

Comprehensive levels of fatty acids in meat: implications for human health

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Abstract: There are strong indicators of the link between diets and increased burdens of obesity, cardiovascular diseases, diabetes and some cancers. Healthy dietary patterns were defined as diets that are high in fruits, vegetables and non-fat dairy (low in saturated and trans fats). The aims of this study were to determine the fatty acid (FA) profile of meat (poultry, pork, lamb and beef) and the calculated atherogenic index (AI) and thrombogenic index (TI). Poultry, pork and lamb contained more monounsaturated fatty acids (MUFAs) than saturated fatty acids (SFAs). Linear discriminant analysis (LDA) demonstrated that the first discriminant explained 56.11% of the total variance and the second discriminant explained 23.85% of the total variance. The established p value of Wilks' test was $p < 0.0001$. By canonical correlation, the first and the second discriminant functions in the LDA were established as 0.995 and 0.995, respectively. AI and TI values of less than 0.5 and 1.0, respectively, were previously advised. The obtained AI values in poultry (0.37-0.45) were lower than those in pork (0.50-0.53), lamb (0.54) and beef (0.93) meats. The obtained TI values in poultry (0.81-0.87) were also lower than in pork (1.09-1.18), lamb (1.44) and beef (1.93) meats. Beneficial nutrition habits, i.e., nutrition according to the food pyramid and a Mediterranean diet, should reduce the rate of coronary heart disease and result in better health outcomes for consumers.

Keywords: meat, fatty acids, linear discriminant analysis, atherogenic index, thrombogenic index

Introduction

The World Health Organization (WHO) has published a guide to proper diet in which they presented the food pyramid (Safe food, 2022). The food pyramid represents the types of foods that should be eaten daily. The base of the pyramid contains vegetables, salads and fruits, which should account for 15-20% of the total daily energy intake (marked in green). The next step in the pyramid consists of whole meal, cereals and bread, potatoes, pasta and rice accounting for 35-45% of the total daily intake (marked in brown). The third step consists of the milk and milk products and the group of meat, fish and eggs, both with 10% of the total daily energy intake (marked in blue and yellow). At the top of the pyramid are oils, fats, and sugars accounting for only 5% of the total daily energy intake (marked in red) and food and drinks high in fat, sugar and salt (Figure 1).

