Chemical composition and antioxidant profile of snails (*Cornu aspersum aspersum*) fed diets with different protein sources under intensive rearing conditions

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ABSTRACT. Snail meat is considered as a valuable nutrient source, due to its high protein and low-fat content, low caloric value and high content of ω-3 and ω-6 fatty acids. Escargot could become an alternative for consumers looking for diets enriched with nutrients beneficial for human health. The aim of this study was to examine the effect of four compound diets containing a different protein source on the chemical composition, the levels of fatty acids and the antioxidant profile of meat of *Cornu aspersum aspersum* reared under intensive farm conditions. Snails of each treatment were fed with a compound diet containing soya (*Glycine max*), organic soya, white lupin (*Lupinus alba*) or pea (*Pisum sativum*). In addition, sexually mature snails were collected directly from nature for comparison purposes. Chemical analyses were conducted on the meat of snails that have reached proper commercial weight and size (mean weight: 16.97 ± 0.29 g; mean diameter: 38.66 ± 0.24 mm). The results revealed that meat from snails fed diet containing organic soya had higher collagen content, whereas meat from snails fed *Pisum sativum* and *Lupinus alba* resulted in better protein and fat content, healthier fatty acid profile and better antioxidant capacity. So, the obtained results provide novel, valuable information on snail farming practices, and may serve as a piece of information for producers how to provide consumers with high-quality snail products.

Introduction

Heliciculture is considered as an alternative farming activity in many countries, offering an additive source of income for farmers and contributing to social cohesion. Snails have been an important nutrient source since prehistoric times. In ancient Rome, garden snails were selected, bred and fattened under controlled conditions (De Grisse, 1991), establishing, therefore, the primitive forms of snail farming and introducing edible snails (escargot) in countries worldwide. Nowadays, escargot is consumed in many countries (i.e., Europe, Africa, Middle East) and is considered as a delicatessen food (Ghosh et al., 2016; 2017).

Snail meat is low in calories and forms a valuable nutrient due to its high protein and low-fat content (Cheney, 1988). The increasing human population growth leading to the need for different protein sources together with the vast interest in healthier

foods of low energy and fat content, render escargot as a promising alternative. According to Murphy (2001), snail meat contains many nutrients required for a balanced and healthy diet. Dietary lipids are important for humans as the composition of fatty acids in the consumed foods plays a pivotal role in human health. Excessive intake of saturated fatty acids in comparison to (poly)unsaturated fatty acids is considered as a crucial factor for developing heart disease and other pathologies (Ander et al., 2003). The development of such pathologies is directly linked with oxidative stress (Rajendran et al., 2014), the effects of which are strongly related to the type and amount of macronutrients consumed (Kitabchi et al., 2013). Thus, the production of foods (i.e., through animal farming) containing higher levels of healthier nutrients (i.e., polyunsaturated fatty acids (PUFA), ω-3/ω-6 fatty acids ratio, etc.) is of utmost importance since they assist human health.

Although heliciculture is considered a difficult farming activity, many snail farms have been established trying to cover market demands. Various farming systems for escargot production are, usually, implemented. These range from the so-called extensive systems, which are characterized by low infrastructure level and feed support based on green vegetables, to the intensive systems, which are characterized by a high level of intensification and the use of compound diet.

Many studies have focused on the biological, technical, management and feeding requirements concerning snail's breeding under farming conditions (FAO, 1986a,b; Bonnet et al., 1990; Blanc, 1993; Iglesias and Castillejo, 1999). Changing the protein source in a diet or enriching diets with various lipid sources is a common practice used in farm animal production either for management purposes or for increasing the nutrient value of the final product. The advantages of using compound diets on snail farming in order to improve the growth rate and hygiene of the farm has been previously documented (Ribas, 1986). The enrichment of snail diet with different vegetable oils revealed statistically significant differences in the levels of fatty acids (Milinsk et al., 2003). However, there is still inadequate information concerning how diets with different protein sources could affect snail meat composition in rearing conditions. So, we hypothesized that the use of different protein sources could lead to changes in the chemical proximate characteristics of the final product, that may further assist to an added value of the product or to meet consumer's demands (i.e., meat enriched in ω-3 and ω-6 fatty acids).

