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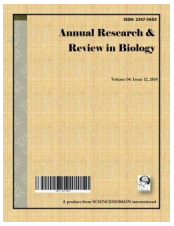

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No Difference in Nutritional Profiles of Wild and Cultured Juvenile Sandfish, *Holothuria scabra*

Asep Ridwanudin[1*], Lisa Fajar Indriana[1] and Andreas Kunzmann[2]

[1]Research and Development Division for Marine Bio Industry, Indonesian Institute of Sciences (LIPI), Lombok, Indonesia.
[2]Leibniz Centre for Tropical Marine Research (ZMT) GmbH, Fahrenheitstraße 6, 28359, Bremen, Germany.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AR and AK designed the study. Author AR performed experiments and wrote the first manuscript. Author LFI managed the literature searches. Author AK improved the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The demand for sandfish, *Holothuria scabra* has rapidly grown in the last decades. In order to better understand the quality of this species as human food, nutritional profiles of farmed and wild juvenile sandfish were investigated in this study by measuring the proximate body compositions of carbohydrates, proteins and lipids, including the amino acid and fatty acid contents. Body wall of juvenile wild sandfish from Medana and Sekotong in Lombok, Indonesia were compared with body wall of juveniles cultivated at Marine Bio Industry LIPI, and fed with mashed sea grass *Enhalus acoroides* leaves for a three months feeding period. The results show that protein, lipid and carbohydrate contents of juvenile farmed sandfish were similar to juvenile wild sandfish. Amino acid compositions of wild and farmed juvenile sandfish predominantly consist of glycine, glutamic acid and alanine. Polyunsaturated fatty acids (PUFAs) were the major fatty acids in the body wall of wild and farmed *H. scabra*. Arachidonic acid (C20:4n6) was recorded as the highest component among

**Corresponding author: E-mail: ase_rid@yahoo.com;*

all PUFAs. The contents of total PUFAs, total omega-3 and total omega-6 in the body wall of farmed *H. scabra* were slightly higher compared to wild *H. scabra*. In summary, both wild and farmed juvenile sandfish contain high amounts of valuable nutrients that have the potential to be used as a functional food for human health due to beneficial FA ratios, besides being adelicious and healthy seafood for human consumption.

Keywords: Body wall; amino acids; fatty acids; aquaculture; Indonesia.

1. INTRODUCTION

Holothuria scabra, known as a sandfish, is one of the tropical holothurians species that are widely distributed in the shallow water throughout Indo-Pacific regions from northern Africa to the central Pacific [1] with the habitat characteristics of sandy bottom and mud or muddy sandy [2]. The larvae and juveniles of *H. scabra* were also found in the leaves of seagrass *Enhalus acoroides* and *Thalassia hemprichii,* particularly after larvae settlement [3]. As a deposit feeder organism, holothurians utilize organic matter from microalgae, bacteria or benthic detritus in the sediment for growth [4,5]. Furthermore, to fulfill their nutrient requirements, some tropical holothurians select the appropriate grain size of sediment with high nutrient contents, and absorb it effectively [6]. On the other hand, decreasing organic matter content in deposited sediment that has been consumed by holothurians could inhibit algal blooms in the water [7], and some holothurians are also able to assimilate "waste" organic matter (excess feed and faeces) produced from aquaculture activities [8]. Therefore, holothurians play an important environmental role by reducing the effect of organic matter enrichment in sediments and recycle nutrient in the costal ecosystems [9].

The populations of sea cucumbers have been reported to decrease in some countries due to overfishing. Additional reasons are their slow growth [10] and that they are easily caught by fishermen [11]. Increasing demand and high value for sea cucumbers as a luxury seafood are also factors that cause depletion of sea cucumber populations [12]. This condition has promoted the development of sea cucumber farming to reduce the dependency on wild specimens and also to meet the growing market demand for sea cucumbers [13]. In addition, global catch and production (including aquaculture) of sea cucumber has increased from 2,300 t in 1950 to 30,500 t in 2006 [11] and consists mostly of Japanese sea cucumber *Apostichopus japonicus,* that is cultured in China [14]. Tropical sea cucumber production contributed 6% to the global total production in 2011, and tends to increase due to improvements in culturing methods [13,14].

