PAPER

A COMPARATIVE STUDY ON FATTY ACID CONTENT OF MAIN ORGANS AND LIPID CLASSES OF LAND SNAILS *ASSYRIELLA ESCHERIANA* AND *ASSYRIELLA GUTTATA* DISTRIBUTED IN SOUTHEASTERN ANATOLIA

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ABSTRACT

In the present work, main organs (digestive gland, cephalopedal, gonad and mantle) and lipid classes (total, neutral and phospholipid) of land snails *Assyriella escheriana* and *Assyriella guttata* from southeastern Anatolia were examined for their fatty acids. The major components detected in both of the species were C16:0, C18:0, C18:1ω9, C18:2ω6, C18:3ω3, C20:2ω6 and C20:4ω6. C18:2ω6 was identified as the primary fatty acid ranging from17.07% to 28.12% in *A. guttata* and 18.02% to 27.43% in *A. escheriana*. The proportions of C20:4ω6 modified to form prostaglandins that are directly involved in regulation of reproduction, ranged from10.01% to 20.30% in *A. escheriana* and 11.05 % to 16.58% in *A. guttata*. Taking into consideration that ΣPUFA levels were always higher than ΣSFA and ΣMUFA levels in all treatments of both species. This was an expected finding for the snails collected during the breeding season because PUFA plays an important role as precursors for signal-transduction involved in the regulation of mating and reproduction. A significant amount of C20:2ω6 was concentrated in the cephalopedal of *A. guttata* (13.42%) and *A. escheriana* (14.93%). Probably, cephalopedal serves as a storage organ of this component. Consequently, the findings revealed that the snail's fatty acid profiles were qualitatively similar, but quantitatively there were some differences. Most important of all, tissues of the snails were good source of essential fatty acids (C18:2ω6 and C18:3ω3) and PUFA, particularly omega 6 fatty acids.

- Keywords: Fatty acids, Assyriella escheriana, Assyriella guttata, organs, lipid classes -

INTRODUCTION

Assyriella escheriana and *Assyriella guttata* (endemic to southeastern Turkey and North Iraq) species are common in moist and calciferous habitats of southeastern Turkey. Although they are not eaten and commercially not exported, they are big enough and fleshy as much as edible relatives *Helix lucorum, Eobania vermiculata* dwelling in the same region. The fatty acid distribution of a large number of commercially important marine and freshwater molluscs have been reported and reviewed in varying degrees of details (ACKMAN, 2000; KARAKOLTSIDIS *et al.*, 1995). Lipids from marine, freshwater and edible land molluscs are more extensively studied (ÖZOĞUL *et al.*, 2005; MILINSK *et al.*, 2006; MILETIC *et al.*, 1991; RAKSHIT *et al.*, 1997; EKİN and BAŞHAN, 2010; EKİN *et al.*, 2012, 2014) than those from nonedible terrestrial members. Nevertheless, nonedible land snails deserve special attention from the point of their evolutional relationship, roles in food chain, nutritional value, taxonomic and possible benefits in cosmetic, medicine and biochemistry. Nutritional, food chain and taxonomic studies are important in understanding interrelationship in marine, freshwater and terrestrial environment, however for the southeastern Anatolia; quite little data were available in the literature on edible and nonedible land snails.

A. escheriana and *A. guttata* species whose edible relatives including *Helix aspersa, H. asemnis, H. cincta* and *H. lucorum, Theba pisana, Eobania vermiculata* and *Cantareus apertus,* living in Turkey (YILDIRIM, 2004), can deserve more detailed studies. Many snail farms are being established in some countries in order to produce good quality snails for consumption and export. So, it seemed useful to make a comparative study of similarity and differences in biochemical and nutritional composition of edible and nonedible snail species.

A comparative biochemical study on fatty acid composition of snails belonging to same classes but living in different habitats is hoped to provide an insight into the adaptive capabilities and influence of the environment on their fatty acid distribution. This paper discusses the fatty acid distribution of main organs and lipid classes in two same genus gastropods from southeastern Anatolia, as data which is hoped to be basic to further comparative biochemical, nutritional, taxonomic and evolutionary studies.

