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THE EFFECTS OF SEASONS ON CHOLESTEROL CONTENT AND FATTY ACID COMPOSITIONS OF MUSCLE OF *HELIX ASPERSA* LIVING IN KONYA, TURKEY

Ozcan Baris Citil[1], Yener Tekeli[2], Hatice Danahaliloglu[2], Serbay Bucak[2]

[1]Selcuk University, Faculty of Veterinary Medicine, Basic Sciences, Selcuklu Konya Turkey, [2]Mustafa Kemal University, Science and Art Faculty, Department of Biochemistry, Hatay Turkey

Corresponding authorE-mail: yenertekeli@gmail.com, ytekeli@mku.edu.tr

Abstract

Background: The aim of the present study is the determination of the effects of seasonal variations on the proximate analysis, cholesterol content and fatty acid compositions of *Helix aspersa.*
Materials and Methods: Garden snails (*Helix aspersa*) were picked up by hand from the Central Anatolia Region of Turkey, in autumn (November) and spring (April) in 2011. Fatty acid methyl esters (FAMEs) and cholesterol analysis were analyzed by gas chromatography (GC). The protein contents of snail muscle were determined with Kjeldahl distillation units. Statistical comparisons were made by using SPSS Software (version 16.0).
Results: Thirty different fatty acids of different saturation levels were detected. As the predominant fatty acids, stearic acid (C18:0), oleic acid (C18:1ω9), linoleic acid (C18:2ω6), palmitic acid (C16:0), arachidonic acid (C20:4ω6), eicosadienoic acid (C20:2) and linolenic acid (C18:3ω3) were found in *Helix aspersa*. Palmitic acid (C16:0) was identified as the major SFA in autumn and spring. Linoleic acid (C18:2ω6), eicosadienoic acid (C20:2) and arachidonic acid (C20:4ω6) have the highest levels among the PUFAs. In the present study, ω3 were found 5.48% and 13.94% in autumn and spring, respectively.
Conclusion: Linolenic acid and omega-3 fatty acid amounts in the spring increased significantly but cholesterol content was not affected in *Helix aspersa* both in season.

Key words: *Helix aspersa*, seasonal variations, fatty acid, SFA, PUFA, cholesterol

Introduction

Snails are small nutrients contain protein and some minerals abundantly and inadequate cholesterol and fat that has linoleic and linolenic acids (known as essential fatty acids) in its composition and polyunsaturated fatty acids with more than twenty C atoms, suggesting that this food can be used for patient nutrition irrespective of total lipid content. *Helix aspersa* contains high levels of polyunsaturated fatty acids (PUFAs) of the omega 6 series and especially omega 3 series such as eicosapentaenoic acid (EPA, C20:5ω3) and linolenic acid (C18:3ω3) naturally these are recognized as essential biochemical components of human diet due to their beneficial effects on human wellness. Because long chain ω3 PUFAs cannot be synthesized by humans (Adeyeye and Afolabi, 2004, Milinsk et al., 2003, Alasalvar et al., 2002, Milinsk et al., 2006). It is known that ω3 fatty acids, or balanced of ω3/ω6 ratios in the diet essential for normal development and development and may take on an important role in prevention and treatment against such as coronary artery disease, diabetes, high blood pressure and cancer. They also affect for the neurodevelopment of infants, glycemic control of fat, improve of learning abilities and visual functions (Kinsella et al., 1990). EPA is the most important essential fatty acid of ω3 series in the human diet because of its precursor of the 3-series eicosanoids (Chen et al., 1995). Arachidonic acid (C20:4ω6), EPA and DHA are important structural parts of cell membranes (Innis, 1991).

Helix aspersa is an abundant small family in Konya, Turkey and one of the most widely cultured snail species all over the globe. However the fatty acid dynamics of this species are not easily recognized. No reports have been yet published about the effects of seasonal variations on the fatty acid makeup of this important species in Konya. The main objective of the present work was to determine the proximate composition, cholesterol content and fatty acid composition of *Helix aspersa* muscle.