Thus, the aim of the present study was to examine the effect of four compound diets containing the different main protein source (conventional genetically modified (GM)-soya (*Glycine max*), organic soya, white lupin (*Lupinus alba*) or pea (*Pisum sativum*)) on meat of snails (*Cornu aspersum aspersum*) reared under intensive rearing conditions. More specifically, we focused on a) the muscle chemical composition; b) levels of the fatty acids in meat; and c) the meat antioxidant profile. Moreover, the results of this study can help implement better farming practices to obtain a final escargot product containing higher levels of human health favourable nutrients (i.e., PUFA) compared to snails collected directly from nature.

Material and methods

Animals and diets

The study was conducted on an intensive snail farm (greenhouse type) located in Central Greece (Prefecture of Thessaly) and lasted 120 days (15th May – 15th September 2019). For the experimental purposes 8 000 snails (*Cornu aspersum aspersum*) of the same age and weight (1-day-old; 0.003 ± 0.001 g each) originated from parents that were farm kept, mated and reared under the same farming conditions, were randomly distributed into four different treatments (experimental parks; Gogas et al., 2003) with five replicates in each treatment (400 snails in each replicate). All animals were artificially reared under the same experimental protocol with optimum farming conditions (mean temperature 25.02 ± 0.98 °C; relative humidity 85–90% using a mist propagation system; light conditions followed climate conditions with a minimum 11 h and maximum 14 h of light). In each treatment, a different diet was implemented. Specifically, the diet given in each treatment differed in the main protein source included in the final ration; however, the final protein content was set at approximately 14 g. The first diet, which was the farm's conventional diet, contained GM-soya flour (*Glycine max*; SYD diet) as the main protein source, the second diet contained organic soya flour (organic *Glycine max*; OSD diet), the third diet contained *Lupinus albus* (white lubin) bean flour (LAD diet) and the fourth diet contained *Pisum sativum* (pea) bean flour (PSD diet). Diets LAD and PSD were chosen as cheaper alternatives to soya and OSD as more organic approach towards consumers. The composition and characteristics of the diets that were implemented in each treatment are presented in Table 1.

Table 1. Composition of experimental diets fed to snails reared under intensive farming conditions

Ration	Feed components		SYD				OSD				LAD				PSD			
	protein, g/kg	total lipids, g/kg	content, %	protein, g/kg	protein, %	lipid, g/kg	content, %	protein, g/kg	protein, %	lipid, g/kg	content, %	protein, g/kg	protein, %	lipid, g/kg	content, %	protein, g/kg	protein, %	lipid, g/kg
Maize	8.3	42	35	2.91	20.73	14.7	35	2.91	20.73	14.7	13	1.08	7.66	5.46	6	0.50	3.53	2.52
Wheat	11.5	20	6	0.69	4.92	1.2	6	0.69	4.92	1.2	12	1.38	9.79	2.4	8	0.92	6.50	1.6
Soybean flour (GM)	44	10	21	9.24	65.86	2.1	-	-	-	-	2	0.88	6.24	0.2	6	2.64	18.67	0.6
Organic soybean flour	44	10	-	-	-	-	21	9.24	65.86	2.1	-	-	-	-	-	-	-	-
Lupinus alba bean flour	31.5	85	-	-	-	-	-	-	-	-	27	8.51	60.35	22.95	-	-	-	-
Pisum sativa bean flour	21.5	15	-	-	-	-	-	-	-	-	-	-	-	-	39	8.39	59.29	5.85
Wheat bran	17	41	7	1.19	8.49	2.87	7	1.19	8.49	2.87	15	2.25	15.96	6.15	10	1.7	12.01	4.1
Calcium carbonate	-	-	30	-	-	-	30	-	-	-	30	-	-	-	30	-	-	-
Minerals and vitamins	-	-	1	-	-	-	1	-	-	-	1	-	-	-	1	-	-	-
Total			100	14.03	100%	20.87	100	14.03	100	20.87	100	14.10	100	37.16	100	14.15	100	14.67
Energy, MJ/kg			8.81				8.81				8.39				8.29			

