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DYNAMIC FATTY ACID PROFILES OF ASIAN SEA BASS (*Lates calcarifer)* FROM SETIU WETLANDS, EAST COAST PENINSULAR MALAYSIA

Profil Dinamik Asid Lemak Ikan Siakap Asia (*Lates calcarifer)* Dari Tanah Bencah Setiu, Pantai Timur Semenanjung Malaysia

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Abstract

The oil lipids from the tissue and liver of locally grown Asian sea bass (*Lates calcarifer*) in an aquaculture of Setiu Wetlands, Terengganu, were extracted and analyzed for their fatty acid composition. The obtained fatty acid profiles revealed that both oils consisted of high amounts of saturated and monounsaturated fatty acids (SFA and MUFA, respectively) ranging within 35-54%, while polyunsaturated fatty acid (PUFA) were detected in lower proportions (10-15%). Among the fatty acids detected in the *L. calcarifer* tissue, C24:1 had the highest proportion (31.14%), followed by C16:0, C18:1n9 (*trans*), and C18:0. In contrast, C16:0 was the predominant fatty acid in the liver oil accounting for 33.88%, followed by C18:1n9 (*cis*), C24:1, and C18:0. The higher content of the extracted tissue and liver oils in MUFAs and SFAs was attributed to the salinity and temperature of the estuary water and the different dietary intake during the monsoon season. The fatty acid profiles were also compared with those obtained for *L. calcarifer* grown in other regions, indicating that the growing area of fish can affect the distribution of oil in the fish body as well as the lipid profiles within the same species.

Keywords: Asian sea bass, eicosapentaenoic acid, docosahexaenoic acid, liver, Setiu Wetlands, tissue

Abstrak

Minyak dari tisu dan hati ikan siakap Asia tempatan (*Lates calcarifer*) yang diternak dalam akuakultur di Tanah Bencah Setiu, Terengganu, telah diekstrak dan dianalisis untuk mengetahui komposisi asid lemak mereka. Profil asid lemak yang diperolehi mendedahkan bahawa kedua-dua minyak terdiri daripada jumlah asid lemak tepu dan mono tak tepu yang tinggi (SFAs dan MUFAs, masing-masing) antara 35-54%, manakala asid lemak poli tak tepu (PUFAs) dikesan dalam kadar yang lebih rendah (10-15%). Antara asid lemak yang dikesan dalam tisu *L.calcarifer*, C24:1 mempunyai bahagian tertinggi (31.14%), diikuti C16:0, C18:1n9 (*trans*), dan C18:0. Sebaliknya, C16:0 adalah asid lemak utama dalam minyak hati memberikan 33.88%, diikuti oleh C18:1n9 (*cis*), C24:1, dan C18:0. Kandungan tinggi MUFAs dan SFAs yang diekstrak dari minyak tisu dan minyak hati dalam

adalah disebabkan oleh perbezaan saliniti dan suhu air muara serta tahap pengambilan pemakanan yang berbeza pada musim tengkujuh. Profil asid lemak juga dibandingkan dengan untuk *L. calcarifer* yang didapati di kawasan lain, menunjukkan bahawa kawasan penternakan ikan boleh memberi kesan terhadap kuantiti minyak di dalam badan ikan serta profil lipid dalam spesies yang sama.

Kata kunci: ikan siakap, asid eikosapentaenoik, asid dokosaheksaenoik, hati, Tanah Bencah Setiu, tisu

Introduction

Natural fatty acids are aliphatic (saturated or unsaturated) monocarboxylic acids derived from animal or vegetable fat, oil, or wax, and they consist of a chain of 4-28 commonly unbranched and even-numbered carbons [1]. Fatty acids in animals are formed from carbohydrates during lactation in the liver, adipose tissue, and mammary glands. Polyunsaturated fatty acids (PUFAs), which may contain either omega-6 (n-6) or omega-3 (n-3) double bonds, are essential dietary fatty acids [2]. In fish and fish oils, they are mainly found as long-chain PUFA (LC-PUFA) with docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and docosapentaenoic acid (DPA, 22:5n-3) being the most significant n-3 PUFA [3]. LC n-3 PUFA in fish have gained particular scientific and public interest due to their benefits to animal and human health [4]. According to Yi et al., EPA and DHA are vital for the prevention of cardiovascular diseases, the reduction of the inflammatory rate after surgery, and the growth and functional development of the infant brain, while they can also benefit the therapeutic potential in mental conditions and maintain the normal function of the adult brain [5]. Although n-3 PUFA are the essential nutrient, n-3 fatty acids, they cannot be synthesized by the human body [4].