Materials and Methods

Sample collection

Garden snails (*Helix aspersa*) were picked up by hand from the Central Anatolia Region of Turkey. The Konya city is situated at between 36°22’ - 39°08’ north latitude and 31°14' - 34°05' east longitude and is the greatest province of Turkey with a surface area of 38.183 km^2. The population of the city is approximately 1.085.000. The survey region is approximately 17.1 km wide from east to west and 25 km long from north to south, which affords a total field of 427.5 km^2.of 427.5 km^2. The snails were collected in autumn (November) and spring (April) in 2011. Ten (n=10) snails of similar size (2 cm distance) were sampled each season for lipid analysis. The temperatures of the weather were 8°C in autumn and 15°C in spring. Collected snails were kept in 80°C water for 30 minutes.

Fatty acid analysis

The basic method of Folch et al. (1957) was observed for the extraction of fatty acids from the tissues. Thus, samples were homogenized in a chloroform/methanol (2/1, v/v) mixture. The method of AOAC (1972), was used in order to obtain the methyl esters of fatty acids by using BF_3 (14%).

Fatty acid methyl esters (FAMEs) were analyzed by Shimadzu 15-A gas chromatography (GC) which equipped with dual flame ionization detector and 1.8 m × 3 mm internal diameter, packed glass column containing 100/120 Chromosorb WAW coated with 10% SP 2330. The oven temperature was programmed with initial temperature of 190°C, was increased at a rate of 30°C per min to 220 °C where it was maintained for 5 min. The injector and flame ionization detector were set at 280 °C. Nitrogen (N_2) was used as a carrier gas. The injection volume

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was 1 µl. Identification of normal fatty acids was carried out by comparing sample FAMEs peak relative retention times with those obtained for Supelco standards.

Proximate composition and cholesterol content analysis

The protein contents of snail muscle were determined according to previously published method with Kjeldahl distillation units. Moisture and ash contents of *Helix aspersa* were determined by according to published by the Association of Official Agricultural Chemists (AOAC, 1990).

Cholesterol analysis of the non-saponifable part of the material was carried out by according to the Naeemi et al. (1995) method with some modifications. The chromatographic conditions were followed as: injector and detector temperatures were 300°C and column temperature was 270°C (isothermal); carrier gas N_2 40 ml/min, H_2 30 ml/min and air 300 ml/min.

Statistical analysis

The proximate compositions and GC analyses were repeated for three times. In fatty acid analysis, ten data (n=10) were obtained for each variety. The results are reported as means ± SD. Statistical comparisons were made by using SPSS Software (version 16.0).

Result and Discussion

In this study, cholesterol contents of *Helix aspersa* were assessed 0.02% and 0.03% in autumn, and in spring, respectively. Similarly, Daniels et al. (1999) reported that the total cholesterol content of *Helix aspersa* was 0.04% in Australia. Cholesterol content was the same in autumn and in spring. Cholesterol contents of the terrestrial snails were not affected by different diets (Wacker, 2005). The proximate compositions are shown in (Table 1). The protein, moisture, ashes were assessed 14.12%, 5.27%, 81.23%, in autumn, 12.31%, 5.11%, 82.68%, in spring, respectively.

Table 1: Proximate compositions and cholesterol content of *Helix aspersa*

Source	Autumn	Spring
Protein (%)	14.12±0.05	12.31±0.04
Moisture (%)	81.23±2.29	82.68±2.25
Ash (%)	5.27±0.03	5.11±0.03
Oil content (%)	0.52±0.00	1.80±0.01
Cholesterol (mg / 100g)	0.02±0.00	0.03±0.01

The variation depends largely on the variety. As can be seen *Helix aspersa* is a good source of protein. These findings were supported by Cagiltay et al. (2011). The results of the protein value of similar studies as follows: 12.2-19.12% , 16.35% , 16.17% (Olmez and Secer, 1998), (Ozogul, 2005), (Yildirim, 1996). Ozden and Erkan, (2011) reported that 66.78 % moisture, 21.08 % protein, 1.96 % ash, 2.54 % fat and 8.64 % carbohydrate for sea snail. It is thought that the higher proximate compositions to autumn than spring. These results can be associated with different latitude.