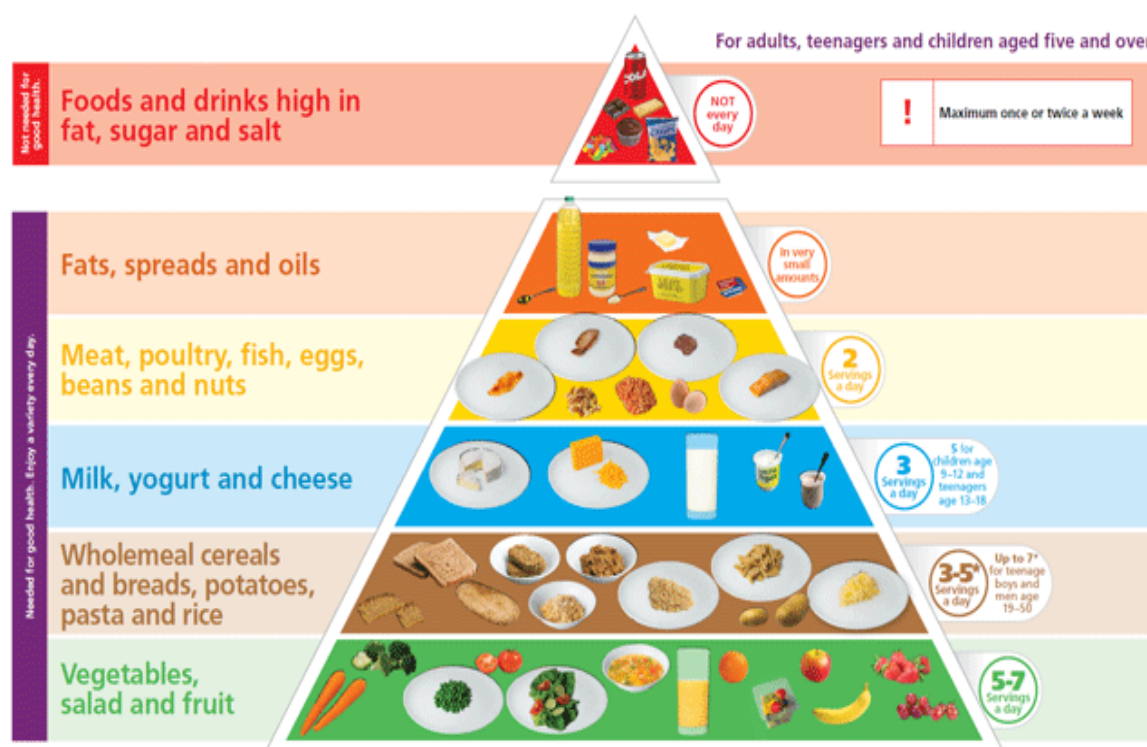


Figure 1. The food pyramid (source *Safe food*, 2022)

However, cardiovascular disease is the leading cause of death in Serbia according to the Health Statistical Yearbook of the Republic of Serbia (2020). Analysis of the global burden of disease (WHO, 2015) showed that diet is the most important factor that determines health. There are

strong indicators of the link between diets and increased burdens of obesity, cardiovascular diseases, diabetes and some cancers. The WHO study concluded that balanced diets maintain healthy body weights. The Mediterranean diet consists of high consumption of fruits and vegetables, legumes and complex carbohydrates (whole grains), a moderate consumption of fish and olive oil, low consumption of red meat, low-to-moderate consumption of red wine, and low-to-moderate consumption of milk and dairy products (Estruch et al., 2013; De Lorgeril et al., 1999). Worldwide, increased consumption of poultry and decreased beef consumption is noted, while consumption of pork and lamb is moderate (OECD/FAO, 2018; Bender, 1992). Vlahović et al. (2011) examined meat consumption in Serbia. They reported meat consumption in Serbia was 41.4 kg per capita (2009), but is decreasing. Consumption was dominated by pork and poultry that together accounted for 77% of total meat consumption, while beef consumption was low. Regionally, higher meat consumption was seen in the province of Vojvodina than in Central Serbia, but decrease meat consumption in both regions were experiencing decreasing meat consumption.

A substantial proportion of the population worldwide, including children and adolescents, is now overweight, with far-reaching consequences in terms of increased risk of chronic illness (WHO, 2015). The overweight epidemic has spurred research into the health consequences of overeating and overweight, and information about this has found its way to the general public that now tends to associate eating with health, especially in the United States of America (USA) (Rozin et al., 1999). The prevalence of obesity in the adult population in Serbia and the USA is presented in Figure 2. Adult obesity occurs in both countries, but the prevalence is lower in Serbia (15-22.5%) than in the USA (25-38%). However, very similar linear increases in adult obesity prevalence in the two countries are seen from 2000 to 2016.

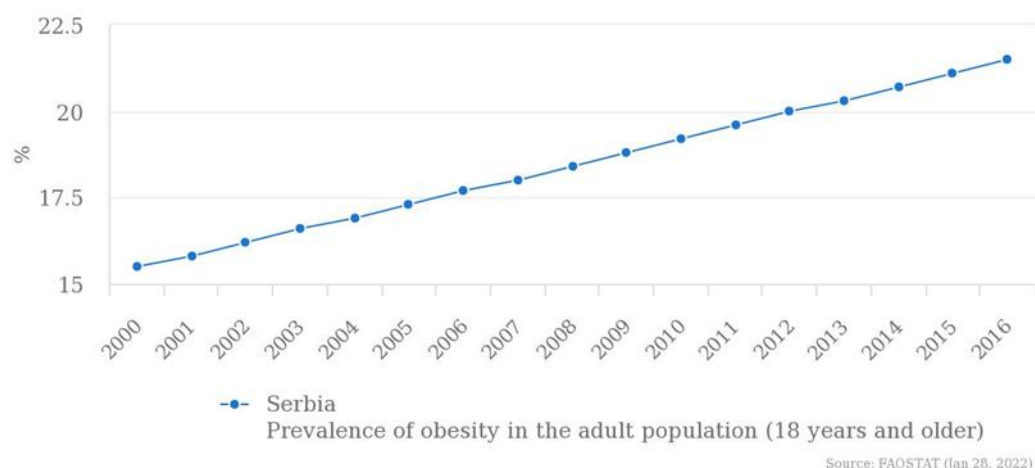


Figure 1. Prevalence of obesity in Serbia (Source FAOSTAT, 2022)

