



Changes in fatty acid profiles of transition milk of Czech Fleckvieh cows milked in automatic milking system

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ABSTRACT

Transition milk is an important part of a newborn calf's nutrition, and its positive effects on the calf's health could be attributed, among others, to its fatty acid (FA) profile. Transition milk can be collected via automated milking systems (AMS). This study aimed to evaluate changes in FA profile of transition milk in dependence on parity, the number of milkings, and the between-milking interval when using AMS for its collection. The on-farm observation was conducted on 18 Czech Fleckvieh cows in a confined system that were in their first (7 cows), second (5 cows), or third or higher parity (6 cows). Starting from the second milking, cows were milked in AMS. Milk was sampled at each milking within 4 days postpartum and analysed for its FA profile using two-dimensional gas chromatography with a vacuum ultraviolet detector. The FA profile changed gradually with the increasing number of milkings postpartum. The relative content of short-chain and long-chain FA increased ($P < 0.05$) at the expense of medium-chain FA, and the content of monounsaturated FA increased at the expense of saturated FA ($P < 0.001$). Parity affected mainly the content of butyric, myristic, stearic, and oleic acids ($P < 0.05$). The interval between milkings did not have a pronounced effect on the FA profile, as only slight changes in the concentration of lauric and linoleic acids were observed ($P < 0.05$). When using AMS, cows' parity and milking number seem to be the main factors influencing the FA profile of transition milk.

Introduction

Bovine colostrum is the first secretion of the cow's mammary gland after the parturition and represents a unique "cocktail" of all components needed for the growth and development of a newborn calf. In addition to high content of macro- and micronutrients and immunoglobulins, colostrum is rich in various bioactive compounds, such as immune factors, antimicrobial peptides, whole immune cells, endocrine factors, signalling molecules, and microorganisms (for details see [Silva et al., 2024](#)). The secretion produced thereafter, transition milk, goes through gradual changes in its physical and chemical properties until mature milk is produced. Although the concentrations of nutrients, immunoglobulins, and bioactive compounds in transition milk gradually decrease compared to colostrum ([Fischer-Tlustos et al., 2020](#)), it is still a very important part of a newborn dairy calf's nutrition. Compared to whole milk or milk replacer, transition milk still has a higher protein

content, mainly due to the increased concentrations of IgG and casein, an increased fat content, and higher concentrations of vitamins A, E, B12, and selenium ([Godden et al., 2019](#)).

Even short-time (2 to 4 days postpartum) administration of transition milk as a total or partial replacement of milk replacer or whole milk is connected with increased body weight gain, reduced likelihood of poorer eye/ear, nose, or faecal scores over the whole rearing period, and improved intestinal development in the first few days of life ([Van Soest et al., 2022](#)). These positive effects can be attributed, at least in part, to specific fatty acids (FA), particularly the essential ones, such as linoleic (LA) and α -linolenic acid (ALA) ([Garcia et al., 2015](#)). In addition, butyric acid and medium- and long-chain FA play a role, as they influence gastrointestinal and immune functions ([Górka et al., 2018](#)).

As summarized by [Silva et al. \(2024\)](#), colostrum seems to have a lower content of saturated FA (SFA), branched chain FA, mono-unsaturated FA (MUFA), and conjugated linoleic acid (CLA) but a higher