SYD – diet based on genetically modified (GM)-soya (*Glycine max*) flour, OSD – diet based on organic soya flour, LAD – diet based on *Lupinus albus* (white lubin) bean flour, PSD – diet based on *Pisum sativum* (pea) bean flour

Animals during the whole experimental period had *ad libitum* access to feed. The amount of the implemented diet per treatment varied depending on the week of the experimental period (fattening period) and ranged from 80 to 200 g from the week 1 to the final week (week 16), respectively. In addition, for comparative purposes, 1 000 sexually mature snails (*Cornu aspersum aspersum*) that lived in nature (NAD group) were collected directly from the field (southwest part of Greece; September 2019). One day (24 h) before the end of the experimental period snails were fasted. Then, 150–200 snails from each treatment were randomly selected for further analyses. The selected animals had reached a proper commercial weight and size (mean weight: 16.97 ± 0.29 g; mean diameter: 38.66 ± 0.24 mm). Before the meat was extracted from the shell the snails were washed with running tap water to remove surface dirt. Approximately, 500 g of homogenized snail meat from each treatment was used for further chemical and antioxidant analyses. Weight was estimated using a digital electronic weighting scale (Mettler PC180, Mettler-Toledo, Greifensee, Switzerland).

Chemical composition and antioxidant analyses

Water content (moisture) of the studied meat samples was measured by desiccation at 105 °C. The difference in weight (g) before and after drying, was expressed as a percentage of the water content. Weight was estimated using a weighting scale as described above. All chemical analyses were conducted in three replicates. Protein content (%) was determined using the Kjeldahl method (% N × 6.25; AOAC, 1990). Collagen content (% of the total protein) was quantified using the ISO standard method (ISO 3496:1994) for hydroxyproline content determination (ISO, 1994).

The total lipid extraction from the meat samples was conducted using the Folch method (Folch et al., 1957) and fatty acid methyl esters (FAMEs) were further prepared. FAMEs were analysed with the use of a gas chromatograph (Hewlett Packard 5880 GC, Hewlett-Packard, Palo Alto, CA, USA) equipped with a flame ionization detector followed the methodology described by Milinsk et al. (2003).

For protein carbonyls (PC; nmol/mg total protein) the methodology described by Patsoukis et al. (2004) was used. In this assay, 50 µl of 20% trichloroacetic acid (TCA) was added to 500 mg of homogenized muscle and the mixture was incubated in an ice bath for 15 min and then centrifuged at 15 000 *g*

for 5 min at 4 °C. The supernatant was discarded and 500 μl of 10 mM 2,4-dinitrophenylhydra-zine (DNPH) were added to the sample, or 500 μl of 2.5 N HCl for the blank, and added in the pellet. The samples were incubated in the dark at room temperature for 1 h with vortexing every 15 min and centrifuged at 15 000 g for 5 min at 4 °C. The supernatant was discarded and 1 ml of 10% TCA was added, vortexed and centrifuged at 15 000 g for 5 min at 4 °C. The supernatant was discarded and 1 ml of ethanol-ethyl acetate mixture (1:1, v/v) was added, vortexed and then centrifuged at 15 000 g for 5 min at 4 °C. This step was repeated twice. The supernatant was discarded and 1 ml of 5 M urea (pH 2.3) was added, vortexed and incubated at 37 °C for 15 min. The samples were centrifuged at 15 000 g for 3 min at 4 °C and the absorbance of the supernatant was read at 375 nm.

Thiobarbituric acid reactive substances (TBARS; nmol/mg total protein) were measured according to the method described by Keles et al. (2001). Briefly, 100 mg of homogenized muscle was mixed with 500 μl of 35% TCA and 500 μl of Tris(hydroxymethyl)aminomethane hydrochloride (200 mM, pH 7.4), and incubated for 10 min at room temperature. One millilitre of 2 M Na_2SO_4 and 55 mM of thiobarbituric acid solution were added and the samples were incubated at 95 °C for 45 min. The samples were cooled on ice for 5 min and vortexed after adding 1 ml of 70% TCA. The samples were centrifuged at 15 000 g for 3 min and the absorbance of the supernatant was read at 530 nm.