Sea cucumbers are often sold as a dried product, known as bêche-de-mer or trepang with the most valuable tropical sea cucumber in dried seafood market being *H. scabra* [12]. Bêche-de-mer are highly consumed by Asian consumers, particularly the people in China [15] as a tonic food to prevent and treat illness [16]. The medical properties of sea cucumbers are associated with the presence of bioactive compounds such as triterpene glycosides [17], saponins [18] lectin [19] and phenolic components [20] that have physiological benefits for human health. The nutritional compositions of sea cucumbers have also been reported to contain high amounts of protein, but low amounts of lipids [21]. Nevertheless, lipids of sea cucumbers are rich in polyunsaturated fatty acids (PUFAs), particularly arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are highly suitable for human consumption [22]. Saito et al. [23] reported that proteins in the body wall of sea cucumbers mainly consist of insolubilized collagen fibers, and that they have a potential to be used in food and cosmetic industries due to good characteristics in solubility and high moisture absorption capabilities [24].

Nutritional profiles of sea cucumbers differ depending on the species [21] and seasonal variations [25]. Aydin et al. [22] found that different locations had also effects on nutritional contents of sea cucumbers, and it was associated with different diet contents in those locations. This was supported by Neto et al. [26], who reported that lipid contents in the body wall of some deep-sea holothurians were affected by diet supply in the sediment. Moreover, Yu et al. [27] and Zacarias-Soto and Olvera-Novoa [28] reported that SFA, MUFA and PUFA contents of the sea cucumber *A. japonicus* and the four-sided sea cucumber *Isostichopus badionotus* changed when fed with different diet sources, respectively. It seems that the nutritional

composition of sea cucumbers is linked to the diet supply, both in the wild and in aquaculture.

In order to successfully increase the sea cucumber production from aquaculture, it is important to understand the nutritional profile and hence the quality of the product sea cucumber. Numerous studies have been carried out to investigate the bioactive compounds and nutritional profiles of sea cucumbers by focusing on different species, seasonal variations or locations [21,22,29]. However, studies regarding nutritional profiles of certain sea cucumbers are still scarce, particularly involving also juvenile stages of *H. scabra* and controlled diet sources. For these reasons, this study aims to determine the nutritional profile of wild and cultured juvenile sandfish *H. scabra,* by analyzing protein and lipid contents of their body wall, including amino acid and fatty acid compositions.

2. MATERIALS AND METHODS

2.1 Sandfish *Holothuria scabra*

Twenty juveniles of *H. scabra* were collected from Medana in North Lombok and Sekotong in West Lombok, Indonesia. Samples were categorized based on the wet weight of *H. scabra* after 24 h starvation, i.e; A for the wet weight ≤ 10 gram, B for the wet weight 10 – 20 gram and C for the wet weight > 20 gram.

One hundred and eighty hatchery-reared juveniles of *H. scabra* with an average body weight of 0.025 gram were distributed into three aquarium tanks with 60 juveniles each. Those juveniles were produced in the hatchery of the Research and Development Division for Marine Bio Industry, Indonesian Institute of Sciences (LIPI) on Lombok. Juvenile *H. scabra* were fed with mashed sea grass *E. acoroides* leaves *ad libitum* once a day. Leaves collected from Medana in North Lombok were cut into small pieces (≈1 cm) and mixed with sea water at a ratio 1:1 (g/g), using a mixer machine until homogenous. The extracts of sea grass leaves were then filtered, and stored in the refrigerator at 4ºC using plastic bags until used. After three months feeding trial, ten juveniles of *H. scabra* from each tank with an average body weight of 1.50 gram were sampled. After pre-trials and some test analysis, without significant differences between the specimens, it was decided to pool samples and homogenize them for subsequent analysis.