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content of polyunsaturated FA (PUFA), n3, n6, and cholesterol compared to mature milk. During the production of transition milk, this profile gradually changes to a profile close to that found in mature milk. However, the period of transition milk production is not clearly defined and differs between studies. While some studies estimate transition milk production based on the number of milkings, others base it on the number of days postpartum. In the first case, transition milk is produced from the second up to the sixth milking (Van Soest et al., 2020; Wilms et al., 2022) while in the second case, transition milk can be produced within the first two days of lactation (Quinn et al., 2020), or up to 5 days postpartum (Commission Regulation No 1662/2006, 2006). The discrepancy in the characterization of transition milk production can be misleading when using automated milking systems (AMS), where cows determine their own milking frequency, resulting in a great variability in the number of milkings between cows within the legislatively established length of transition milk production. This means that when using AMS with an average milking frequency of 1.9 to 3.2 milkings per day (Jacobs and Siegford, 2012), the number of milkings within 5 days postpartum can be up to 16, which, when milking regularly twice a day, would be achieved on the 8th day after calving, when the milk is already considered mature (Godden et al., 2019; Lashkari et al., 2025). Although the effect of AMS on mature milk yield and composition has already been described (e.g., De Marchi et al., 2017), information on the effect of AMS on FA profile of transition milk is still lacking.

The aim of the study was to evaluate the changes in FA profiles in transition milk of Czech Fleckvieh cows, as a representative of a dual-purpose breed, in dependence on parity, number of milkings, and between-milking interval when using an automated milking system.

Material and methods

Animals and feeding

The on-farm observation was carried out on 18 Czech Fleckvieh cows in a private dairy farm (L. Klíčová, Božice, Czech Republic). The cows involved in the experiment calved within 3 months (February – April 2022) and were on their first (P1, 7 cows), second (P2, 5 cows), or third and higher parity (P3+, 6 cows). Cows were group-housed in a free-stall pen together with cows not involved in the experiment. Cows had free access to water, the feed bunk, and the laying space (mattress stalls bedded with wood shavings). After the delivery, the cows were milked individually in the calving pen to obtain colostrum for the first calf feeding. Since then, the cows have been milked in the automatic milking system (AMS; Lely Astronaut A3, Lely Industries NV, Maassluis, The Netherlands), which was freely accessible to them and visited voluntarily. The cows were milked in AMS since the 2nd milking (M2), with the AMS software limiting the maximum number of milkings (M) per day to 4. The between-milking interval was monitored and classified as Short (<12 h) or Long (>12 h).

The cows were fed once daily a total mixed ration consisting of maize silage (15 kg), lucerne haylage (3 kg), brewers spent grain silage (3 kg), lucerne hay (0.5 kg), rolled oats (0.65 kg), a mineral mixture (0.2 kg), and a commercial supplemental feed (2.5 kg, all as fed) that was provided individually via feeding box (Lely Cosmix, Lely Industries NV, Maassluis, The Netherlands) in close-up cows or via AMS in lactating cows for the initial milk production of 20 kg of milk. To stimulate milk production, the dose of the supplemental mixture was gradually increased from the 3rd to the 40th day postpartum to up to 8.5 kg. The nutrient contents and FA profiles of the diets were described in our previous study (Farková et al., 2024) and are given in Table 1.

Sampling and sample analysis

Colostrum samples were collected from the second milking until the milk was considered suitable for sale, covering the first four days of lactation. Following collection, the samples were stored at –20°C until

Table 1

Content of nutrients (g/kg, on dry matter basis unless otherwise stated) and fatty acid profile (relative abundance, %) of diets fed to cows during dry period and early lactation.

Item	TMR dry cows	TMR lactating cows	Pellets
Dry matter (g/kg)	497.6	389.5	919.8
Crude protein	112.4	119.7	219.1
Crude fat	25.1	32.5	54.1
Crude fibre	296.2	208.5	71.6
Neutral-detergent fibre	533.3	472.1	272.3
Crude ash	81.5	74.6	88.4
Fatty acids ¹			
C4	9.40	9.18	2.04
C14	1.32	1.50	0.32
C16	21.61	21.89	13.42
C16:1	1.73	1.88	0.88
C18	2.95	3.19	1.82
C18:1n9c	11.16	12.04	35.25
C18:2n6c	29.28	32.12	37.49
C18:3n3	13.88	12.79	4.56
C20	1.15	1.20	0.62
C20:1n9	1.26	0.83	0.88
C20:5n3	0.95	0.25	0.52
C22	1.21	1.02	0.39
C22:6n3	1.20	0.50	0.00
C24	1.19	1.15	0.38

Abbreviations: TMR – total mixed ration

¹ for names see Nonstandard abbreviations

analysis.

