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Determination of fatty acids from phospholipid subclasses in the total body and cephalopedal tissues from edible snail *Helix lucorum*

[Yenilebilir salyangoz *Helix lucorum*'un sefalopedal ve total vücut dokusundan elde edilen fosfolipit alt sınıflarına ait yağ asitlerinin belirlenmesi]

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ABSTRACT

Objective: The objective of the study was to examine the nutritive value of *Helix lucorum* meat and to create awareness about fatty acid composition of phospholipid subclasses of the total body and cephalopedal tissues of the snail.

Methods: Thin layer chromatography plates contained lipid samples placed in the chromatography tank containing: chloroform/ethanol/water/triethylamine. The phospholipid subclasses were dissolved in about 5 ml of methanol and 5 drop of sulfuric acid. The mixture was refluxed for 2 h to form fatty acid methyl esters at 85 °C. Fatty acids were detected by Gas chromatography.

Results: The most noteworthy result was the high level of C20:2ω6 in PE (10.49%-11.35%) and PC (17.33%-12.96%). Appreciable quantity of essential fatty acid C18:2ω6 was determined in PC (20.85%-17.46%) and PE (16.88%-17.53%) from both tissues. Precursor of eicosanoids, C20:4ω6 was found apparently high in PI, PS and PE of the total body. The highest level of ΣPUFA was 63.90% in PE from total body whereas the highest level of ΣSFA was 60.79% in PI from the cephalopedal. ΣMUFA level was pretty low in PI, PS and PE.

Conclusion: The study is a guide for biochemical and nutritional value of edible snails and can be useful for further investigation on physiological and systematic studies of other species.

Key Words: Phospholipid subclasses, fatty acids, *Helix lucorum*, edible land snail

Conflict of Interest: The authors have no conflict of interest.

ÖZET

Amaç: Bu çalışmanın amacı, *Helix lucorum*'un besin değerini incelemek ve salyangozun total vücut ve sefalopedal dokularına ait fosfolipid alt sınıflarının yağ asidi kompozisyonu ile ilgili farkındalık yaratmaktır.

Metod: Lipit örnekleri içeren ince tabaka kromatografi plakaları, kloroform/etanol/su/trietilamin içeren kromatografi tankına yerleştirildi. Elde edilen fosfolipid altsınıfları, yaklaşık 5 ml metanol ve 5 damla sülfürik asit içinde çözünür hale getirildi. Karışım, yağ asidi metil esterlerini oluşturmak için 2 saat boyunca 85 °C de karıştırılıp ısıtıldı. Yağ asitleri Gaz kromatografi cihazı ile tespit edildi.

Bulgular: C20:2ω6 nin, PE (%10.49-%11.35) ve PC (%12.96-%17.33) deki yüksek oranı en dikkat çekici sonuçtu. Her iki dokunun PC (%20.85-%17.46) ve PE (%16.88-%17.53) sinde, esansiyel yağ asidi olan C18:2ω6 nın oranı yüksekti. Eikosanoidlerin öncü bileşeni olan C20:4ω6, total vücut dokusuna ait PI, PS ve PE (%28.37, %30.91 ve %26.48) de yüksek bulundu. ΣPUFA en yüksek oranı %63.90 ile total vücut PE sinde, ΣSFA en yüksek oranı ise %60.79 ile sefalopedal PI sinde tespit edildi. ΣMUFA oranı ise PI, PS ve PE de oldukça düşüktü.

Sonuç: Bu çalışma, yenilebilir salyangozların besinsel ve biyokimyasal değeri için rehber niteliğindedir ve diğer türler ile ilgili ilerde yapılacak sistematik ve fizyolojik araştırmalar için yararlı olacağına inanılmaktadır.

Anahtar Kelimeler: Fosfolipid altsınıfları, yağ asitleri, *Helix lucorum*, yenilebilir kara salyangozu

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Molluscs constitute an excellent source of protein and lipid; therefore demand for qualified food research is increasing day by day. Mollusc lipids are characterized by a great variety of fatty acids especially in major lipid classes, because of a large number of molecular species exist for each class [1]. Lipids and their compounds are of great importance for humankind. For example, the consumption of lipids rich in saturated fatty acids (SFA) and cholesterol increases atherogenesis, while lipids rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) reduce atherogenesis, thrombogenesis and the risk of cardiovascular diseases [2]. Functioning of the immune system and hormonal systems of an organism is directly related to fatty acid content and variety. The deficiency of C22:6ω3 affects neurotransmission, membrane-bound enzyme and ion channel activities and leading to neurodegenerative diseases [3] as well as C20:5ω3 and C20:4ω6 play a major role in modulating the biosynthesis of eicosanoids.