The total lipid content of *Helix aspersa* was determined throughout the two seasons. It is presented in Table 1. For this species total lipid contents of autumn were determined higher than in spring. This difference can be explained by the seasonal eating habits in of snail. Fat content is influenced by species, seasonal variations, geographical regions, age and maturity (Piggot and Tucker, 1990).

The results of fatty acid analyses were shown in (Table 2). Thirty fatty acids were identified among the muscle lipids of *Helix aspersa*. The highest fatty acids of *Helix aspersa* in two seasons were C18:2ω6 linoleic acid, C18:0 stearic acid, C18:1ω9 oleic acid, C20:4 arachidonic acid and C20:2 eicosadienoic acid, C16:0 palmitic acid, respectively. In the present study, polyunsaturated fatty acids (PUFAs) were higher than total monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs). The ratio of total PUFAs ranged between 39.65% and 43.21%. Linoleic acid was the major PUFA (12.17 - 19.49%) for *Helix aspersa* in two seasons. Similar results for other snail species have also been reported in literature (Ekin et al., 2011). According to Fried et al. (1993), stearic acid is the major SFA in Gastropoda . The leading fatty acids have been found palmitic acid in SFAs and oleic acid in MUFAs (Zhu et al., 1994). Similar results for stearic acid were identified in the present study as 18.91%, 16.26% for autumn and spring respectively. C6:0 was found in autumn and spring with low rates (0.02–0.02%). C8:0, C10:0, C12:0, C15:0, C21:0 were investigated low levels rate the SFA fractions of the muscle. There were no differences between seasons in terms of C14:0, C17:0, C20:0 and C22:0 in SFA ($p < 0.05$). Oleic acid was identified as the major MUFA of *Helix aspersa* (67.94–70.56% of total MUFAs). Oleic acid in muscle tissue of *Helix aspersa* was found 14.45%, and 17.57% in autumn and spring, respectively. Similarly Ozogretmen, (2006) found that C18:1ω9 was the major MUFA in muscle tissue of *Helix aspersa*. Gadoleic acid was the second most abundant MUFA (2.41–3.41%) in the present study. The high levels of MUFAs were reported as a characteristic property of snail oils.

There were no differences between autumn and spring in terms of C16:1ω7 ($p < 0.05$). Although *Helix aspersa* had the most stable fatty acid compositions, there were quantitative differences. C14:1ω5 and C22:1ω9 were found too low levels among MUFA fractions of the muscle. On the other hand, MUFA contents were higher than SFAs and PUFAs in autumn and spring. In spring a high ratio of C18:1ω9 (17.57%) increased the MUFA content and a high ratio of C18:2 increased the PUFA content in the autumn. Variations between the fatty acid compositions are might be related to changes in nutritional habits of the snail. PUFAs of *Helix aspersa* were observed significant constituent as 50.2–57.0% according to the season. Linoleic acid, eicosadienoic acid, arachidonic acid and linolenic acid were assessed as predominant PUFAs. In the present study, the percentages (in total lipid) of EPA and DHA, which have a vital role in human nutrition were between 1.50–3.70% and 0.45–2.97%, respectively, according to the seasons. Thus, among the ω3 series, the *Helix aspersa* are good sources of EPA and DHA in two seasons. The amounts of EPA and DHA for daily ingestion have been suggested to be in the range 200–1000 mg (Simopoulos, 1991). In this study, the authors reported that relatively high amounts of DHA have been found in *Helix aspersa*. An increase in the human dietary ω3/ω6 fatty acid ratio is essential to help prevent coronary heart disease by reducing plasma lipids and reduce the risk of cancer (Gokce, 2004). The amounts and balance of these fats in a person's diet will affect the body's eicosanoid-controlled functions. Eicosanoids have great significance. Eicosanoids mediate several pathophysiological events in mammals, insects, and other invertebrates by influencing ion transport and mediating cellular defense mechanisms (Stanley, 2000). The ratio of ω3/ω6 PUFAs ranged from 0.14% to 0.20% and showed variation in many species as reported by Hearn et al. (1987). In the present study, the ratio of ω3/ω6 PUFAs was found within the range for *Helix aspersa*, except for spring (0.54%). The ω6/ω3 ratios were recommended as maximum of 4.0 (HMSO, 1994). Similarly, Milinsk et al. (2003) identified this ratio in *Helix aspersa* as

