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IMPACT OF MILKING SEQUENCE AND LACTATION NUMBER ON THE FATTY ACID COMPOSITION OF SHEEP MILK

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Abstract

The fatty acid profile of ewe milk is a critical indicator of its nutritional value and technological quality, being influenced by various physiological factors, including the stage of lactation and the order of milking. The aim of this study was to evaluate the evolution of the fatty acid composition in ewe milk across three consecutive lactations (L1, L2, and L3) and to determine the impact of milking sequence on these parameters. A total of 45 samples were collected over three milking sequences, from family farm of 15 heads of the Tsurcana (Țurcană) sheep breed from Șieuț locality, Bistrița Năsăud county. The findings revealed a significant improvement in the nutritional quality of the milk as the lactation rank advanced. A favorable decrease in the n-6/n-3 polyunsaturated fatty acid ratio was observed, shifting from 3.21 in the first lactation (L1) to 2.88 in the third lactation (L3). The analysis confirmed that the biochemical composition of ewe milk undergoes progressive modifications, with the lipid profile becoming more nutritionally balanced.

Key words*: ewe milk, fatty acid profile, lactation, lipid quality, n-6/n-3 ratio.*

INTRODUCTION

Consumption of dairy products has benefits in lowering blood pressure and cholesterol, helps in muscle development, prevents cancer (colorectal and bladder cancer), diabetes, obesity, has protective effects on coronary and cardiovascular diseases, stroke, dementia (Kliem & Givens, 2011; Huth & Park, 2012; Tunick & Van Hekken, 2014; Kanekanian, 2014). Milk is a complete and complex food that contains a variety of essential nutrients to ensure a healthy and balanced diet, being rich in valuable amino acids, lipids, carbohydrates, vitamins, minerals, enzymes, immunoglobulins (Kumar et al., 2017). It is also rich in functional ingredients such as immunoglobulins, α-lactalbumin, β-lactoglobulin, lactoferrin, lactoperoxidase, caseins and calcium (Anastasova et al., 2019). Bone health is ensured by the consumption of dairy products due to the valuable intake of calcium, vitamin D, protein and potassium (Rizzoli, 2014). The main factors influencing the fatty acid profile in milk are biological (breed, lactation, individuality, lactation: parity and stage) and external: animal nutrition (presence of concentrates, fat content and oil supplementation) and management (maintenance system, season, milking) (Hanuš et al., 2018). Fatty acids in milk are the result of the feed administered to the animals and the microbial activity of the rumen. Of all natural fats, the most structurally complex is milk fat because it is made up of approximately 400 different fatty acids (Lindmark, 2008). Sheep and goat milk is rich in medium-chain fatty acids: caproic (C6:0), caprylic (C8:0) and capric (C10:0), which is why its flavor is different compared to other species. Adding fresh grass to the animal diet increases the proportion of unsaturated fatty acids in milk fat (Djordjevic et al., 2019). Grazing has the effect of increasing milk production by 30% compared to feeding sheep in shelter (de Renobales et al., 2014). The largest seasonal variations were observed within the season, namely polyunsaturated fatty acids

with maximum values were identified in spring (44%) and summer and minimum values were observed in winter (De La Fuente et al., 2009). The main sources of fatty acids are found in oleaginous feeds, fish oil and fat supplements (Mansbridge & Blake, 1997). Grazing and the use of supplements rich in vegetable oils have an influence on the biohydrogenation process of fatty acids in the rumen, therefore improving the nutritional value of the fatty acid profile (Nudda et al., 2014). Other categories of vegetable lipids are flaxseed, soybean, safflower and sunflower which have positive effects on the content of conjugated linoleic acid and unsaturated fatty acids in milk fat (Luna et al., 2005; Bodas et al., 2010). The objective of this work was to investigate the dynamics of the fatty acid profile in sheep milk, with a focus on the sequential impact of milking and the evolution of these parameters during lactations I, II and III.