MATERIALS AND METHODS

Sample collection and preparation

Fifteen adult *A. escheriana* species were collected from a woodland near Tizyan (Elmabahçe) village, 20 km north of Mardin (N 37° 49' / E 40° 68') at an altitude of 985 m and fifteen adult endemic *Assyriella guttata* species were collected from stony and rocky region of Diyarbakır city walls (N 37° 55.2' / E 40° 13.8') at an altitude 675 m. Both species were collected in April 2012. Similar size (length: 4 ± 0.60 cm, wet flesh weight: 13 ± 0.50 g) snail species were sampled for lipid analyses. The snails' shells were removed and divided into seven groups (digestive gland, cephalopedal, gonad, mantle, total lipid, neutral lipid and phospholipid) and their organs were dissected out. Then, tissues of each experimental set were conditioned in polyethylene bags and kept at -80°C until chemical analysis.

Extraction of fatty acids and GC analysis

Digestive gland, gonad, mantle, cephalopedal and whole body samples were homogenized in chloroform/ methanol (2:1, v/v) solution in order to extract total body lipids (BLIGH and DYER, 1959). Organ's lipid, total lipid, phospholipid and neutral lipid fractions were obtained according to the method of STANLEY-SAMUELSON and DADD, 1983.

Fatty acids methyl esters (FAMEs) were provided by capillary gas chromatography (GC) using Hewlett Packard (Wilmington, DE) gas chromatograph (model 6890), a DB-23 capillary column (60 m × 0.25 mm i.d. × 0.250 μm film thickness and Bonded 50% cyanopropyl) (J & W Scientific, Folsom, CA), a flame ionization detector, and Hewlett-Packard ChemStation software. The injection port and the detector temperatures were 270°C and 280°C, respectively. The split ratio was 1:20. The flow rates of compressed air and hydrogen were 300 ml/min, 30 ml/min, respectively. Carrier gas was helium (2.8 ml/min). The oven temperature was programmed at a rate of 6.5°C/min from 130°C (1 min hold) to 170°C, then increased at a rate of 2.75°C/min to a 215°C, then again increased at a rate of 40°C/min to 230°C, was held for 12 minutes. Each tissue fatty acids percentages and spectra of FAMEs are obtained by HP 3365 ChemStation computer program. FAMEs existence and retention times were determined by comparing the spectra of authentic standards (Sigma-Aldrich Chemicals). Individual FAME was identified by comparisons with the chromatographic behaviors of authentic standards.

Statistical analyses

The results were expressed as mean values ± SD (Standard Deviation). All analytical determinations were performed in triplicate and the mean values were reported. The analyses were performed using a commercial statistical program (SPSS 20). The percentages of fatty acid were compared by ANOVA variance analysis with 5% significance level. TUKEY's test was used for cooperation of average values.

RESULTS

In both snails, C16:0, C18:0, C18:1ω9, C18:2ω6, C18:3ω3, C20:2ω6 and C20:4ω6 were presented as predominant fatty acids. Of the detected fatty acids, amount of C18:2ω6 was the highest in all analyses from both *A. escheriana* and *A. guttata* (Tables 1 and 2).

Compared to the fatty acids of the species' organs, in *A. escheriana,* highest level of C16:0 (11.81%) and C18:1ω9 (20.56%) were presented in the gonad; C18:0 (15.18%) and C20:2ω6 (14.93%) were in the cephalopedal; C18:2ω6 (26.67%) and C18:3ω3 (7.36%) were in the digestive gland and C20:4ω6 (17.86%) was in the mantle (Table 1). In *A. guttata* highest level of C16:0 (9.72%), C18:1ω9 (19.65%) and C18:3ω3 (4.76%) were found in the gonad; C18:0 (15.21%) and C20:4ω6 (15.68%) were in the mantle; C18:2ω6 (28.12%) was in the digestive gland and C20:2ω6 (13.42%) was in the cephalopedal (Table 2). On the other hand, the fatty acids from lipid classes of the species showed some differences, in *A. escheriana,* highest level of C16:0 (10.16%) and C18:3ω3 (8.42%) were identified in the total lipid; C18:0 (11.21%), C18:1ω9 (13.33%) and C20:2ω6 (11.79%) were in the neutral lipid; C18:2ω6 (27.43%) and C20:4ω6 (20.30%) were in the phospholipid (Table 1). In *A. guttata,* highest level of C16:0 (9.61%) and C20:4ω6 (16.58%) were presented in the total lipid; C18:0 (13.01%), C20:2ω6 (10.99%) and C18:1ω9 (16.03%) were in the neutral lipid; C18:2ω6 (22.34%) and C18:3ω3 (10.70%) were in the phospholipid (Table 2).

