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The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Content of orotic acid and selected bioactive compounds in ovine milk during lamb rearing

W. Wojtak[1,*], M. Białek[1], A. Białek[1,2], E. Molik[3] and M. Czauderna[1]

[1] The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Instytucka 3, 05-110 Jabłonna, Poland
[2] University of Economics and Human Sciences in Warsaw, 01-043 Warsaw, Poland
[3] University of Agriculture in Krakow, Faculty of Animal Science, Department of Animal Nutrition and Biotechnology, and Fisheries, 30-059 Krakow, Poland

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* Corresponding author:
e-mail: w.wojtak@ifzz.pl

ABSTRACT. Early lactation is very demanding on the ovine maternal body and represents a critical stage for development of newborn lambs. Therefore, our study aimed to investigate the composition of sheep's milk during the initial phase of lactation, when it undergoes the greatest alterations due to adaptation to the changing nutritional needs of lambs. Twenty-one Polish Mountain sheep were assigned to three experimental groups. Milk samples were collected at day 20 (n = 7, L20), 30 (n = 7, L30), and 40 (n = 7, L40) of lactation. Parameters such as fatty acid (FA) profile, concentrations of orotic acid (OA), malondialdehyde (MDA), total cholesterol (Ch) and selected chemical forms of vitamin E were determined using gas and liquid chromatography methods. Multivariate statistical analysis was used to detect subtle changes in milk composition between short sampling intervals. FA profile analysis revealed that the L30 samples were characterised by the highest content of all examined FA. The most abundant were palmitic, stearic, oleic, linoleic, rumenic, α-linolenic, γ-linolenic, and eicosapentaenoic acids. The highest levels of Ch and MDA were recorded in the L20 samples, while OA was predominant in milk from the L30 group. No significant differences in tocopherols content were found at individual lactation days. The research allowed to conclude that milk of Polish Mountain sheep has a nutritionally beneficial composition, especially in the early lactation stage, which can support the proper growth of lambs.

Introduction

Polish Mountain sheep are well adapted to harsh climate conditions and life in mountainous areas. Sheep are economically significant farm animals with diverse uses, primarily in the meat and dairy industries (Kawęcka and Sikora, 2022). Sheep's milk is highly suitable for producing traditional foods (e.g., cheese) rich in health-promoting compounds (Molik et al., 2018). Polish regional cheeses, such as oscypek, redykołka and bryndza podhalańska are registered under the Protected Designations of Origin (PDO) approved by the European Commission and must be made exclusively from the milk of Polish Mountain sheep. At present, there is a growing trend of using sheep for alternative purposes, such as grazing in landscape parks, fallow lands, agricultural wasteland, and in nature reserves. These practices are intended to promote landscape care and ensure its diversification. Mountain sheep is one of the representatives of native sheep breeds found in Poland, and is covered by the 'Preservation

of endangered animal genetic resources in agriculture' programme implemented under the Common Agricultural Policy for 2023–2027. This initiative continues the European Union's efforts in protecting animal genetic resources, which have been ongoing since 2005. Over the course of the project, the number of protected sheep has increased more than eightfold (Kawęcka and Sikora, 2022).

Milk production is one of the most important uses of sheep in Poland, as the components of ovine milk can exert beneficial effects on the health of both young lambs and humans. Sheep's milk has a superior composition compared to the milk of other ruminants due its high content of dry matter (approx. 16–22%), fat (approx. 7–9%), and protein (approx. 5–7%) (Kawęcka and Sikora, 2022). It also has a favourable fatty acid profile: high content of omega-3 (n-3), omega-6 (n-6) polyunsaturated fatty acids (PUFAs), conjugated linoleic acid (CLA), short chain saturated fatty acids (SCSFAs), and branched chain fatty acids (BCFAs) (Sinanoglou et al., 2015). Moreover, ovine milk is characterised by a higher content of water- and fat-soluble vitamins (especially A and D), and minerals compared to cow's milk (Mohapatra et al., 2019; Flis et al., 2022). Sheep's milk fully covers human requirement for essential amino acids, has lower allergenic potential, and may be a suitable substitute for individuals allergic to cow's milk (Michlová et al., 2015; Kawęcka and Sikora, 2022).

The CLA isomers most frequently found in ruminant milk include *cis*(*c*)9 *trans*(*t*)*11*C18:2 and *t10c12*C18:2. These compounds are known for their bioactive properties, such as anti-inflammatory and anti-cancer effects. These isomers stimulate certain populations of T cells and immunoglobulins, and may increase the body's resistance to infections. Their role in preventing neurodegenerative disorders, such as Alzheimer's disease should also not be overlooked. CLA, n-3, and n-6 PUFAs inhibit the development of atherosclerosis by reducing the synthesis of cholesterol (Ch) and triglycerides, as well as by lowering blood glucose levels (Govari et al., 2020; Flis et al., 2022). Isomers of CLA may also act as antioxidants (Molik et al., 2020), similarly to vitamin E. Vitamin E is a group of several separate compounds with similar structure known as tocopherols (Ts) and tocotrienols (T3s). These compounds occur in various chemical forms, among which the most common include α-, β-, γ- and δ-Ts and T3s, with α-T being the main and most biologically active form (Czauderna et al., 2024). Antioxidants protect living organisms against free radicals (FR), reactive oxygen species (ROS), and reactive nitrogen species (RNS). These free radicals, ROS, and RNS are by-products of oxidation and reduction reactions, and cause permanent damage to the genetic material and inhibit the functions of proteins, enzymes, and lipids (Michlová et al., 2015; Tashla, 2021). Another important compound present in milk is orotic acid (OA), also known as vitamin B_{13}. This compound supports muscle build-up and the development of the microbiome in the digestive system of ruminants. Additionally, it influences the formation and maintenance of healthy intestinal flora in newborns (Löffler et al., 2015). It is known that OA can affect the regulation of gene transcription in prokaryotic and eukaryotic organisms. Potential anticancer agents containing OA in the form of metal orotates (mainly palladium, platinum and zinc) are used and widely screened in medicine (Marynowicz et al., 2023). OA exerts a preventive effect against cardiovascular diseases, increasing heart contractility and inhibiting the retention of cholesterol in blood vessels (Güler et al., 2018; Czauderna et al., 2021). Ch, a component of sheep's milk, is particularly abundant in the postpartum period and early lactation. Despite its well-known negative effect on health when consumed in excessive amounts, Ch is an important and essential component of mammalian cells (Tufarelli et al., 2023).

The composition of ovine milk, in terms of the content of all mentioned bioactive compounds, depends on many endo- and exogenous factors, including the age and breed of sheep, breeding conditions and feeding regimen. However, the primary factor regulating milk composition is the lactation phase (Molik et al., 2018; Youssef et al., 2022). Lactation in Polish Mountain sheep lasts about 150 days and is divided into two stages: the lamb rearing period and the sheep's milking period. The rearing period lasting until about day 56 of lactation. Directly after weaning the lambs, sheep are milked to obtain milk for consumption (ICAR, 2018). Milk composition and production, especially during the first stage of lactation, are important for the survival and health of growing and developing lambs. Appropriate amounts and types of nutrients and hormones are crucial to ensure proper foetal growth and development. The end of pregnancy, parturition and lactation are very demanding processes for the mother's body, leading to significant physiological and biochemical changes (Selmi et al., 2019). After lambing, the increased energy demand results in a negative energy balance, and the period of increased milk production elevates the risks of metabolic and homeostasis disorders. These factors collectively contribute to the development of oxidative stress (OS) in the sheep's body (Tashla, 2021). The most common indicator of OS is malondialdehyde

Table 1. Total composition of ovine diet[a] from preparation for mating to the end of the experiment

Item	From preparation for mating to 4th month of pregnancy			From the 4th month of pregnancy to end of the experiment	
	Forage pasture	Silage	Hay[b]	Concentrate feed[c]	Hay[a]
Portion, kg/per sheep/day	5	4	*ad libitum*	0.6[d]	*ad libitum*
Dry matter, g/kg	214	382	882	886	882
Crude protein, g/kg	49	58	185	220	185
Net energy, MJ/kg	1.24	1.95	3.24	7.5	3.24

[a] content per kg of diet: 20 g of the mineral and vitamin mixture (premix); the mineral and vitamin mixture comprised per kg: g: Ca 285, P 16, Na 56, Fe 1 (as sulphate), Cu 0.5 (as sulphate), Mn 5.8 (as sulphate), Zn 7.5 (as sulphate); mg: Co 42 (as carbonate), I 10 (as iodate), Se 6 (as sodium selenite); IU: vit. A 500,000; vit. D_3 125,000; and vit. E 25,000 (as α-tocopherol); [b] hay was harvested from a meadow at the Experimental Station of the University of Agriculture in Krakow (Poland); [c] pelleted granulate (containing: cereal grains, rape, dried legume plants, dried beet pulp, maize flour) produced by the Polish CJ company; [d] animals' daily requirements were divided into two portions: morning (300 g of pelleted granulate) and evening (300 g of pelleted granulate)

(MDA), formed as a decomposition product during the lipid oxidation. Monitoring of MDA levels is crucial for assessing the health of sheep because OS itself does not produce visible symptoms and is difficult to detect (Tashla, 2021). Fatty acids, especially SCSFAs absorbed in the rumen, have a positive effect on the development of the digestive tract in newborn lambs. Additionally, n-3 PUFAs, particularly docosahexaenoic acid (DHA), affect the formation and development of the nervous system in lambs. These PUFAs are involved in the formation of brain tissue, and signal transmission between nerve cells (Echeverría et al., 2017).