MATERIALS AND METHODS

The study was conducted on a flock of sheep belonging to a private family farm. As milk sampling was performed during the usual milking technological flow, without subjecting the animals to invasive experimental procedures, dietary changes or additional stress, specific approval from an ethics committee was not required (according to Directive 2010/63/EU on the protection of animals used for scientific purposes). All animal handling procedures complied with national farm animal welfare standards.

Animals

The research was conducted on a family sheep farm located in the Transylvania region: Șieuț locality, Bistrița-Năsăud county. The biological material was represented by Tsurcana sheep, Brează variety. The geographical coordinates of the pasture are: 46º58'37.50"N and 24º39'00.10"E. Lactating females are fed in shelter during the winter and in the summer they graze in pastures. The feed received by the animals during the winter is diversified, consisting of good quality hay, vetch, alfalfa and corn in a quantity of 0.5 kg/head. The meadow consists of grasses and spontaneous legumes (wild clover). Drinking water is provided at discretion since the pastures have natural springs. Milking is done manually, twice a day, starting with April and until October. The biological material is represented by 15 lactating female heads grouped into 3 categories: 5 primiparous females, 5 multiparous females (lactation 2) and 5 multiparous females (lactation 3).



Figure 1. Tsurcana sheep (original source)

Sampling

Milk samples collected for fatty acid analysis were collected individually on 3 milking sequences (sequence 1 - beginning of milking; sequence 2 - middle milking; sequence 3 - end of milking). Milking sequences were determined based on the milking duration that were previously recorded by a milking test, then they were divided equally. Milk was collected from the two quarters as a single sample. Subsequently, the samples were taken in sterile tubes and frozen until the analysis was determined. The samples were collected in June 2025.



Figure 2. Milk samples (original source)

Gas chromatographic analysis of fatty acids

Fatty acid composition was analyzed using a gas chromatograph system (GC-FID) from Agilent Technologies, model 6890N, located in Wilmington, DE, USA. This system included a DB-WAX capillary column with dimensions 30 meters long, 0.25 mm in diameter, and a 0.25 micrometer thick layer of polyethylene glycol as the stationary phase, along with a flame ionization detector. Initially, lipids were extracted from the samples. A volume of 50 mL of the sample was mixed with 50 mL of a chloroform:methanol solution in a 2:1 ratio by volume. The mixture underwent ultrasonic extraction for 30 minutes at room temperature using an ultrasonic bath, model Sonorex RK 512H, made by BANDELIN electronic GmbH & Co. KG, based in Berlin, Germany. The bath had internal dimensions of 150 × 138 × 65 mm and a working volume of 1.3 liters. It operated at a frequency of 40 kHz and a power of 60 W. After extraction, the mixture was filtered using Whatman No. 40 filter paper, sourced from Sigma-Aldrich, located in St. Louis, MO, USA. The organic layer was then collected. To purify the lipid extract, the sample was washed with 20 mL of a 0.74% solution of potassium chloride. The mixture was then centrifuged at a speed of 5000 revolutions per minute for 10 minutes to aid in the separation of phases. The organic layer was passed through anhydrous sodium sulfate to absorb any residual water. The solvent was evaporated using a rotary evaporator, model Laborota 4010, from Heidolph. The resulting oil was dried at a temperature of 60°C in an oven. To prepare fatty acid methyl esters (FAME), about 30 ± 0.1 mg of the extracted oil was dissolved in 2 mL of isooctane. Transesterification was then conducted by adding 200 microliters of a 2 mol/L methanolic potassium hydroxide solution while vigorously stirring. The organic layer was then treated with sodium hydrogen sulfate. Following phase separation, the supernatant was collected and used directly for gas chromatographic analysis.

Chromatographic analysis was performed by injecting 1 microliter of the prepared solution in split mode (1:20). The temperatures of the injector and detector were kept constant at 250°C. The oven temperature program started at 60°C and was held for 1 minute, then it increased from 60 to 200°C at a rate of 10°C per minute, with a 2-minute hold. The temperature was then raised from 200 to 220°C at a rate of 5°C per minute and held for 20 minutes. The detector gases used were hydrogen at 40 mL/min, air at 450 mL/min, and helium at 30 mL/min. Fatty acids were identified by comparing the retention times of the peaks from the sample with those from a certified reference standard mixture, Supelco 37-component FAME mix, CRM47885, from Sigma-Aldrich. The relative composition of each fatty acid was determined by normalizing the chromatographic peak areas internally and expressed as a percentage of the total identified fatty acids.