Total antioxidant capacity (TAC; μmol/mg total protein) was determined as described by Janaszewska and Bartosz (2002). In this assay, 20 mg of muscle were added to 480 μl of 10 mM sodium potassium phosphate (pH 7.4) and 500 μl of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, and the samples were incubated in the dark for 30 min at room temperature. The samples were centrifuged for 3 min at 20 000 g and the absorbance of the supernatant was read at 520 nm. Glutathione content (GSH; μmol/mg total protein) was measured according to Reddy et al. (2004). Homogenized muscle (100 mg) treated with 5% TCA was mixed with 660 μl of 67 mM sodium potassium phosphate (pH 8) and 330 μl of 1 mM 5,5'-dithiobis(2-nitrobenzoate) (DTNB). The samples were incubated in the dark at room temperature for 45 min and the absorbance was read at 412 nm. Catalase activity (U/mg total protein) was determined using the methodology described by Aebi (1984). Homogenized muscle (10 mg, diluted 1:10) was added to 2 990 μl of 67 mM sodium potassium phosphate (pH 7.4) and the samples were incubated at 37 °C for 10 min. Hydrogen peroxide (H_2O_2, 30%, 5 μl) was added to the samples and the change in absorbance was immediately read at 240 nm for 130 s. Calculation of catalase activity was based on the molar extinction coefficient of H_2O_2.

Statistical analysis

One way analysis of variance (ANOVA) was used to examine the effect of diet on the determined chemical and antioxidant parameters. The average values were compared using the Tukey test ($P < 0.05$). The SPSS ver. 25 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis purposes.

Results

The results of chemical analyses of muscle of snails fed different diets as well as of those collected directly from nature are presented in Table 2. No statistically significant differences were observed between four examined treatments in regard to water content; however, NAD snails showed statistically lower levels ($P < 0.001$) of water content in their muscle. Moreover, all snails fed under the intensive farming system with the implemented compound diets (SYD, OSD, LAD, PSD) showed higher levels of protein in their muscle in comparison with NAD group. Snails fed SYD diet showed approximately 7.1–9.1% lower levels of protein content in comparison with snails fed OSD, LAD and PSD diets. Additionally, snails fed PSD diet showed approximately 14.7 and 28.4% higher ($P < 0.001$) fat content than snails fed SYD diet or NAD snails, respectively. In regard to collagen levels, snails fed OSD diet were characterised by higher ($P < 0.001$) collagen levels in comparison with other snails.

Table 2. Chemical composition and water content of muscle of snails (*Cornu aspersum aspersum*) fed either experimental diets with different protein sources or collected in nature

Group	Water content, %	Protein, %	Fat, %	Collagen, %
NAD	78.94 ± 2.10[a]	10.63 ± 0.20[a]	0.83 ± 0.03[a]	1.10 ± 0.02[a]
SYD	82.97 ± 0.09[b]	11.64 ± 0.18[b]	0.99 ± 0.04[b]	1.04 ± 0.02[a]
OSD	83.19 ± 0.24[b]	12.53 ± 0.19[c]	1.05 ± 0.03[bc]	1.57 ± 0.08[b]
LAD	83.26 ± 0.09[b]	12.81 ± 0.21[c]	1.07 ± 0.04[bc]	1.20 ± 0.03[a]
PSD	83.37 ± 0.18[b]	12.57 ± 0.12[c]	1.16 ± 0.03[c]	1.22 ± 0.02[a]
P-value	0.001	0.001	0.001	0.001

NAD – snails collected in nature, SYD – snails fed diet based on genetically modified *Glycine max* (soya) flour, OSD – snails fed diet based on organic soya flour, LAD – snails fed diet based on *Lupinus albus* (white lupin) bean flour, PSD – snails fed diet based on *Pisum sativum* (pea) bean flour; analyses were conducted in triplicates; results are presented as mean ± standard error; [a-c] – means with different superscripts in the same column are significantly different at *P* < 0.001

The fatty acid profile of snail muscles is shown in Table 3. Snails fed SYD diet showed lower (*P* < 0.001) levels of unsaturated (UFA) to saturated (SFA) fatty acids ratio in comparison with

higher (*P* < 0.001) ω-3 fatty acid content in comparison with the rest examined groups.