Phospholipids are essential constituents of all biological membranes and play important roles in biological processes, cellular messengers, enzyme activators and precursors of eicosanoids. Both the nature of phospholipid head groups and the esterified fatty acids influence the physico-chemical properties and the associated cellular functions of the membranes [4]. The main phospholipid subclasses of organisms are phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC). Generally, phospholipid subclasses, polar lipids, glycolipids and phosphonolipids can be observed in gastropod molluscs; however, bivalve molluscs often lack sphingomyelin class which is present in all mammals and marine vertebrates [5,6].

Although Turkey is one of the important regions in terms of gastropods, especially endemic species, there is a limited data about physiological, nutritional, molecular and biochemical properties of edible gastropods. *Theba pisana, Eobania vermiculata*, *Helix aspersa*, *Cantareus apertus*, *H. asemnis*, *H. cincta* and *H. lucorum* are mostly distributed edible gastropods in Turkey. Today many tons of Turkish edible snails, in special *H. aspersa*, *H. pomatia* and *H. lucorum* are raised on the snail farms or collected from nature and exported European countries for consumption. Fatty acid compositional data for widely consuming organisms such as fish, squid, octopus, crustacean, livestock and poultry are available in the literature; however, fatty acids content of phospholipid subclasses in edible land snails is meager. Most of the studies about fatty acid of terrestrial and aquatic mollusc of Turkey are published by us [7-9]. In the current study, the fatty acid composition of two tissues from *H. lucorum* was analyzed to assess the nutritive value of its meat and thereby to create awareness about fatty acid distribution of phospholipid subclasses including PE, PC, PI and PS. Information obtained herein adds to our knowledge of the comparative biochemistry of the fatty acid composition of representative edible gastropods.

Material and Methods

Sample collection and lipid extraction

Snail samples were collected from the Hevsel Gardens, in Diyarbakır (Altitude: 583 m, Coordinate: N 37° 55.2'/E 40° 13.8'), Turkey and transported to laboratory for sample preparation within 1 h of collection. The samples were received in the month of May, 2013. Individually, eighteen snails (nine for cephalopedal, nine for total body) similar size (length: 4±1.20 cm, wet flesh weight: 12±0.50 g) were sampled for each tissue lipid analysis. Samples were washed with distilled water and their shells were removed. Then, the total body and cephalopedal parts were dissected out and immediately used for analyses. Total lipids were extracted with 10 ml of chloroform-methanol (2:1). During the extraction process, autoxidation of unsaturated fatty acids was minimized by adding 50 ml of 2% butylated hydroxytoluene (BHT) in chloroform to each sample. Nonlipid contaminants were removed by extraction with 5 ml of 0.88% aqueous KCl [10]. The lipid containing lower phase was separated and evaporated under a stream of nitrogen at room temperature just to dryness.

Separation of phospholipid subclasses by one-dimensional TLC

Thin layer chromatography plates (TLC) were air-dried in a fume hood and placed in the preservation tank until used. They were thoroughly wetted with boric acid solution in ethanol (2.3% w/v), drained 5 min in a fume hood and dried for 15 min at 100°C in an oven prior to using. Lipid samples were quickly deposited on the plates as 1 or 2 cm parallel streaks in the concentration zone and placed in the chromatography tank containing: chloroform/ethanol/water/triethylamine (30/35/7/35, v/v). The migration time was about 2 hours. Then, plates were dried in a fume hood and sprayed with 0.2%, 2'7 dichlorofluorescein in ethanol. After viewing under UV light, spots belonging to phospholipid subclasses were scraped into methylation container [11].