The aim of the present study was to investigate the composition of milk from Polish Mountain sheep during the initial phase of lactation, when it undergoes the greatest changes due to adaptation to the evolving nutritional needs of the lambs. Therefore, milk samples were collected immediately after lambing, when ewes were feeding and rearing their young. Multivariate statistical analysis methods, such as chemometric methods, were applied to discern subtle changes and dependencies in milk composition across various time points in the same lactation phase.

Material and methods

Animals, diet and sample collection

According to the 2nd Local Institutional Animal Care and Use Committee (IACUC) in Cracow, the research did not require the consent of the ethics committee.

Twenty-one Polish Mountain sheep were selected for the experiment based on age (3rd or 4th lactation) and body weight (45 ± 5 kg). The animals originated from the Experimental Station of the University of Agriculture in Cracow (Poland) and were housed in a sheepfold under natural light conditions (50°04′ N, 19°57′ E) during the experiment. All sheep were fed according to the feeding recommendation for ruminants (Strzetelski et al., 2014) to meet nutritional requirements appropriate to their physiological conditions. The detailed composition of the ovine diet is given in Table 1. Fresh tap water and mineral salt blocks were freely available for animals throughout the whole experimental period. Zoohygienic and weather conditions were the same for all animals, and the body condition score was 3 or 4 (Russel et al., 1969). Milk samples were collected from each ewe on day 20 (L20), 30 (L30) and 40 (L40) of lactation, with 7 milk samples collected on each lactation day. After collection, the samples were immediately frozen and stored at −30 °C until analysis.

Milk sample preparation and chromatographic analyses

Fatty acid profile determination

Fatty acids in milk samples were determined using capillary gas chromatography coupled with mass spectrometry (GC-MS). The Shimadzu GC-MS-QP2010 Plus EI model (Tokyo, Japan) used in the analysis consisted of an injection port, a BPX70, fused silica column (120 m × 0.25 mm i.d. × 0.25 μm film thickness; SHIM-POL) and a quadruple mass selective detector (Model 5973 N, Shimadzu, Tokyo, Japan). Helium was used as the carrier gas. All biological samples were prepared according to the procedure described by Czauderna et al. (2023). The method involved mild base- and acid-catalysed methylations to obtain fatty acid methyl esters (FAME), with nonadecenoic acid added to each sample as an internal standard (IS). Detailed chromatographic conditions, including column parameters, gradient temperature programme, flow rate, and other have been described previously (Czauderna et al., 2023). The identification of FAs was based on electron impact ionisation spectra of FAs and subsequently compared to authentic FAME standards (Sigma, St. Louis, MO, USA) and the NIST 2007 reference mass spectra

library (National Institute of Standard and Technology, Gaithersburg, MD, USA).

Analysis of total cholesterol, tocopherols and malondialdehyde contents

The ultra-fast liquid chromatography (UFLC) system (Shimadzu, Tokyo, Japan) was used to quantify total Ch (TCh), selected forms of tocopherols (Ts) (α-, γ-, and δ-) and α-tocopheryl acetate (αTac). The chromatographic instrument consisted of two LC-20ADXP pumps (UFLCXR), a SIL-20ACXR autosampler (LFLCXR), a CBM-20A communication bus module (UFLC), a CTO-20A column oven, a DGU-20A5 degasser, and a SPD photodiode array detector (PDA). Analysis was carried out using reversed-phase (RP) conditions, following the protocol outlined by Czauderna et al. (2009).

MDA level was also determined using the UFLC system (Shimadzu, Tokyo, Japan) coupled with a PDA. Analysis and milk samples preparation (saponification and derivatisation) were conducted following the procedure described previously (Czauderna et al., 2011).

Determination of orotic acid content

Determination of OA concentration in ovine milk samples was performed using an ultra-fast liquid chromatograph (UFLC-PDA) (Shimadzu, Tokyo, Japan). Chromatographic separations were carried out using two serially connected analytical C18 columns (Kinetex®; 1.7 µm, 100 Å, 150 mm × 2.1 mm, Phenomenex). All analysis and pre-column sample treatments were performed according to the protocol published by Czauderna et al. (2021).

Statistical analysis

All statistical analyses were performed using Statistica software, version 13.3 (Statsoft Inc., 2016). Results are presented as means ± standard deviation (SD). The normality of distribution was assessed using the Shapiro-Wilk test, and the homogeneity of variance was evaluated with the Levene test. The content of analytes in milk samples collected on individual lactation days for variables with a normal distribution, and homogeneous variance was tested using one-way ANOVA and the *post-hoc* HSD RIR Tukey test. Variables without homogeneous variance were tested with the F Welch test, and also the *post-hoc* HSD RIR Tukey test. For variables that did not follow a normal distribution, the non-parametric Kruskal-Wallis with *post-hoc* multiple comparison tests were applied. Differences were considered statistically significant at $P \leq 0.05$.

As all samples were obtained during the same lactation phase, chemometric analyses were used to detect subtle changes in milk composition and relationships between bioactive constituents. All original data were auto-scaled (standardised) before analyses. The method of grouping features and objects was used to analyse similarity and create heat maps. Cluster analysis (CA) was performed using the agglomeration method, with Euclidean distance for determining similarity and the Ward method for agglomeration. A more restrictive Sneath's criterion (33%) was used for dendrogram analysis and cluster distinguishing due to the similarity in the FA profiles of sheep's milk. For dendrogram analysis and cluster distinction based on the similarity of remaining substances (TCh, MDA, OA, α-T, γ-T, δ-T and α-TAc), a less restrictive Sneath's criterion (66%) was applied. The non-parametric Kruskal-Wallis test with a *post-hoc* multiple comparison test were used to determine differences between the revealed clusters. The significance level was set at $P \leq 0.05$.

Results

Fatty acid profile in milk samples

Milk samples were characterised by a high content and diversity of FAs. The use of the GC-MS method allowed for identification and quantification of 73 saturated (SFAs), unsaturated (UFAs) and branched chain fatty acids. In total, the FA profile of the milk samples under study consisted of 17 SFAs, 29 monounsaturated fatty acids (MUFAs), 16 PUFAs and 11 BCFAs. The total content of FAs in all samples increased in the following order: BCFAs < PUFAs < MUFAs < SFAs, regardless of the day of lactation. The results showing the content of individual FAs at days 20, 30 and 40 of lactation are presented in Table 2. In the analysed samples, the dominant SFAs were: (i) SCSFAs: caproic (C6:0), caprylic (C8:0), capric (C10:0) and lauric (C12:0) acids, and (ii) myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids among long chain saturated fatty acids (LCSFAs). All samples were characterised by a particularly high content of C16:0, which on average accounted for half of all determined SFAs. Oleic acid (*c9*C18:1) was particularly prominent among MUFAs. Other MUFAs identified included decenoic acid isomer (*c9*C10:1), tetradecenoic acid isomer (*c9*C14:1), pentadecenoic acid isomer (*c10*C15:1), palmitoleic acid isomer (*c9*C16:1), margaroleic acid isomer (*c9*C17:1), and two octadecenoic acid isomers (*t11*C18:1 and *c11*C18:1).