Antioxidant biomarker levels in the snail muscle are shown in Table 4. Statistically significant differences (*P* < 0.001) in protein carbonyls were observed between all the examined treatments. The highest value was observed in snails fed LAD diet, while the lower value was observed in NAD snails followed by those fed OSD diet. In NAD snails TBARS value was lower in comparison with snails from experimental groups. However, snails fed soya flour (either organic or genetically modified) showed lower (*P* < 0.001) TBARS values in comparison with snails fed LAD or PSD diets (Table 3). Catalase levels were higher (*P* < 0.001) in snails fed LAD or PSD diets in comparison with the rest examined cases. No statistical differences were observed for GSH levels. However, snails fed LAD diets showed higher values (P < 0.001) of TAC in comparison with snails fed SYD, OSD diets or NAD ones.

Table 3. Lipid profile of muscle of snails (*Cornu aspersum aspersum*) fed either experimental diets with different protein sources or collected in nature

Group	UFA/SFA	PUFA/MUFA	ω-6/ω-3	ω-3, %
NAD	1.85 ± 0.03[c]	3.15 ± 0.03[a]	1.91 ± 0.05[a]	14.04 ± 0.06[a]
SYD	1.55 ± 0.02[a]	3.45 ± 0.03[b]	2.42 ± 0.04[b]	16.75 ± 0.16[b]
OSD	1.70 ± 0.02[b]	3.43 ± 0.04[b]	2.25 ± 0.04[b]	16.60 ± 0.13[b]
LAD	2.00 ± 0.04[d]	5.07 ± 0.05[c]	2.76 ± 0.07[c]	16.78 ± 0.12[b]
PSD	1.90 ± 0.01[cd]	5.68 ± 0.06[d]	2.89 ± 0.09[c]	20.59 ± 0.18[c]
P-value	0.001	0.001	0.001	0.001

NAD – snails collected in nature, SYD – snails fed diet based on genetically modified *Glycine max* (soya) flour, OSD – snails fed diet based on organic soya flour, LAD – snails fed diet based on *Lupinus albus* (white lubin) bean flour, PSD – snails fed diet based on *Pisum sativum* (pea) bean flour; UFA – unsaturated fatty acids, SFA – saturated fatty acids, PUFA – polyunsaturated fatty acids, MUFA – monounsaturated fatty acids; analyses were conducted in triplicates; results are presented as mean ± standard error; a-d – means with different superscripts in the same column are significantly different at *P* < 0.001

Table 4. Antioxidant profile of muscle of snails (*Cornu aspersum aspersum*) fed either experimental diets with different protein sources or collected in nature

Group	Protein carbonyls, nmol/mg total protein	TBARS, nmol/mg total protein	Catalase activity, U/mg total protein	Glutathione content, nmol/mg total protein	Total antioxidant activity, µmol/ml total protein
NAD	31.34 ± 0.12[a]	29.54 ± 0.12[a]	111.84 ± 0.32[a]	0.049 ± 0.001	0.599 ± 0.006[a]
SYD	33.68 ± 0.05[c]	31.34 ± 0.22[b]	143.67 ± 0.44[b]	0.042 ± 0.002	0.587 ± 0.006[a]
OSD	32.28 ± 0.12[b]	31.03 ± 0.26[b]	148.98 ± 0.42[c]	0.045 ± 0.001	0.661 ± 0.016[b]
LAD	36.00 ± 0.48[e]	34.82 ± 0.35[c]	178.05 ± 0.28[d]	0.050 ± 0.001	0.715 ± 0.019[c]
PSD	34.86 ± 0.18[d]	33.88 ± 0.18[c]	181.79 ± 0.30[e]	0.048 ± 0.008	0.682 ± 0.015[bc]
P-value	0.001	0.001	0.001	>0.05	0.001