In all treatments, results showed that concentration of ω6 (omega 6) always higher than concentration of ω3 (omega 3) family fatty acids. In *A. escheriana*, the ratio of Σω6 / Σω3 was 4.86, 6.71, 5.44 and 7.66 in the digestive gland, cephalopedal, gonad and mantle, respectively (Table 1). In *A. guttata* it was 5.13, 8.49, 5.29 and 4.84 in the digestive gland, cephalopedal, gonad and mantle, respectively (Table 2). Additionally, Σω6 / Σω3 ratio were 5.19 in the total lipid, 4.86 in the neutral lipid and 6.59 in the phospholipid of *A. escheriana* (Table 1). This ratio was observed 4.00 in the total lipid, 3.91 in the neutral lipid

Table 1 - Fatty acid profile of main organs total lipid and lipid classes from *Assyriella escheriana.*

	Fatty acid compositions of total lipid from *A. escheriana* organs				**Fatty acid compositions of lipid classes from *A. escheriana***		
Fatty Acids	**Digestive gland (Mean*±S.D.)****	**Cephalopedal (Mean*±S.D.)****	**Gonad (Mean*±S.D.)****	**Mantle (Mean*±S.D.)****	**Total lipid (Mean*±S.D.)****	**Neutral lipid (Mean*±S.D.)****	**Phospholipid (Mean*±S.D.)****
C10:0	0.12±0.02a	0.05±0.01b	0.08±0.02ab	-	0.02±0.01c	0.04±0.01b	0.10±0.02a
C12:0	0.03±0.01a	0.07±0.02b	0.04±0.01a	0.05±0.01ab	0.06±0.01ab	0.07±0.01b	0.09±0.02b
C13:0	0.08±0.01a	-	0.09±0.02a	0.03±0.01b	0.10±0.02a	-	0.05±0.01b
C14:0	1.31±0.11a	0.29±0.04b	0.49±0.06c	0.81±0.09d	0.58±0.07cd	0.73±0.08d	0.44±0.06c
C15:0	0.39±0.04a	0.19±0.03b	0.23±0.04b	0.30±0.04a	0.23±0.04b	0.68±0.06c	0.37±0.04a
C16:0	**8.41±0.67a**	**9.33±0.83a**	**11.81±1.03b**	**9.72±0.80a**	**10.16±0.91ab**	**9.99±0.82a**	**7.05±0.61c**
C17:0	1.63±0.14a	2.03±0.15b	0.87±0.09c	2.48±0.25b	1.60±0.14a	1.11±0.10d	1.08±0.09d
C18:0	**10.66±0.84a**	**15.18±1.05b**	**10.11±0.79a**	**11.06±0.87a**	**10.30±0.73a**	**11.21±0.91a**	**9.67±0.69a**
C14:1ω9	0.45±0.05a	0.90±0.10b	0.32±0.04a	-	0.15±0.03c	-	0.07±0.02c
C16:1ω7	0.88±0.09a	0.63±0.07b	1.07±0.09c	0.99±0.08c	1.80±0.16d	1.02±0.12c	0.98±0.09c
C18:1ω9	**19.50±1.41a**	**15.20±1.30b**	**20.56±1.52a**	**14.53±1.21b**	**12.68±1.10c**	**13.33±1.11c**	**13.04±1.10c**
C20:1ω9	0.60±0.05a	0.75±0.07a	0.76±0.06a	0.46±0.04b	0.34±0.04b	0.64±0.05a	0.96±0.08c
C22:1ω9	0.03±0.01a	0.05±0.01a	0.12±0.03b	0.14±0.03b	-	0.06±0.01a	0.18±0.03b
C18:2ω6	**26.67±1.72a**	**18.02±1.41b**	**24.42±1.55c**	**22.32±1.49d**	**20.45±1.39e**	**19.77±1.43e**	**27.43±1.66a**
C18:3ω3	**7.36±0.69a**	**1.74±0.15b**	**5.66±0.44c**	**3.96±0.29d**	**8.42±0.71a**	**8.00±0.70a**	**6.60±0.51e**
C20:2ω6	**7.35±0.68a**	**14.93±1.26b**	**8.51±0.75a**	**10.91±0.96c**	**10.72±0.94c**	**11.79±1.01d**	**7.25±0.64a**
C20:3ω6	1.41±0.12a	0.66±0.05b	1.42±0.13a	0.88±0.07b	0.73±0.06b	0.88±0.09b	1.30±0.10a
C20:4ω6	**11.14±1.04a**	**14.37±1.12b**	**10.01±0.94a**	**17.86±1.27c**	**19.51±1.39d**	**17.12±1.25c**	**20.30±1.44d**
C20:5ω3	1.07±0.09a	4.11±0.31b	1.90±0.13a	1.91±0.15a	1.03±0.14a	1.75±0.15a	1.00±0.09a
C22:2ω6	0.09±0.02a	0.22±0.03b	-	0.06±0.01a	-	-	0.05±0.01a
C22:5ω6	0.21±0.03a	0.45±0.04b	1.05±0.08c	0.73±0.05d	0.89±0.08d	0.78±0.06d	1.02±0.10c
C22:6ω3	1.22±0.12a	1.40±0.13a	0.78±0.06b	1.02±0.09a	0.63±0.05b	0.60±0.05b	1.10±0.10a
Σω6 / Σω3	4.86	6.71	5.44	7.66	5.19	4.86	6.59
ΣSFA	22.63±1.50a	27.14±1.63b	23.72±1.56a	24.45±1.58c	23.05±1.49a	23.83±1.51a	18.85±1.41d
ΣMUFA	21.46±1.47a	17.53±1.25b	22.83±1.44a	16.12±1.23b	14.97±1.17c	15.05±1.19c	15.23±1.18c
ΣPUFA	**56.52±2.28a**	**55.90±2.27a**	**53.75±2.20b**	**59.65±2.48c**	**62.38±2.55d**	**60.69±2.52c**	**66.05±2.61e**