Preparation of fatty acid methyl ester and GC conditions

The lipid sample was dissolved in about 5 ml of methanol, and 5 drop of sulfuric acid was added. The mixture was refluxed for 2 h to form fatty acid methyl esters (FAME) at 85°C. Then, FAME was extracted from the mixture with n-hexane and concentrated on a Rota evaporator at 40°C to reduce their volume to 1 ml for analysis. The content of FAME was analyzed by capillary gas chromatography using a Shimadzu GC-2010 Plus equipped with a flame ionization detector (FID) and a fused silica capillary column (DB-23) (Bonded 50% cyanopropil, 30 m×0.25 mm×0.25 mikrom film thickness, J&W Scientific, Folsom, CA, USA).The flow rates of compressed air and hydrogen were 300 ml/min, 30ml/min, respectively. Helium was used as

Table 1. Fatty acid profiles of phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidylethanolamine (PE) of **total body** from *Helix lucorum*

	Phosphatidylcholine (PC)	**Phosphatidylinositol (PI)**	**Phosphatidylserine (PS)**	**Phosphatidylethanolamine (PE)**
Fatty Acids	Total body (Mean*±SD)	Total body (Mean*±SD)	Total body (Mean*±SD)	Total body (Mean*±SD)
C14:0	0.39±0.04a	0.12±0.01a	0.80±0.07b	0.11±0.01a
C15:0	0.32±0.03a	0.09±0.08b	0.04±0.01b	0.11±0.01b
C16:0	15.12±1.27a	4.86±0.53b	5.58±0.64b	3.38±0.36c
C17:0	1.63±0.15a	1.76±0.16a	1.45±0.13a	0.96±0.09b
C18:0	5.12±0.54a	41.79±2.19b	42.75±2.23b	20.97±1.75c
C16:1ω7	0.62±0.05a	0.08±0.01b	0.26±0.02a	3.25±0.31c
C18:1ω9	19.58±1.44a	4.18±0.42b	3.94±0.40b	6.83±0.60c
C20:1ω9	0.07±0.01a	0.56±0.04b	1.26±0.14c	0.48±0.05b
C18:2ω6	20.85±1.71a	7.78±0.75b	6.75±0.69b	16.88±1.26c
C18:3ω3	9.76±0.95a	2.88±0.24b	0.78±0.07c	5.77±0.50d
C20:2ω6	17.33±1.38a	4.74±0.45b	1.50±0.14c	10.49±0.94d
C20:3ω6	0.83±0.07a	1.16±0.12a	1.71±0.16b	1.12±0.11a
C20:4ω6	6.87±0.64a	28.37±1.94b	30.91±2.04b	26.48±1.74b
C20:5ω3	0.84±0.08a	1.25±0.12b	1.84±0.14b	2.23±0.19c
C22:5ω6	0.45±0.04a	0.28±0.03a	0.56±0.05a	0.83±0.07a
C22:6ω3	0.21±0.02a	0.12±0.01a	0.58±0.06b	0.10±0.01a
Σω6/Σω3	**4.60**	**9.96**	**12.95**	**6.89**
ΣSFA	**22.58±1.67a**	**48.62±2.46b**	**50.62±2.48b**	**25.53±1.72a**
ΣMUFA	**20.27±1.54a**	**4.82±0.41b**	**5.46±0.49b**	**10.56±0.94c**
ΣPUFA	**57.14±2.67a**	**46.58±2.33b**	**44.63±2.25b**	**63.90±2.91c**

*Data represent the means±SD (Standard Deviation) of three replicate samples; values with different letters in one line are significantly different (ANOVA, Tukey HSD test, $P<0.05$). SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; Σω6: Total of omega 6 fatty acids; Σω3: Total of omega 3 fatty acids.

carrier gas with a flow rate of 0.50 ml/min. The oven program was: initial temperature, at 170°C (initial time, 2 min), ramping at 2°C/min, final temperature, 210°C and held for 10°C. The injection and the detector temperatures were 250°C. The split ratio was 20:1. FAME was identified by comparisons of the retention times with those of Standard purified fatty acids (SigmaChemical Co., St., Louis, MO, USA). Determinations were in triplicate and results were expressed as FID response area relative percentages.

Statistical analyses

The samples were each analyzed in triplicate, and the results of fatty acid composition were expressed as mean±SD (Standard Deviation) for each fatty acid, representing a percentage of their total. A one-way ANOVA analysis was done on each lipid class (PI, PC, PE and PS) to determine the differences between selected parameters (p-value). Comparisons between means were performed using Tukey's test. Differences between means were considered to be significant at $P<0.05$.