Feed samples were sampled prior to the experiment, and the content of basic nutrients was analysed as follows. The dry matter (DM) was determined after drying at 105°C for 24 h. The crude protein (CP) was determined by Kjeldahl method using Buchi analyser (Centec Automation, Czech Republic) according to (ČSN EN ISO 5983-1, 2012; ČSN EN ISO 5983-2, 2012). The crude fat was determined by Soxhlet method (ČSN 46 7092-7, 1998). The crude fibre and neutral detergent fibre were determined using ANKOM 220 fibre analyser (O.K. Servis BioPro, Czech Republic) according to ČSN EN ISO 16472 (2012) and ČSN EN ISO 6865 (2001). The crude ash was determined after burning at 550°C under prescribed conditions.

The FA profile of feed and transition milk samples was analysed as described previously by Farková et al. (2024) based on Fina et al. (2022). The fatty acids were identified using fatty acid methyl ester (FAME) reference standards (Supelco C37 FAME MIX) from MilliporeSigma (Darmstadt, Germany).

Briefly, the samples were extracted and derivatized by microwave-assisted extraction into cyclohexane with HCl in MeOH and analysed by Shimadzu GCMS-TQ8050 NX system (Shimadzu, Duisburg, Germany) with GC2030 fitted with a vacuum-ultraviolet detector (VUV), and a VGA-101 Analyzer (VUV Analyzer, Texas, USA). The system was equipped with an INSIGHT reversed fill/flush flow modulator with a bleeding line flow controller (SepSolve, Peterborough, UK). The GC×GC analysis utilized two SepSolve columns: 1D-FAMES polar fused silica capillary column (20 m × 0.18 mm i.d. × 0.1 µm film thickness) with a flow rate of 0.4 mL/min and 2D-FAMES non-polar fused silica capillary column (5 m × 0.25 mm i.d. × 0.1 µm film thickness) with a flow rate of 20 mL/min. The equivalent bleeding line flow was maintained at 0.45 mL/min. Helium was employed as the carrier gas. The modulation period was 3 s, with a reinjection time of 100 ms. The oven temperature program began with an initial hold at 35°C for 3 minutes, followed by a temperature ramp to 260°C at a rate of 9°C/min, with a final hold of 2 minutes at 260°C. Sample injection was conducted in split mode with a split ratio of 1:10. A 1 µL sample was injected at an inlet temperature of 250°C, utilizing pulsed mode at 60 psi for 1 min to accommodate the overpressure. The VUV wavelength detection range was from 125 nm to 430 nm, with a detection frequency of 25 Hz. We used VUVision 3.4.0 software (VUV analytics, USA) for the data acquisition and ChromSpace

2.1.4 software (SepSolve) for processing, utilizing the 125–160 nm wavelength range.

Calculation of indexes

The stearyl-CoA desaturase indexes (DI) for following SFA and their MUFA counterparts were calculated according to Schennink et al. (2008).

$$DI14 = [C14 : 1cis - 9 / (C14 : 0 + C14 : 1cis - 9)] \times 100$$

$$DI16 = [C16 : 1cis - 9 / (C16 : 0 + C16 : 1cis - 9)] \times 100$$

$$DI18 = [C18 : 1cis - 9 / (C18 : 0 + C18 : 1cis - 9)] \times 100$$

where C14 – myristic acid; C14:1 cis-9 – myristoleic acid; C16 – palmitic acid; C16:1 cis-9 – palmitoleic acid; C18 – stearic acid; C18:1 cis-9 – oleic acid

Statistical analysis

First, normality was tested by a normal probability plot, and homogeneity of variances was tested by Levene's test. The effect of parity on the milk yield and FA profile was assessed using ANOVA with Tukey post-hoc test. The effect of the number of milkings on the milk yield and FA profile was assessed using a general linear model. The effect of the between-milking interval on the milk yield and FA profile was assessed as the difference between milk samples obtained after an interval lower than 12 hours and those obtained after an interval higher than 12 hours by Student's t-test. The interactions of these factors were assessed by factorial ANOVA.