Results expressed as percentage of total fatty acids methyl esters.

*Values are means ± S.D (Standard Deviation) for three samples of triplicate analysis.

Means followed by different letters in the same line are significantly different (P** < 0.05) by Tukey's test.

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 acid.

Table 2 - Fatty acid profile of main organs total lipid and lipid classes from *Assyriella guttata*.

Fatty Acids	Fatty acid compositions of total lipid from *A. guttata* organs				Fatty acid compositions of lipid classes from *A. guttata*		
	Digestive gland (Mean*±S.D.)**	Cephalopedal (Mean*±S.D.)**	Gonad (Mean*±S.D.)**	Mantle (Mean*±S.D.)**	Total lipid (Mean*±S.D.)**	Neutral lipid (Mean*±S.D.)**	Phospholipid (Mean*±S.D.)**
C10:0	-	0.09±0.02a	0.12±0.03b	0.08±0.02a	0.07±0.02a	0.24±0.04c	-
C12:0	0.70±0.08a	-	0.87±0.10b	0.95±0.10b	0.60±0.07a	0.77±0.08ab	-
C13:0	1.35±0.13a	1.16±0.12a	1.09±0.10a	1.30±0.12a	1.11±0.12a	1.05±0.11a	0.09±0.02b
C14:0	2.22±0.21a	1.47±0.16b	1.10±0.12a	1.78±0.19ab	1.85±0.20ab	2.03±0.21a	0.64±0.08c
C15:0	0.99±0.10a	1.02±0.11a	1.32±0.14a	1.44±0.15a	1.32±0.14a	1.82±0.20b	0.47±0.07c
C16:0	**7.01±0.64a**	**9.01±0.72b**	**9.72±0.78b**	**7.82±0.66ab**	**9.61±0.73b**	**7.78±0.58ab**	**5.92±0.41c**
C17:0	0.13±0.03a	0.33±0.06b	0.16±0.03a	0.18±0.04a	0.06±0.02c	0.09±0.02ac	0.08±0.02ac
C18:0	**8.62±0.64a**	**13.58±0.91b**	**10.11±0.74c**	**15.21±1.04d**	**12.33±0.86bc**	**13.01±0.94b**	**8.76±0.59a**
C14:1ω9	0.35 ±0.05a	0.21±0.04a	0.23 ±0.04a	-	0.25±0.04a	-	0.67±0.06b
C16:1ω7	1.34±0.12a	0.77±0.09b	0.87±0.10b	0.89±0.16b	0.40±0.03c	0.44±0.03c	0.68±0.07bc
C18:1ω9	17.42±1.32a	16.32±1.25a	19.65±1.49b	15.35±1.21c	15.06±1.21c	16.03±1.30a	16.02±1.29a
C20:1ω9	0.60±0.07a	0.75±0.08a	0.67±0.06a	0.16±0.03b	0.43±0.05c	0.31±0.05c	0.69±0.08a
C22:1ω9	-	-	0.21±0.04a	0.04±0.01b	-	0.09±0.02b	0.08±0.02b
C18:2ω6	**28.12±1.75a**	**21.60±1.48b**	**24.24±1.55c**	**20.23±1.45b**	**18.54±1.38d**	**17.07±1.35d**	**22.34±1.65a**
C18:3ω3	**4.06±0.31a**	**2.43±0.19b**	**4.76±0.44a**	**2.90±0.32a**	**6.24±0.51c**	**7.01±0.57c**	**10.70±0.41ac**
C20:2ω6	**6.14±0.53a**	**13.42±1.16b**	**9.15±1.02c**	**8.81±0.96c**	**9.22±1.04c**	**10.99±1.16bc**	**8.52±0.84c**
C20:3ω6	0.71±0.62a	0.61±0.56a	0.24±0.03b	0.19±0.03b	0.13±0.03c	0.08±0.02c	0.80±0.09a