Statistical Methods

The data were analyzed using a General Linear Model (GLM) approach. A Two-Way Analysis of Variance (ANOVA) was conducted to assess the effects of lactation stage (L1, L2, L3) and milking sequence (S1, S2, S3), as well as their interaction (L × S). Since multiple samples were obtained from the same animals, 'Sheep ID' was included as a random effect in a mixed model to account for individual variation. The normality of the data distribution and homogeneity of variance were checked using the Shapiro-Wilk and Levene tests, respectively. If significant effects were found ($P < 0.05$), means were compared using Tukey's Honestly Significant Difference (HSD) post-hoc test. All results obtained were reported as mean and standard error of the mean (SEM). Statistical significance was set at $P < 0.05$. Calculations for Health Indices (Atherogenicity Index - AI and Thrombogenicity Index - TI) were based on the formulas given by Ulbricht & Southgate in 1991.

RESULTS AND DISCUSSIONS

The results indicate that the number of lactations exerts a direct influence on the quality of the lipid profile of sheep milk. ANOVA analysis confirmed significant differences ($p < 0.05$) in the case of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Table 1 presents the distribution of fatty acids in sheep milk according to lactation stage (L1, L2, L3). Data are expressed as mean ± SEM, and the statistical significance of variations between

physiological stages is highlighted by the calculated p-values.

Table 1. Fatty acid profile according to lactation (Means, Standard Error of the Mean)

Indicator (g/100 g)	Lactation 1 (Mean ± SEM)	Lactation 2 (Mean ± SEM)	Lactation 3 (Mean ± SEM)	p-value (ANOVA)
SFA (Saturated Fatty Acids)	71.42± 0.85	69.85± 0.72	68.12± 0.94	p<0.05
MUFA (Monounsaturated Fatty Acids)	22.15± 0.45	23.60± 0.51	24.85± 0.62	p<0.05
PUFA (Polyunsaturated Fatty Acids)	6.43± 0.22	6.55± 0.18	7.03± 0.31	p>0.05 (NS)
Report n-6/n-3	3.21± 0.12	3.05± 0.10	2.88± 0.14	p<0.05

Legend: *** - highly significant (P < 0.001); * - significant (P < 0.05); ns - not significant (P > 0.05)

An improvement in nutritional value was found as lactation progressed, highlighted by the reduction of the n-6/n-3 ratio (from 3.21 in L1 to 2.88 in L3). The favorable fatty acid profile observed in our study, especially in the third lactation (L3), can be attributed to the synergistic effect of animal maturity and the diet exclusively with green forage during the summer season. Grazing is an advantageous option for obtaining a higher concentration of phenolic compounds and antioxidant capacity of sheep milk (Ruiz de Gordoa et al., 2011; Chávez-Servín et al., 2018). The grazing-based agricultural system produces milk rich in fat and microcomponents beneficial to human health (fatty acids and vitamins) and volatile components (aroma, terpenes) (Morand-Fehr et al., 2007). Sheep farmed extensively on pasture have a higher content of cis-9, trans-11 and the milk fat profile is more beneficial for human health due to the high content of PUFA (Tsiplakou et al., 2006; Mierlita & Vicas, 2015). Natural pastures are a primary source of polyunsaturated fatty acids (PUFA), especially alpha-linolenic acid (C18:3 n-3), which acts as a precursor for the synthesis of beneficial lipids in milk (Mierlița, 2016). Sheep kept on pasture generally produce higher values of milk fat such as C18:3 linolenic acid, and unsaturated fatty acids MUFA and PUFA while feedlot sheep have a higher protein content (Atti et al., 2006; Ptáček et al., 2019). Standard Error (SEM) values confirm the precision of the measurements and the stability of the composition at the group level. Statistical analysis demonstrates that the maturity of the animal (expressed by the number of lactations) improves the quality of sheep milk by reducing the share of saturated fatty acids and optimizing the n-6/n-3 ratio. The differences observed between L1 and L3 are statistically significant ($p < 0.05$), confirming the hypothesis that the lipid profile evolves with the physiological age of the animal. The distribution of the main fatty acid classes (SFA, MUFA and PUFA) according to the milking sequence is presented in Table 2. The data (mean ± SEM) reflect the dynamics of lipid fractions during milking, the statistical significance of the variations being indicated by p-values.