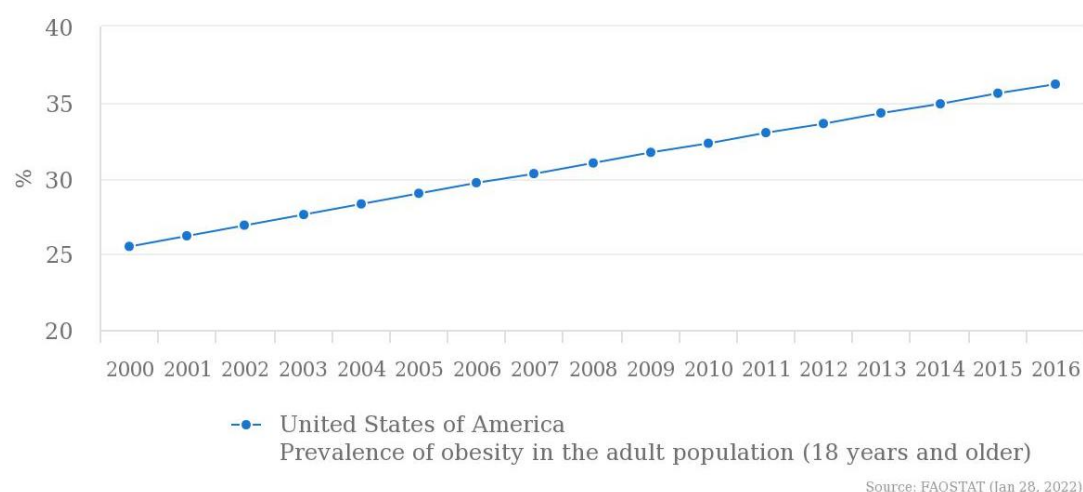


Figure 2. Prevalence of obesity in USA (Source FAOSTAT, 2022)

In Serbia, overweight and obesity estimates from 2008 showed that 58.6% of the adult population (≥ 20 years old) in Serbia were overweight and 24.8% were obese. The prevalence of overweight was higher among men (66.5%) than women (51.0%). The model predicts in 2030 that 48% of men and 31% of women will be obese (WHO, 2022). Šarčević et al. (2013) concluded that in Serbian cuisine, poultry is the most commonly consumed meat, followed by pork, beef and lamb. On the other hand, Baltić et al. (2018) concluded that pork was the most

common type of meat in the diet of the Serbian population. The prevalence of obesity could be partly due to preparation of meat.

So, this study aimed to determine the fatty acid (FA) profile of the meats traditionally consumed in Serbia (poultry, pork, lamb and beef) as well as the calculated atherogenic index (AI) and thrombogenic index (TI).

Materials and methods

Animal breeds, housing and feeding conditions

All animals were kept in commercial farms at the Institute for Animal Husbandry (Belgrade, Serbia), with a covered (pigs), semi-covered (cattle, sheep) or pan system (broilers). During the entire experimental period, animals were fed commercial feed mixtures for conventional production, the structure, composition and nutritive value of which were adapted to animal species and stage of growth. The diets consist of barley and soybeans, corn, vegetable oil, cellulose, mineral and vitamin supplements. All mixtures were based on corn as the basic energy source and soybean meal as main source of protein. The amount of feed was varied according to the metabolic weight of the animal. Feeding of animals was *ad libitum*.

Slaughtering and meat sampling

After reaching the targeted body weight of approximately 550 kg for cattle, 100 kg for pigs, 30 kg for sheep and 4 kg (or 42 days of age) for broilers, all animals were transported to the commercial slaughterhouse (Institute for Animal Husbandry Belgrade, Serbia). They were denied food 12 h prior to slaughtering (6 h for broilers), but had free access to water. After the removal of skin and head (for cattle and sheep), defeathering (for broilers) and scalding (for pigs), and evisceration, carcasses were placed in cold storage at 4°C for the next 24 h.

After chilling, from each carcass the following samples were collected: *longissimus dorsi* (for pigs, cattle and sheep), and breasts and drumstick (for broilers). After the removal of any visible fat and connective tissues, meat samples were sealed in plastic bags under vacuum and stored at -20°C until further analyses. Analyses (in batches of 42 per day) were conducted on each meat sample, after thawing at 4°C a day before and homogenizing in a laboratory mixer (CombiMax 600, Braun, Germany).