Lates calcarifer, also known as Barramundi or Asian sea bass (in Malay: Kakap Putih or Siakap), can be normally found in Southeast Asia and Northern Australia. Its abundance in individual nature basins depends on the river morphology and the fishing pressure. In such microhabitats, *L. calcarifer* is much more affected, allowing the collection of more quantitative information. The spawning grounds of *L. calcarifer* are generally complex, but they are most likely determined by the presence of high salinity such as in estuaries, coastal mudflats headlands, and other nearshore waters. In particular, the spawning grounds can be found in shallow (up to 2 m deep) lateral gutters near river mouths, but not close to the main channel to be protected against high tidal sand and/or mud flows—[6-8]. Interestingly, the LC-PUFA biosynthesis in diadromous fish, such as *L. calcarifer*, has caught the attraction of researchers as the dietary PUFA requirement is different and the capabilities of converting PUFA to LC-PUFA are within that of marine and freshwater fish species [9]. Hence, in this study, the fatty acid compositions in fish oil extracted from *L. calcarifer* tissue and liver was analyzed. In addition, the results were compared with those obtained for *L. calcarifer* grown in other regions.

Materials and Methods

Study area and sample collection

Aqua cultured *L. calcarifer* (n = 10) of the same weight (~300 g) were caught during Northeast Monsoon season at a fish farm in Setiu Wetland, Estuary of Terengganu, East Coast Peninsular Malaysia (Figure 1), and stored at 4 °C in the ice box and kept frozen at -8 °C in the laboratory until further analysis.

Lipid extraction

The collected fish were gutted, washed, and dissected. For each sample, 20 g of flesh and liver were weighted and prepared for lipid extraction. The Bligh–Dyer method [10] was used with some modifications for the lipid extraction using a sample/chloroform/methanol mixture of 1:1:2. In brief, each fish flesh or liver sample was homogenized in 20 mL chloroform and 40 mL methanol, forming a monophasic system. The mixture was then homogenized again using 20 mL chloroform, followed by the addition of 20 mL distilled water. After filtration of the mixture, a biphasic system was finally obtained, which was allowed to separate. The lower layer of the mixture was collected, and the remaining sample was mixed with 10 mL chloroform and filtered. The lipid content in each sample was determined

gravimetrically by evaporating the chloroform layer in the presence of nitrogen.

Transesterification of fatty acid methyl ester (FAME)

Transesterification reaction of fish oils was carried out using a one-step reaction using methoxide reagent. In brief, a solution of sodium methoxide (5.402 g) was prepared in 100 mL. *n*-Hexane (1 mL) was then added to 0.1 mL of the oil sample. Afterward, 1 mL of the sodium methoxide solution was added to the oil solution and stirred vigorously for 30-60 s using a vortex stirrer. The mixture was then allowed to stand for 30 min to separate the fatty acid methyl ester (FAME) from the cloudy aqueous layer.

Fatty acid analysis by gas chromatography–flame ionization detector

A Shimadzu GC-2011 gas chromatographic (GC) system equipped with a flame ionization detector (FID) (Shimadzu Corp., Japan) and a BPX 70 capillary column (30 m × 0.25 mm × 0.25 mm i.d.) (SGE Analytical Science, Australia) were used to determine and quantify the FAME chemical composition. The injection volume was 1 µL in split injection mode with a split ratio of 1:20. The total airflow and column flow rates were set at 400 and 0.92 mL/min, respectively, and the hydrogen and helium (carrier gas) gas flow rates were set at 40 and 30 mL/min, respectively. Moreover, the temperatures of the injection port and the detector were set at 250 °C and 255 °C, respectively, while the injector port pressure was set at 75.4 kPa. The oven temperature program involved heating at 40 °C for 1 min, followed by a temperature increase to 140 °C at a rate of 10 °C/min, where it was maintained for 5 min. Finally, the oven temperature was increased to 240 °C at a rate of 4 °C/min, where it was maintained for another 5 min. Supelco 37 Components FAME Mix was used as external standard in determining the identity of the FAME chemical composition obtained from GC-FID.