Table 2. Impact of milking sequence on fatty acid profile (Mean ± SEM)

Indicator (g/100 g)	S1 (Initial Milking)	S2 (Middle Milking)	S3 (Final Milking)	p-value (ANOVA)
Total Fat (estimated)	Low	Average	High	p<0.01
SFA (Saturated Fatty Acids)	70.25± 0.90	69.90± 0.85	69.25± 0.95	p>0.05 (NS)
MUFA (Monounsaturated Fatty Acids)	23.10± 0.55	23.45± 0.50	23.95± 0.60	p>0.05 (NS)
PUFA (Polyunsaturated Fatty Acids)	6.65± 0.25	6.65± 0.22	6.80± 0.28	p>0.05 (NS)

Legend: *** - highly significant (P < 0.001); * - significant (P < 0.05); ns - not significant (P > 0.05)

Analysis of the milking sequences (S1-S3) indicates a remarkable stability of the fatty acid profile. Although the literature confirms an increase in total lipid content towards the end of milking, the percentage distribution of the SFA, MUFA and PUFA groups did not undergo statistically significant changes ($p > 0.05$). This result suggests that the milking process influences the amount of fat extracted, but does not alter the intrinsic quality of the lipid profile established at the level of mammary alveolar metabolism. To assess the nutritional quality of the lipid fraction of sheep milk and its potential impact on human health, atherogenicity (AI) and thrombogenicity (TI) indices were calculated, along with the n-6/n-3 fatty acid ratio. The mean values of these health parameters, recorded according to lactation rank, are summarized in Table 3. The results presented highlight a progressive improvement in the bioactive

properties of milk, suggesting a direct correlation between the physiological maturity of the animal and the reduction of the atherogenic and thrombogenic potential of milk fat.

Table 3. Lipid health indices according to lactation

Parameter	L1 (Media ± SEM)	L2 (Media ± SEM)	L3 (Media ± SEM)	p-value
n-6/n-3 ratio	3.21±0.04	3.05±0.05	2.88±0.04	<0.001
AI (Atherogenicity Index)	2.85±0.05	2.62±0.04	2.41±0.06	<0.001
TI (Thrombogenicity Index)	2.58±0.03	2.34±0.04	2.21±0.05	<0.01

Legend: *** - highly significant ($P < 0.001$); * - significant ($P < 0.05$); ns - not significant ($P > 0.05$)

Cardiovascular health indicators (AI and TI) showed a favorable evolution correlated with the number of lactations. The atherogenic index decreased from 2.85 (L1) to 2.41 (L3), indicating an improvement in lipid quality as the animals reach productive maturity. This decrease is determined by the rebalancing of the ratio between atherogenic fatty acids (C12:0-C16:0) and the unsaturated fraction. The stability of AI and TI indices across milking sequences (S1-S3) suggests that although fat content increases towards the end of milking due to the displacement of large fat globules from the alveolar lumen (Ontsouka et al., 2003), the health-promoting properties of milk remain constant throughout the milking process. The results demonstrate that although the milking sequence (S1-S3) modifies the lipid density, the AI and TI indices remain constant, the health profile of the milk being an intrinsic feature of the physiological stage of the sheep. The study confirms that lactation rank is a determining factor in establishing the nutritional value of sheep milk fat. The third lactation (L3) offers the most balanced lipid profile, characterized by the lowest values of the atherogenic and thrombogenic indices, as well as the most optimal n-6/n-3 ratio. The graphical analysis highlights a restructuring of the lipid composition, illustrating in detail the evolution of the major classes: SFA in Figure 1, MUFA in Figure 2 and PUFA in Figure 3, highlighting the trends in the modification of the fatty acid profile throughout the lactation cycles. The results of the ANOVA analysis revealed a significant effect of the lactation number on the main groups of fatty acids ($p < 0.05$). A progressive decrease in saturated fatty acids was observed along with an optimization of the n-6/n-3 ratio towards the end of the productive cycle of the animals (L3), a fact supported by the low values of the standard error (SEM), which indicates a stability of the lipid profile in mature animals.