Ethical issues

The animal feeding and slaughtering was performed in normal production systems, with no special interventions during the experiment, in agreement with national and EU legislation on animal husbandry and with respect to high welfare standards (Council Directive 98/58 EC). All the sampling took place *postmortem*.

Fatty acid analysis

Total fat content was determined by extraction with petroleum ether in a Soxhlet apparatus after the acid hydrolysis of the sample according to ISO 1443:1973. Based on the ISO 12966-3:2016 method, fatty acid methyl esters (FAMES) from extracted lipids were transesterified using 0.25 M trimethylsulfonium hydroxide (TMSH; Fluka, Buchs, Switzerland). Before transesterification, 0.05 mL of heneicosanoic acid methyl ester (p.a. $\geq 99\%$, Fluka, Buchs, Switzerland) ($c = 10 \text{ mg mL}^{-1}$) was added as an internal standard. The obtained FAMES were analysed on a Shimadzu 2010 gas chromatograph (Kyoto, Japan) with a split injector, HP-88 column (J&W Scientific, Santa Clara, USA) (length = 100 m, i.d. = 0.25 mm, film thickness = 0.20 μm) and flame ionization detector. The injector and detector temperatures were set at 250 °C and 280 °C, respectively. Column oven temperature was programmed starting at 125 °C and ending at 230 °C. Injected volume was 1 μL with 1:50 split ratio. Identification of the chromatographic peaks in the samples was performed by comparing their relative retention times to the FAME retention times in the Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA). The fatty acid (FA) peak areas in the samples were corrected by applying response factors calculated by the ratios between the peak area of the individual FAME and of the internal standard. The quantities of FAs were expressed as weight percentages of the total FAs.

Statistical analysis

Data on intramuscular FA composition were subjected to analysis of variance (ANOVA) to evaluate the effect of different animal species. Data were presented as mean and standard deviations. Differences between means were assessed using Tukey's HSD test. Linear discriminant analysis (LDA) was performed to differentiate meat samples. The statistical

analysis was performed with a XLSTAT2022 (for Microsoft Excel, Addinsoft, NY, USA). AI and TI indices were calculated as given by Ulbricht and Southgate, 1991.

Results

The FA compositions, AIs and TIs of the different meats are presented in Table 1.

Table 1. Fatty acid composition (%) and atherogenic (AI) and thrombogenic (TI) indices of meats

Fatty acids	Chicken breast (n=6)	Chicken drumstick (n=6)	Pork fresh ham (n=8)	Pork tenderloin (n=8)
C14:0	0.39±0.05 ^C	0.36±0.03 ^C	1.18±0.12 ^B	1.22±0.10 ^B
C15:0	0.09±0.03 ^B	0.09±0.01 ^B	0.06±0.02 ^B	0.08±0.02 ^B
C16:0	24.71±3.23 ^{BC}	23.39±1.16 ^{BC}	25.47±1.46 ^{BC}	26.16±1.08 ^B
C16:1	3.73±0.53 ^A	4.33±0.37 ^A	2.54±0.43 ^B	2.23±0.49 ^B
C17:0	0.13±0.04 ^B	0.15±0.01 ^B	0.29±0.07 ^B	0.38±0.08
C18:0	7.72±1.38 ^C	7.73±0.49 ^C	11.96±1.03 ^B	13.45±0.99 ^B
C18:1n-9	37.36±3.29 ^B	39.19±1.50 ^B	44.99±1.73 ^A	40.27±2.59 ^B
C18:2n-6	21.55±2.44 ^A	21.29±2.33 ^A	9.70±2.66 ^B	9.86±4.99 ^B
C18:3n-6	0.12±0.10 ^A	0.11±0.03 ^A	nd	nd
C18:3n-3	1.10±0.55 ^{AB}	0.85±0.16 ^{AB}	1.69±0.89 ^A	1.74±0.70 ^A
C20:0	0.10±0.05 ^{AB}	0.10±0.01 ^{AB}	0.23±0.05 ^{AB}	0.34±0.07 ^A
C20:1	0.45±0.02 ^C	0.50±0.01 ^{BC}	0.69±0.04 ^A	0.63±0.08 ^{AB}
C20:2	0.45±0.10 ^A	0.47±0.11 ^A	0.34±0.04 ^{AB}	0.35±0.04 ^A
C20:3n-6	0.33±0.17 ^B	0.55±0.09 ^A	0.10±0.02 ^C	0.11±0.04 ^C
C20:3n-3	0.22±0.16 ^A	0.10±0.01 ^{AB}	0.17±0.08 ^{AB}	0.22±0.13 ^A