Table 2. Fatty acids composition (μg/ml) of milk samples on individual days of lactation

Fatty acid	L20	L30	L40	*P*-value
C6:0	153 ± 90	208 ± 120	277 ± 181	0.2214^{AN}
C7:0	8.6 ± 2.8^{A}	4.1 ± 1.5^{B}	6.5 ± 2.4^{AB}	0.0141^{K}
C8:0	145 ± 96	211 ± 138	185 ± 60	0.3568^{K}
C9:0	4.35 ± 2.02	5.6 ± 3.6	6.4 ± 2.2	0.3293^{AN}
C10:0	360 ± 282	550 ± 334	431 ± 147	0.1987^{K}
C11:0	5.5 ± 1.3	7.9 ± 3.5	5.4 ± 1.3	0.2194^{K}
C12:0	181 ± 149	321 ± 188	219 ± 87	0.1261^{K}
C13:0	8.9 ± 4.8	8.4 ± 4.1	9.0 ± 3.9	0.9672^{AN}
C14:0	484 ± 352	891 ± 418	798 ± 516	0.1065^{K}
C15:0	37 ± 15^{B}	85 ± 39^{A}	92 ± 58^{A}	0.0169^{F}
C16:0	984 ± 329^{B}	2030 ± 758^{A}	1873 ± 1068^{AB}	0.0315^{AN}
C17:0	66 ± 35^{B}	117 ± 46^{A}	76 ± 20^{AB}	0.0295^{AN}
C18:0	550 ± 282^{B}	860 ± 233^{A}	651 ± 108^{AB}	0.0450^{AN}
C20:0	7.1 ± 1.4	10.3 ± 4.3	9.2 ± 2.6	0.0893^{F}
C21:0	2.24 ± 0.44^{B}	2.55 ± 0.85^{B}	4.4 ± 1.4^{A}	0.0029^{K}
C22:0	3.7 ± 1.9	4.2 ± 1.9	5.8 ± 1.9	0.1210^{AN}
C24:0	1.40 ± 0.19^{B}	1.84 ± 0.24^{B}	3.8 ± 1.9^{A}	0.0020^{F}
ΣSFAs	3002 ± 1248	5318 ± 2133	4653 ± 2039	0.0612^{AN}
*c9*C10:1	13 ± 10	18 ± 10	16 ± 11	0.4666^{K}
*c11*C12:1	7.56 ± 3.08	6.8 ± 2.3	6.3 ± 2.1	0.4347^{K}
*c7*C14:1	4.00 ± 0.35	5.5 ± 2.3	4.8 ± 1.8	0.5429^{K}
*c9*C14:1	12.74 ± 9.09	17.0 ± 8.9	13.84 ± 9.01	0.6492^{AN}
*c7*C15:1	4.6 ± 1.8	5.4 ± 1.5	6.5 ± 1.9	0.1514^{AN}
*c10*C15:1	13.6 ± 2.4^{B}	26.0 ± 7.7^{A}	26 ± 12^{A}	0.0107^{AN}
*t7*C16:1	3.2 ± 1.3^{B}	4.28 ± 0.98^{AB}	5.9 ± 1.5^{A}	0.0033^{K}
*t9*C16:1	4.1 ± 1.4^{B}	10.0 ± 4.1^{A}	11.7 ± 3.7^{A}	0.0008^{F}
*c7*C16:1	14.1 ± 4.8^{B}	40 ± 18^{A}	33 ± 12^{A}	0.0006^{K}
*c8*C16:1	3.54 ± 1.03^{B}	9.3 ± 5.3^{A}	7.7 ± 1.1^{A}	0.0006^{K}
*c9*C16:1	46 ± 28	97 ± 47	78 ± 39	0.0511^{AN}
*c11*C16:1	1.589 ± 0.028^{B}	3.5 ± 1.4^{A}	3.6 ± 1.4^{A}	0.0006^{K}
*t7*C17:1	2.1 ± 0.0^{AB}	4.1 ± 1.8^{A}	0.00 ± 0.00^{B}	<0.0001^{K}
*t9*C17:1	0.965 ± 0.070^{B}	2.07 ± 0.77^{A}	1.98 ± 0.33^{A}	0.0007^{K}
*c6*C17:1	3.8 ± 1.7	4.3 ± 2.0	4.3 ± 1.8	0.6076^{K}
*c9*C17:1	20.5 ± 8.2	40 ± 21	29 ± 12	0.0533^{AN}
*t9*C18:1	15.8 ± 8.2^{B}	31 ± 14^{A}	25.3 ± 7.7^{AB}	0.0441^{K}
*t10*C18:1	8.3 ± 3.4^{B}	15.2 ± 7.2^{A}	13.1 ± 3.7^{AB}	0.0355^{F}
*t11*C18:1	73 ± 48^{B}	135 ± 47^{A}	129 ± 43^{AB}	0.0330^{AN}
*c6*C18:1	7.0 ± 5.1^{B}	16.7 ± 8.7^{A}	12.5 ± 3.8^{AB}	0.0234^{AN}
*c9*C18:1	1238 ± 534^{B}	2331 ± 914^{A}	1791 ± 589^{AB}	0.0253^{K}
*c10*C18:1	6.4 ± 2.7^{B}	13.2 ± 5.6^{A}	10.2 ± 2.8^{AB}	0.0100^{AN}
*c11*C18:1	24 ± 10^{B}	45 ± 18^{A}	34 ± 11^{AB}	0.0175^{AN}
*c12*C18:1	4.7 ± 1.5^{B}	9.0 ± 2.8^{A}	6.7 ± 1.9^{AB}	0.0099^{F}
*c14*C18:1	2.46 ± 0.00^{A}	1.43 ± 0.18^{B}	1.52 ± 0.25^{B}	0.0004^{K}
*c16*C18:1	10.6 ± 4.6^{B}	19.5 ± 7.7^{A}	15.6 ± 3.7^{AB}	0.0202^{AN}
*c9*C20:1	1.02 ± 0.11^{AB}	3.4 ± 1.5^{A}	0.00 ± 0.00^{B}	0.0001^{K}
*c11*C20:1	2.93 ± 0.30^{B}	4.2 ± 1.3^{AB}	5.3 ± 1.7^{A}	0.0074^{F}
*c15*C24:1	2.39 ± 0.35	2.92 ± 0.98	3.6 ± 1.2	0.0700^{K}
ΣMUFAs	1552 ± 637^{B}	2921 ± 1141^{A}	2297 ± 704^{AB}	0.0177^{K}
*t9t12*C18:2	2.78 ± 0.99	3.6 ± 1.3	3.28 ± 0.83	0.3044^{AN}
*t9c12*C18:2	14.98 ± 6.06^{B}	34 ± 22^{A}	22.9 ± 3.7^{AB}	0.0176^{K}
*t11c15*C18:2	6.1 ± 1.2^{B}	12.8 ± 7.4^{A}	8.8 ± 1.1^{A}	0.0035^{K}
*t13c16*C18:2	2.10 ± 0.28^{B}	4.4 ± 1.7^{A}	2.95 ± 0.48^{B}	0.0019^{F}
*t14c17*C18:2	9.4 ± 1.4	10.0 ± 4.4	7.7 ± 2.4	0.4882^{K}