NAD – snails collected in nature, SYD – snails fed diet based on genetically modified soya flour, OSD – snails fed diet based on organic soya flour, LAD – snails fed diet based on *Lupinus albus* (white lupin) bean flour, PSD – snails fed diet based on *Pisum sativum* (pea) bean flour; PUFA/MUFA – polyunsaturated fatty acids to monounsaturated fatty acids ratio; TBARS – thiobarbituric acid reactive substances; analyses were conducted in triplicates; results are presented as mean ± standard error; a-e – means with different superscripts in the same column are significantly different at *P* < 0.001

the other examined treatments. Moreover, in snails fed PSD diet the highest ratio of polyunsaturated (PUFA) to monounsaturated (MUFA) fatty acids was noted, followed by snails fed LAD diet. The lowest level (*P* < 0.001) of PUFA/MUFA ratio was observed in the NAD snails. In addition, snails fed PSD and LAD diets revealed higher (*P* < 0.001) levels of ω-6 to ω-3 ratio in comparison with the respective values obtained from snails fed OSD and SYD diets or even NAD snails (approximately 16.2, 22.1 and 33.9% higher values, respectively). On the contrary, snails reared under natural conditions (NAD) showed the lowest levels of the respective ratio (ω-6/ω-3). Snails from PSD group had

Discussion

The world population is expected to reach about 9.7 billion human beings in 2050 (FAO, 2021) projecting the need for animal products to be doubled. In addition, a majority of the consumers is expected to turn their preferences into functional foods focusing on specific nutrients that may assist and protect human health (i.e., PUFA, ω-3, ω-6 fatty acids etc.). Heliciculture could, auxiliary, contribute to such an approach, as snails are considered a valuable nutrient source for human health in respect to their high level of protein content, low-fat content and beneficial fatty acid profile. In such

an approach, the implemented farming practices could also assist, like changes in diet composition, which is widely used in farm animals. However, little research has been conducted on snails reared under intensive rearing conditions on this aspect. In the present study, we report the effect of four implemented diets consisting of a different type of major protein source (soya flour, organic soya flour, *Lupinus albus* flour, *Pisum sativum* flour) on muscle chemical composition and fatty acid profile of snails reared under controlled farming conditions.

It was observed that the implemented diets affected water, protein, fat and collagen contents in snails. Better and controlled rearing conditions that were under intensive farming in comparison with natural conditions could be obviously attributed to the observed differences in muscle chemical composition. Interestingly, in snails fed organic soya (OSD diet) an increase of protein content in muscle was observed in comparison to those fed non-organic soya (SYD diet). As little research has been conducted on the nutrition physiology of snails, there is no profound reason why this was noted. Generally, dietary energy, protein concentration and essential amino acids balance are important factors for daily weight gain and further muscle development. Taking into account that both diets (SYD and OSD) had the same protein level and energy, it can be assumed that differences in the amino acids' concentrations could possibly have attributed to the observed protein content difference, either as a result of an increase in feed intake by animals or changes in the amino acid profile resulting from genetically modified soya processing. In pigs, according to Li and Patience (2017), imbalances in branched-chain amino acids can be detected and further affect negatively feed intake. Experiments conducted on mice fed a GM soya-based diet, revealed mitochondrial modifications – an indicator of reduced metabolic rate (Malatesta et al, 2008). Further, changes in microscopic and ultramicroscopic levels due to GM-soya intake have been reported (Cisterna et al., 2008), and could possibly lead to a lower content of metabolized nutrients. Moreover, although soya beans contain a higher protein content than *Pisum sativum*, the differences in the protein muscle content observed in snails fed LAD or PSD diets could be possibly attributed to a better protein digestibility of the animal itself. Moreover, the higher fat content observed in muscle of animals fed compound diets than in NAD animals can be related to the higher fat content of these diets. Although PSD diet had a lower fat content in comparison with SYD diet, similarly

to protein content the meat of snails fed this diet had significantly higher fat content. This may indicate a possibly better digestibility of fats contained in PSD diet and further better nutrient intake and absorbance in comparison with soya-based diets. Generally, it has been reported that in the land snails, the feed assimilation efficiency ranges between 30–78% depending on the type of feed (Richardson, 1975). However, as the snail physiology and diet absorbance are not very well studied, further research on snail farming conditions is needed for clarifying the aforementioned assumptions with respect to novel compound protein sources.