Wild and cultured *H. scabra* were starved for 24 h for depuration. Sandfish were killed by immersing in ice water before dissection, then the body wall and internal organs were separated. The body wall of *H. scabra* and *E. acoroides* leaves were dried using a freeze dryer at -45ºC for two days. Samples were ground into powder and stored in the refrigerator at 4ºC before protein, lipid, amino acid and fatty acid analyses.

2.2 Chemical Analysis

The total protein, total lipid and total carbohydrate contents of sea cucumbers and seagrass were determined from 300 µl of homogenate, and measured with the plate reader infinite M200 Pro (Salzburg, Austria) using colorimetric methods. The total protein content was determined according to Bradford [30] using bovine serum albumin as a reference standard and the absorbance was recorded at 592 nm. Homogenate samples were precipitated in 100 µl of TCA and incubated at -20ºC for 10 minutes. Samples were then centrifuged (3500 rpm for 10 minutes at 15ºC), and the remaining pellets were re-suspended by adding 500 µl of NaOH 1M. Samples were heated at 60ºC for 30 minutes and then neutralized with HCl. The supernatant fraction after centrifugation was used for the carbohydrate analysis.

Total lipid content was determined according to Bligh and Dyer [31]. Total lipid from homogenate samples were extracted using 500 µl spectrophotometric grade of chloroform and methanol, respectively, and 250 µl of distilled water. The top phase of homogenates was then discarded after centrifugation (3500 rpm for 5 minutes at 15ºC). 500 µl of H_2SO_4 were added into 100 µl of lipid extract, and then incubated at 200ºC for 20 minutes using an oven. After cooling down to room temperature, 1.5 ml of distilled water were added into the lipid extract. Total lipid content was measured through absorbance at 375 nm using tripalmitine as a reference standard.

Carbohydrate contents were analyzed by adding 50 µl of phenol and 200 µl of H_2SO_4 into 50 µl of supernatant fraction. Samples were then incubated for 30 minutes at room temperature (dark condition). Total carbohydrate content was measured at 492 nm using glucose as a reference standard according to DuBois et al. [32].

Ash was determined by combustion at 540ºC in the muffle furnace for 24h based on the

procedures of the AOAC [33]. Samples were weighed (1-2 mg) in tin cups for total carbon and total nitrogen analyses, and in Ag cups for organic carbon and organic nitrogen analyses. The organic carbon and organic nitrogen contents were determined by acidification with 300 µL of 1M HCl and dried overnight at 40ºC to remove inorganic carbon and nitrogen from the samples. The percentage of total and organic carbon and nitrogen contents was measured using a CN analyzer EA3000 (Eurovector, Pavia, Italy).

Samples were hydrolyzed with 4 ml of 6N HCl for 22h at 110ºC for total amino acid analysis. After hydrolyzation, 1 ml of supernatant was evaporated at 60ºC and 60 mbar for 2h to remove HCl, and then diluted with 2 ml of acidic sodium citrate buffer (pH 2.65). Amino acid contents were determined using an amino acid analyzer (Biochrom 30 series; Biochrom, Ltd., UK).

