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Investigation of the Fatty Acid Contents of Edible Snails *Helix lucorum, Eobania vermiculata* and Non-Edible Slug *Limax flavus*

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Abstract: The purpose of this work is to reveal the fatty acid composition of phospholipids, neutral and total lipid content of edible land snails *Helix lucorum*, *Eobania vermiculata* and non-edible land slug *Limax flavus* (Gastropoda: Pulmonata). The analyses revealed that the snails and slug are rich in C16:0, C18:0, C18:1ω9 and C18:2ω6 acids. Phospholipid fractions contained very high amounts of C20:4ω6 (15.37% in *H. lucorum*, 12.37% in *E. vermiculata* and 8.59% in *L. flavus*) which is the most important precursor of eicosanoids. The level of ΣPUFA, in edible snails, was found to be higher than ΣSFA and ΣMUFA. However, in the slug, ΣMUFA level was higher than ΣSFA and ΣPUFA levels in neutral and total lipid contents. Σω6 / Σω3 (Omega 6 / Omega 3) ratio was defined to be very high in the phospholipid of *H. lucorum*, *E. vermiculata* and *L. flavus*; 6.07, 8.29 and 5.39, respectively. Furthermore, the study showed that all three species are valuable sources of omega 6 and essential fatty acids; C18:2ω6 and C18:3ω6.

Keywords: Fatty acids, *Helix lucorum*; *Eobania vermiculata*; *Limax flavus.*

1. Animal Source

H. lucorum (L. 1758) and *L. flavus* (L. 1758) were collected from Hevsel Gardens and residential gardens located in Diyarbakır, respectively and *E. vermiculata* (O. F. Müller 1774) was collected from residential gardens located in Şanlıurfa, Türkiye, on March 2014. Individually, three snails and slugs of similar size (*H. lucorum*: length: 6±1.00 cm, wet flesh weight: 7±1.25 g; *E. vermiculata*: 4±0.50 cm, wet flesh weight: 3±0.75; *L. flavus*: length: 7±1.00 cm, wet flesh weight: 5±1.25 g) were sampled for each lipid analysis, 27 samples in total, for three replicates of all lipid analyses.

2. Previous Studies

Cultivated terrestrial gastropods mostly consist of *Helix pomatia*, *H. aspersa maxima* and *Achatina achatina* all over the world. In Türkiye, the edible land snails are represented by *Theba pisana*, *Eobania vermiculata*, *Cantareus apertus*, *H. asemnis*, *H. cincta* and *H. lucorum*. Although, these snails are not consumed by Turkish people, they are exported mainly to European countries such as France, Germany, Greece, Italy, and Spain for consuming [1]. Together with taste, snail meat (escargot) has several advantages over others such as calorific values, rich mineral, essential amino acids and fatty acids content, especially, PUFA (polyunsaturated fatty acids). Fatty acid contents of marine molluscs [2-4] have been more extensively studied then land representatives. Few

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investigations have been carried out on edible terrestrial forms [1, 5-8], particularly, on the content of phospholipid, neutral and total lipids. However, in Türkiye, little is known about nutritional value of land gastropods and their fatty acid compositions.

Tablo 1. Fatty acid composition of phospholipid, neutral lipid and total lipid of *Helix lucorum*

Fatty acids	Phospholipid	Neutral lipid	Total lipid
	(Mean*±S.D.)**	(Mean*± S.D.)**	(Mean*± S.D.)**
C10:0	0.05±0.02a	0.05±0.02a	0.03±0.01a
C12:0	0.03±0.01a	-	0.04±0.01a
C13:0	0.02±0.01a	0.02±0.01a	0.05±0.02b
C14:0	0.94±0.11a	0.60±0.08b	0.53±0.06b
C15:0	0.60±0.07a	0.36±0.05b	0.69±0.06a
C16:0	12.92±1.48a	9.29±1.05b	9.66±1.10b
C17:0	1.90±0.20a	1.00±0.14b	1.25±0.10b
C18:0	9.02±1.02a	7.00±0.72b	6.75±0.55b
C20:0	0.18±0.06a	0.10±0.03b	0.20±0.04a
C22:0	2.40±0.35a	2.00±0.23a	2.50±0.24a
ΣSFA	**28.06±1.70a**	**20.42±1.35b**	**21.70±1.44b**
C14:1ω9	-	0.19±0.04a	0.25±0.06a
C16:1ω7	1.94±0.30a	1.93±0.28a	1.86±0.24a
C17:1ω7	0.30±0.04a	0.20±0.04a	-
C18:1ω9	11.29±1.14a	24.17±1.66b	22.02±1.48b
C20:1ω9	3.34±0.30a	1.28±0.13b	1.50±0.16b
C22:1ω9	0.50±0.06a	-	0.90±0.08a
ΣMUFA	**17.37±1.18a**	**27.77±1.60b**	**26.53±1.55b**
C18:2ω6	20.25±1.30a	21.28±1.25a	21.76±1.27a
C20:2ω6	8.33±.0.90a	7.02±0.72b	6.90±0.71b
C20:3ω6	2.14±0.25a	1.72±0.18a	1.87±0.18a
C20:4ω6	15.37±1.19a	9.64±1.02b	9.85±1.06b
C22:5ω6	0.75±0.09a	0.55±0.05a	0.60±0.07a
Totalω6	46.84	40.21	40.98
C18:3ω3	6.09±0.70a	10.50±0.90b	10.08±0.84b
C20:5ω3	0.92±0.08a	0.66±0.06b	0.71±0.07b
C22:6ω3	0.70±0.06a	0.44±0.04b	-
Totalω3	7.71	11.60	10.79
Σω6/Σω3	6.07	3.47	3.80
ΣPUFA	**54.55±2.32a**	**51.81±2.14b**	**51.77±2.08b**
ΣFatty Acids	**99.98**	**100.00**	**100.00**