C20:4n-6	1.23±0.87 ^A	0.70±0.31 ^{AB}	0.31±0.08 ^{BC}	0.32±0.18 ^{BC}
C20:5n-3	0.09±0.11 ^{NS}	0.08±0.07 ^{NS}	0.04±0.03 ^{NS}	0.07±0.05 ^{NS}
C22:5n-3	0.10±0.16 ^{NS}	nd	0.19±0.04 ^A	0.17±0.09 ^{NS}
C22:6n-3	0.06±0.09 ^{NS}	nd	0.01±0.02 ^{NS}	0.01±0.02 ^{NS}
SFA	35.39±2.99 ^{CD}	32.15±1.60 ^D	39.20±2.16 ^{BC}	41.70±1.33 ^B
MUFA	41.55±3.56 ^C	44.03±1.69 ^{BC}	48.33±1.85 ^{AB}	45.19±3.37 ^A
PUFA	24.03±3.36 ^A	23.45±2.69 ^A	12.24±2.94 ^B	12.82±2.89 ^B
n-6	22.46±3.63 ^A	22.43±2.52 ^A	10.14±2.65 ^B	10.59±2.62 ^B
n-3	1.57±0.77 ^{AB}	1.02±0.18 ^{BC}	2.11±0.99 ^A	2.17±0.54 ^A
n-6/n-3	16.59±2.09 ^B	22.14±1.81 ^A	5.87±3.16 ^C	5.10±1.68 ^C
AI	0.40±0.08 ^{CD}	0.37±0.03 ^D	0.50±0.04 ^{BC}	0.53±0.02 ^B
TI	0.91±0.24 ^D	0.87±0.07 ^D	1.09±0.16 ^{CD}	1.18±0.08 ^C

n, number of samples; results are represented as mean ± SD; nd = not detected. Values in the same row with the same letter are not significantly different ($P \geq 0.05$).

fatty acids; PUFA, polyunsaturated fatty acids; atherogenic (AI) and thrombogenic (TI) indices

Poultry, pork and lamb contained more monounsaturated FAs (MUFAs) than saturated FAs (SFAs). Poultry meats (chicken breast and drumstick) were characterized by lower amounts of SFAs (35.39-32.15%) than pork (fresh ham and tenderloin) (39.20-41.70%), lamb (46.05%) and beef (48.66%). The levels of SFAs were significantly different ($P < 0.05$) in the different meat species. The level of MUFAs was the highest in lamb (48.82%) followed by pork fresh ham (48.33%) and pork tenderloin (45.19%), then by poultry meats (breast and drumstick) (41.55-44.03%) and beef (42.72%). The MUFA contents were significantly different ($P < 0.05$) in the different meat species. The most common MUFA was oleic acid (C18:1n-9). The level of polyunsaturated fatty acids (PUFAs) was higher in poultry (23.45-24.03%) than in the other meats. The levels of PUFAs in pork were 12.24-12.82%, but were much lower in lamb, 3.07%, and beef, 4.18%. The most common n-6 PUFA was linoleic acid (C18:2n-6). The most common n-3 PUFA was α -linolenic acid (C18:3n-3), which was more abundant in pork and poultry, while it occurred in lower amounts in lamb and beef. Generally, significantly higher contents of eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA) ($P < 0.05$) were found in poultry followed by pork and beef and lamb. The obtained AI values in poultry (0.37-0.45) were lower than in pork (0.50-0.53), lamb (0.54) and beef (0.93). The obtained TI values in poultry (0.87-0.91) were lower than in pork (1.09-1.18), lamb (1.44) and beef (1.95). AI and TI values were higher than are recommended, except for these indices in poultry and pork tenderloin. High AI and TI in beef were caused by naturally high contents of SFAs, especially C14:0 and C16:0.