C20:4ω6	**15.09±1.29a**	**13.73±1.12b**	**11.05±1.10c**	**15.68±1.25a**	**16.58±1.30d**	**15.71±1.28a**	**16.13±1.33ad**
C20:5ω3	4.53±0.39a	2.16±0.21b	2.45±0.23b	4.85±0.35c	3.75±0.38c	3.54±0.34c	4.76±0.41a
C22:2ω6	0.14±0.03a	0.02±0.01b	0.04±0.01b	-	-	0.59 ±0.05c	0.08±0.02ab
C22:5ω6	0.15±0.03a	0.13±0.03a	0.55±0.06b	0.37±0.04c	0.98±0.10d	0.67±0.08b	0.82±0.09d
C22:6ω3	1.23±0.13a	1.24±0.12a	1.38±0.14a	1.60±0.16a	1.36±0.15a	1.00±0.10a	2.08±0.24b
Σω6 / Σω3	**5.13**	**8.49**	**5.29**	**4.84**	**4.00**	**3.91**	**2.28**
ΣSFA	21.02±1.51a	26.66±1.62b	24.49±1.56ab	28.76±1.74c	26.95±1.67b	26.79±1.59b	15.96±1.32c
ΣMUFA	19.71±1.37a	18.05±1.33a	21.63±1.42b	16.44±1.28c	16.14±1.22c	16.87±1.30c	18.14±1.33a
ΣPUFA	60.17±2.49a	55.34±2.25b	53.86±2.15c	54.63±2.18b	56.80±2.39d	56.66±2.36d	66.23±2.51e

Results expressed as percentage of total fatty acids methyl esters.

*Values are means ± S.D (Standard Deviation) for three samples of triplicate analysis.

Means followed by different letters in the same line are significantly different (P** < 0.05) by Tukey's test.

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.

and 2.28 in the phospholipid of *A. guttata* (Table 2). These high levels of Σω6/Σω3 were mostly on account of higher concentration of C18:2ω6 and C20:4ω6.

The most notable result was significantly high level of ΣPUFA (total polyunsaturated fatty acids) and low level of ΣSFA (total saturated fatty acids) and ΣMUFA (total monounsaturated fatty acids) in all organs and lipid classes. Among the organs, the maximum level of ΣPUFA was obtained in the mantle (59.65%) of *A. escheriana* (Table 1) and in the digestive gland (60.17%) of *A. guttata* (Table 2). On the other hand, maximum level of ΣSFA was detected in the cephalopedal (27.14%) of *A. escheriana* (Table 1) and in the mantle (28.76%) of *A. guttata* (Table 2). The level of ΣMUFA in all treatments was found significantly lower than ΣPUFA and ΣSFA. It ranged from 16.12% to 21.46% in *A. escheriana* and 16.44% to 21.63% in *A. guttata.* It was noteworthy that, the amount of ΣPUFA was significantly high; 66.05% in the phospholipid, 62.38% in the total lipid and 60.69% in the neutral lipid of *A. escheriana* (Table 1) and 66.23% in the phospholipid, 56.80% in the total lipid and 56.66% in the neutral lipid of *A. guttata* (Table 2).