For fatty acids analysis, the extraction of total lipids was carried out using the solvent mixture dichloromethane/methanol (2:1, v/v, CH_2Cl_2/MeOH). Samples of sandfish and sea grass were weighed to the nearest 0.1 g in glass test tubes, respectively, and then 4 ml of CH_2Cl_2/MeOH and 200 µl of internal standard (23:0) were added. Samples were homogenized using a potter homogenizer for 2 minutes at 1200 rpm, and then homogenized using ultrasonic for 30 seconds (on pulses: 0.2s; pulses to off: 1.0s; amplitude: 20%). After that, samples were extracted again using 4 ml of CH_2Cl_2/MeOH, and then homogenized using a potter homogenizer for 1.5 minutes at 1200 rpm. 2 ml of KCl 0.2% were added after homogenization using ultrasonic for 30 seconds (on pulses: 0.2s; pulses to off: 1.0s; amplitude: 20%). Samples were then gentlly hand agitated for 30 seconds, and the upper layer was removed after centrifugation (10 minutes, 3000 rpm, 2ºC). Trans-esterification was followed by adding 200 µl of internal standard (17:0) into lipid extract. 200 µl of hexane and 1 ml of H_2SO_4/MeOH 1% were then added, and heated for 4h at 80 ^{0}C. Lipid extracts were then strong hand agitated for 30 seconds after 4 ml of distilled water and 1.5 ml of hexane were added. The upper phase was transferred into a new test tube after centrifugation (10 minutes, 3000 rpm, 2ºC). The lower phase was then washed two times with 1.5 ml of hexane, followed by hand agitation and centrifugation. The upper phases were pooled and then evaporated completely using N_2 as a fatty acid methyl ester (FAME). FAME samples were then stored at -80ºC until analysis. Analysis and quantification of FAME were conducted using gas chromatography (Agilent 5977A MSD with 7890B GC-FID, USA) in the chemical laboratory of ZMT, Bremen, Germany.

3. RESULTS

The present study shows that the average contents of protein and lipid of *H. scabra* from Sekotong were lower than those from Medana. We did not find significant differences between the three size classes (Table 1). Furthermore, protein, lipid and carbohydrate contents in the body wall of farmed *H. scabra* were similar to the body wall of wild *H. scabra* (Table 2). However, the contents of ash, total carbon and total nitrogen of farmed *H. scabra* were higher compared to wild *H. scabra*. The percentage of organic carbon and organic nitrogen contents ranged from 4.8 to 12.9% and from 1.3 to 3.6%, respectively. The proximate body composition of sea cucumbers is presented in Table 1. In contrast, the essential amino acids (EAA) and non-essential amino acids (NEAA) compositions of farmed sandfish were lower compared to wild sandfish, except for arginine, glycine and alanine. In addition, total non-essential amino acids (TNEAA) were twice as high compared to total essential amino acids (TEAA), both in wild and farmed juvenile sandfish (Table 3).

The fatty acids content of *H. scabra* is given in Table 4. Farmed *H. scabra* had a higher content of arachidic acid (C20:0), lignoceric acid (C24:0), eicosenoic acid (C20:1), erucic acid (C22:1n9), nervonic acid (C24:1), linoleic acid (C18:2n6), alpha linolenic acid (C18:3n3), eicosapentaenoic acid (C20:5n3) and eicosadienoic acid (C20:2) than wild *H. scabra*. Except for the location Medana C, total PUFAs of wild and farmed *H. scabra* were higher compared to total SFA and total MUFA. The predominant PUFA was arachidonic acid (C20:4n6) with a range from 6.47 to 112.30 mg/g. Furthermore, the highest total PUFA content was achieved in the body wall of farmed *H. scabra* (194.25 mg/g). The contents of EPA (C20:5n3) were higher than DHA (C22:6n3), both in the body wall of wild and cultured *H. scabra* with a range from 1.91 to 44.89 mg/g and from 1.00 to 2.41 mg/g, respectively. In addition, total omega-3 were lower than total omega-6 in the body wall of wild and farmed *H. scabra* with a ratio of omega-6 to omega-3 fatty acid of *H. scabra* ranging from 0.29 to 0.55.

Table 1. Body composition, ash, carbon and nitrogen contents of dried body wall in wild and cultured sandfish *Holothuria scabra*[a]. A, B, C refers to wet weights of ≤ 10, 10-20 and > 20 g, farmed animals were also ≤ 10 g

Location	Wild					Farmed
	Sekotong A	Sekotong B	Medana A	Medana B	Medana C	
Proximate body compositions (mg/g)						
Protein	1.96	3.59	4.46	4.98	2.69	4.36
Lipid	1.57	2.06	2.46	3.26	2.05	2.83
Carbohydrate	0.87	1.23	1.1	0.75	0.79	1.39
Ash, carbon and nitrogen (%)						
Ash	54.52	53.63	48.13	55.40	45.58	58.14
Total carbon	15.30	14.55	16.83	17.10	15.72	17.75
Total nitrogen	1.29	1.13	2.30	2.61	1.35	3.54
Organic carbon	6.74	4.83	9.06	8.64	5.24	12.86
Organic nitrogen	1.81	1.24	2.42	2.39	1.29	3.55

