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Analysis of fatty acid profile in milk fat of Wallachian sheep during lactation

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Abstract

In this Research Communication we evaluate the fatty acid (FA) profile of Wallachian sheep milk. The study was performed on 38 ewes in Beskydy Mountains. Samples were collected 4 times during the lactation, at monthly intervals. FA profile as well as groups of saturated, monounsaturated (MUFA), and polyunsaturated (PUFA) FAs were investigated. Considerable increase over the lactation was detected for lauric, myristic, myristoleic and palmitic acids, while stearic acid showed the opposed tendency. Variability, supported by significant differences among particular days of milk collection, was demonstrated for oleic acid; its highest distribution occurred at the beginning and at the end of the trial. The highest distribution of CLA was at the second sample day. Milk of Wallachian sheep naturally grazed at permanent pasture areas showed higher content of PUFA and MUFA in contrast with intensive or semi-intensive sheep breeds reported in the literature.

The Wallachian sheep is an original Czech breed with a triple production purpose (milk, meat, wool) which is characterized by a medium body size, long coarse hairs with short kemps and lower performance parameters accompanied by adaptability and resistance to harsh climatic conditions (Jandurová *et al.*, 2005; Milerski, 2013). The breed reached the area of Beskydy Mountains during the Wallachian colonization of Carpathians in 15th and 16th centuries (Milerski, 2013). In the second half of 20th century Wallachian sheep were widely crossed with imported sheep breeds, primarily for wool quality and production improvement and by the mid-1980s only a few individuals of original Wallachian sheep survived. Since 1999 Wallachian sheep have been classified among the genetic resources of the Czech Republic. The number of Wallachian sheep increased again and in 2017 a total of 1100 purebred ewes were registered. At present, Wallachian sheep in the Czech Republic are usually not milked, although in the past milk production used to be a substantial part of their combined utilization. The population of milked sheep has been growing during last years in the Czech Republic; such that these sheep represent about 15% of the total recorded flock. Therefore, the importance for sheep milk monitoring increases. The use of Wallachian sheep milking potential would improve the survival probability of this breed and reduce its dependence on governmental subsidies. Wallachian sheep cannot compete with specialized breeds such as Lacaune or East-Friesian in milk yield, but can be promoted for their ability to use mountain pastures and to provide milk of valuable composition and quality. Monitoring of fatty acid (FA) profile in milk fat is important for its close connection with human nutrition and health, or for its contribution to the characteristic flavors of ripened cheese (Haenlein, 2004; Haug *et al.*, 2007).

The aim of the present study was to analyze the FA profile in milk fat of Wallachian ewes at different lactation periods and age categories and to compare these results with literature for other breeds of sheep and under other production systems. To the best of the author's knowledge, information about the FA composition of indigenous (unimproved) Wallachian sheep is not available in the literature although Mierlita *et al.* (2011) have examined the FA profile of the phylogenetically related breed Turcana in Romania.

Material and methods

Animals and data collection

The milk samples were collected from 38 purebred Wallachian ewes selected from the flock on 4 occasions from April to August 2015: 27th April, average 42nd day of lactation, 25th May, average 71st day of lactation, 23rd June, average 100th day of lactation, and 4th August, average 142nd day of lactation. The ewes were selected with regard to different age and number of lambs, but all had lambed during a 15 d period. The ewes were suckling their own lambs and were not milked. During the experiment, all animals were kept at pasture in one flock under identical conditions without any differences in nutrition (apart from natural variation in pasture quality) or management. All were healthy and showed no signs of mastitis or other health problems. More information about flock management is given in on-line Supplementary Material and Methods (Flock location and management). Information about milk production in particular collection days and number and sex of lambs were recorded.

Data collection

Milk was sampled according to Doney *et al.* (1979). The lambs were separated from their mothers and oxytocin was injected intravenously at a dose of 2 UI to ewes to achieve complete milk ejection (including alveolar milk). Sheep were milked individually at the pasture using simple fixation and single bucket milking machine system (Interpuls, vacuum level 38–40 kPa, 100 cycles/min, pulsation ratio 60 : 40). This milk was discarded. After around 6 h (from 317 to 501 min) ewes were again injected with oxytocin and milked to achieve their total milk production, after which they were reunited with their lambs. The exact time interval was recoded for each ewe, to allow calculation of their total 24 h milk production. Milk samples (30 ml) from this second milking were collected in accordance with standard protocols (ICAR, 2012). Samples were frozen (−20 °C) before transfer to laboratories of Czech University of Life Sciences in Prague for FA estimation.