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Table 2. continued

Fatty acid	L20	L30	L40	*P*-value
*c9c12*C18:2	81 ± 32^{B}	163 ± 80^{A}	135 ± 65^{AB}	0.0487^{AN}
*c6c9c12*C18:3	2.64 ± 0.79^{B}	4.0 ± 1.3^{A}	3.01 ± 0.73^{AB}	0.0470^{AN}
*c9c12c15*C18:3	41 ± 29	82 ± 37	59.3 ± 10.0	0.0525^{K}
*c9t11*C18:2 CLA	30 ± 17^{B}	69 ± 36^{A}	51 ± 15^{AB}	0.0276^{F}
*t9t11*C18:2 CLA	3.41 ± 0.97^{B}	3.62 ± 0.88^{AB}	4.04 ± 0.01^{A}	0.0432^{K}
*c11c14*C20:2	1.89 ± 0.29^{B}	1.84 ± 0.32^{B}	2.36 ± 0.30^{A}	0.0057^{K}
*c8c11c14*C20:3	2.13 ± 0.59^{B}	2.81 ± 0.95^{AB}	4.1 ± 1.4^{A}	0.0050^{K}
*c5c8c11c14*C20:4	6.5 ± 1.8	11.3 ± 6.6	8.9 ± 3.8	0.1383^{F}
*c5c8c11c14c17*C20:5	3.9 ± 1.2^{B}	7.4 ± 3.0^{A}	8.4 ± 2.4^{A}	0.0021^{F}
*c7c10c13c16c19*C22:5	6.4 ± 3.2	10.5 ± 7.2	8.9 ± 4.3	0.3073^{AN}
*c4c7c10c13c16c19*C22:6	3.77 ± 0.73	3.14 ± 1.00	3.8 ± 1.1	0.1709^{K}
ΣPUFAs	217 ± 84^{B}	423 ± 204^{A}	335 ± 89^{AB}	0.0277^{F}
Σn-3 PUFAs	55 ± 31^{B}	103 ± 47^{A}	80 ±11^{AB}	0.0312^{K}
Σn-6 PUFAs	83 ± 32^{B}	167 ± 80^{A}	138 ± 65^{AB}	0.0452^{AN}
iso C13:0	1.86 ± 0.31^{B}	2.19 ± 0.41^{AB}	4.43 ± 2.07^{A}	0.0140^{K}
iso C14:0	5.6 ± 1.1^{B}	10.95 ± 4.03^{AB}	12.5 ± 5.0^{A}	0.0044^{K}
iso C15:0	8.1 ± 2.4^{B}	16.1 ± 6.8^{A}	13.1 ± 4.2^{AB}	0.0151^{F}
anteiso C15:0	13.0 ± 3.5^{B}	30 ± 13^{A}	27.5 ± 6.5^{A}	0.0007^{F}
iso C16:0	2.33 ± 0.51^{B}	4.020 ± 0.105^{AB}	6.02 ± 0.13^{A}	0.0001^{K}
anteiso C16:0	28.8 ± 8.5^{B}	67 ± 28^{A}	48 ± 11^{AB}	0.0026^{F}
iso C17:0	15.8 ± 7.6^{B}	35 ± 14^{A}	25.0 ± 5.8^{AB}	0.0132^{K}
2,6,10,14-metyl C15:0	2.4 ± 1.5	3.5 ± 1.2	4.3 ± 1.5	0.0511^{AN}
3,7,11,15-metyl C16:0	1.02 ± 0.20^{B}	1.29 ± 0.14^{A}	1.51 ± 0.83^{AB}	0.0311^{K}
iso C18:0	5.3 ± 1.1	7.0 ± 1.9	6.2 ± 1.8	0.1780^{AN}
anteiso C18:0	2.65 ± 0.26^{B}	3.05 ± 0.56^{AB}	3.45 ± 0.26^{A}	0.0016^{K}
ΣBCFAs	87 ± 16^{B}	179 ± 65^{A}	152 ± 29^{A}	0.0006^{F}

SFAs – saturated fatty acids; MUFAs – monounsaturated fatty acids; PUFAs – polyunsaturated fatty acids; BCFAs – branched chain fatty acids; CLA – conjugated linoleic acid; n-3 – omega-3; n-6 – omega-6; L20 – 20th day of lactation; L30 – 30th day of lactation; L40 – 40th day of lactation; C6:0 – hexanoic acid; C7:0 – heptanoic acid; C8:0 – octanoic acid; C9:0 – nonanoic acid; C10:0 – decanoic acid; C11:0 – undecanoic acid; C12:0 – dodecanoic acid; C13:0 – tridecanoic acid; C14:0 – tertradecanoic acid; C15:0 – pentadecanoic acid; C16:0 – heksadecanoic acid; C17:0 – heptadecanoic acid; C18:0 – oktadecanoic acid; C20:0 – eicosanoic acid; C21:0 – heneicosanoic acid; C22:0 – docosanoic acid; C24:0 – tetracosanoic acid; *c9*C10:1 – *cis-9*-decenoic acid; *c11*C12:1 – *cis-11*-dodecenoic acid; *c7*C14:1 – *cis-7*-tetradecenoic acid; *c9*C14:1 – *cis-9*-tetradecenoic acid; *c7*C15:1 – *cis-7*-pentadecenoic acid; *c10*C15:1 – *cis-10*-pentadecenoic acid; *t7*C16:1 – *trans-7*-heksadecenoic acid; *t9*C16:1 – *trans-9*-heksadecenoic acid; *c7*C16:1 – *cis-7*-heksadecenoic acid; *c8*C16:1 – *cis-8*-heksadecenoic acid; *c9*C16:1 – *cis-9*-heksadecenoic acid; *c11*C16:1 – *cis-11*-heksadecenoic acid; *t7*C17:1 – *trans-7*-heptadecenoic acid; *t9*C17:1 – *trans-9*-heptadecenoic acid; *c6*C17:1 – *cis-6*-heptadecenoic acid; *c9*C17:1 – *cis-9*-heptadecenoic acid; *t9*C18:1 – *trans-9*-octadecenoic acid; *t10*C18:1 – *trans-10*-octadecenoic acid; *t11*C18:1 – *trans-11*-octadecenoic acid; *c6*C18:1 – *cis-6*-octadecenoic acid; *c9*C18:1 – *cis-9*-octadecenoic acid; *c10*C18:1 – *cis-10*-octadecenoic acid; *c11*C18:1 – *cis-11*-octadecenoic acid; *c12*C18:1 – *cis-12*-octadecenoic acid; *c14*C18:1 – *cis-14*-octadecenoic acid; *c16*C18:1 – *cis-16*-octadecenoic acid; *c9*C20:1 – *cis-9*-eicosaenoic acid; *c11*C20:1 – *cis-11*-eicosaenoic acid; *c15*C24:1 – *cis-15*-tetracosaenoic; *t9t12*C18:2 – *trans trans-9,12*-octadecadienoic acid; *t9c12*C18:2 – *trans cis-9,12*-octadecadienoic acid; *t11c15* C18:2 – *trans cis-11,15*-octadecadienoic acid; *t13c16*C18:2 – *trans cis-13,16*-octadecadienoic acid; *t14c17*C18:2 – *trans cis-14,17*-octadecadienoic acid; *c9c12*C18:2 – *cis cis-9,12*-octadecadienoic acid; *c6c9c12*C18:3 – *cis cis cis-6,9,12*-octadecatrienoic acid; *c9c12c15*C18:3 – *cis cis cis-9,12,15*-octadecatrienoic acid; *c9t11*C18:2 – *cis trans-9,11*-octadecadienoic acid; *t9t11*C18:2 – *trans trans-9,11*-octadecadienoic acid; *c11c14*C20:2 – *cis cis-11,14*-eicosadienoic acid; *c8c11c14*C20:3 – *cis cis cis-8,11,14*-eicosatrienoic acid; *c5c8c11c14*C20:4 – *cis cis cis cis-5,8,11,14*-eicosatetraenoic acid; *c5c8c11c14c17*C20:5 – *cis cis cis cis cis-5,8,11,14,17*-eicosapentaenoic acid; *c7c10c13c16c19*C22:5 – *cis cis cis cis cis-7,10,13,16,19*-docosapentaenoic acid; *c4c7c10c13c16c19*C22:6 – *cis cis cis cis cis cis-4,7,10,13,16,19*-docosaheksaenoic acid; 2,6,10,14-methyl C15:0 – 2,6,10,14-metylpentadecanoic acid; 3,7,11,15-metyl C16:0 – 3,7,11,15-metylheksadecenoic acid. All results are presented as mean values ± standard deviation (SD). ^{AN} one-way ANOVA with *post-hoc* HSD RIR Tukey test. ^{F} F Welch with *post-hoc* HSD RIR Tukey test. ^{K} Non-parametric Kruskal-Wallis with *post-hoc* multiple comparison test. ^{AB} means within a row with different superscripts are significantly different at $P \leq 0.05$