[a] *Values are averages of triplicate determinations*

Table 2. Protein and lipid compositions, ash, carbon and nitrogen contents of sea grass *Enhalus acoroides* leaves[a]

	Content
Proximate compositions (mg/g)	
Protein	3.17
Lipid	14.63
Carbohydrate	0.86
Ash, carbon and nitrogen (%)	
Ash	47.58
Total carbon	22.69
Total nitrogen	2.15
Organic carbon	20.49
Organic nitrogen	2.09

[a] *Values are averages of triplicate determinations*

4. DISCUSSION

In this study we aimed at a comparison of the proximate body composition of sea cucumbers from aquaculture with wild specimens. Some studies on sea cucumbers have reported that the percentage of protein in the body wall (7.41 to 8.34%) were higher compared to lipid contents (0.23 to 0.50%) [34,35]. However, in the present study, the results showed that the contents of protein were low, and close to the lipid contents of wild and farmed juvenile *H. scabra* with an average of 3.7 and 2.4 mg/g, respectively. This difference might be attributed to the size of *H. scabra*. Most of the previous studies used commercial size of *H. scabra* (more than 50 grams on wet weight basis) for their experiments. Comparison of the present results with previous studies is difficult due to few studies about juvenile body compositions. On the other hand, the results of the present study showed that the average lipid contents of juvenile farmed and wild *H. scabra* were twice as high compared to lipid contents in adult (1.1%) *H. scabra* reported by Ibrahim et al. [36]. Although lipid content in the sediment was not analyzed in this study, lipid contents in seagrass *E. acoroides* were the most predominant nutrient content amongst others. Therefore, it could be assumed that high contents of lipids in *H. scabra* might be related to the high content of lipids in the sediment and seagrass *E. acoroides* that are used as a diet for *H. scabra* in this study. This result is in accordance with the results of Neto et al. [26] who reported that high lipid contents in the body wall of some deep-sea holothurians *Oneirophanta mutabilis*, *Pseudostichopus villosus* and *Psychropotes longicauda* were highly correlated with the lipid content in the surface of sediment as their food sources.

Low content of carbohydrate was detected in the body wall of wild and farmed juvenile *H. scabra* in this study, ranging from 0.75 to 1.23 mg/g. Our results are similar with Fei et al. [29] who reported that carbohydrate contents of *A. japonicus* ranged from 0.25 to 0.55%. They noted that seasonal variations significantly affect carbohydrate, lipid and protein contents of *A. japonicus*. The average ash content in this study was 52.6%, and similar with ash content in the body wall of *H. arguinensis* (50.2%) [35]. However, ash content in this study was twice as high than ash content in the body wall of processed *H. scabra* (after boiling, gutting and drying) [36]. Juvenile *H. scabra* in this study were gutted and dried without boiling, and it seems that the processing method can change the ash content of *H. scabra.* Haider et al. [37] reported that sea cucumbers *H. arenocila* and *Actinopyga mauritiana* contain high levels of sodium (5485 mg/100 g), potassium (570 mg/100 g), calcium (4155 mg/100 g) and magnesium (3310 mg/100 g), and it is likely that the high content of ash in this study is related with the mineral content of *H. scabra*.