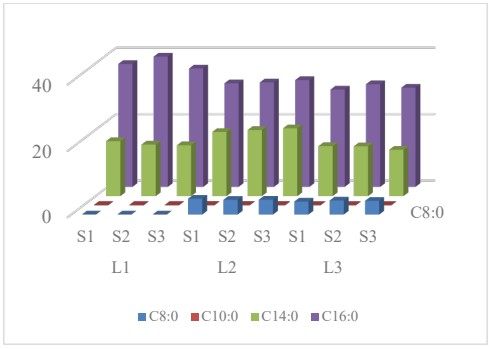

Figure 1. SFA milking sequence
Lactation stage (L1, L2, L3) and milking sequence (S1, S2, S3)

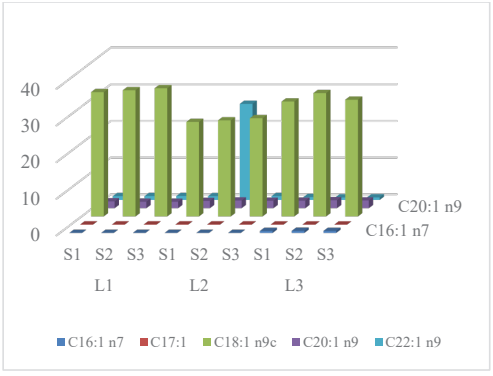

Figure 2. MUFA milking sequence

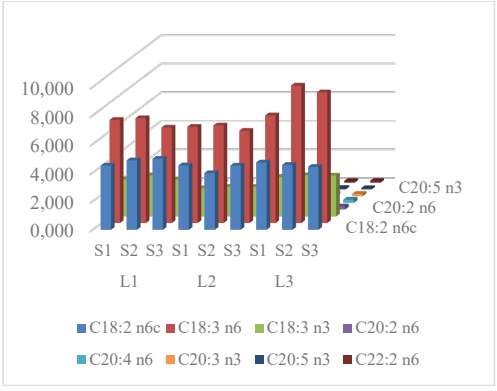

Figure 3. PUFA milking sequence

The fatty acid profile differs depending on the season, for example during the summer there are higher concentrations of α-linolenic acid, cis-9, trans 11-CLA, trans 11, C18:1 and PUFA and a low content of saturated fatty acids C12:0, C14:0 and C16:0 (Papaloukas et al., 2016). Altomonte et al. (2019) consider the milking period of sheep as a factor influencing milk characteristics and find that in the spring season there is a higher level of trans-11 C18:1, cis-9, cis-12, cis-15 C18:3 compared to the winter season. Mierliță et al. (2011a) observed that during the lactation period, milk production decreases while fat and protein content increases. With advancing lactation, the concentration of fatty acids C10:0, C14:0, C16:0, C16:1, C18:0, C18:3n3 and PUFA increases, while a decrease in fatty acids C4:0, C6:0, C12:0, C13:0, C15:0, C18:2n6 is observed. In the early lactation period, milk recorded the highest oleic acid content, which decreased during advancing lactation (Inostroza et al., 2020). Tsurcana sheep have a healthy fatty acid profile due to the low concentration of SFA (60.9%) and the high concentration of MUFA (31.0%) and PUFA (8.06%), as well as the C18:1, trans 11 C18:1, cis 9, trans 11 - CLA isomers (Mierlita & Dărăban, 2011, Mierliță et al., 2011b). Hemp seeds improve milk production, fat content by increasing polyunsaturated fatty acids and α-linolenic acid, while reducing unsaturated fats (Mierlita & Vicas, 2015). Adding soy to sheep diets increases the values of C18:0 and C18:1 isomers threefold compared to the control sample (Gómez-Cortés et al., 2008). At the same time, flaxseed supplementation increases milk production by 8%, increases C18:3 and CLA, decreases the proportion of unsaturated fatty acids without affecting the fat and protein components of milk, while the addition of sunflower seeds had no positive effects on milk production (Zhang et al., 2006).