Results and Discussion

It is stated that phospholipids are quickly hydrolyzed by phospholipase during storage and that lyso-type phospholipids are also hydrolyzed by lysophospholipase [12]. On account of this, it is considered that the content of phospholipids will be probably decreased after storage because of enzymatic hydrolysis. For we know it, we immediately used the tissues of *H. lucorum* in the analyses after collecting to get reliable results. Due to the insufficient studies on fatty acids of phospholipid subclasses from terrestrial gastropods, the comparison of the present paper with terrestrial forms are not possible, just to compare the results with their close relatives such as clams, mussels, squids and fish.

According to the results, there were some significant differences in term of the quantity of fatty acids. For instance, level of C18:0 in both cephalopedal and total body was detected considerably high in PI, PS and PE, ranging from 41.79% to 44.32%; 42.75% to 48.88% and 20.97% to 27.58% respectively (Table 1, 2). It was reported in a study on fatty acids from marine and freshwater clams and mussels, the quantity of C18:0 from fatty aldehydes of PS (25.90%-52.03%) and from plasmalogens of PE (28.9%-46.10%) was found significantly high in eight mollusc species; however, strangely, in the same study, the level of C18:0 from fatty acids of PE, PS and PC was declared very

Table 2. Fatty acid profiles of phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidylethanolamine (PE) of **cephalopedal tissue** from *Helix lucorum*

	Phosphatidylcholine (PC)	**Phosphatidylinositol (PI)**	**Phosphatidylserine (PS)**	**Phosphatidylethanolamine (PE)**
Fatty Acids	Cephalopedal (Mean*±SD)	Cephalopedal (Mean*±SD)	Cephalopedal (Mean*±SD)	Cephalopedal (Mean*±SD)
C14:0	0.63±0.05a	0.38±0.03a	0.32±0.03a	0.48±0.04a
C15:0	0.43±0.04a	0.21±0.02a	0.12±0.01b	0.34±0.03a
C16:0	17.81±1.33a	14.46±1.20b	8.13±0.89c	7.61±0.87c
C17:0	1.79±0.16a	1.42±0.12a	1.79±0.16a	1.24±0.11a
C18:0	8.58±0.85a	44.32±2.27b	48.88±2.41c	27.58±1.82d
C16:1ω7	0.33±0.03a	0.04±0.01b	0.41±0.03a	4.48±0.41c
C18:1ω9	30.30±2.03a	5.39±0.51b	8.21±0.71c	7.93±0.71c
C20:1ω9	0.07±0.01a	0.31±0.03b	0.55±0.06b	0.49±0.05b
C18:2ω6	17.46±1.39a	7.10±0.71b	11.53±0.96c	17.53±1.32a
C18:3ω3	1.80±0.17a	0.49±0.41b	0.26±0.02b	0.92±0.09ab
C20:2ω6	12.96±1.06a	4.46±0.41b	1.32±0.11c	11.35±1.08a
C20:3ω6	0.81±0.08a	0.71±0.06a	0.42±0.03a	0.97±0.08a
C20:4ω6	4.84±0.42a	15.85±1.26b	14.15±1.21b	14.46±1.22b
C20:5ω3	1.09±0.11a	3.85±0.35b	3.16±0.35b	2.73±0.24ab
C22:5ω6	0.68±0.06a	0.53±0.05a	0.44±0.04a	1.61±0.15b
C22:6ω3	0.44±0.04a	0.48±0.05a	0.31±0.03a	0.28±0.03a
Σω6/Σω3	**11.04**	**5.94**	**7.47**	**11.68**
ΣSFA	**29.24±1.83a**	**60.79±2.88b**	**59.24±2.71b**	**37.25±2.13c**
ΣMUFA	**30.70±1.95a**	**5.74±0.53b**	**9.17±0.89c**	**12.90±1.07d**
ΣPUFA	**40.08±2.20a**	**33.47±2.08b**	**31.59±1.97b**	**49.85±2.46c**

*Data represent the means±SD (Standard Deviation) of three replicate samples; values with different letters in one line are significantly different (ANOVA, Tukey HSD test, $P<0.05$). SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; Σω6: Total of omega 6 fatty acids; Σω3: Total of omega 3 fatty acids.