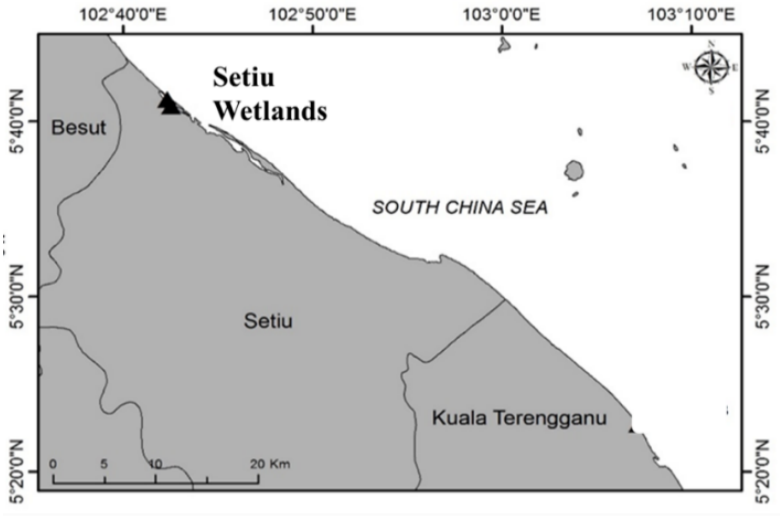

Figure 1. Location of Setiu Wetland, Estuary of Terengganu, East Coast Peninsular Malaysia.

Results and Discussion

Fatty acid composition of the *L. calcarifer* tissue and liver obtained from Setiu Wetlands

Eight types of major fatty acids were detected in the *L. calcarifer* tissue obtained from Setiu Wetlands, Terengganu using a GC-FID system (Figure 2, Table 1). Among them, the highest proportion (31.14%) was found for C24:1, followed by C16:0 (19.42%), C18:1n9 (*trans*) (18.02%), and C18:0 (11.36%). Moreover, in the total content of total fatty acids (TFA) (17.86%) in the *L. calcarifer* tissue, C20:2, C18:2n6 (cis), C24:0, and C20:3n3 accounted for 5.86%, 5.49%, 4.11%, and 2.4%, respectively. In addition, the total content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and PUFA was 35.73 ± 0.01%, 49.81 ± 0.01%, and 14.45 ± 0.01%, respectively, while the n-3/n-6 ratio of PUFAs was 0.43.

Meanwhile, as shown in Figure 3 and Table 1, nine types of major fatty acids were detected in the *L. calcarifer* liver collected from Setiu Wetland, Terengganu. C16:0 was the predominant fatty acid corresponding to 33.88% of the total fatty acid content, followed by C18:1n9 (cis) (16.67%), C24:1 (13.30%), and C18:0 (12.73%). Moreover, in the total content of TFA (15.35%) in the *L. calcarifer* liver, 6.48%, 3.22%, 2.35%, 2.31%, and 0.99% were attributed to C18:2n6 (trans), C16:1, C14:0, C22:1n9, and C22:2, respectively. Moreover, the total content of SFA, MUFA, and PUFA was 53.77%, 35.94%, and 9.74%, respectively, and the n-3/n-6 ratio of PUFA was 0.11.

The fatty acids determined in the *L. calcarifer* tissue and liver were compared, indicating that the tissue of *L. calcarifer* contained the largest amount of MUFA (49.81 ± 0.01%), while the SFA were the predominant fatty acids (53.77%) in the *L. calcarifer* liver. Moreover, large amounts of palmitic acid (C16:0) were found in both fish body parts, whereas α-linoleic acid (C18:3n-3) was the least detectable fatty acid (up to 0.87%). The finding is supported by Daniela in his report in 2015, as he reported that the lipids are stored in the form of SFA and MUFA in fish to generate energy and that both SFA and MUFA can be synthesized de novo [11]. It has also been found that the different lipid classes are affected by the water salinity [12]. Herein, the water salinity of Setiu Wetland was 17.8 ppt, i.e., within the range of fresh water and seawater salinity (0.5–30.0 ppt), as the estuary is a mixture of fresh water and seawater (brackish water). Likewise, when the water salinity is 5–18 ppt, the water is called mesohaline water. Moreover, the water temperature has a significant effect on the fish lipid classes. It suggests that the high water temperature is not suitable for the production of long-chain PUFA, as Malaysia is located near to equatorial with tropical climate. The long-chain PUFA are mainly found in high amounts at low water temperatures [13] and a similar result has been previously reported, where a large amount of UFA was clearly observed in the colder regions [14]. However, *L. calcarifer* from Setiu Wetland live in a mesohaline environment (17.8 ppt) with a warm water temperature (31.7 °C), which explains the predominance of MUFA over PUFA in the *L. calcarifer* tissue. Furthermore, Rabeh et al. have reported that the content of SFA and MUFA is significantly higher in seawater than in freshwater reared fish, while n-3 PUFA predominate in freshwater reared fish [12]. The different fatty acid compositions in freshwater and marine fish may be related to the physiological adaption of fish to the environment. In fresh water, SFA and UFA are used to produce energy or synthesize long chain-PUFA, whereas marine fish need these fatty acids to regulate the osmotic pressure [12, 15].