Table 3. Amino acid composition of wild and cultured sandfish *Holothuria scabra*, and sea grass *Enhalus acoroides* leaves. A, B, C refers to wet weights of ≤ 10, 10-20 and > 20 g, farmed animals were also ≤ 10 g. TEAA = Total essential amino acids, TNEAA = Total non-essential amino acids

Amino acids	Wild					Farmed	Sea grass
	Sekotong A	Sekotong B	Medana A	Medana B	Medana C		
Essential amino acids							
Methionine	1.18	1.02	0.98	1.01	1.01	0.00	1.03
Lysine	3.34	3.44	2.67	3.28	3.50	3.15	4.97
Threonine	5.61	5.93	5.62	5.59	5.96	5.31	6.29
Valine	4.68	5.15	4.75	4.54	5.15	4.50	5.14
Phenylalanine	2.94	3.14	2.91	2.81	3.23	2.72	4.70
Histidine	1.59	1.60	1.54	1.48	1.56	1.44	2.15
Tryptophan	2.57	2.61	2.45	2.43	2.68	2.33	3.07
Isoleucine	3.18	3.34	3.20	3.02	3.35	2.94	4.40
Leucine	5.20	5.47	5.31	5.17	5.59	4.89	8.15
Arginine	5.78	5.64	5.92	5.85	5.63	5.99	5.00
TEAA	*36.08*	*37.34*	*35.35*	*35.18*	*37.66*	*33.27*	*44.90*
Lys/Arg	*0.58*	*0.61*	*0.45*	*0.56*	*0.62*	*0.52*	*0.99*
Non-essential amino acids							
Serine	5.65	5.94	5.69	5.65	5.99	5.37	7.12
Glutamic acid	11.71	11.65	12.05	11.93	12.09	11.55	10.33
Glycine	21.69	20.25	21.69	22.64	19.68	25.23	10.96
Alanine	11.10	10.76	11.15	11.35	10.75	11.84	9.62
Asparagine	9.22	9.84	9.37	9.27	9.94	8.80	11.47
Taurine	0.91	0.78	0.78	0.75	0.75	0.79	0.90
Ornithine	1.69	1.45	1.11	1.44	1.37	1.46	1.73
Cystein	1.10	1.21	1.07	1.03	1.04	0.91	1.09
TNEAA	*63.07*	*61.88*	*62.91*	*64.06*	*61.61*	*65.95*	*53.22*
TEAA/TNEAA	*0.57*	*0.60*	*0.56*	*0.55*	*0.61*	*0.54*	*0.84*

Table 4. Fatty acid composition of wild and cultured sandfish *Holothuria scabra*, and sea grass *Enhalus acoroides* leaves. A, B, C refers to wet weights of ≤ 10, 10-20 and > 20 g, farmed animals were also ≤ 10 g. TEAA = Total essential amino acids, TNEAA = Total non-essential amino acids

Fatty acids	Wild					Farmed	Sea grass (area %)
	Sekotong A	Sekotong B	Medana A	Medana B	Medana C		
C14:0	6.42	5.80	2.33	4.60	7.05	5.97	4.27
C16:0	35.32	30.61	2.19	20.08	45.71	37.67	28.64
C18:0	28.90	24.67	1.46	24.90	29.92	29.34	3.32
C20:0	22.95	20.14	2.77	16.88	24.14	30.99	0.38
C24:0	4.94	3.42	0.00	3.05	5.00	7.38	0.48
C16:1	25.19	22.74	1.14	10.82	35.01	20.79	7.14
C18:1n9	11.12	11.35	0.27	6.99	18.48	14.38	2.19
C18:1n7	31.01	38.57	0.67	17.07	40.33	23.72	8.80
C20:1	4.45	3.23	1.81	4.03	2.22	5.94	0.13
C22:1n9	4.97	3.16	1.91	2.33	2.49	5.09	0.13
C24:1	23.74	21.78	0.00	17.94	23.24	37.55	0.19
C18:2n6	2.10	1.59	0.00	1.12	2.69	4.63	18.67
C18:3n3	13.57	11.28	0.39	9.70	13.20	23.00	8.64
C20:4n6	100.00	103.50	6.47	112.30	45.46	101.73	1.70
C20:5n3	25.21	19.89	1.91	33.57	14.26	44.89	0.78
C20:2	5.44	5.87	8.27	6.21	5.01	18.51	0.65
C22:6n3	1.75	1.10	2.41	1.81	1.00	1.49	nd
∑SFA	*98.53*	*84.64*	*8.75*	*69.51*	*111.82*	*111.35*	*37.09*
∑MUFA	*100.48*	*100.83*	*5.80*	*59.18*	*121.77*	*107.47*	*18.58*
∑PUFA	*148.07*	*143.23*	*19.45*	*164.71*	*81.62*	*194.25*	*30.44*
∑ω3	*40.53*	*32.27*	*4.71*	*45.08*	*28.46*	*69.38*	*9.42*
∑ω6	*107.54*	*110.96*	*14.74*	*119.63*	*53.16*	*124.87*	*21.02*
ω6/ω3	*2.65*	*3.44*	*3.12*	*2.65*	*1.87*	*1.80*	*2.23*