As many of the indexes did not meet the normality assumption, the effect of parity on indexes was assessed using the Kruskal-Wallis test. Thus, although not all derived indexes deviated from normality, non-parametric tests were applied to all indexes to ensure methodological consistency. The effect of the number of milkings on indexes was assessed using a generalized model. The effect of the between-milking interval on indexes was assessed as the difference between milk samples obtained after an interval lower than 12 hours and those obtained after an interval higher than 12 hours by Mann-Whitney test.

Samples from the first milking were excluded. Outliers were tested for FAs by Grubbs test, but they were not excluded from the analyses. The number of samples was $n = 55$ for parity value 1, $n = 37$ for parity value 2, and $n = 38$ for parity value 3 and higher. For milking numbers 2–10, the number of samples was $n = 18, 12, 16, 13, 14, 14, 16, 10$, and 17, respectively. For short between-milking interval, the number of samples was $n = 82$ and for long interval, the number of samples was $n = 47$. The threshold for statistical significance was set at $P = 0.05$. The analyses were done in the data analysis software system STATISTICA (StatSoft, Inc.), version 14.

Results

The average number of milkings per day during days 1 to 4 postpartum was 2.21 ± 0.09 in P1 cows, 2.50 ± 0.15 in P2 cows, and 2.17 ± 0.10 in P3+ cows (for details, see Supplementary Table S1).

Effect of parity

Generally, the P1 cows had a lower milk yield per milking (8 ± 0.4 L) than the other cows (11 ± 0.7 L, Supplementary Table S2). Furthermore, milk from the P1 cows had a slightly different FA profile, characterized by a higher percentage of butyric (7% and 3% for P1 and P2, respectively, Table 2) and oleic acid (17% and 14% for P1 and other parities, respectively, Table 3), and a lower percentage of myristic (10% and 12% for P1 and other parities, respectively) and stearic acid (7% and 8% for

P1 and P3+, respectively) and a higher content of short-chain fatty acids (SCFA, 21% and 18% for P1 and P2, respectively) and a lower content of medium-chain fatty acids (MCFA, 50% and 55% for P1 and P2 cows, respectively), a lower content of SFA (73% and 76% for P1 and other parities, respectively), and a higher content of MUFA (22% and 19% for P1 and other parities, respectively, Table 4). Consequently, DI of C14 was higher during the first lactation (11% and 8% for P1 and P3+, respectively) and DI of C18 decreased gradually from 70% (P1) through 66% (P2) to 63% (P3+, Table 4).

Effect of milking number

The milk yield per milking gradually increased as the number of milkings increased (Supplementary Table S1). Similarly, the FA profile changed with an increasing number of milkings. While the relative contents of caproic, margaric, stearic, and oleic acids increased, the relative contents of palmitic, myristic, and myristoleic acids decreased (Fig. 1, Tables 2 and 3).

Overall, the relative content of SCFA and long-chain FA (LCFA) increased, while the relative content of MCFA decreased (Fig. 1 C) and the relative content of SFA decreased, while the relative content of MUFA increased. The relative content of preformed FA increased at the expense of mixed FA, while the relative content of de novo FA remained unchanged (Fig. 1 D). Furthermore, the DI18 decreased slightly with an increasing number of milkings (Fig. 1 D).