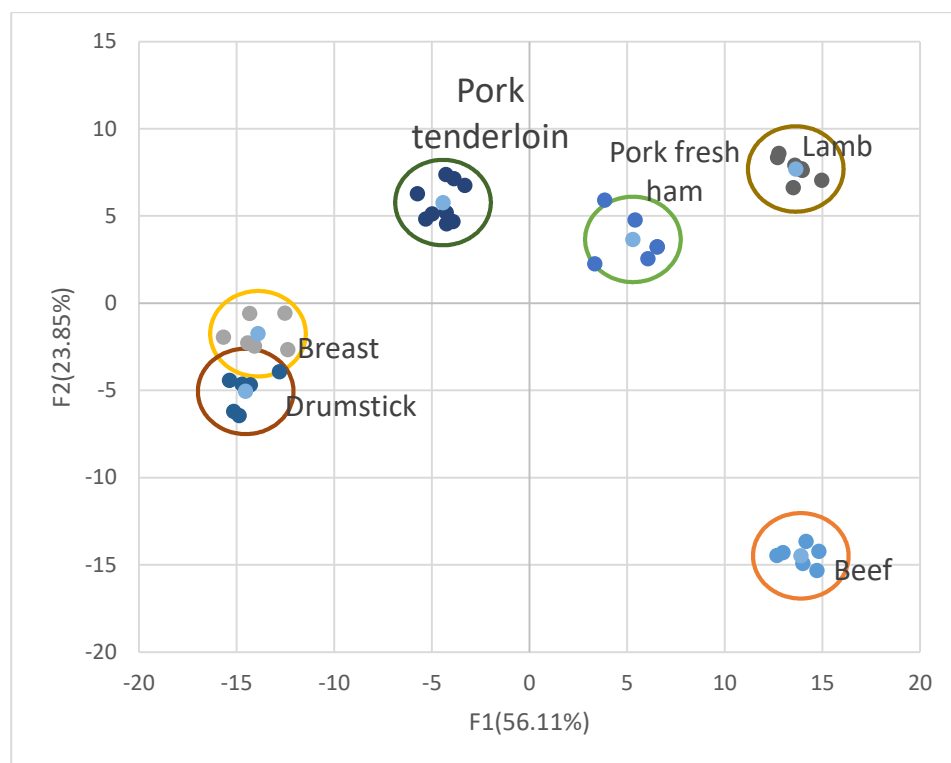


Figure 3. Linear discriminant analysis showing individual observation discriminant scores of meats from different animal species

LDA demonstrated that the first discriminant explained 56.11% of the total variance and the second discriminant explained 23.85% of the total variance (Figure 3). The established p value of Wilks' test was $p < 0.0001$. By canonical correlation, the first and the second discriminant functions were established as 0.995 and 0.995, respectively.

The results of the classification were very satisfactory and allowed 100% of the meat samples to be correctly grouped. Distribution of data was expressed as discriminant scores along two eigenvectors, as a function of group provenance, regarding FA content. The shortest distance between the points on the canonical plot in Figure 3 represents the smallest difference in the FA profiles of the samples. In terms of FA content, beef meat was very distant from poultry, pork and lamb meats, and lamb meat was very distant from pork meat; this correlates to the type of feed ingested by the animals

Discussion

Our measured FA in chicken breast was lower than that in breast muscle reported by Yu *et al.* (2021), but was similar to the FA contents of chicken breast reported by Barido *et al.* (2022), of

broiler chickens reported by Omid et al. (2020) and of chicken breast and drumstick (Milićević et al., 2014). It is widely accepted that the n-6/n-3 ratio in chicken meat can decrease if the birds access supplementary diets with n-3 fat sources, such as fish oil and flax seed oil/seed (Turner et al., 2014; Okrouhlá et al., 2013; Corino et al., 2014).

FA profile in pork was similar to of the major FA profile of intramuscular neutral lipids of pork reported by Olivares et al. (2009), Chen et al. (2021), He et al. (2012), and similar in fatty acid groups of muscle tissue of Kasprzyk et al. (2015). Our results for FAs in pork were higher than in the studies by Janiszewski et al. (2016), fatty acid composition of crossbred pigs of Supakankul and Mekchay (2016) and pork back fat in the study of Scerra et al. (2022).