FAs profile estimation

Methods of milk fat extraction, and fatty acid estimation are reported in on-line Supplementary Material and Methods (FAs profile estimation). Percentage content (%) was determined for 45 individual FA, while further evaluation included only FA with more than 0.1% (30 FA). Nevertheless, all the determined FA were used to create groups of SFA, MUFA and PUFA that were also investigated as a part of the evaluation.

Statistical evaluation

Statistical analyses were performed using MIXED procedure of SAS 9.3. (SAS/STAT, 2011). Variables were corrected for the day of milk collection, ewe age category (both fixed effects), nested effect of days in milk within particular days of milk collection and animal as random effects. The description of model equation used for all FA or FA group evaluations is presented in on-line Supplementary Material and Methods. Significance level $P < 0.05$ was used to evaluate differences among groups.

Results

The basic compositional analysis of the Basic characteristic of Wallachian sheep milk composition is reported in on-line Supplementary File Table S1. Average calculated yield was 0.85 kg/d at a protein, lactose and fat concentration of 5.18%, 5.01% and 6.98%, respectively.

Model description

The model was significant for all the evaluated FA groups, and for all the individual FA.

The FA profile varied significantly according to stage of lactation (sampling day), but the effect of ewe age category was non-significant. Nested effect of days in milk within particular sample day was significant for SFA and MUFA. Significance of factors in model for individual FA are reported in Table 1. Results related to factor of ewe age category are excluded, due to non-significance of this factor in the model for all the evaluated traits.

FA groups

Figure 1 shows influence of stage of lactation on FA groups. The SFA percentage increased significantly from the 1st sampling to the 2nd and 3rd (+9.03%); and then slightly decreased (−1.12%). Nevertheless, results obtained during the 1st half of the lactation (1st and 2nd samples) were significantly lower than those detected during the 2nd half of the lactation (3rd and 4th samples). The MUFA showed the opposed tendency to SFA percentage, with significantly lower MUFA percentage at 3rd and 4th samples. PUFA content did not vary between sample days apart from the second sample being lower than later samples ($P < 0.05$).

FAs profile

The overview of FA profile, having more than 0.1% percentage content in the milk fat, is reported in on-line Supplementary File Table S2. Oleic acid ($C_{18:1n9c} = 22.00\%$), palmitic acid ($C_{16:0} = 20.04\%$), stearic acid ($C_{18:0} = 15.05\%$), myristic acid ($C_{14:0} = 7.43\%$), and capric acid ($C_{10:0} = 4.20\%$) were the predominant FA. Significantly lowest butyric acid ($C_{4:0}$) content was demonstrated for the 4th day of milk collection, as presented in Table 1. No significant differences were detected among all the other sampling days for butyric acid. Caproic ($C_{6:0}$), caprylic ($C_{8:0}$), and capric acid ($C_{10:0}$) percentages were characterized by mainly significantly higher values during sample days 2 and 3, while generally lower values were detected in the beginning and at the end of the lactation. A significant increase over the lactation was detected for lauric acid ($C_{12:0}$), myristic acid ($C_{14:0}$), myristoleic acid ($C_{14:1}$) and palmitic acid ($C_{16:0}$). Stearic acid ($C_{18:0}$) showed the opposite change (from 17.00% in early lactation to 12.17%). Oleic acid ($C_{18:1n9c}$) was variable throughout lactation, with higher values at the beginning and at the end of sampling. The highest content of CLA was seen at the 2nd sample day (2.91%), significantly higher than any other sample.