Among the polyunsaturated fatty acids (PUFAs), the following FAs can be distinguished: linoleic (*c9c12*C18:2, LA), rumenic (*c9t11*C18:2, *c9t11* CLA, RA), and α-linolenic (*c9c12c15*C18:3, ALA) acids. Additionally, two isomers of CLA were detected (*c9t11*CLA and *t9t11*CLA). The level of *c9t11*CLA was the highest on day 30 of lactation (L30) (69 ± 36 µg/ml), and the lowest on day 20 of lactation (L20) (30 ± 17 µg/ml). Meanwhile, the highest content of *t9t11*CLA was observed on day 40 of lactation (L40) (4.04 ± 0.01 µg/ml). Omega-3 (n-3) and omega-6 (n-6) PUFAs were also determined in the sheep's milk samples. The n-3 PUFAs include ALA as

a precursor of n-3 PUFAs family, and its metabolites: eicosapentaenoic acid (*c5c8c11c14c17*C20:5, EPA), docosapentaenoic acid (*c7c10c13c16c19*C22:5, DPA), and docosahexaenoic acid (*c4c7c10c13c16c19*C22:6, DHA). The n-6 PUFAs include LA as a precursor of n-6 PUFAs family, accompanied by γ-linolenic acid (*c6c9c12*C18:3, GLA), arachidonic acid (*c5c8c11c14*C20:4, AA) and *c4c7c10c13c16*C22:5 (DPA). EPA was the only n-3 PUFA for which significant differences were observed between the experimental groups. The content of EPA increased during lactation from 3.9 ± 1.2 μg/ml on day 20 (L20) to 8.4 ± 2.4 μg/ml on day 40 (L40). A consistent dependency of n-3 PUFAs < n-6 PUFAs was observed during all days of lactation (Table 2). The presence of BCFAs with both *iso*- (i) and *anteiso*- (a) isomers was confirmed in the samples. The highest share of BCFAs in all groups constitute *a*-C15:0, *a*-C16:0, and *i*-C17:0. Additionally, significant amounts of *i*-C14:0 and *i*-C15:0 were found in milk samples collected on days 30 (L30) and 40 (L40). The concentrations of *iso* forms of C13:0, C14:0 and C16:0 acids showed an increasing tendency among the experimental groups. The content of *anteiso* C18:0 slightly increased with the progression of lactation period. The remaining BCFAs, whose contents varied significantly between the groups, showed the highest values on L30. The greatest differences in values for all BCFAs were observed between L20 and L30.

TCh, MDA, OA, α-T, γ-T, δ-T and α-Tac contents in ovine milk

The results concerning the content of the tested compounds in sheep's milk are presented in Table 3. While the levels of α-, γ-, and δ-Ts did not display significant differences among the groups (L20, L30 and L40), the highest level of α-TAc was recorded in L40. Ch levels were the highest in L20, and decreased throughout lactation (318 ± 32 μg/ml in L20, 312 ± 16 μg/ml in L30 and 252 ± 51 μg/ml in L40). Minimal variations were observed in MDA levels, with no significant differences among the groups. The highest content of OA was determined at the beginning of lactation (L20 and L30), amounting to 87 ± 36 μg/ml in L20, 96 ± 67 μg/ml in L30, and 62 ± 24 μg/ml in L40.

Table 3. Contents of cholesterol, malondialdehyde, orotic acid and selected forms of vitamin E in ovine milk samples at individual days of lactation, μg/ml

Compound	L20	L30	L40	*P*-value
δ-T	1.08 ± 0.19	1.06 ± 0.19	0.87 ± 0.23	0.1250^{AN}
γ-T	0.39 ± 0.12	0.44 ± 0.32	0.313 ± 0.063	0.2700^{F}
α-T	0.88 ± 0.54	1.70 ± 1.05	1.40 ± 0.52	0.1186^{AN}
α-TAc	0.90 ± 0.33^{B}	1.58 ± 0.95^{AB}	3.1 ± 2.6^{A}	0.0402^{AN}
Ch	318 ± 32^{A}	312 ± 16^{A}	252 ± 51^{B}	0.0040^{AN}
MDA, ng/ml	7714 ± 1614	6338 ± 2120	6145 ± 2057	0.2477^{AN}
OA	87 ± 36	96 ± 67	62 ± 24	0.2441^{K}

δ-T – delta-tocopherol, γ-T – gamma-tocopherol, α-T – alpha-tocopherol, α-TAc – α-tocopheryl acetate, Ch – cholesterol, MDA – malondialdehyde, OA – orotic acid, L20 – day 20 of lactation, L30 – day 30 of lactation, L40 – day 40 of lactation. All results are presented as mean values ± standard deviation (SD), ^{AN} one-way ANOVA with *post-hoc* HSD RIR Tukey test. ^{F} F Welch with *post-hoc* HSD RIR Tukey test. ^{K} non-parametric Kruskal-Wallis with *post-hoc* multiple comparison test. ^{AB} means within a row with different superscripts are significantly different at $P \leq 0.05$

Chemometric analysis

A heat map (Figure 1) was prepared to illustrate the differences in the levels of FAs between subsequent days of lactation. The method of grouping features and objects was used to create a heat map for FAs that differed significantly between the experimental groups ($P \leq 0.05$). The heat map clearly demonstrated that the highest content of FAs was found in milk obtained from sheep at L30. Additionally, the L30 samples contained the highest amounts of CLA isomers. Milk samples from the L20 group had the lowest amounts of determined FAs, except for C7:0 and *c14*C18:1. On day 40, the main acids were: C24:0, C21:0, BCFAs (*i*-C13:0; *i*-C16:0; *a*-C18:0), *t7*C16:1, *c11*C20:1, *c11c14*C20:2, and *c8c11c14*C20:3. The concentrations of C16:0, C15:0, *i*-C14:0, *a*-C15:0, *c5c8c11c14c17*C20:5 (EPA) and certain C16:1-MUFAs (*t9*, *c7* and *c11* isomers), *t9*C17:1, and *t11*C18:1 were similar in L30 and L40. Despite the narrow time intervals in sheep's milk collection, the differences in the FAs contents were explicit.

The dendrogram (Figure 2) shows the division of individual sheep participating in the experiment into clusters based on similarities in the FA profiles of milk. Cluster analysis (CA) was employed to generate the dendrogram, utilising Sneath's criterion of 33%, resulting in the division of sheep into 4 clusters (G1–G4). In the first cluster (G1), sheep 17 and 18 from L30 were grouped together. The second cluster (G2) consisted of sheep 12, 22, 10, and 9 from L40, along with sheep 8 from L30. The third and largest cluster (G3) included sheep 21, 20, 11 from L40, 19, 6, 7, and 5 from L30, and 4 from L20. Sheep 1, 2, 3, 13, 14, 15, and 16 from L20 formed the fourth cluster (G4). It should be noted that sheep clustering using this method differed from the division into the three experimental groups.

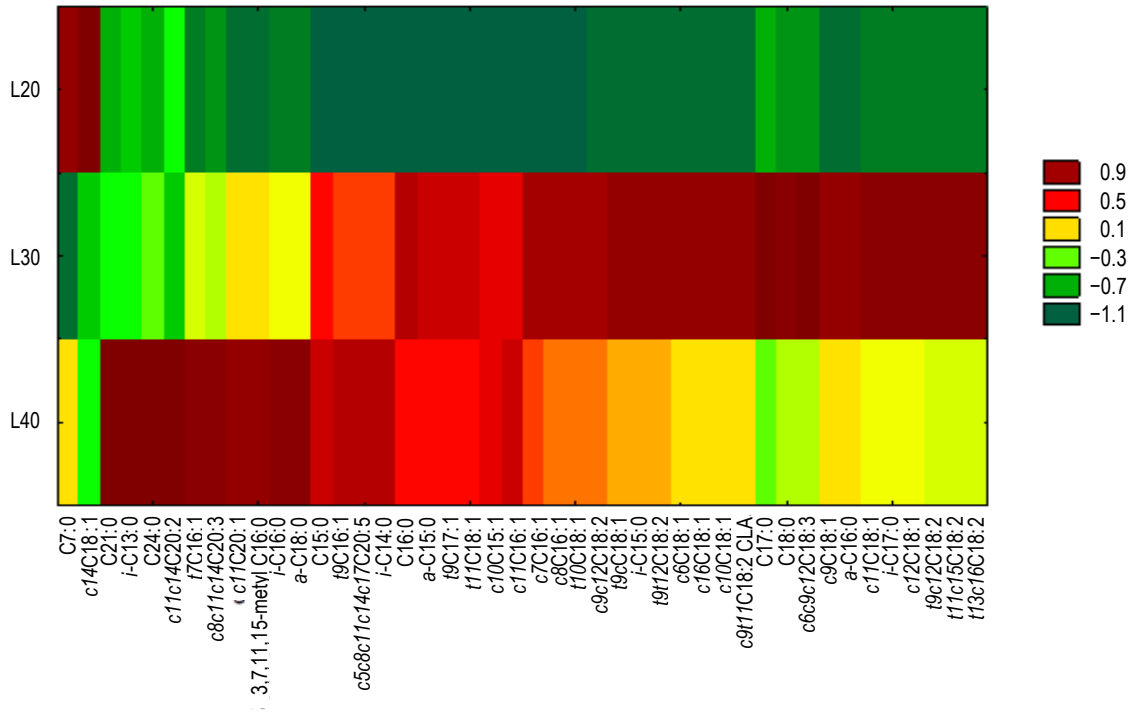

Figure 1. Heat map of the fatty acid profile in ovine milk of 3 experimental groups (L20 – day 20 of lactation; L30 – day 30 of lactation; L40 – day 40 of lactation)