low (Table 3) [13]. In Pectin maximus gonads, the concentration of C18:0 was detected also high in PI (33.80%), PS (44.60%) and PE (28.10%) [14] as well as in edible land snail *Eobania vermiculata*, the level of C18:0 was found to 16.39% in PE and 18.65% in PC from total body (Table 3) [15]. Additionally, in fish, this saturated fatty acid was determined in high percentages, too. For example, it was 22.50% in PS, 22.80% in PI and 8.4% in PE from the testes of skipjack fish [16] and in PI of the liver and muscle from wild and captive *Diplodus sargus*, it was in the range of 25.66%-41.63% [17]. Intercalarily, in PI from the dorsal meat, livers and ovaries of cultured Japanese catfish *Silurus asotus*, C18:0 was detected between 23.0% and 36.40% [18]. Generally, PC is the most abundant phospholipid class in both plants and animals, counting over 50% in total phospholipids and PE is the second one [3]; therefore, PC is the mostly studied subclass among organisms and as emphasized in most of the studies, PC contained high level of C16:0. In *H. lucorum*, the highest level of C16:0 was predictably detected in PC from the total body (15.12%) and cephalopedal (17.81%). This high proportion of C16:0 in PC fractions probably reflects structural and physiological necessities. As noted in several studies, this saturated fatty acid became dominant in PC fraction.

In *P. maximus* gonad, the percentages of C18:1ω9 and C18:2ω6 were detected at low rates in four phospholipid subclasses; the sum of two fatty acids did not exceed 5% [14]. Likewise, in PC of seven squid samples, the proportion of C18:1ω9 (2.8% to 5.0%) and C18:2ω6 (0.0% to 0.5%) were also reported at low concentrations (Table 4) [19]. Additionally, Hanus *et al*. [13] stated that the percentages of C18:1ω9 and C18:2ω6 were very minor in PC fractions, insomuch that they did not detect these components in PE and PS fractions from eight clams and mussel species (Table 3). Surprisingly, in *H. lucorum*, the percentages of C18:1ω9 and C18:2ω6 were found significantly at high rate in PC fractions; C18:1ω9 accounted for 19.58% in PC from the total body, 30.30% in PC from the cephalopedal and C18:2ω6 accounted for 20.85% in PC from the total body, 17.46% in PC from the cephalopedal (Table 1, 2). C18:2ω6 (16.88%-17.53%) was also present abundantly in PE. As in *H. lucorum*, the amount of C18:1ω9 and C18:2ω6 were stated at high level in terrestrial snail *E. vermiculata* (Table 4) [15]. On the other hand, in fish samples, the content of the fatty acids differentiated, for example, in PI, PC and PE from liver and muscle of *D. sargus*, the levels of C18:1ω9 and C18:2ω6 were moderate amount [17]; in PI from the dor-

Table 3. Composition of main fatty acids from phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylcholine (PC) of clam and mussel bivalves; *Donax trunculus* (1), *Mactra coralline* (2), *Mytilus galloprovincialis* (3), *Callista florida* (4), *Pteria aegyptia* (5), *Corbicula fluminalis* (6), *Potanida littoralis semirugatus* (7), *Unio terminalis* (8) [13] and land snail *Eobania vermiculata* (9) [15]

Fatty acids	Marine bivalves					Freshwater bivalves			Land snail
	1	2	3	4	5	6	7	8	9
PE									
C16:0	-	2.3	-	1.2	1.6	2.1	3.4	3.9	2.21
C18:0	1.2	1.4	1.1	-	-	1.3	1.1	1.5	16.39
C18:1ω9	-	-	-	-	-	-	-	-	5.01
C18:2ω6	-	-	-	-	-	-	-	-	10.65
C20:2ω6	-	-	-	-	-	-	-	-	12.07
C20:4ω6	2.1	1.8	2.6	1.5	1.9	3.2	2.8	2.6	-
C20:5ω3	30.5	28.7	29.8	31.5	36.7	12.8	13.8	16.1	-
C22:6ω3	6.8	4.7	4.9	5.1	5.9	23.1	28.3	26.9	-
PS									Not
C16:0	1.5	-	1.8	1.4	2.1	2.7	2.4	3.1	studied
C18:0	-	3.3	4.1	3.6	-	1.2	1.8	2.2	
C20:4ω6	2.3	3.1	4.5	1.8	2.2	3.8	4.1	2.5	
C20:5ω3	42.2	44.8	39.2	53.2	56.8	29.1	22.8	26.3	
C22:6ω3	8.6	5.2	6.9	7.7	9.4	36.2	32.4	38.9	
PC									
C16:0	13.2	14.1	16.9	12.2	12.4	17.4	18.2	19.8	18.54
C18:0	2.3	2.5	1.7	3.8	3.1	5.9	4.4	6.7	18.65
C18:1ω9	2.2	1.4	2.5	2.1	3.2	3.3	2.8	1.5	24.89
C18:2ω6	-	3.2	2.6	1.5	-	-	-	-	3.62
C20:4ω6	3.9	2.6	4.8	6.1	5.2		2.2	1.3	-
C20:5ω3	18.3	16.6	15.2	19.9	15.3	8.4	11.9	10.7	-
C22:6ω3	13.4	18.7	16.8	11.2	15.8	24.7	22.1	28.3	-