To assess the contribution of experimental factors to lipid profile variability, two-way ANOVA was applied. Table 4 summarizes the statistical indicators of this model, including sums of squares (SS), degrees of freedom (df), and F-test values. This approach allows the identification of statistical significance for the main effects - lactation rank (L) and milking sequence (S) - as well as for the interaction between them (L x S). In this context, parameters such as sums of squares (SS) and F-test value become predictive indicators for the stability of the lipid profile under the given experimental conditions.

Table 4. Statistical indicators of lipid fraction variability according to experimental factors (Lactation and Milking Sequence)

Source of Variation	SS (Sum of Squares)	df (Deg. of Freedom)	MS (Mean Square)	F-value	p-value	Meaning
Lactation (L)	82.45	2	41.22	12.45	p<0.01	***
Sequence (S)	6.12	2	3.06	0.92	p>0.05	NS
Interaction (LxS)	2.34	4	0.58	0.17	p>0.05	NS
Error (Residual)	119.2	36	3.31	-	-	-
Total	210.11	44	-	-	-	-

Legend: *** - highly significant (P < 0.001); * - significant (P < 0.05); ns - not significant (P > 0.05)

The results of the analysis of variance (Table 4) confirm that lactation stage is the main source of variation in the lipid profile ($p < 0.001$), exerting a determining influence on the proportion of fatty acid classes. In contrast, milking sequence and the interaction between the two factors (L x S) did not generate statistically significant differences ($p > 0.05$). This indicates that, although the composition of milk changes as the animal matures, the distribution of fatty acids remains remarkably stable throughout the milk ejection process from the mammary gland, regardless of lactation stage.

CONCLUSIONS

The third lactation (L3) offered the most favorable nutritional profile, characterized by the lowest levels of saturated fatty acids (SFA) and the most beneficial values for the Atherogenicity (2.41) and Thrombogenicity (2.21) indices. This suggests that mature ewes exhibit superior metabolic efficiency in the synthesis of unsaturated lipids when maintained on exclusive summer pasture. Although fat concentration increases significantly towards the end of the milking process (S3), the quality of the lipid profile (AI, TI and n-6/n-3 ratio) does not very significantly between the cisternal and alveolar fractions. It is confirmed that the sanogenic properties of sheep milk are constant throughout the milking process. The exclusive

summer grazing system acts as a determining factor for the high concentration of polyunsaturated fatty acids, resulting in an optimal n-6/n-3 ratio (<3.0) in mature animals. The results demonstrate that, although the milking sequence (S1-S3) modifies lipid density, the AI and TI indices remain constant, the health profile of the milk being an intrinsic feature of the physiological stage of the ewe.

REFERENCES

Altomonte, I., Conte, G., Serra, A., Mele, M., Cannizzo, L., Salari, F., & Martini, M. (2019). Nutritional characteristics and volatile components of sheep milk products during two grazing seasons. *Small Ruminant Research*, 180, 41–49.

Anastasova, L., Petreska Ivanovska, T., Petkovska, R., & Petrusevska-Tozi, L. (2019). Concepts, benefits and perspectives of functional dairy food products. *Macedonian Pharmaceutical Bulletin*, 64(02), 73–83.

Atti, N., Rouissi, H., & Othmane, M. H. (2006). Milk production, milk fatty acid composition and conjugated linoleic acid (CLA) content in dairy ewes raised on feedlot or grazing pasture. *Livestock Science*, 104(1–2), 121–127.