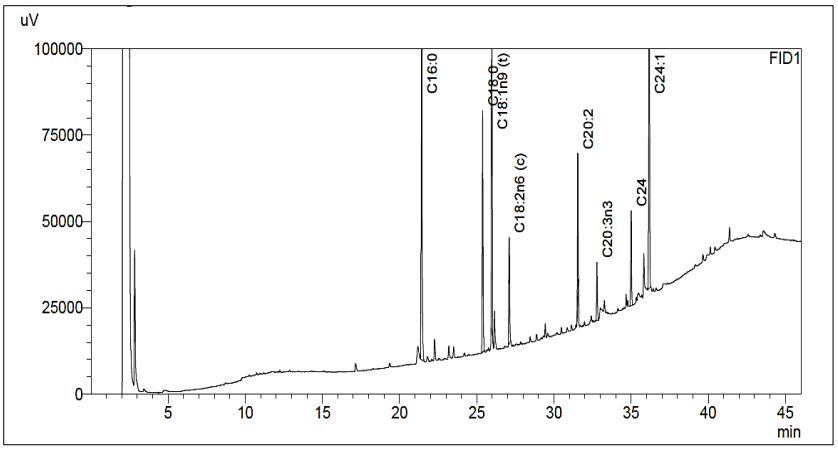

Figure 2. Chromatogram of the *L. calcarifer* tissue oil.

Table 1. Main fatty acids identified in the *L. calcarifer* tissue and liver

Fatty Acids	**Name**	**Percentage (%)**	
		Tissue	**Liver**
C14:0	Myristic acid	n.d.	2.35
C16:0	Palmitic acid	19.42	33.88
C18:0	Stearic acid	11.36	12.73
C24:0	Lignoceric acid	4.11	0.46
Total SFA		35.73	53.77
C16:1	Palmitoleic acid	0.42	3.22
C18:1n9 (*trans*)	Elaidic acid	18.02	n.d.
C18:1n9 (*cis*)	Oleic acid	0.05	16.67
C22:1n9	Erucic acid	0.05	2.31
C24:1	Nervonic acid	31.14	13.30
Total MUFA		49.81	35.94
C18:3n3	α-Linolenic acid	n.d.	0.87
C20:3n3	*cis*-11,14,17-Eicostrienoic acid	2.40	n.d.
C20:5n3	*cis*-5,8,11,14,17-Eicosapentaenoic acid	0.08	n.d.
C18:2n6 (*trans*)	Linolelaidic acid	0.10	6.48
C18:2n6 (*cis*)	Linoleic acid	5.49	n.d.
C20:2	*cis*-11,14-Eicocadienoic acid	5.86	0.56
C22:2	*cis*-13,16-Docosadienoic acid	0.29	0.99
Total PUFA		14.45	9.74
n-3/n-6 PUFA ratio		0.43	0.11

n.d.: not detectabl

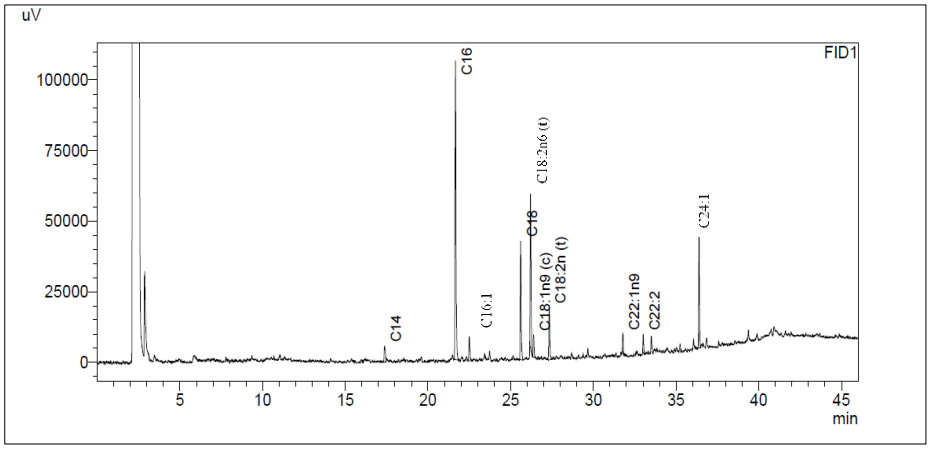

Figure 3. Chromatogram of the *L. calcarifer* liver oil.