nd (nPot detectable)

Table 3 shows that the values of essential and non-essential amino acid compositions of wild *H. scabra* were close to farmed *H. scabra* fed with seagrass. Diet did not change the contents of amino acid of sea cucumbers in this study and also in the previous study reported by Seo et al. [38], who found that amino acid contents in the body wall of *A. japonicus* were not significantly different when fed with various diet sources. Among amino acid compositions, glycine was the most abundant in the body wall of wild and farmed juvenile sandfish *H. scabra*. High content of glycine has also been found in the body wall of sea cucumbers *Stichopus japonicus*, *Thelenota ananas*, *Thelenota anax*, *H. fuscogilva*, *H. fuscopunctata*, *Actinopyga mauritiana*, *Actinopyga caerulea* and *Bohadschia argus* [21]. The average content of glycine in the body wall of juvenile *H. scabra* in this study was 22.5% of the total amino acids and it was 15% higher than the glycine content of adult *H. scabra* (38.1%) reported by Omron [39]. It indicates that juvenile *H. scabra* contain higher amounts of glycine than adult *H. scabra*. Glycine is a non-essential amino acid that has various physiological functions and crucial roles in nutrition and metabolism [40]. It has been reported that glycine could act as a therapeutic immune-nutrient to prevent and treat chronic liver diseases, e.g. alcoholic liver disease [41]. Senthilkumar et al. [42] also reported that dietary glycine could decrease lipid peroxidation in erythrocyte membranes, hepatocytes and plasma in rats by increasing antioxidant enzymes' activities.

Glutamic acid and alanine were also abundant in the body wall of juvenile *H. scabra* in this study with an average of 11.8 and 11.2%, respectively. This was similar with the results reported by Ibrahim et al. [36] who found that the contents of glutamic acid and alanine in the body wall of adult *H. scabra* were 10.3 and 13.5%, respectively. Roggartz et al. [35] also found that alanine was the highest content among amino acids composition in the body wall of *H. arguinensis* (12.7%), followed by glycine 10.9% and glutamic acid 7.6%. Different species of sea cucumber showed different contents of glycine, glutamic acid and alanine. However, high contents of those amino acids were mostly found in the body wall of sea cucumbers. Glutamic acid is naturally found in the food either free or bound with other amino acids to form structural protein [43]. Heat cooking or fermentation processes could hydrolyse structural proteins and then produce free glutamate that provides the umami taste in the food [44]. Another important aspect of amino acid compositions related to beneficial effects on human health is the low ratio of lysine to arginine in this study (average 0.56) and also in the previous studies in some species of sea cucumbers [21,35,37]. Low ratios of lysine to arginine could decrease cholesterol levels in the blood plasma of humans [45]. High contents of glycine and glutamic acid, and low ratios of lysine to arginine in the body wall of *H. scabra* in this study indicate the potential of *H. scabra* not only as tonic seafood, but also as delicious and healthy seafood for human consumption.