* Values are means ± S.D (Standard Deviation) for three replicates. Results were expressed as percentage of total fatty acids methyl esters. **Means followed by different letters in the same line are significantly different. ($P \leq 0.05$) by Tukey's test. (Results expressed as % of whole body lipid fatty acids).

3. Present Study

Preparation of Gastropods and Lipid Extraction: The shells and the digestive organs of the gastropods were carefully removed and fleshy parts were analyzed. They were washed and cut into tiny pieces. Each group of samples was transferred into chloroform / methanol (2:1, v / v) bottles and kept frozen (-80 °C) until use. The phospholipids, neutral and total lipids of the snails and slugs were extracted according to the method of Bligh and Dyer [9].

Gas Chromatography (GC) Conditions: Fatty acid methyl esters (FAME) of the total, neutral and phospholipids were prepared according to an existing method [10].

Tablo 2. Fatty acid composition of phospholipid, neutral lipid and total lipid content of *Eobania vermiculata*

Fatty acids	Phospholipid (Mean*±S.D.)**	Neutral lipid (Mean*± S.D.)**	Total lipid (Mean*± S.D.)**
Saturated			
C10:0	-	0.05±0.01a	0.05±0.01a
C12:0	0.33±0.04a	0.21±0.03b	0.12±0.02c
C13:0	1.08±0.10a	0.78±0.09b	0.81±0.09b
C14:0	0.63±0.05a	0.37±0.04b	0.30±0.03b
C15:0	0.88±0.09a	0.22±0.03b	0.33±0.03b
C16:0	14.61±1.08a	12.49±0.95b	10.49±0.90c
C17:0	0.98±0.09a	0.91±0.09a	1.02±0.14a
C18:0	10.37±0.95a	7.12±0.73b	7.76±0.75b
C20:0	0.20±0.03a	0.25±0.03a	-
C22:0	1.35±0.15a	1.20±0.14a	1.80±0.17a
ΣSFA	**30.43±1.80a**	**23.60±1.35b**	**22.68 ±1.34b**
C14:1ω9	0.10±0.02a	-	0.07±0.01a
C16:1ω7	2.80±0.26a	1.08±0.18b	1.60±0.15b
C17:1ω7	0.09±0.02a	0.08±0.02a	0.12±0.03b
C18:1ω9	13.09±0.96a	20.04±1.26b	19.07±1.18b
C20:1ω9	2.24±0.26a	0.39±0.04b	0.21±0.03b
C22:1ω9	0.30±0.03a	0.27±0.03a	0.70±0.05b
ΣMUFA	**18.62±1.18a**	**21.86±1.36b**	**21.77±1.30b**
C18:2ω6	25.87±1.36a	34.92±1.99b	37.67±1.83c
C20:2ω6	6.29±0.60a	5.44±0.52b	5.43±0.50b
C20:3ω6	0.77±0.05a	0.61±0.04b	0.56±0.04b
C20:4ω6	12.37±0.95a	5.05±0.42b	3.54±0.37c
C22:5ω6	0.15±0.02a	0.14±0.02a	0.17±0.03a
Total ω6	45.45	46.16	47.37
C18:3ω3	4.14±0.40a	7.49±0.72b	7.31±0.74b
C20:5ω3	1.02±0.16a	0.48±0.04b	0.37±0.03b
C22:6ω3	0.32±0.03a	-	0.51±0.04a
Total ω3	5.48	7.97	8.19
Σω6/Σω3	8.29	5.79	5.78
ΣPUFA	**50.93±2.02a**	**54.53±2.14b**	**55.56±2.18b**
ΣFatty Acids	**99.98**	**99.99**	**100.01**

* Values are means ± S.D (Standard Deviation) for three replicates. Results were expressed as percentage of total fatty acids methyl esters. **Means followed by different letters in the same line are significantly different. ($P \leq 0.05$) by Tukey's test. (Results expressed as % of whole body lipid fatty acids).