Our mean FA profile in lamb meat was higher than results raw Australian lambs in the study of Flakemore et al. (2017), lamb meat allowed to graze Vasta et al. (2012) and pasture and stable production system of Margetín et al. (2018), but different to fat-tailed lamb in the study by Atti and Mahounachi, (2009), and similar to results in the studies of Greek breeds by Arsenos et al. (2006) and lamb fed on North Macedonia of study of Vasilev et al. (2020). Regarding individual FAs, the proportions of linoleic acid and α -linolenic acid in lamb were previously found to be higher in a pasture production system (6.42 and 2.38%, respectively) than in lamb fed on concentrate or hay/silage (2.73 and 0.91%, respectively) (Margetín et al., 2018). Accordingly, our results for linoleic acid and α -linolenic acid, 2.09% and 0.69%, respectively, confirm that our lambs were fed on concentrate mainly during the winter period. Díaz et al. (2005) differentiated lamb meat from pasture and concentrate production systems, and found pasture feeding increased n-3 PUFA and decreased the n-6/n-3 ratio in the meat. However, the FA profiles of our lamb meat were similar to those of meat from German lambs that were fed grass and concentrate (Díaz et al., 2005).

Our mean FA content in beef meat was lower than was found in the study by Nikolić et al. (2009), similar to results reported by Kerth et al. (2015), but lower than in the study by Lengyel et al. (2003) in Psoas major and Semitendinosus and Longissimus dorsi of beef meat. Our mean FA content in beef was also lower than in the study by Alfaia et al. (2010) and lower than reported by Salami et al. (2021).

According to a statement by Simopoulos (2002), the recommended ratio of n-6/n-3 PUFAs in human diets should be between 1 and 4. However, in our study, the best ratio was obtained in lamb (2.23) followed by beef (3.62), pork (5.10-5.87) and poultry (16.53-22.14).

AI and TI less than 0.5 and 1.0, respectively, in human diets are recommended according to some researchers (Fernandes et al., 2014; Sinanoglou, 2013). AI and TI need to be sufficiently low values which indicate the suitability of the food to prevent cardiovascular diseases in

humans. A significant decrease in AI or TI were established ($P < 0.05$) in statistical analysis of our results. The range of measured AIs in poultry meat in our study was similar to that reported by Skřivanova et al. (2017) (0.35-0.45) but lower than in the study by Liu et al. (2020) (0.42-0.44). However, our range of TIs in poultry was higher than reported by Skřivanova et al. (2017) (0.64-0.79) and by Liu et al. (2020) (0.62-0.91). In contrast, the ranges of AI and TI in our poultry were lower than in the study by Puerto et al. (2017) (0.48-0.49 and 1.09-1.14, respectively).

The ranges of AI and TI values in pork in our study were higher than those in the study by Scerra et al. (2022) (0.76-0.84 and 1.09-1.18, respectively). Our results for mean AI and TI in lamb meat were in accordance with those in lamb published by Flakemore et al. (2017) (0.5 and 1.2, respectively). The ranges of AI and TI in lamb were higher than in the study of de Souza et al. (2022) (0.18-0.19 and 1.04-1.12, respectively, although the experiments differed), and were similar to results from the study by Margetín et al. (2018) (0.97 and 1.24, respectively). The ranges of our AI and TI in beef were higher than previously reported by Salami et al. (2021) (0.82-0.83 and 1.78-1.87, respectively), but lower than in the study by Pilarczyk and Wójcik (2015) (1.62-1.85 and 3.84-3.98, respectively). AI and TI values in the meats we studied were generally closely recommended for healthy human diets.

Conclusion

Myristic (C14:0) and palmitic (C16:0) FA, which are recognized as health risks, were lower in poultry meat than in the other meats, whereas PUFA was significantly higher in poultry meat, which is positively reflected in the AI and TI for this meat. High health indices (AI and TI) in beef were caused by naturally high contents of SFAs, especially C14:0 and C16:0. Beneficial nutrition habits, i.e., nutrition according the food pyramid and a Mediterranean diet, should reduce the rates of coronary heart disease and contribute to better health in meat consumers. We think that our results will help in that decision.

Acknowledgements

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CONFLICT OF INTEREST

We declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

DT, ML, DV, JĆ, VĐ, NS and NP established the study, contributed to the design and reviewing the manuscript. DT, ML, DV and JĆ acquired and selected the samples for the analysis and wrote the manuscript. DT, JĆ and DV performed statistical analysis, and evaluate the literature. All authors have read and given their permission to publish the final manuscript.

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