Table 4. Main fatty acid composition of phosphatidylcholine (PC) of seven squid muscle [19]

Squid Samples	**C16:0**	**C18:0**	**C18:1**	**C20:1**	**C18:2ω6**	**C20:4ω6**	**C20:5ω3**	**C22:6ω3**
1. Rhobust clubhook squid	31.6	1.3	4.5	6.9	0.5	1.7	9.8	33.3
2. Boreopacific gonate squid	34.7	0.7	5.0	3.8	0.3	0.7	5.9	39.3
3. Ditto squid	34.3	0.4	4.8	2.1	0.5	0.5	6.7	39.9
4. Boreal clubhook squid	38.4	0.7	4.9	5.7	0.2	1.2	5.1	34.0
5. Neon flying squid	42.9	2.9	2.8	4.8	0.2	0.3	4.3	35.3
6. Argentine short fin squid	39.9	0.9	3.1	1.3	0.2	0.6	6.6	40.2
7. Japanese flying squid	18.1	7.9	3.8	5.9	0.0	3.3	20.8	35.7

sal meat, livers and ovaries of *S. asotus*, C18:1ω9 ranged from 4.0% to 13.4% and C18:2ω6 ranged from 0.6% to 1.4% [18]. In PC from liver of *Spondyliosoma cantharus*, C18:1ω9 was detected between 9.08% and 15.22%, and C18:2ω6 was between 0.24% and 3.76%, and in PE, C18:1ω9 was between 7.74% and 10.33%, and C18:2ω6 was between 0.22% and 0.79% [20]. Understandingly, the high percentages of C18:1ω9 in PC and C18:2ω6 in PE and PC from *H. lucorum* are important findings. Additionally, C18:3ω3 was observed in high concentration in PC of the total body (9.76%). Probably, this high concentration of the fatty acids was concluded from structural and physiological metabolism of the snail. C18:2ω6 and C18:3ω3 are essential fatty acids and cannot be synthesized in animal bodies and must be obtained from diet. These basic components, mostly found in plants, are used to build specialized fatty acids called omega-3 (ω3) and omega-6 (ω6) fatty acids. Deficiencies in these fatty acids

lead to a host of symptoms and disorders including, reduced growth rates, reproduction rates, immune malfunction, abnormalities in the organs, physiological disorders and dryness of the skin.

The level of C20:2ω6 which is not reported in most of the sea mollusc species such as P. maximus [14], seven squid species [19] and *Donax trunculus*, *Mactra corallina*, *Mytilus galloprovincialis*, *Callista florida*, *Pteria aegyptia*, *Corbicula fluminalis*, *Potanida littoralis semirugatus*, *Unio terminalis* bivalves [13] was high in *H. lucorum* similar to terrestrial representatives; the level of C20:2ω6 in PC was found to be 17.33% in the total body and 12.96% in the cephalopedal as well as it was detected 10.49% in the total body and 11.35% in the cephalopedal from PE (Table 1, 2). Similarly, in the studies on terrestrial gastropods, C20:2ω6 has been identified at good amount, for example in edible land snail *E. vermiculata*, it was detected as 12.07% in PE [15] and it was reported at a good rate in the total lipid from *Arion ater* (9.9%), *Limax maximus* (7.9%), *Prophysaon andersoni* (8.9%) slugs and *Helix sp.* (12.1%), *Haplotrema sportella* (10.5%), *Vespericola columbiana* (9.1%) land snails [21]. Conversely, in commonly consumed snail, *H. aspersa maxima* feed with vegetable oils added diets, C20:2ω6 was not identified [22]. It is unable to make any comparison with phospholipid subclasses of other land snails because of not finding studies on phospholipid subclasses. But, in fish species, C20:2ω6 was either absent or detected trace amounts [17,18,20]. We do not foresight the origin of C20:2ω6 identified in high proportion in PC and PE from the tissues of *H. lucorum*. However, it is likely to theorize that it could accumulate from snail diets or synthesis from other fatty acids due to metabolic activities. Corroborating this hypothesis necessitate additional studies.