Comparison of *L. calcarifer* tissue fatty acid profiles from Setiu Wetland and other regions

The fatty acid profile of the *L. calcarifer* tissue oil obtained from Setiu Wetland were also compared with the corresponding results reported for *L. calcarifer* from other regions. As shown in Table 2, C16:0 dominated in all *L. calcarifer* tissue oils obtained from Setiu Wetland and other regions (9.14-39.41%), except for the oil extracted from the tissue of Kelantan and Australia aqua cultured *L. calcarifer* [8, 16]. In these regions, the major fatty acids were C18:1n-9 and C18:1n-9 (*cis*). Instead, for wild estuary fish species including from Vietnam [16], Sri Lanka [17] and Myanmar [18], C16:0 and C18:0 were the major SFAs, while C18:1 was the major MUFA (Table 2). Moreover, C18:3n-3 was detected in most regions as a minor fatty acid (0.06%–1.9%) but was not detectable in *L. calcarifer* from Setiu Wetland and Selangor. This difference could be attributed to the inability of vertebrates, including fish, to produce PUFAs de novo [13, 15, 19]. Additionally, the lack of Δ12 and Δ15 double bonds in n-3 would reduce the chance to be desaturate C18:1n9 to C18:2n6 and subsequently to C18:3n3 by fatty acid desaturases [20].

Furthermore, *L. calcarifer* from all compared regions had a high content of C18 PUFAs, indicating that despite the growing area, the fatty acid elongase of *L. calcarifer* had a similar pattern to the elongation of very long chain fatty acids protein 5 (known as Elovl5) with similar elongation activity toward C18 and C20 PUFAs, but lower toward C22, as previously reported [8]. Moreover, as already mentioned, C16:0 was the SFA with the highest percentage in the *L. calcarifer* tissue fish oil obtained from Setiu Wetland accounting for 19.42%. A similar trend was also observed in the *L. calcarifer* tissue oils obtained from the other compared areas, which was in line with the results reported by Pervin et al. in 2012 [7]. In contrast, C18:1, which is one of the main substrates for mitochondrial β-oxidation catabolized through the tricarboxylic acid cycle to generate energy, was the major MUFA, in which C16:0 and C18:1n-9 fatty acids are key components for energy production in fish, as reported previously [21]. It is worth to note that the amount of C18 PUFAs was relatively high in the species obtained Setiu Wetlands and Kelantan, whereas both located at the South China Sea region. Nevertheless, this finding was in agreement with previously reported study [21] indicated that *L. calcarifer* obtained from East Peninsular Malaysia had a high total content of C18 PUFAs, indicating the greater amount of C18 PUFAs and the lower content of C20 and C22 fatty acids in local freshwater fish than in seawater fish. Moreover, C16:0 was the most dominant SFA, corresponding to 33.88% and 19.4 ± 0.6% in Setiu Wetland and Kelantan aquacultures, respectively,

complying with the findings of Nath et al. conducted in 2014 [22].

In comparison, C18:3n-3 was the minor fatty acid in the *L. calcarifer* liver oil extracted from all regions (ranging from 0.17% and 1.9%), except for the present study obtained from the Setiu Wetlands. This result was supported by Ackman in his findings in 1967 [14], who reported that the fish cannot synthesize C18:3n-3, and in agreement with Tu and his co-worker results obtained in 2013 [9], who found that the C18:3n-3 level remained low although *L. calcarifer* was fed a C18:3n-3-rich diet. As previously mentioned, the low C18:3n-3 content could be further explained by the lack of Δ12 and Δ15 double bonds in the n-3 PUFA, which serve as initiators of the C18:1n-9 desaturation to C18:2n-6 and C18:3n-3. This earlier study also showed that *L. calcarifer* lacks pathways for long chain-PUFAs biosynthesis, further limiting the amount of C18:3n-3 formed [20]. Thus, it was revealed that the predominant fatty acids in fish tissue oil and in general the lipid content may vary between different growing areas of the same species.