Bodas, R., Manso, T., Mantecón, A. R., Juárez, M., De La Fuente, M. A., & Gómez-Cortés, P. (2010). Comparison of the fatty acid profiles in cheeses from ewes fed diets supplemented with different plant oils. *Journal of Agricultural and Food Chemistry*, 58, 10493-10502.

Chávez-Servín, J. L., Andrade-Montemayor, H. M., Velázquez Vázquez, C., Aguilera Barreyro, A., García-Gasca, T., Ferríz Martínez, R. A., Olvera Ramírez, A. M., & de la Torre-Carbot, K. (2018). Effects of feeding system, heat treatment and season on phenolic compounds and antioxidant capacity in goat milk, whey and cheese. *Small Ruminant Research*, 160, 54–58.

De La Fuente, L. F., Barbosa, E., Carriedo, J. A., Gonzalo, C., Arenas, R., Fresno, J. M., & San Primitivo, F. (2009). Factors influencing variation of fatty acid content in ovine milk. *Journal of Dairy Science*, 92(8), 3791–3799.

de Renobales, M., Amores, G., Arranz, J., Virto, M., Barrón, L. J. R., Bustamante, M. A., Ruiz de Gordoa, J. C., Nájera, A. I., Valdivielso, I., Abilleira, E., Beltrán de Heredia, I., Pérez-Elortondo, F. J., Ruiz, R., Albisu, M., & Mandaluniz, N. (2012). Part-time grazing improves sheep milk production and its nutritional characteristics. *Food Chemistry*, 130(1), 90–96.

Djordjevic, J., Ledina, T., Baltic, M. Z., Trbovic, D., Babic, M., & Bulajic, S. (2019). Fatty acid profile of milk. *IOP Conference Series: Earth and Environmental Science*, 333, 01205.

Gómez-Cortés, P., Frutos, P., Mantecón, A. R., Juárez, M., de la Fuente, M. A., & Hervás, G. (2008). Milk Production, Conjugated Linoleic Acid Content, and In Vitro Ruminal Fermentation in Response to High Levels of Soybean Oil in Dairy Ewe Diet. *Journal of Dairy Science*, 91(4), 1560–1569.

Hanuš, O., Samková, E., Křížová, L., Hasoňová, L., & Kala, R. (2018). Role of Fatty Acids in Milk Fat and the Influence of Selected Factors on Their Variability-A Review. *Molecules*, 23(7).

Huth, P. J., & Park, K. M. (2012). Influence of Dairy Product and Milk Fat Consumption on Cardiovascular Disease Risk: A Review of the Evidence. *Advances in Nutrition*, 3(3), 266–285.

Inostroza, K., Bravo, S., Larama, G., Saenz, C., & Sepúlveda, N. (2020). Variation in Milk Composition and Fatty Acid Profile during the Lactation of Araucana Creole Ewes in a Pasture-Based System. *Animals*, *10*(1), 92.

Kanekanian, A. (2014). The Health Benefits of Bioactive Compounds from Milk and Dairy Products. *Milk and Dairy Products as Functional Foods*, 1–22.

Kliem, K. E., & Givens, D. I. (2011). Dairy Products in the Food Chain: Their Impact on Health. *Annual Review of Food Science and Technology*, 2(1), 21–36.

Kumar, N., Raghavendra, M., Tokas, J., & Singal, H. R. (2017). Flavor Addition in Dairy Products. *Nutrients in Dairy and their Implications on Health and Disease*, 123–135.

Lindmark Månsson, H. (2008). Fatty acids in bovine milk fat. *Food & Nutrition Research*, 52(1). 1821.

Luna, P., Fontecha, J., Juárez, M. & de La Fuente, M. A. (2005). Changes in the milk and cheese fat composition of ewes fed commercial supplements containing linseed with special reference to the CLA content and isomer composition. *Lipids*, 40, 445-454.

Mansbridge, R. J., & Blake, J. S. (1997). Nutritional factors affecting the fatty acid composition of bovine milk. *British Journal of Nutrition*, 78(1), S37–S47.