A high percentage of C20:4ω6 in PI (28.37%), PS (30.91%) and PE (26.48%) from the total body is another remarkable finding (Table 1). So that, PI, PS and PE from the total body is characterized by C20:4ω6. This data is in agreement with PI fraction of *P. maximus*, accounting for 36.70% [14]. In contrast, PC from seven squid samples [19] and PE, PS and PC from eight freshwater clams and mussels contained low level of C20:4ω6 [13]. However, it was apparently high in PI fractions of fish, for example, in skipjack tuna [16], *D. sargus* [17] and *Siluris asotus* [18], it was accounted for 20.05%, 26.65% and 33.50% respectively. PI is an important component in cellular regulatory mechanisms due to its role as a precursor of inositoltriphosphate (IP3) which activates an intracellular cascade [23] and containing high amount of C20:4ω6 [24] necessary for the synthesis of prostaglandins [25]. Prostaglandins appear to be considerably important in basic physiological functions in molluscs such as renal function, ion regulation, reproductive physiology [26] and stimulate egg production in gastropod [27]. The maintenance of high amount of C20:4ω6 in PI, PS and PE of *H. lucorum* can reflect a storage function for prostaglandin precursors involved in reproduction. Notably, land snails have two fertile periods per year in spring and autumn and their digestive glands accumulate lipids and mobilized them to obtain essential energy for sexual maturation and breeding [28]. It is known that gonad and digestive gland contained higher amount of unsaturated fatty acids than other parts of organism. Probably that is why, content of C20:4ω6 in PI, PS and PE from total body of *H. lucorum* which including both gonad and digestive gland, was detected significantly high. To be remembered, *H. lucorum* snails were collected in spring just before breeding periods. Probably, high content of C20:4ω6 is related to reproduction process. Furthermore, C20:4ω6 composition of PI appears to have a wide distribution across the animal kingdom and hence, suggests a common cellular role. In human, it is known to have significant functions: the production of second messengers (Diacylglycerol, IP3) and precursor of prostaglandins, thromboxanes and leukotrienes [29]. Castell *et al.* [30] defined that C20:4ω6 had growth promoting effects in juvenile turbot when included at levels between 0.3 and 1.0% of dietary dry mass. In *P. maximus*, C20:5ω3 level was 6.2%, 11.5%, 30.4% and 18.4% in PI, PS, PE and PC from gonad, respectively [14] and in seven squid samples, its content varied from 4.3% to 20.8% (Table 4) [19]. Above all, PS fraction of freshwater clams and mussels, *D. trunculus* (42.2%), *M. coralline* (44.8%), *M. galloprovincialis* (39.2%), *C. florida* (53.2%), *P. aegyptia* (56.8%), *C. fluminalis* (29.1%), *P. littoralis semirugatus* (22.8%), *U. terminalis* (26.3%) contained comparatively high amount of C20:5ω3 as well as PE and PC fractions of those species also contained noticeable quantity of C20:5ω3 (Table 3) [13]. In *H. lucorum*, it did not exceed 4% in all fractions (Table 1, 2). The PUFA (polyunsaturated fatty acids) with 20 and 22 carbon atoms and more than three double bonds containing are essential for survival, growth and reproduction of molluscs [31]. Zhu *et al.* [22] stated that slugs and snails have relatively high content of ω6 long-chain polyunsaturated fatty acids including C20:4ω6, C22:4ω6 and C22:5ω6 and they can synthesize C18:2ω6 from acetate, probably, the presence of C22:5ω6 in *H. lucorum* indicated synthesis from dietary C18:2ω6. It is usually accepted that C22:6ω3 is formed from the reaction of Δ4 desaturase on C22:5ω3, a precursor fatty acid present in both slugs and snails [22]. In PC, PE, PS and PI from the testes of skipjack, C22:6ω3 level varied from 21.6% to 52.8%, while that in PE and PC from the ovaries of the same fish (PI and PS were not studied), its level differed from 35.2% to 48.9% [16]. In PE from the liver and muscle of wild and captive *D. sargus*, C22:6ω3 was found between 29.12% and 37.65% in PC, 6.14% and 25.45% in PI fraction [17]. Furthermore, the level of C22:6ω3 in marine and freshwater mollusc varies from species to species. For instance, in PE, PC, PI and PS of *P. maximus* gonads, the percentages were 6.1%, 12.7%, 8.2% and 17.8% respectively [14]. Conversely, in seven squid muscle, this fatty acid percentage was re-