DISCUSSION

The significance of fatty acids drives from their role as fuel to provide metabolic energy, their usage for storage products, eicosanoids, physiological activities, structural components such as membrane lipids particularly phospholipids and sterol esters. Most importantly, they fulfill a structural role and are also very important intermediates in cell physiology, formation of prostaglandins and other eicosanoids from ω3 and ω6 fatty acids (STANLEY-SAMUELSON, 1994). The importance of specific fatty acids as dilatory compounds for animals is partly because of almost all animals to introduce second or third double bond into fatty acids to synthesize polyunsaturated fatty acids (BEENAKKERS *et al.*, 1985).

The seriousness of fatty acids was mostly emphasized for freshwater molluscs. However, lipid data correlated with nutritional, physiologi-

cal, structural and environmental factors in terrestrial snails is notably limited in literatures and relatively little is known about terrestrial snails' fatty acid composition, particularly on their organs. There are only a few studies regarding of fatty acids of edible, nonedible snails and land slugs such as *H. aspersa* (ÇAĞILTAY *et al.*, 2011), *H. aspersa maxima* (MILINSK *et al.*, 2006), *H. pomatia* (ÖZOĞUL *et al.*, 2005), *E. vermiculata* (STAVRAKAKIS *et al.*, 1989), *Helix sp., Haplotrema sportella, Vespericola columbiana, Arion ater, Limax maximus, Prophysaon andersoni* (ZHU *et al.*, 1994). Furthermore, only few studies are present on fatty acid distribution of mollusc organs and tissues. *Macoma balthica* (WENNE and POLAK, 1989), *Telescopium telescopium* (RAKSHIT *et al.*, 1997), *Argopecten purpuratus* (CAERS *et al.*, 1999), *Bellamya bengalensis*, *Pila globosa* (MISRA *et al.*, 2002), *Unio elongatulus* (EKİN and BAŞHAN, 2010), *Corbicula fluminalis* (EKİN, 2012), *H. lucorum* (EKİN 2014) are some of known mollusc species, tissue and organs studied.

Qualitatively, fatty acid profiles of two species were similar. Similarity of the fatty acid content of both species is not surprising. Because they are close relatives and derived from the same origin. However, quantitative differences in the fatty acid profile were likely due to environmental, nutritional and physiological effects.

Generally, molluscs are well known to contain C16:0, C18:0, C18:1ω9, C18:2ω6, C18:3ω3 and C20:4ω6 as major fatty acids. These fatty acids have previously reported in most of the mollusc species and explored for their potential use in food chain studies. They were identified as predominant components in *Theodoxus jordani, Melanoides tuberculata, Pyrigula barroisi, Melanopsis praemorsum* freshwater snails (GO *et al.*, 2002); in *Helix sp. H. sportella, V. columbiana* (ZHU *et al.*, 1994), *H. aspersa* (ÇAĞILTAY *et al.*, 2011), *H. pomatia* (ÖZOĞUL *et al.*, 2004), *H. lucorum* (EKİN, 2014) land snails; in *T. telescopium* marine snail (RAKSHIT *et al.*, 1997); in *U. elongatulus* (EKİN and BAŞHAN, 2010), *C. fluminalis* (EKİN, 2012), *B. bengalensis*, *P. globosa* (MISRA *et al.*, 2002), *A. purpuratus* mussels (CAERS *et al.*, 1999); in *P. andersoni, A. ater, L. maximus* (ZHU *et al.*, 1994) slugs. As highlighted above, *A. escheriana* and *A. guttata* also contained high amount of C16:0, C18:0, C18:1ω9, C18:2ω6, C18:3ω3 and C20:4ω6, concentrated in the organs and fractions. In previous studies, it was stated that C20:4ω6 is more characteristic of sea urchins and starfish, C20:5ω3 is characteristic of invertebrates that feed on single-celled algae and occurs in almost all classes, additionaly C22:6ω3 is more characteristic of fish and crustacea (SINANOGLOU and MINIADIS-MEIMAROGLOU, 1998). Notably, C16:0, C18:0 and C18:1ω9 can be found in most of the animal tissues and very common among fatty acids.