i – *iso*; *a* – *anteiso*; C7:0 – heptanoic acid; C13:0 – tridecanoic acid; C14:0 – tertradecanoic acid; C15:0 – pentadecanoic acid; C16:0 – heksadecanoic acid; C17:0 – heptadecanoic acid; C18:0 – oktadecanoic acid; C21:0 – heneicosanoic acid; C24:0 – tetracosanoic acid; *c10*C15:1 – *cis-10*-pentadecenoic acid; *t7*C16:1 – *trans-7*-heksadecenoic acid; *t9*C16:1 – *trans-9*-heksadecenoic acid; *c7*C16:1 – *cis-7*-heksadecenoic acid; *c8*C16:1 – *cis-8*-heksadecenoic acid; *c11*C16:1 – *cis-11*-heksadecenoic acid; *t9*C17:1 – *trans-9*-heptadecenoic acid; *t9*C18:1 – *trans-9*-octadecenoic acid; *t10*C18:1 – *trans-10*-octadecenoic acid; *t11*C18:1 – *trans-11*-octadecenoic acid; *c6*C18:1 – *cis-6*-octadecenoic acid; *c9*C18:1 – *cis-9*-octadecenoic acid; *c10*C18:1 – *cis-10*-octadecenoic acid; *c11*C18:1 – *cis-11*-octadecenoic acid; *c12*C18:1 – *cis-12*-octadecenoic acid; *c14*C18:1 – *cis-14*-octadecenoic acid; *c16*C18:1 – *cis-16*-octadecenoic acid; *c11*C20:1 – *cis-11*-eicosaenoic acid; *t9t12*C18:2 – *trans trans-9,12*-octadecadienoic acid; *t9c12*C18:2 – *trans cis-9,12*-octadecadienoic acid; *t11c15*C18:2 – *trans cis-11,15*-octadecadienoic acid; *t13c16*C18:2 – *trans cis-13,16*-octadecadienoic acid; *c9c12*C18:2 – *cis cis-9,12*-octadecadienoic acid; *c6c9c12*C18:3 – *cis cis cis-6,9,12*-octadecatrienoic acid; *c9t11*C18:2 – *cis trans-9,11*-octadecadienoic acid; *c11c14*C20:2 – *cis cis-11,14*-eicosadienoic acid; *c8c11c14*C20:3 – *cis cis cis-8,11,14*-eicosatrienoic acid; *c5c8c11c14c17*C20:5 – *cis cis cis cis cis-5,8,11,14,17*-eicosapentaenoic acid; 3,7,11,15-metyl C16:0 – 3,7,11,15-metylheksadecenoic acid

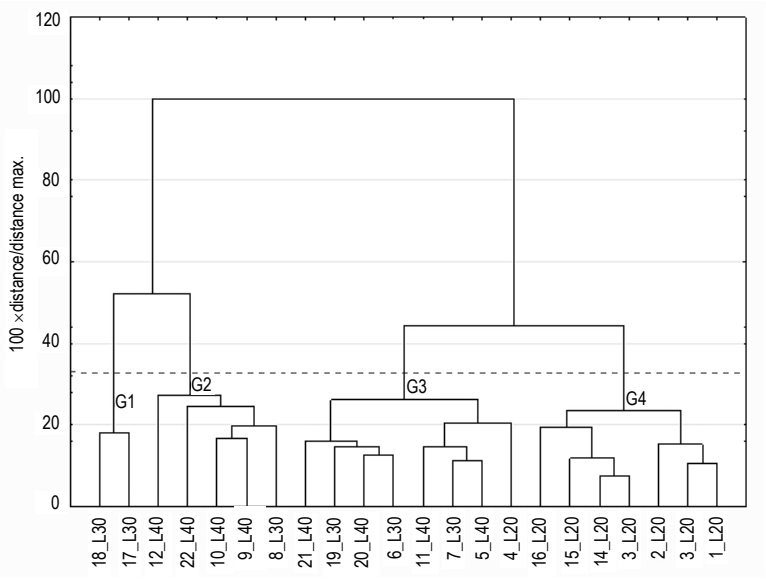

Figure 2. Dendrogram of the division of experimental animals according to the similarity of the fatty acids profiles in sheep's milk

G1 – first cluster, G2 – second cluster, G3 – third cluster, G4 – fourth cluster, L20 – day 20 of lactation, L30 – day 30 of lactation, L40 – day 40 of lactation, numbers from 1 to 22 indicate individual sheep numbers

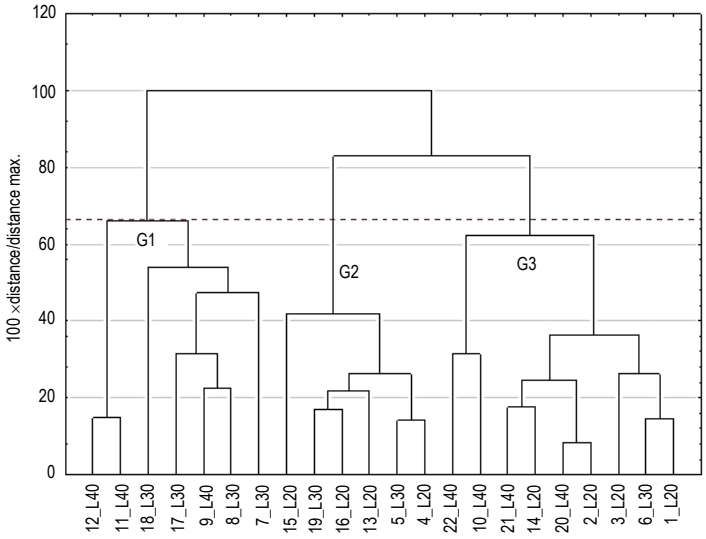

Figure 3. Dendrogram of the division of experimental animals based on the similarity in the content of orotic acid, α–tocopherol, γ-tocopherol, δ-tocopherol, α-tocopheryl acetate, cholesterol and malondialdehyde in the milk

G1 – first cluster, G2 – second cluster, G3 – third cluster, G4 – fourth cluster, L20 – day 20 of lactation, L30 – day 30 of lactation, L40 – day 40 of lactation, numbers from 1 to 22 indicate individual sheep numbers

Cluster analysis was also performed for other components determined in milk, including OA, α-T, γ-T, δ-T, α-Tac, Ch and MDA. The less restrictive Sneath's criterion (66%) was applied for this analysis. The division of individual sheep involved in the study into 3 clusters is presented on the dendrogram (Figure 3). The first cluster (G1) consisted of sheep 9, 11, 12 from L40, and 7, 8, 17, and 18 from L30. Sheep 4, 13, 15, and 16 from L20 together with 5, and 19 from L30 formed the second cluster (G2). The third cluster (G3) consisted of sheep 1, 2, and 3 from L20, 6 from L30 and 10, 14, 20, 21, and 22 from L40. It should be noted that this division differed from the one adopted in this work. The clusters formed based on the content of the aforementioned substances showed less organisation. However, some regularities could be observed in G1 and G2, where milk samples from sheep in L20 and L30 predominated in G1, and those from L30 and L40 in G2. Unfortunately, G3 cluster included samples from all lactation days.

Among compounds other than FAs, statistical analysis revealed significant differences only for the contents of α-T, δ-T, Ch and MDA. The heat map (Figure 4) demonstrated differences between the contents of α-T, δ-T, Ch and MDA in the formed clusters (G1–G3). Cluster G1 contained the highest levels of Ch, MDA and δ-T.