Discussion

We focused on determination of the milk fatty acid profile of Wallachian sheep under extensive Carpathian production system based on grazing and on evaluation of factors connected with lactation stage and the age of ewes. Previous studies have reported about the negative effect of SFA on human health (Haug *et al.*, 2007). Distribution of SFA content in the present study was 57.99% in average. Other authors reported similar (Carta *et al.*, 2008) or higher content of SFAs in sheep milk (Markiewicz-Kęszycka *et al.*, 2013; Aguilar *et al.*, 2014). The SFA percentage increased as lactation progressed, which agrees with data for Assaf sheep (Mihaylova *et al.*, 2005; Salari *et al.*, 2018). By contrast, gradually decreasing SFA content related to month of

Table 1. Influence of collection day throughout the lactation on fatty acid profile in milk fat of original Wallachian sheep (LSM ± SEM)

	DAY 1	DAY 2	DAY 3	DAY 4	DAY	AGE	DIM (DAY)
C4 : 0	1.85 ± 0.043^{A}	1.88 ± 0.043^{A}	1.89 ± 0.043^{A}	1.58 ± 0.046^{B}	***	n.s.	*
C6 : 0	1.38 ± 0.054^{A}	1.71 ± 0.054^{B}	1.76 ± 0.054^{B}	1.40 ± 0.056^{A}	***	n.s.	n.s.
C8 : 0	1.23 ± 0.065^{A}	1.74 ± 0.065^{B}	1.74 ± 0.065^{B}	1.40 ± 0.068^{A}	***	n.s.	n.s.
C10 : 0	3.07 ± 0.217^{A}	5.00 ± 0.218^{BC}	5.34 ± 0.218^{B}	4.53 ± 0.227^{C}	***	n.s.	*
C11 : 0	0.11 ± 0.011^{A}	0.17 ± 0.011^{B}	0.17 ± 0.011^{B}	0.22 ± 0.011^{C}	***	n.s.	*
C12 : 0	1.88 ± 0.117^{A}	3.01 ± 0.117^{B}	3.17 ± 0.117^{B}	3.18 ± 0.123^{B}	***	n.s.	**
C14 : 0R	0.12 ± 0.006^{A}	0.17 ± 0.006^{B}	0.17 ± 0.006^{B}	0.15 ± 0.007^{C}	***	n.s.	n.s.
C14 : 0	5.18 ± 0.215^{A}	7.35 ± 0.215^{B}	8.87 ± 0.215^{C}	9.75 ± 0.224^{D}	***	n.s.	**
C15 : 0R	0.36 ± 0.011^{A}	0.46 ± 0.011^{B}	0.37 ± 0.011^{A}	0.44 ± 0.011^{B}	***	n.s.	n.s.
C14 : 1	0.07 ± 0.014^{A}	0.09 ± 0.014^{A}	0.11 ± 0.014^{A}	0.20 ± 0.015^{B}	***	n.s.	n.s.
C15 : 0	0.93 ± 0.028^{A}	1.22 ± 0.028^{B}	1.21 ± 0.028^{B}	1.26 ± 0.030^{B}	***	n.s.	n.s.
C16 : 0R	0.29 ± 0.017^{A}	0.39 ± 0.017^{B}	0.36 ± 0.017^{BC}	0.32 ± 0.018^{AC}	***	n.s.	n.s.
C16 : 0	18.42 ± 0.256^{A}	18.59 ± 0.256^{A}	21.50 ± 0.256^{B}	23.03 ± 0.266^{C}	***	n.s.	n.s.
C16 : 1T	0.58 ± 0.015^{A}	0.61 ± 0.015^{AB}	0.66 ± 0.015^{BC}	0.68 ± 0.016^{C}	***	n.s.	n.s.
C16 : 1	0.81 ± 0.043^{A}	0.74 ± 0.043^{A}	0.82 ± 0.043^{A}	1.08 ± 0.044^{B}	***	n.s.	n.s.
C17 : 0	0.87 ± 0.021^{A}	0.76 ± 0.021^{B}	0.76 ± 0.021^{B}	0.92 ± 0.021^{A}	***	n.s.	n.s.
C17 : 1	0.35 ± 0.011^{A}	0.28 ± 0.011^{B}	0.29 ± 0.011^{B}	0.34 ± 0.012^{A}	***	n.s.	n.s.
C18 : 0	17.00 ± 0.489^{A}	15.59 ± 0.490^{B}	14.23 ± 0.490^{C}	12.17 ± 0.508^{D}	***	n.s.	n.s.
∑C18 : 1elaivak	7.07 ± 0.361^{A}	10.20 ± 0.362^{B}	7.66 ± 0.362^{A}	5.43 ± 0.376^{C}	***	n.s.	n.s.
C18 : 1n9c	27.67 ± 0.580^{A}	19.78 ± 0.581^{B}	17.91 ± 0.581^{C}	20.90 ± 0.605^{B}	***	n.s.	n.s.
∑C18 : 1C	2.14 ± 0.063^{A}	1.88 ± 0.063^{B}	1.97 ± 0.063^{B}	1.87 ± 0.065^{B}	***	n.s.	n.s.
∑C18 : 2T	1.83 ± 0.064^{A}	1.56 ± 0.064^{B}	1.75 ± 0.064^{A}	1.83 ± 0.066^{A}	***	n.s.	n.s.
C18 : 2n6c	1.81 ± 0.056^{A}	1.28 ± 0.057^{B}	1.93 ± 0.057^{AC}	2.04 ± 0.059^{C}	***	n.s.	n.s.
C20 : 0	0.29 ± 0.008^{A}	0.23 ± 0.008^{B}	0.30 ± 0.008^{A}	0.28 ± 0.008^{A}	***	n.s.	*
C18 : 3n3	1.65 ± 0.061^{AC}	1.20 ± 0.061^{B}	1.79 ± 0.061^{A}	1.53 ± 0.064^{C}	***	n.s.	n.s.
C21 : 0	0.07 ± 0.035^{A}	0.07 ± 0.035^{A}	0.11 ± 0.035^{A}	0.18 ± 0.037^{B}	***	n.s.	n.s.
C22 : 0	0.14 ± 0.005^{A}	0.15 ± 0.005^{AB}	0.19 ± 0.005^{C}	0.16 ± 0.005^{B}	***	n.s.	**
C20 : 4n6	0.11 ± 0.004^{A}	0.08 ± 0.004^{B}	0.11 ± 0.004^{A}	0.14 ± 0.004^{C}	***	n.s.	n.s.
C20 : 5n3	0.12 ± 0.003^{A}	0.10 ± 0.003^{B}	0.12 ± 0.003^{A}	0.13 ± 0.003^{C}	***	n.s.	*
CLA	1.85 ± 0.140^{A}	2.91 ± 0.141^{B}	1.90 ± 0.141^{A}	1.82 ± 0.146^{A}	***	n.s.	n.s.