Table 2. Comparison of selected fatty acids identified in the tissue oil of *L. calcarifer* obtained from Malaysia (local) and other (overseas) regions

		Content of Fatty Acids (%)								Total Content (%)			n-3/n-6 PUFAs Ratio
		C16:0	C18:0	C18: 1n-7	C18: 1n-9 (*trans*)	C18: 1n-9 (*cis*)	C18: 3n-3	C20: 5n-3	C22: 6n-3	SFAs	MUFAs	PUFAs	
Present study	Setiu Wetland, Terengganu	19.42	11.36	n.d.	18.02	0.05	n.d.	0.08	n.d.	35.73	49.81	14.45	0.43
Locals	Kelantan[8] (Aquaculture)	19.5 ± 0.6	7.4 ± 0.6	2.6 ± 0.1	19.6 ± 1.1[a]		1.2 ± 0.2	4.4 ± 0.3	10.2 ± 1.6	30.7 ± 1.0	28.7 ± 1.7	40.6 ± 1.6	1.0 ± 0.1
	Selangor[19]	9.14	4.17	-	7.20[b]		n.d.	-	-	14.71	17.97[c]		-
Overseas	Sri Lanka[17] (Wild)	22.90	-	12.44	-	11.53	-	-	27.59	26.67	25.73	47.60	2.67
	India[22]	39.41	13.68	-	12.96	2.76	0.17	0.19	0.21	66.23	28.69	5.05	1.03
	Vietnam[16] (Wild)	26.6 ± 1.13	9.5 ± 0.40	2.5 ± 0.09	-	17.3 ± 2.52	0.5 ± 0.08	2.5 ± 0.20	13.3 ± 1.29	41.0	24.8	25.0	15.80
	Vietnam[16] (Aquaculture)	22.8 ± 2.43	8.3 ± 2.61	3.0 ± 0.58	-	15.6 ± 5.44	1.1 ± 0.34	3.0 ± 1.06	9.2 ± 4.75	36.9	24.6	26.0	12.20
	Australia[16] (Wild)	26.9 ± 0.61	6.7 ± 0.57	2.5 ± 0.06	-	12.8 ± 0.73	1.9 ± 0.07	3.3 ± 0.17	4.1 ± 0.31	28.0	43.6	18.5	7.40
	Australia[16] (Aquaculture)	19.3 ± 0.47	5.4 ± 0.26	2.6 ± 0.04	-	33.7 ± 0.53	1.5 ± 0.06	3.0 ± 0.11	3.1 ± 0.33	43.5	25.6	21.2	6.2
	Myanmar[18] (Wild)	23.69	9.36	3.22	8.09[d]		1.86	2.97	18.68	34.15	18.91	39.64	1.80

n.d.: not detectable; -: not reported; [a,d]Total percentage of C18:1n9; [b]Total percentage of C18:1; [c]Total percentage of U

Conclusion

The lipids of the tissue and liver of *L. calcarifer* grown in an aquaculture in Setiu Wetland were extracted to determine their fatty acid profiles. The results showed that the tissue oil had a high content of MUFA (44.81%), while the highest amount of SFA was found in the *L. calcarifer* liver (53.71%). However, both tissue and liver had the lowest PUFA content, accounting for 14.45% and 9.74%, respectively. Similar lipid composition results have been reported in studies using wild or aqua cultured *L. calcarifer* grown in other regions. The comparison of the findings in the *L. calcarifer* tissue of the current study with those of other regions indicated that most of the compared regions had a high content of either SFA or MUFA. Further comparison of the lipid composition in the *L. calcarifer* liver revealed that fish from Setiu Wetland had the highest amount of MUFA (33.88%) compared to the *L. calcarifer* liver from the Kelantan aquaculture, where PUFA predominated (37%). These differences were attributed to the effect of temperature, different dietary intake, and salinity of estuary water on the fatty acid profiles, as well as on the unique properties of SFA and MUFA. Nevertheless, C18:1n-9 was found to be the predominant MUFA in all regions. Given that C18:1 is one of the main sources for energy production and that C18:1n-9 can decrease the risk of breast cancer and cardiovascular diseases, it could be used to synthetically prepare fish oil with high nutritional value. However, further research studies are needed, as there are some arguments regarding the benefits of C18:1n-9 to human health.

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