Statistical Analyses: The data of analyses were obtained by statistical program (SPSS 12.0). All analytical values were performed in triplicate and the mean values were calculated. The statistical analyses of fatty acid proportions were performed by analysis of variance (ANOVA) and comparisons between means were performed with Tukey's test. Differences between means were evaluated as significant at $P \leq 0.05$.

The fatty acids percentages of total, neutral and phospholipid contents from the snail samples and slug samples are presented in the Tables 1, 2, and 3. All species contained about 5 to 9 times higher content of ω6 (omega 6) than ω3 (omega 3) (Table 1, 2, 3) and C18:2ω6 and C20:4ω6 lipids constitutes the highest proportions of ω6. The level of ΣPUFA in edible snails was found to be higher than ΣSFA (saturated fatty acids) and ΣMUFA (monounsaturated fatty acids). However, in the slug samples, ΣMUFA level found to be higher than ΣSFA and ΣPUFA levels in terms of neutral and total lipid content.

Table 3. Fatty acid composition of phospholipid, neutral lipid and total lipid content of *Limax flavus.*

Fatty acids	Phospholipid	Neutral lipid	Total lipid
	(Mean*±S.D.)**	(Mean*±S.D.)**	(Mean*±S.D.)**
C10:0	1.30±0.12a	0.18±0.03b	1.95±0.24a
C12:0	1.42±0.16a	0.68±0.05b	1.03±0.10a
C13:0	-	0.29±0.03a	0.50±0.04b
C14:0	4.44±0.38a	3.30±0.25b	4.62±0.36a
C15:0	0.56±0.04a	0.50±0.04a	0.55±0.04a
C16:0	23.10±1.21a	22.13±1.20a	20.53±1.18b
C17:0	0.20±0.02a	0.40±0.04b	0.20±0.02a
C18:0	7.01±0.65a	5.18±0.45b	5.54±0.47b
C20:0	0.15±0.03a	0.20±0.04a	0.10±0.03a
C22:0	3.10±0.25a	3.20±0.35a	2.70±0.27a
ΣSFA	**41.28±2.07a**	**36.06±1.90b**	**37.72 ±1.91b**
C14:1ω9	0.30±0.03a	0.29±0.03a	-
C16:1ω7	2.71±0.28a	5.18±0.49b	4.75±0.42b
C17:1ω7	0.70±0.06a	0.60±0.05a	0.70±0.06a
C18:1ω9	20.06±1.20a	32.20±1.75b	31.94±1.70b
C20:1ω9	1.01±0.11a	1.03±0.12a	1.02±0.12a
C22:1ω9	0.30±0.02a	0.50±0.04a	-
ΣMUFA	**25.08±1.24a**	**39.80±1.95b**	**38.41±1.83b**
C18:2ω6	15.18±1.11a	14.81±1.08a	13.03±1.04a
C20:2ω6	2.70±0.23a	2.26±0.20a	2.49±0.22a
C20:3ω6	1.08±0.18a	0.21±0.02b	0.30±0.02b
C20:4ω6	8.59±0.75a	2.24±0.20b	3.48±0.29c
C22:2ω6	0.82±0.09a	0.76±0.06a	0.50±0.04b
Total ω6	28.37	20.28	19.80
C18:3ω3	2.56±0.28a	2.26±0.26a	2.13±0.20a
C20:5ω3	2.20±0.21a	1.15±0.13b	1.92±0.18a
C22:6ω3	0.50±0.04a	0.45±0.03a	-
Total ω3	5.26	3.86	4.05
Σω6/Σω3	5.39	5.25	4.89
ΣPUFA	**33.63±1.34a**	**24.14±1.18b**	**23.85±1.17b**
ΣFatty Acids	**99.99**	**100.00**	**99.98**

* Values are means ± S.D (Standard Deviation) for three replicates. Results were expressed as percentage of total fatty acids methyl esters. **Means followed by different letters in the same line are significantly different. ($P \leq 0.05$) by Tukey's test. (Results expressed as % of whole body lipid fatty acids).

The results of the current study declared that the fatty acid profiles of the snails and slug revealed common patterns of fatty acids and common characteristics to gastropods in general [4-6, 11-14].