Mierliță, D., Hilma, E., Dărăban, S., & Lup, F. (2011a), Influence of Lactation Stage on Milk Yield and Milk Fatty Acid Profile in Dairy Ewes, *Bulletin USAMV Animal Science and Biotechnologies*, 68(1-2). 279-286.

Mierliță, D., Padeanu, I., Maerescu, C., Chereji, I., Hilma, E., & Lup, F. (2011b). Comparative study regarding the fatty acids profile in sheep milk related to the breed and parity. *Analele Universității din Oradea, Fascicula: Ecotoxicologie, Zootehnie și Tehnologii de Industrie Alimentară*, 221-232.

Mierlita, D. (2016). Fatty acid profile and health lipid indices in the raw milk of ewes grazing part-time and hemp seed supplementation of lactating ewes. *South African Journal of Animal Science*, 46(3), 237–246.

Mierlita, D., & Vicas, S. (2015). Dietary effect of silage type and combination with camelina seed on milk fatty acid profile and antioxidant capacity of sheep milk. *South African Journal of Animal Science*, 45(1). 1-11.

Mierlita, D., Daraban, S., & Lup, E. (2011). Effects of breed on milk fatty acid profile in dairy ewes, with particular reference to cis-9, trans-11 conjugated linoleic acid. *South African Journal of Animal Science*, 41(3). 308-317.

Morand-Fehr, P., Fedele, V., Decandia, M., & Le Frileux, Y. (2007). Influence of farming and feeding systems on composition and quality of goat and sheep milk. *Small Ruminant Research*, 68(1–2), 20–34.

Nudda, A., Battacone, G., Boaventura Neto, O., Cannas, A., Francesconi, A. H. D., Atzori, A. S., & Pulina, G. (2014). Feeding strategies to design the fatty acid profile of sheep milk and cheese. *Revista Brasileira de Zootecnia*, *43*(8), 445–456.

Ontsouka, C. E., Bruckmaier, R. M., & Blum, J. W. (2003). Fractionized Milk Composition During Removal of Colostrum and Mature Milk. *Journal of Dairy Science*, 86(6), 2005–2010.

Papaloukas, L., Sinapis, E., Arsenos, G., Kyriakou, G., & Basdagianni, Z. (2016). Effect of season on fatty acid and terpene profiles of milk from Greek sheep raised under a semi-extensive production system. *Journal of Dairy Research*, *83*(3), 375–382.

Ptáček, M., Milerski, M., Ducháček, J., Schmidová, J., Tančin, V., Uhrinčať, M., Stádník, L., & Michlová, T. (2019). Analysis of fatty acid profile in milk fat of Wallachian sheep during lactation. *Journal of Dairy Research*, *86*(2), 233–237.

Rizzoli, R. (2014). Dairy products, yogurts, and bone health. *The American Journal of Clinical Nutrition*, 99(5). 1256S–1262S.

Ruiz de Gordoa J.C., Bustamante M, Arranz J, Virto M., Barrón L.J.R., Beltrán de Heredia I., Amores G., Abilleira E., Nájera A.I., Ruiz R., Albisu M., Pérez-Elortondo F.J., Mandaluniz N., & de Renobales M., (2011), Increase in water-soluble total antioxidant capacity of sheep's milk as a result of increased grazing time, *Options Méditerranéennes: Série A. Séminaires Méditerranéens*, 99, 267-271.

Tsiplakou, E., Mountzouris, K. C., & Zervas, G. (2006). Concentration of conjugated linoleic acid in grazing sheep and goat milk fat. *Livestock Science*, 103(1–2), 74–84.

Tunick, M. H., & Van Hekken, D. L. (2014). Dairy Products and Health: Recent Insights. *Journal of Agricultural and Food Chemistry*, 63(43), 9381–9388.

Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: seven dietary factors. *The Lancet*, 338(8773), 985–992.

Zhang, R. H., Mustafa, A. F., & Zhao, X. (2006). Effects of feeding oilseeds rich in linoleic and linolenic fatty acids to lactating ewes on cheese yield and on fatty acid composition of milk and cheese. *Animal Feed Science and Technology, 127*(3–4), 220–233.