DAY = control day of milk collection; DAY 1 = 1st day of milk collection; DAY 2 = 2nd day of milk collection; DAY 3 = 3rd day of milk collection; DAY 4 = 4th day of milk collection; AGE = ewe age category; DIM (DAY) = nested effect of days in milk within particular days of milk collection; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s. = non-significant; Different letters among rows (A, B, C, D) indicate significant differences at $P < 0.05$ level of significance.

collection was reported for improved Wallachian, Tsigai, Lacaune, and their crossbreds (Meľuchová *et al.*, 2008). In general, we observed a lower SFA percentage for our pasture fed sheep than previously published studies. The putative negative health effect of SFA is mainly related to lauric ($C_{12:0}$), myristic ($C_{14:0}$), and palmitic ($C_{16:0}$) FA (Haenlein, 2004), and we did observe high levels of palmitic and myristic FA in this study. This agrees with earlier data for ovine (De La Fuente *et al.*, 2009) and goat milk (Maroteau *et al.*, 2014). Some individual SFA such as butyric ($C_{4:0}$), caprylic ($C_{8:0}$), capric ($C_{10:0}$) and lauric acid ($C_{12:0}$) have been associated with possible health benefits (delayed tumor growth, cancer prevention or antiviral and antibacterial function: Haug *et al.*, 2007). These FA were also detected at lower concentration than in earlier reports (De La Fuente *et al.*, 2009; Maroteau *et al.*, 2014).

PUFAs or MUFAs have been suggested to have favorable effects on human health (Haenlein, 2004). We observed higher PUFA and MUFA percentage than earlier reports for sheep milk (Markiewicz-Kęszycka *et al.*, 2013; Aguilar *et al.*, 2014, both unspecified breed), sheep milk of Improved Wallachian, Tsigai, Lacaune sheep, and their crossbreds kept under different feeding regimes (Ostrovský *et al.*, 2009) or sheep milk from Spanish genetic resources (Signorelli *et al.*, 2008). MUFA and PUFA concentrations decreasing across lactation has been reported by Mihaylova *et al.* (2005) and our data agree with this, in contrast to the increase reported by Meľuchová *et al.* (2008) or lack of consistent variation reported by Salari *et al.* (2018) for Assaf ewes.