According to the result of a slug study, in the slugs C16:0 (6.7% - 10.1%), C18:0 (9.1% - 10.7%), C18:1ω9 (12.1% - 15.7%), C18:2ω6 (11.6% - 15.2%) and C20:4ω6 (13.5% - 14.3%); in the snails C16:0 (7.1% - 9.6%), C18:0 (8.9% - 10.6%), C18:1ω9 (10.0% - 12.2%), C18:2ω6 (11.0% - 15.7%), C20:2ω6 (9.1% - 12.1%) and C20:4ω6 (13.8% - 16.9%) were determined as predominant components of land slugs *Arion ater, Limax maximus, Prophysaon andersoni* and land snails *Helix sp. Haplotrema sportella, Vespericola columbiana* [6]. In another study, C16:0 (6.14% - 10.79%), C18:1ω9 (17.12% - 29.22%) and C18:2ω6 (2.87% - 8.40%) in *Valvata baicalensis*; C16:0 (5.48% - 12.32%), C18:1ω9 (16.13% - 31.33%) and C18:2ω6 (1.59% - 9.42%) were reported as principal components of *Valvata piligera* [15]. Similarly, high percentages of these fatty acids were also found in freshwater gastropods *Melanopsis praemorsum, Melanoides tuberculata, Theodoxus jordani* and *Pyrigula barroisi* [13] and they were also detected as main fatty acids in *H. aspersa* [8] and *H. pomatia* [7]. In the present study, the levels of C16:0, C18:1ω9 and C18:2ω6 were similarly found higher. The other noteworthy data of the study was the detection of C13:0, C15:0, C17:0, C14:1ω9 and C17:1ω7 from all lipid analyses. These fatty acids were not detected in the slugs and snails mentioned in the study of Zhu *et al*., [6] and most of the other studies.

The ratio of Σω6 / Σω3 was reported higher in *P. andersoni, A. ater, L. maximus* slugs and *H. sp. H. sportella, V. columbiana* snails [6]. In most of the previous studies, the percentage of Σω3 was observed higher than Σω6 in marine molluscs and the ratio of Σω6 / Σω3 was lower than terrestrial forms [2, 4]. The differences in the omega fatty acid profiles of molluscs is potentially due to dietary differences since marine plankton are rich in ω3 acids while terrestrial and freshwater plants and planktons are rich in ω6 acids [16].

According to analyses of our samples, C18:2ω6 and C20:4ω6 were mostly abundant in the phospholipids fractions of three species. C20:4ω6 is an important component of phospholipid, particularly in phosphatidylinositol subclass [17] and it is necessary for series 2 prostaglandins synthesizing mostly from C20:4ω6 of membrane phospholipids [2]. Prostaglandins appear to be considerably significant component of molluscs in basic physiological functions, particularly in ion regulation, renal function and reproduction [18]. Probably, higher levels of these fatty acids have been related to prostaglandin synthesis of the species. Also, the quantity of C20:4ω6 was reported much higher levels than the most of the terrestrial and freshwater gastropods [6, 13, 19] which are close relatives of land snails and slugs. In the present study, the percentages of C20:5ω3 did not exceed 2.20% in any sample studied. Lower levels of C20:5ω3 in the snails and slug is expected since, Isay and Busarova [20] mentioned C20:5ω3 is a characteristic component of marine invertebrates as a result of fatty acid analysis of 51 species of marine invertebrates (most of them were molluscs) from Japanese Sea.

The levels of C22:5ω6 and C22:6ω3 were mentioned in most of the studies on gastropods and bivalves such as *Coretus carneus, Viviparus viviparus, Radix auricularia, Limnaea stagnalis, Dreissena polymorpha, Unio sp.* [19], *Assyriella escheriana* and *A. guttata* [21]. Additionally, in some studies, the high content of C22:6ω3 (10% - 27%) was reported in marine oysters, clams and scallops [13]. In this study, C22:6ω3 were detected in all species; however, C22:5ω6 was only found in *H. lucorum* and *E. vermiculata,* furthermore, the quantities of C22:6ω3 and C22:5ω6 did not exceed 1%. The type and composition of fatty acids is found different between mollusc species because of their environmental conditions, nutrient habits, food availability and physiological activities.

Non-methylene interrupted dienoic (NMID) fatty acids were reported in both marine [2, 4], and some freshwater [14] molluscs, as well as in some freshwater fish [22]. Their structures were known as C20:2$\Delta^{5\text{-}11}$, C20:2$\Delta^{5\text{-}13}$, C22:2$\Delta^{7\text{-}13}$ and C22:2$\Delta^{7\text{-}15}$. Some authors suppose that in aquatic invertebrates, these fatty acids have an endogenous origin [23, 24]. As it has been mentioned in the most of the other freshwater mollusc studies, none of the NMID fatty acids were detected in the current study.

The fatty acid compositions of selected tissues of *H. lucorum* including gonad, mantle, digestive gland, cephalopedal and whole body of *H. lucorum* were studied in detail in our previous studies [25]; however, the fatty acid composition of lipid classes of *E. vermiculata* and *L. flavus* were not studied. As a result, current study can be a significant guide for nutritional value and quantitative analysis of edible snails of Türkiye. On the other hand, the study can be useful for comparison of further investigations on systematic and physiological studies of new species.

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