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5.01–7.05. The ω6/ω3 ratios found by Zhu et al. (1994), for *Helix* sp. was 7.8 for *Haplotrema sportella* 6.9 and for *Vespericola columbiana* 5.2. A minimum value of the PUFA / SFA ratio recommended as 0.45 (HMSO, 1994) which were obtained from *Helix aspersa* with values of 1.22%, 1.12%, in autumn, spring, respectively. The fatty acid compositions, cholesterol contents and proximate compositions of *Helix aspersa* were determined and compared. The results clearly indicate that there are differences in fatty acid compositions, cholesterol contents and proximate compositions among them. These differences are thought to arise from latitude in the spring. However, ω6/ω3 and PUFA/SFA ratios were found better than according to HMSO (1994).

Table 2: Fatty acid composition of *Helix aspersa*

Fatty acid	Autumn	Spring	Fatty acid	Autumn	Spring	Fatty acid	Autumn	Spring
C 6:0	0.02±0.01	0.02±0.01	C 14:1ω5	0.01±0.00	0.14±0.02	C 18:2ω6	19.49±0.41	12.17±0.20
C 8:0	0.13±0.01	0.04±0.01	C 16:1ω7	0.78±0.02	1.04±0.09	C 18:3ω3	2.39±0.12	5.83±0.55
C 10:0	0.1±0.01	0.12±0.01	C 17:1ω8	1.04±0.03	1.19±0.02	C 20:2ω6	8.06±0.05	6.84±0.61
C 12:0	0.15±0.01	0.14±0.01	C 18:1ω9	14.45±0.50	17.57±0.65	C 20:3ω3	0.48±0.01	0.43±0.21
C 14:0	0.95±0.02	1.25±0.02	C 20:1ω9	2.41±0.15	3.41±0.36	C 20:4ω6	9.23±0.1	5.61±0.48
C 15:0	0.24±0.02	0.38±0.03	C 22:1ω9	0.02±0.01	0.03±0.01	C 20:5ω3	1.50±0.22	3.70±0.32
C 16:0	8.81±0.57	8.3±0.470	C 24:1ω9	2.57±0.33	1.54±1.49	C 22:3ω3	0.44±0.03	0.03±0.01
C 17:0	1.26±0.01	1.32±0.23				C 22:4ω6	0.96±0.18	1.09±0.20
C 18:0	18.91±0.37	16.26±0.92				C 22:5ω3	0.22±0.01	1.00±0.26
C 20:0	1.87±0.06	1.94±0.09				C 22:6ω3	0.45±0.03	2.97±0.19
C 21:0	0.31±0.02	0.70±0.18				**ω3**	**5.48±0.16**	**13.94±0.73**
C 22:0	1.83±0.3	2.72±0.61				**ω6**	**37.73±0.68**	**25.71±0.27**
C 24:0	0.97±0.12	2.3±0.040				**ω3/ω6**	**0.15±0.00**	**0.54±0.030**
SFA	**35.53±0.81**	**35.45±0.67**	**MUFA**	**21.27±0.01**	**24.90±1.13**	**PUFA**	**43.21±0.82**	**39.65±0.47**

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