Oleic acid has been reported to decrease during the course of lactation (Signorelli *et al.*, 2008; De La Fuente *et al.*, 2009) but our

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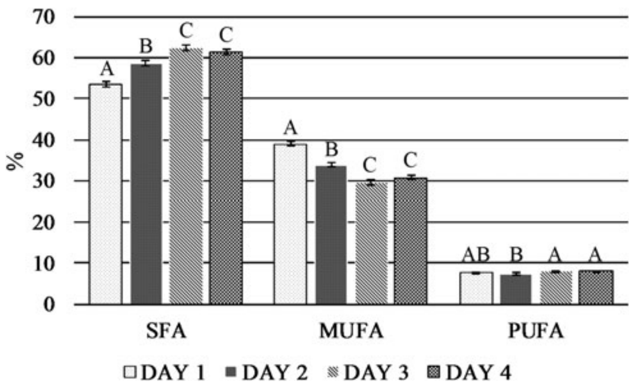

Fig. 1. Influence of collection day throughout the lactation on fatty acid groups according to their saturation of original Wallachian sheep. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; DAY 1 = 1st day of milk collection; DAY 2 = 2nd day of milk collection; DAY 3 = 3rd day of milk collection; DAY 4 = 4th day of milk collection; Different letters among bars within FAs groups (A, B, C, D) indicate significant differences at $P < 0.05$ level of significance.

results and those of Meľuchová *et al.* (2008) and Martini *et al.* (2013) do not support any significant effect of lactation stage. The main representatives of PUFAs in milk are the linoleic and alpha-linolenic FA (Haug *et al.*, 2007); both increased across lactation in our study in agreement with De La Fuente *et al.* (2009), however, Martini *et al.* (2013) saw no such effect in sheep of the Massese breed.

CLA has been reported to lower plasma cholesterol status and has putative anticarcinogenic effects (Haug *et al.*, 2007). De La Fuente *et al.* (2009) found an increased CLA content across the lactation period in Churra ewes, whereas we observed higher levels overall, but no effect of stage of lactation. Our CLA levels were also higher than Sarda sheep (Buccioni *et al.*, 2015) or than sheep and goats naturally grazed in mountainous highlands of Greece (Talpur *et al.*, 2009). Still higher levels have been reported for crossbred Tsigai ewes reared in Rodopi Mountains (Mihaylova *et al.*, 2005) and the phylogenetically related Turcana breed (Mierlita *et al.*, 2011).

These differences in FA profile may relate to specific genotypes but will also be influenced by environmental effects, especially plane of nutrition (Manso *et al.*, 2016). Green pasture, an important source of unsaturated FA, positively affects PUFA and MUFA content in milk fat (Nudda *et al.*, 2005), increasing the content of alpha-linolenic (Joy *et al.*, 2012) or CLA fatty acids (De Renobales *et al.*, 2012) amongst others. As pasture matures the content of unsaturated FA decreases (Lock and Bauman, 2004). Additionally, some phenolic compound in plants, such as tannis, affect ruminal fermentation, and thus might also affect the fatty acid profile of milk (Vasta *et al.*, 2008). However, the traditional Carpathian system did not allow us to monitor pasture intake as the animals were able to browse at will. The pasture contained grasses and herbs in the typical botanical composition characterized for the Eastern location of the White Carpathians and for the Beskydy Mountains.

In conclusion, the milk FA profile of Wallachian sheep kept in mountain conditions of Moravian-Silesian Beskydy were changing in the course of the grazing period. Content of SFAs increased from May to August, while proportion of MUFAs showed a downward trend as the phase of lactation progressed. The main cause of these changes may relate to pasture quality. Compared to literature reports, we observed a better saturated: unsaturated FA ratio and a higher content of CLA. Even though we are not able to separate genetic and environmental effects explicitly, the consequence of their common impact shows, that Wallachian ewes grazed on mountain pastures provide high nutritional and health valuable milk. This could help to increase the population size of this animal genetic resource and its wider use for milk production.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029919000244

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