabolisms during reproducing periods. Previous studies reported that essential amino acids (lysine and tyrosine) have important roles in reproductive functions and nutrition of developing gametes[32,33] while alanine, proline and glycine are considered as osmolytes[34]. The amino acids profiles recorded in this study revealed the potential roles of laor worms for sustainable marine ecosystem, because amino acid in polychaetes play an important role for nitrogen cycling in marine sediment[35] and also for nutritional supplement and pharmaceutical industry. Rapid increase resistant bacteria make necessary to develop a new antimicrobial agent[36]. In recent studies revealed that amino acids are potential for marine antimicrobial peptide[37], for instance phenylalanine and leucine which act as the hydrogelator and antimicrobial building block respectively for Gram-positive bacteria[38]. Seasonal changes in biochemical compositions of marine polychaete were found in Alexandria coastal water. For example fatty acid polyunsaturated fatty acids (PUFAs) were represented mainly by C20:5n-3, which attained the maximum percentage (76.8%) in winter and the minimum (49.6%) in summer, while amino acid were fluctuated seasonally[39]. The variation in biochemical compositions of laor worms between this study and other tropical locations are might be correlated with eco-physiological factors.

CONCLUSION

This study revealed the possibilities of species richness of laor worms in Tobelo coastal water. The fatty acids profile of laor worms showed that nonadecanoic acid (C 19:0) was higher in SFA group, eicosadienoic acid (C 20:2) was higher in MUFA group while docosahexaenoic acid (C 22:6) and arachidonic acid (C 20:4) were higher for PUFA group. There was totally of 15 amino acids recorded for the sample, consist of 8 Essential Amino Acids (EAA) and 7 Non-Essential Amino Acids (NEAA). Higher percentage was found in non-essential compared to essential amino acids. Overall there was a variation in composition of fatty acid and amino acid profiles in laor worms from place to place. Totally fatty acid and amino acid concentrations of laor worms in this study were higher than laor worms collected from other coastal water in Indonesia.

SIGNIFICANCE STATEMENT

Overall this study revealed that Maluku region especially in Tobelo waters have the availability and diversity in laor worm species. The biochemical profile of laor worms varied between places. Amino acids compositions (essential and non essential) in laor worms were almost all available, therefore it's potential as marine protein source for commercial and industrial purposes. The laor worms contained essential long chain omega-3 polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA). Until now, omega-3 polysaturated fatty acids are mostly and mostly obtained from fish or fish oil. This study showed the potential of laor can be used instead of fish for a source of fatty acids.

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