PUFAs may be further modified to form prostaglandins that are directly involved in regulation of reproduction, renal function, ion regulation as known from mollusc species, (STANLEY-SAMUELSON, 1987). It is stated that egg production in freshwater snail *Helisoma durgi* was stimulated by prostaglandins (KUNIGELIS and SALEUDDIN, 1986). C20:4ω6 precursors of prostaglandins was found to be 15.09% in the digestive gland, 13.73% in the cephalopedal, 11.05% in the gonad, 15.68% in the mantle of *A. guttata* and 11.14% in the digestive gland, 14.37% in the cephalopedal, 10.01% in the gonad, 17.86% in the mantle of *A. escheriana*. Probably, this high value is related to reproduction and other physiological activities of the snails. In animal cells prostaglandins precursor C20:4ω6 is mostly obtained from phospholipid main source of PUFA. To remember, the phospholipids of the snails contained high level of C20:4ω6, 20.30% in *A. escheriana* and 16.13% in *A. guttata*. As a matter of fact, ΣPUFA levels in the phospholipid were detected much higher than other lipid fractions and organs both in *A. escheriana* and *A. guttata*. In the phospholipid of *A. escheriana* and *A. guttata*, ΣPUFA levels were presented to be 66.05% and 66.23%, respectively. Recognizing that snail species in this study are herbivores, therefore containing high proportion of PUFA was expected result. Because plant based diet is containing much more PUFA than flesh based diet.

In the present study, ΣPUFA levels were always found to be higher than ΣSFA and ΣMUFA. This finding was in agreement with garden snail *H. aspersa* stating that PUFA was most abundant fatty acids (ÇAĞILTAY *et al.*, 2011). It was also declared that C18:2ω6, C20:4ω6, C18:3ω3 and C20:5ω3 were the dominant fatty acids. Snails frequently feed on decaying plant materials to avoid high concentration of deterrent or toxic plant metabolites (SPEISER *et al.*, 1992). Aging of plant material results in a decrease of its PUFA content (KIS *et al.*, 1998), suggesting that snails eating old plant material may suffer from a shortage of PUFA. Therefore, it can be said that natural food sources vary seasonally in the composition of ingredients (WACKER, 2005). In the present study, the snails were collected in spring season and they mostly fed on fresh plant materials. Maximization of PUFA levels in all organs and fractions were probably because of fresh plant diets. The snails' mating activities are significantly reduced when snails were fed the PUFA-deficient diet. It is stated that PUFA played important role in reproductive allocation (WACKER, 2005).

A. escheriana species were collected from woodland, whereas *A. guttata* species were collected from stony and rocky region of city walls which is containing decaying organic matter, garbage, sediments, grass, shrubs and etc. Decaying organic matter containing places mostly shelters bacteria, protozoa, mold, invertebrates

and other microorganisms. C13:0, C14:0, C15:0, C17:0 and other short-chain saturated fatty acids are common in bacteria (WACKER, 2005). In the analyses, it was observed that *A. guttata* contained slightly higher amount of short-chain fatty acids than *A. escheriana*. C14:0 ranged from 1.10% to 2.22% in *A. guttata* organs and 0.49% to 1.31% in *A. escheriana* organs. C15:0 varied from 0.99% to 1.44% in *A. guttata* and from 0.19% to 0.39% in *A. escheriana*. Probably, it was stem from habitats of *A. guttata* which is suitable for living for microorganisms.

In all fractions and organs, C18:2ω6 essential fatty acid was the main components followed by C18:1ω9 and C20:4ω6. The highest concentration of the fatty acid was found to be 28.12% and 26.67% in the digestive gland of *A. guttata* and *A. escheriana*, respectively. Among the snails' lipid fractions, the phospholipid contained 27.43% in *A. escheriana* and 22.34% in *A. guttata* of C18:2ω6 (Table 1, 2). On the contrary, this fatty acid was found in low level in *T. telescopium* freshwater snail's organs; 2.5% in the digestive gland, 4.3% in mantle and 4.95% in the cephalopedal (RAKSHIT *et al.*, 1997). On the other hand, in edible snail *H. aspersa maxima*, C18:2ω6 was found rather a lot, between 44.79%-51.19% (MILINSK *et al.*, 2006) and this fatty acid was also found good amount in edible snail *H. lucorum* (EKİN, 2014). Most likely, these different data stem from the requirement of the fatty acid for snail species. This essential fatty acid plays central role in production of other PUFA and most animals cannot synthesize it, for this reason they are dependent on taking it from their diets.