The average content of arachidonic acid (C20:4n6) in this study was 78.2 mg/g, and it was identified as a major fatty acid component in the body wall of wild and farmed *H. scabra*. Aydin et al. [22] also reported that arachidonic acid is abundant in fresh sea cucumber *H. tubulosa* and *H. polii* with values ranging from 10.6 to 14% and from 12.9 to 23.1%, respectively. They found that different locations had significantly higher arachidonic acid contents in sea cucumbers. High amount of arachidonic acid (31%) was also found in the body wall of sea cucumber *H. arguinensis* [35]. Arachidonic acid is known as a precursor in the synthesis of EPA [46]. Therefore, high contents of arachidonic acid were in line with the high contents of eicosapentaenoic acid (C20:5n3; EPA) in this study. In addition, dietary arachidonic acid is important in the treatment of coronary artery disease [47] due to its potential to decrease platelet aggregation and increase bleeding times [48]. Arachidonic acid (ARA) and eicosapentaenoic acid (EPA) contents of farmed *H. scabra* fed with seagrass *Enhalus acoroides* in this study were slightly higher than wild *H. scabra.* It might be related with high content of linoleic acid (C18:2n6; LA) (18.67%) and alpha linolenic acid (C18:3n3; ALA) (8.64%) in seagrass *E. acoroides*. The chemical reactions in the body could transform linoleic acid (LA) into arachidonic acid (AA), and alpha linolenic acid (ALA) into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [49]. This is also supported by Zacarias-Soto and Olvera-Novoa [28], who reported that sea cucumber *Isostichopus badionotus* was able to desaturate and elongate fatty acids, when fed with diets containing high amounts of LA and ALA.

Eicosapentaenoic acid (C20:5n3; EPA) and docosahexaenoic acid (C22:6n3; DHA) contents of wild and farmed *H. scabra* in this study ranged

from 1.9 to 44.9 mg/g and from 1.00 to 2.4 mg/g, respectively. The content of EPA was higher compared to DHA in *H. scabra*, and it was also found in other species of sea cucumbers reported by Zhong et al. [34] who found that fresh sea cucumber *Curcumaria frondosa* contain 46.1% EPA and 5.0% DHA. High amount of EPA (10%) was also found in the body wall of sea cucumber *H. arguinensis* [35]. However, sea cucumbers *H. tubulosa*, *H. polli* and *H. mammata* were containing higher amounts of DHA than EPA, with average values of 12.4, 8.0 and 10.3%, respectively [22]. This clearly indicates that sea cucumbers are a good source for EPA and DHA. Eicosapentaenoic acid (C20:5n3; EPA) and docosahexaenoic acid (C22:6n3; DHA) belong to the omega-3 series of polyunsaturated fatty acids that influence physiological and pathological processes in humans [46]. EPA has potential to be used as wound healing agent due to being involved in prostaglandin inhibition and being associated with the ability to initiate tissue repair [50], while docosahexaenoic acid (C22:6n3; DHA) has been used to prevent and treat some diseases such as hypertension, arthritis, diabetes mellitus, thrombosis, heart disease and some cancers, and it is also essential for growth and development of brain function [47]. In addition, *H. scabra* is also good for human health due to a balanced ratio of omega-6 to omega-3 fatty acids, with values ranging from 1.8 to 3.4. High ratios of omega-6 to omega-3 in the diet could increase the pathogenesis of cardiovascular diseases, however, low ratios of omega-6 to omega-3 could protect against degenerative diseases [49].

5. CONCLUSION

The present study found that protein and lipid compositions, amino acid contents, total PUFAs, total omega-3 and total omega-6 of farmed *H. scabra* were slightly higher in farmed, as compared to wild *H. scabra* from different locations. This also demonstrates that the artificial feed provided in aquaculture is suitable. With regard to amino acids, *H. scabra* predominantly consist of glycine, glutamic acid and alanine. Polyunsaturated fatty acids (PUFAs) were the major fatty acids in the body wall of wild and farmed *H. scabra* with arachidonic acid (C20:4n6) being the highest component among all PUFAs. Both, wild and farmed juvenile *H. scabra* are an ideal human food due to a balanced nutritional profile with clear benefits for human health.

ETHICAL APPROVAL

Written ethnical approval has been collected and preserved by the authors as the institutional standard.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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