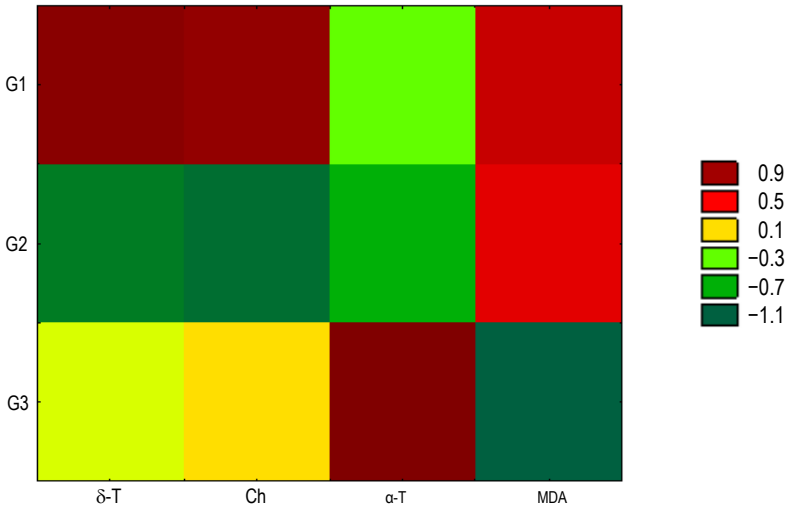

Figure 4. Heat map of α–tocopherol (α–T), δ-tocopherol (δ–T), cholesterol (Ch) and malondialdehyde (MDA) contents in ovine milk G1 – first cluster, G2 – second cluster, G3 – third cluster

Interestingly, both G1 and G2 displayed low levels of α-T, thus contributing to a higher MDA content. Conversely, the trend was reversed in G3, with higher α-T levels corresponding to lower MDA levels.

Discussion

Although the basic composition of milk (water, lactose, proteins, fat, minerals, and vitamins) is well known, it also contains components that exhibit functional properties beyond those expected from nutrient content alone. Fatty acids, in particular, are milk components well known for their bioactive properties. The presence of SCSFAs in sheep's milk contributes to its distinctive taste and smell, as well as improves fat digestibility (Miltko et al., 2016). High levels of SCSFAs increase the acidity of milk, and caproic acid (C6:0) is especially known for its cheese-like, rancid odour (Wu et al., 2023). Excessive SCSFAs content can alter the taste and texture of raw milk, making it unsuitable for consumption and processing (Wu et al., 2023). Ruminal microorganisms are responsible for the biosynthesis of SCSFAs, mainly acetates, propionates and butyrates (Takizawa et al., 2023). These FAs are easily absorbed from the digestive tract, ensuring the proper functioning of the colon epithelium and inhibiting the multiplication of pathogenic microorganisms. They are also believed to possess unique biological properties, such as preventing tumour formation and potentially acting against atherosclerosis development (Rutkowska et al., 2011). Dysbiosis, a change in the composition of microorganisms in the digestive system, may reduce the SCSFAs levels, making the body more susceptible to inflammatory factors, such as certain microorganisms and particles of bacterial origin that penetrate the intestinal wall. SCSFAs can delay cancer development by inhibiting the proliferation of cancer stem cells. Additionally, they are less toxic to the body when combating cancer cells compared to other compounds, as they do not damage the surrounding tissues (Feitelson et al., 2023). SCSFAs absorbed by the ruminal epithelium are a vital energy source for ruminants, supporting their growth and development (He et al., 2020). Butyrate plays a special role in the development of the rumen, maintaining the level of colonisation of the rumen by microorganisms, stimulating fermentation and supporting epithelial cell development (Zhen et al., 2023). At birth, lamb's digestive tract is sterile, but in the first days of life, it is colonised by a large number of bacteria, starting from the forestomach. These bacteria primarily come from the mother's mammary gland and the lamb's living environment. Anaerobic bacteria developing first (Diao et al., 2019). Sheep's milk contains SCSFAs levels that are up to twice as high as those in cow's milk. Brożek et al. (2022) reported the contents of C6:0, C8:0 and C10:0 in sheep's milk to be 2.38, 2.58, and 8.01 g/100 g total FAs (ΣFAs), respectively, while in cow's milk these levels were 1.68 g/100 g ΣFAs, 1.09 g/100 g ΣFAs, and 2.81 g/100 g ΣFAs, respectively. According to Markiewicz-Keszycka et al. (2013), goat milk contains C6:0, C8:0, and C10:0 in amounts of 2.78, 2.92, and 9.59 g/100 g ΣFAs, respectively, making it the richest source of SCSFAs. Lambs, which do not consume solid food, obtain SCSFAs only from their mother's milk. An extended period of milk feeding of young lambs ensures the proper development and function of their digestive tracts. Early weaning can inhibit the digestive capacity of the intestines and the production of ruminal epithelial cells, leading to reduced nutrient absorption. Additionally, early weaning is associated with stress for young animals (Wang et al., 2019).

The digestive tract of ruminants contains a multi-chambered stomach with the rumen being the most important element, where biohydrogenation of UFAs takes place with the participation of bacteria (Szczechowiak et al., 2014). The main substrates for this process are unsaturated FAs with 18 carbon atoms (LA, ALA and oleic acid), which are converted indirectly to stearic acid. CLA isomers are by-products of this process, arising as a result of activity of various ruminal microorganisms, especially *Butyrivibrio fibrisolvents*, *B. proteoclasticus* and *Propionibacterium acnes*. Rumenic acid, the predominant CLA isomer, constitutes approximately 90% of the total CLA produced. The transformations of ALA begin with bacterial isomerisation to rumelenic acid (*c9t11c15*C18:3, RLnA). Subsequently, biohydrogenation of double bonds results in the formation of dienoic vaccelenic FAs (*t11c15*C18:2), vaccenic acid (*t11*C18:1, VA) and *c15*C18:1, which are eventually converted to C18:0. During ALA isomerisation, RLnA isomers such as *t10c12c15*C18:3 and *c9t13c15*C18:3 may be formed (Białek et al., 2017). There was almost a twofold increase in both VA and vaccelenic acid concentrations between L20 and L30, with VA rising from 73 ± 48 to 135 ± 47 μg/ml, and vaccelenic acid from 6.1 ± 1.2 to 12.8 ± 7.4 μg/ml. The rumen is colonised by many types of protozoa, fungi and bacte-

ria that facilitate proper food metabolism, nutrient absorption, and digestive tract development (Zhang et al., 2022). Nutrients absorbed by the ruminal epithelium are derived from the transformation of proteins and plants rich in dietary fibre (Yang et al., 2023). Maintaining the correct pH of the ruminal fluid is very important for the rumen's functionality. It varies depending on the rate of food fermentation, the proper absorption of SCSFAs (Wang et al., 2019), and diet composition, especially the level of dietary non-structural carbohydrates poor in neutral detergent fibre. Natural CLA isomers present in milk are characterised by versatile effects. They stimulate the immune system by increasing the production of immune bodies, mainly lymphocytes, and enhancing phagocytic capacity (Flis et al., 2022). They also help prevent diabetes by increasing tissue sensitivity to insulin (Kowalska and Cichosz, 2013) and combat obesity by reducing the level of free FAs in the blood and inhibiting fat tissue deposition (Daniel and Florin, 2016). Additionally, CLA isomers possess antioxidant properties, protecting structural lipids against free radicals (FR) more effectively than α-T. Processes in the rumen alter the proportion of UFAs to SFAs also in products of animal origin, such as meat or milk (Szczechowiak et al., 2014). EPA and DHA are also biohydrogenated in the rumen, resulting in intermediates with 5 to 6 double bonds in their structure. The transformations of EPA and DHA are mainly based on reduction or isomerisation reaction of the *cis* double bond closest to the carboxyl group (Białek et al., 2017). Long-chain n-3 PUFAs have several important functions: (i) they are involved in signal transmission between nerve cells; (ii) they perform a structural function in the construction of neurotransmitters; (iii) and act as a source of eicosanoids (broad-spectrum lipid mediators). n-3 PUFAs play an extremely important role in the early life of lambs, contributing to the formation and development of the brain and nerve fibres. They aid in neuron maturation and the formation of dendritic spines during synaptogenesis (Drąg et al., 2014). Maintaining a balance between the levels of n-3 and n-6 PUFAs is crucial for overall health, as an excess of n-6 PUFAs can inhibit n-3 PUFAs metabolism (Bojkowski and Mojs, 2016). The fatty acid composition of sheep's milk typically comprises approximately 60% SCSFAs, 28% MUFAs, and 6% PUFAs (Molik et al., 2021). In research of Matar et al. (2023), the fatty acid profile was determined in the milk of Najdi breed ewes. Milk was collected at days 30, 60 and 90 of lactation, and the highest PUFAs levels were observed on day 30. Moreover, in the latter study, n-6 PUFAs were found to be twice as abundant as n-3 PUFAs at all stages of lactation. Similarly, research by Chen et al. (2024) revealed that the total amount of PUFAs in cow's milk was lower compared to milk obtained from goats and camels. Additionally, the levels of n-3 PUFAs in milk of all the aforementioned animals were several times lower than those of n-6 PUFAs. The results of the cited studies indicate that among ruminants, the milk of goats and camels is the richest source of PUFAs, albeit with health-promoting n-3 PUFAs being in the minority in all cases.