ported strikingly high, ranged from 33.3% to 40.02% (Table 4) [19]. In comparison with those in fish and marine mollusc, *H. lucorum* had low proportion of C22:6ω3 and did not exceed 1% (Table 1, 2). Probably, land snails and other terrestrial organism had this component at low rate in their tissues due to environmental, nutritional or physiological factors. It is emphasized that fatty acid profiles of each phospholipid subclasses are composed characteristically of synthesized enzymatic to phospholipid molecular species, although this depends on the fatty acid content of external food sources [32]. On the other hand, the high level of C22:6ω3 (between 10% and 27%) was reported in marine clams, oysters and scallops [33]. According to Katter *et al.* [34], this fatty acid is predominant in polar lipids (up to 31%) of sea slug *Clione limacina* living in both Arctic and Antarctic waters.

Recently, ω3 PUFA have been acclaimed for greater potency in amelioration of heart and cardiovascular disorders than ω6 PUFA. It is noteworthy that the Σω6/Σω3 ratio is a marker of biomedical significance for fish and could be an index of biomedical application [2]. The highest level of Σω6/Σω3 in the cephalopedal of *H. lucorum* was detected 11.04 in PC and 11.68 in PE. In the total body, the highest value was 9.96 and 12.95 in PI and PS, respectively. In our previous studies on terrestrial and freshwater molluscs, ω6 PUFA levels were found higher than ω3 PUFA [7-9]. In agreement with our findings, Σω6/Σω3 ratio was also defined to be high in *A. ater*, *L. maximus*, *P. andersoni*, slugs and *H. sp.*, *H. sportella*, *V. columbiana* snails [22]. But, in marine molluscs, percentage of Σω6 was found to be lower than Σω3. It is expressed that ω6 was known as characteristic to terrestrial and freshwater organisms, whereas ω3 was characteristic to marine organism [26,35].

In comparison with ΣPUFA, total monounsaturated fatty acids (ΣMUFA) and total saturated fatty acids (ΣSFA) in phospholipid subclasses of *H. lucorum*, appreciable quantity of ΣPUFA was identified in PE from the total body (63.90%) and cephalopedal (49.85%), followed by PC from the total body (57.14%) and cephalopedal (40.08%), whereas the high level of ΣSFA was observed in PI (60.79%) and PS (59.24%) from the cephalopedal (Table 2). In the analyses, ΣMUFA levels were much lower in comparison with ΣPUFA and ΣSFA, except for PC. The high quantity of ΣPUFA in *H. lucorum* stemmed from high proportion of C18:2ω6, C20:2ω6 and C20:4ω6. In contrast, the ΣPUFA content in *P. maximus* was always determined higher than ΣMUFA and ΣSFA, moreover the level of ΣMUFA was quite low, in the range of 4.0%-12.4% [14]. As in *H. lucorum*, in fish studies, ΣPUFA compromised large portion of PE and ΣSFA compromised large portion of PI fractions [17]. In *H. lucorum*, ΣSFA of PC was largely dominated by C16:0, whereas ΣSFA of PI, PS and PE was dominated by C18:0.

Consequently, it has been defined that fatty acid composition of the phospholipid subclasses from *H. lucorum* showed both differences and similarities with those molluscs (mussels, clams, land snail, land slugs, squids) and fish species. The results revealed that two tissue of *H. lucorum* had substantial quantitative differences in terms of fatty acid distribution of PI, PE, PS and PC. The most important data obtained in this study was comparatively high level of C20:2ω6 in PC and PE from both cephalopedal and total body and high level of C20:4ω6 in PI, PS and PE from the total body. Furthermore, relatively little is known about fatty acid composition of phospholipid subclasses from edible snails and knowledge mostly comes from studies on fish and marine molluscs. For this reason, this study can be significant guide for nutritional and biochemical value of edible snails particularly on distribution of fatty acids from phospholipid subclasses and can be useful for further investigation on systematic and physiological studies of other species to be able to compare.

Conflict of Interest

There are no conflicts of interest among the authors.

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