Collagen is a high molecular protein that in the last two decades is intensively used by the industry for the production of various types of (para-)pharmaceutical products. Thus, snails could form a natural alternative collagen source. In snails fed OSD higher collagen content was observed. There is no profound explanation as to why snails fed GM-soya (SYD diet) did not have similar high collagen content. Previous reports indicate that soaybean peptide apart from anti-fatigue and antioxidant activities, has also the ability to increase type I collagen (Sekine et al., 2008; Tokudome et al., 2012; Zhang et al., 2020). We can, therefore, assume that the influence of the genetic modification process of soya could have decreased the efficiency of the soybean peptide attributing, possibly, to the observed lower levels of collagen stated in snails fed SYD diet.

The fatty acid profile was, also, differentiated according to the protein source in the diets. Generally, the fatty acid profile could act as a protective vehicle for human health, depending on the ratio of their profile. PUFA, MUFA and SFA, as well as ω-6 and ω-3 fatty acids, are considered as indices of major importance for the nutritional evaluation of fat (British Department of Health, 1994). Dietary ratio of ω-6/ω-3 lower than 4:1 in atherosclerosis prevention is recommended (Williams, 2000; Raes et al., 2004; Orellana et al., 2009). Moreover, ω-3 fatty acids form an integral part of cell membranes, and they have also anti-inflammatory properties. Therefore, they are considered as useful molecules in the management of many inflammatory and chronic diseases. In the present study, a higher ratio of UFA/SFA was observed in snails fed *Lupinus alba*-based diet (LAD). It probably resulted from the higher lipid content in this diet. The observed higher ratio indicates a healthier profile of fatty acids, which is also enhanced by the fact that the ratio of PUFA/MUFA was high. Taking these two ratios together, it can be assumed that snails fed LAD diet developed

a healthier fatty acid balance, possibly due to the higher content of the total lipids in the implemented diet. However, a higher ω-6/ω-3 fatty acids ratio in snails fed experimental diets was noted in comparison with snails collected from nature, as a result of the more elaborated feeds and diets implemented under farming conditions. Although the ω-6/ω-3 ratio was higher in the experimental groups, it did not reach the highest value recommended for human diet in total (4:1). In addition, snails fed PSD diet had better (higher) ω-3 content (% total lipids) that could be attributed to the better lipid profile of *Pisum sativum*. Thus, both diets including *Pisum sativum* or *Lupinus alba* as the main protein source had a healthier profile of ω-6 and ω-3 fatty acids.

It was, also, revealed that compound diets influenced the antioxidant profile of snail meat. Higher levels of the determined antioxidant indices were observed in snails fed LAD or PSD diets in comparison with other groups. Both diets resulted in a better profile of catalase and total antioxidant activity, offering a proxy for ameliorating the antioxidant capacity of the final product. This could be attributed to the higher lipid content, higher ω-6/ω-3 ratio and higher content of ω-3 fatty acids in these diets, which as molecules have a well-established antioxidant activity. However, also, higher levels of protein carbonyls and TBARS can be attributed to the higher levels of protein and fat contents observed in snails fed LAD and PSD diets, as a result of higher catabolism of proteins and lipids. Although elevated levels of both TBARS and protein carbonyls have been related to many pathological conditions including atherosclerosis, obesity and metabolic diseases (Williams, 2000; Rajendran et al., 2014), the overall antioxidant profile noted in LAD and PSD was better in contrast to that of snails fed with SYD, OSD or snails collected from nature.

Conclusions

It was observed that the snail diet composition affected the chemical composition as well as the fatty acid profile and the antioxidant capacity of snail muscle. The diet based on organic *Glycine max* (soya) as the main protein source resulted in higher collagen content in snail meat, whereas animals fed diets containing *Pisum sativum* (pea) and *Lupinus alba* (white lupin) had higher protein and fat content as well as a healthier fatty acid profile (i.e., better proportion of polyunsaturated fatty acids to monounsaturated ones or higher total ω-3 fatty acid content) and a better antioxidant capacity. The results provide novel, valuable information about snail farming practices,

and also how to improve the final product to be suitable for consumer dietary preferences. Nevertheless, further studies on Cornu species reared under farming conditions are needed to explore their digestive processesand nutrition physiology.

Ethical note

All rearing conditions and handling procedures were in accordance with the European Commission Recommendation 2007/526/EC and Directive 2010/63/UE on revised guidelines for the accommodation and care of animals used for experimentation and other scientific purposes.

Conflict of interest

The authors declare that there is no conflict of interest.

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