The FA profiles of Olkuska and Polish Mountain sheep milk in the early lactation were shown to contain notably high levels of palmitic and oleic acids. Moreover, together they accounted for more than half of all determined FAs (Molik et al., 2023), which seems to confirm observations made in this study. Similar results regarding the FA profiles in milk of other native sheep breeds (Araucana Creole and Wallachian ewes) were reported by Hrkovic-Porobija et al. (2019), Ptáček et al. (2019) and Inostroza et al. (2020). On the other hand, the method of FAs analysis applied in the present study allowed to determine a higher number of FAs. Moreover, results of BCFAs determination in cheeses produced from sheep's and goats' milk by Białek et al. (2020) were comparable to those obtained in the milk samples under study. The presence of numerous BCFAs is a characteristic feature of ruminant milk, and their content depends on the microorganisms inhabiting the rumen. BCFAs are biosynthesised from SFAs and through the deamination of certain amino acids by rumen microbiota. The composition of the animal's diet affects the microbial population, and consequently, the BCFAs profile, based on which, it is possible to determine the proportions of rumen microorganisms. The main BCFAs of protozoa origin include *i*- and *a*-C17:0, whereas those in bacteria comprise *i*- and *a*-C13:0, *i*-C14:0, and *a*-C15:0 FAs (Taormina et al., 2020).

The presence of tocopherols significantly enhances the taste, the smell and stability of ruminant meat and milk. The esterification of α-T with acetic acid results in the formation of α-TAc. This ester exhibits greater chemical stability and superior absorbability compared to synthetic homologues. Tocopherols play a crucial role in preventing lipid peroxidation not only within the body but also in animal tissues and animal products. Vitamin E homologs are also found in lipoproteins and act against the oxidation of PUFAs, proteins, cholesterol molecules, triacylglycerols and phospholipids (Białek and Czauderna, 2016). The average content

of vitamin E in milk from sheep of other breeds, including East Friesian, Romanov and Lacaune sheep, determined by Michlová et al. (2014), ranged from 2.11 to 4.26 mg/kg. The average total content of α-, γ- and δ-Ts determined in the present study ranged from 2.35 to 3.20 μg/ml, showing consistency with literature data. Moreover, findings from the study cited above have indicated that the lowest amounts of vitamin E were observed at the beginning of lactation. Graulet (2014) suggested that ovine milk is richer in vitamins compared to other ruminants' milk. This author has also claimed that the main factor affecting vitamin E concentration is diet composition and milk fat composition, with the stage of lactation is not affecting much on vitamin E content. The current results seem to confirm this observation, as no significant differences were observed in tocopherols content between lactation days.

In the work of Pietrzak-Fiećko and Kamelska-Sadowska (2020), the highest level of Ch among ruminants was observed in cow's (approx. 20.58 mg/dl) and ovine' milk (approx. 17.07 mg/dl). The results obtained in the present study indicated higher values (Table 3). The higher Ch levels in mother's milk can affect Ch metabolism in lambs' body and potentially serve as a determinant of their future metabolism and health. Ch is also known to be a precursor for steroid hormones (such as progesterone, testosterone, estrogen, cortisol and aldosterone), as well as being involved in the production of certain vitamins and bile acids. Ch is also part of cell membrane structure and regulates its fluidity and permeability (Zampelas and Magriplis, 2019).

The subtle changes in MDA levels may result from the high total Ts and CLA content during the following days of lactation. Inhibition of lipid oxidation by antioxidants results in reduced MDA formation. Similar changes in MDA levels have been observed primarily in dairy cows. MDA levels reported by Castillo et al. (2006) at 2, 4 and 6 weeks of lactation were very similar to ours and did not differ significantly at successive time points. Research by Yehia et al. (2021) showed that MDA levels in the early phase of lactation was higher compared to the middle phase. Cows, just like sheep, experience significant metabolic changes in early lactation. The increased energy demand during this period is stressful for animals, making them more susceptible to metabolic disorders and diseases (Tufarelli et al., 2023).

In the present study, high levels of OA were detected across the experimental groups. This is expected, as young lambs need more OA in the early days after birth for proper growth and development. OA plays a crucial role in the biosynthetic pathway of pyrimidine derivatives of nucleobases, such as thymidine, cytidine and uridine, which indirectly contributes to the biosynthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Czauderna et al., 2021). Increase in OA levels from day 0 to 15 of lactation has been reported in the literature for two Jersey cows, with levels rising from 4.7 to 36.6 μg/g in one cow and from 7.5 to 46.0 μg/g in the other (Indyk and Woollard, 2004). Güler et al. (2018) determined OA concentrations in the milk of two goat breeds, and showed that its levels increased slightly in the experimental group at the beginning of lactation, followed by significant decreases in subsequent measurements. The presence of OA is a characteristic feature of ruminant milk, while in other animals, it is either absent or present only in trace amounts (Indyk and Woollard, 2004). OA is particularly important in lamb growth, as it helps to gain body weight by increasing muscle mass and connective tissue. Additionally, it accelerates the regeneration of the epithelium and protects organs such as heart, liver and kidneys from degeneration (Milewski, 2006). Positive effects of administering OA to sick dogs and rats have been reported, including increased cardiac tolerance to global ischemia after a recent myocardial infarction (Rosenfeldt et al., 1998), which confirms the role of OA in the control of cardiovascular disorders.

Ambiguous CA results may be due to factors beyond the lactation phase that influenced the fatty acid profile and content of the examined components. The differences may also result from the individual characteristics of each sheep. Although all ewes were fed the same food ration, some may have consumed different amounts of the food provided. Similarly, although sheep usually give birth to one lamb, twin births can also occur. In such cases, the nutrient content in milk must be higher to ensure proper development of both lambs (Kawęcka and Sikora, 2022). We presume that the abovementioned factors might explain the differences in the allocation of animals to groups obtained in CA.

The obtained results also confirmed the antioxidant capacity of α-T. Despite the high level of δ T, no decrease in MDA levels was observed, clearly indicating that α-T has stronger antioxidant properties than δ-T. The intensity of OS can be indirectly measured using MDA levels, where high amounts of MDA indicate increased levels of OS. In the initial stage of lactation, the animal begins to consume less food, which is associated with metabolic disorders and consequently, a negative energy balance and an increase in basic oxygen consumption

(Yehia et al., 2020). The growing foetus competes with the mother's body for the availability of nutrients. Most of the energy obtained from the metabolism of fatty acids, glucose and free amino acids is transferred to the foetus. Restrictions in metabolite availability may adversely affect birth weight and increase perinatal mortality (Pesántez-Pacheco et al., 2019a; Pesántez-Pacheco et al., 2019b). Additionally, FR, RNS and ROS begin to accumulate in the sheep's body due to the inhibition of the antioxidants ability to remove them. Reactive oxygen species are produced by both the foetus and the mother, and support the replication, differentiation and maturation of foetal cells. Non-esterified FAs synthesised in the liver also contribute to the production of ROS. This make maintaining homeostasis difficult and results in the oxidation of important molecules, leading to a state of OS (Yehia et al., 2021).

Conclusions

The current study shows that milk of Polish Mountain sheep, particularly in the early lactation stage, is characterised by a high content of compounds crucial for the proper growth and development of lambs. The ovine milk under study had a very rich composition, as it contained nutritionally beneficial profile of fatty acids, high content of antioxidants (tocopherols and conjugated linoleic acid), as well as orotic acid. All these bioactive compounds may also be beneficial for human health, suggesting that sheep milk could serve as a viable alternative to cow's milk. Considering the multipurpose utility of Polish Mountain sheep, not only for their milk but also, e.g., very tasty meat, wool and skin, it is justified to remain this breed of sheep in the current European Union programme for the protection of genetic resources in agriculture.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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