An interesting fact was that snails had C20:2ω6 with high concentrations varying from 6.14% to 13.42% in *A. guttata* and from 7.25% to 14.93% in *A. escheriana*. In particular, the highest level of the fatty acid was detected in the cephalopedal of both species. In some studies, it is stated that snail cephalopedal served as a storage organ (JOHNS *et al.*, 1979), probably, the cephalopedal stored this fatty acid for further metabolic activities.

In comparison with *A. escheriana* and *A. guttata*, it was observed some strange results in *T. telescopium* snail, for instance C18:3ω3 was not detected in the digestive gland and mantle, but it was found 10.7% in the cephalopedal tissue as well as C16:1ω7 was found to be 11.3% in the digestive gland, 6.1% in the mantle, 4.9% in the cephalopedal (RAKSHIT *et al.*, 1997). In *A. guttata* and *A. escheriana*, C16:1ω7 was found at low concentrations, did not exceed 1.80%. However, C18:3ω3 was presented 7.36% in the digestive gland, 3.96% in the mantle of *A. escheriana* and 4.06% in the digestive gland, 2.90% in the mantle of *A. guttata*. For *A. escheriana,* the highest proportion of C18:3ω3 was found to be 8.42% in the total lipid in comparison with 10.70% in the phospholipid of *A. guttata*. C18:3ω3 was another essential fatty acid and its content was expected to be high in the phospholipid fractions, because phospholipid contains much more PUFA than MUFA and SFA. It is also noteworthy that, the content of fatty acids may differ from year to year, season to season, and depend on the nutrition of organism. Above all, the distribution of an organism is mostly influenced by many factors including temperature, reproduction season, growth, nutrient availability, genetic, physiology and etc.

In the treatments, neutral lipid and total lipid fatty acid distribution are more similar to each other than phospholipids. In particular, ΣSFA, ΣMUFA and ΣPUFA contents in neutral lipid and total lipid were detected so close to each other in both species. This kind of determination is very normal, because neutral lipids and total lipids are structurally and contently similar.

Both ω3 and ω6 fatty acids are important components of biomembranes and are precursors to many other substances in organisms. Researches indicate that omega fatty acids especially ω3 fatty acids reduce inflammation and may help lower risk of chronic diseases such as heart disease, cancer, and arthritis. The ratio of Σω6/Σω3 is usually received to be useful indicator for comparing nutritional values of the samples. In *A. escheriana*, the highest value of Σω6/Σω3 was in the phospholipid (6.59), whereas the lowest value was in the neutral lipid and digestive gland (4.86). On the other hand, in *A. guttata* the highest value of Σω6/Σω3 was in the cephalopedal (8.49), the lowest value was in the phospholipid (2.28). This wide difference between snails' phospholipids fractions was due to the high proportion of C18:2ω6 in *A. escheriana.* In agreement with our findings, Σω6/Σω3 ratio was also found to be high in *A. ater, L. maximus, P. andersoni,* slugs and *V. columbiana, H. sp. H. sportella* (ZHU *et al.*, 1994) and in *H. lucorum* land snails (EKİN, 2014). But, in marine molluscs, percentage of Σω6 was found to be lower than Σω3 (ABAD *et al.*, 1995; PAZOS *et al.*, 2003).

Eventually, the results showed that species were rich in PUFA, totally always over 50% in all analyses and maximization of C16:0, C18:0, C18:1ω9, C18:2ω6, C18:3ω3, C20:2ω6 and C20:4ω6 were observed. Particularly, the organs and lipid fractions of both snails contained good amount of essential fatty acid, C18:2ω6 taking role in the synthesis of other fatty acids. Moreover, Σω6/Σω3 and ΣPUFA/ΣSFA+ ΣMUFA ratios were found in good range. Herewith, the results can be important guide for further investigation on nutritional, physiological, biochemical and taxonomic studies of molluscs. Commercially some important edible snails *Cryptomphalus aspersus (H. aspersa), H. asemnis, H. cincta, H. lucorum, T. pisana, E. vermiculata* and *C. apertus* dwell in Turkish territories (YILDIRIM, 2004). Although *A. escheriana* and *A. guttata* are not edible snails; however they are very common in

the southeastern Anatolia region. It should not be forgotten, snails collected from the wild environment may accommodate poisonous chemicals, heavy metals, drugs, alkaloids and agricultural chemicals. Perhaps, *A. escheriana* and *A. guttata* land snails will be used as edible after the pathological and biochemical detailed studies in the future; however we can offer no adequate explanation about edibility at present.

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