Effect of milking interval

The average number of daily milkings per cow during the first four days postpartum was 2.33, with the average milking interval of 10.7 ± 3.4 h. Due to the increased milking frequency per day, about 55% of cows were milked more than 8 times during the four days (Supplementary Figure S1). The milking interval reflected a high degree of individual variability in this parameter among cows and across lactations, ranging from 1.7 to 19.7 h, with the majority of cows (~ 60%) being milked in the interval 6 to 12 h (Supplementary Figure S2 and S3). The milk yield per milking was significantly lower (9 L on average) when the interval between milkings was Short (8.67 hours on average) and higher (13 L on average) when the interval was Long (14.33 hours on average, $P < 0.001$, Supplementary Table S2). On the other hand, there were only subtle changes in the FA profile depending on the interval between milkings. The relative content of SCFA decreased slightly (from 21% to 19% in Short and Long interval, respectively), while the relative content of MCFA (namely lauric acid) increased slightly (from 51% to 54% in Short and Long interval, respectively) when the interval was longer (Tables 2 and 3, Fig. 2 and 3). Furthermore, there was a slight decrease in the relative content of trans-FA connected with the longer interval between the milkings (Table 4).

Discussion

Recent studies, e.g., Ostendorf et al. (2024) or Van Soest et al. (2022) and authorities, such as the National Academies of Science, Engineering, and Medicine (NASEM, 2021) recommend the administration of transition milk to neonatal calves in addition to colostrum feeding due to its positive effects on early growth and development. When studying these effects, attention is primarily focused on bioactive components, such as IgG, and less attention is paid to fat, the composition of which can also be affected by the management of dairy cows in the postpartum period.

Effect of parity

Generally, the effect of parity on the FA profile of transition milk in Czech Fleckvieh cows was not as strong as described in Holstein or Holstein-Friesian cows, where parity substantially affected a broader spectrum of FA (Contarini et al., 2014; O'Callaghan et al., 2020; Wilms

Table 2

Content of saturated fatty acids (SFA, % from total FA) in transition milk collected by automatic milking system (AMS) during first four days postpartum in dependence on cow parity, number of milkings, and between-milking interval.

		Saturated fatty acids (% expressed as mean and SEM) ¹																			
Item	n	C4		C6		C8		C10		C12		C14		C15		C16		C17		C18	
Parity (P)																					
P1	55	7.4	0.8	5.8	0.2	3.6	0.2	4.6	0.2	4.7	0.2	10.4	0.4	2.0	0.1	26.1	1.2	1.3	0.1	7.1	0.3
P2	37	3.5	0.7	6.2	0.3	3.6	0.2	4.7	0.2	5.0	0.2	12.3	0.4	2.0	0.1	29.9	0.9	1.1	0.1	7.5	0.4
P3+	38	6.2	0.7	5.8	0.4	3.8	0.2	5.4	0.5	5.0	0.2	12.5	0.4	1.9	0.2	26.8	1.3	1.0	0.1	8.1	0.3
Milking (M)																					
M2	18	5.4	1.3	3.9	0.3	2.9	0.2	3.8	0.2	4.9	0.2	14.4	0.7	2.1	0.1	32.6	2.2	0.9	0.1	5.5	0.5
M3	12	5.3	1.5	5.3	0.4	3.5	0.2	4.2	0.2	4.8	0.3	12.4	0.7	2.3	0.2	29.9	0.9	1.1	0.1	6.5	0.4
M4	16	5.3	1.2	6.1	0.3	3.7	0.3	5.0	0.2	5.2	0.2	12.4	0.5	2.2	0.2	29.8	0.9	0.9	0.1	6.4	0.2
M5	13	5.1	1.1	6.7	0.7	3.9	0.4	6.8	1.3	5.1	0.3	11.6	0.6	1.7	0.3	26.2	2.0	1.2	0.2	7.2	0.4
M6	14	4.4	1.2	5.7	0.6	4.4	0.4	4.9	0.5	4.9	0.4	10.5	0.8	2.6	0.4	26.9	4.3	1.3	0.1	7.5	0.6
M7	14	6.6	1.5	6.9	0.5	3.5	0.4	5.2	0.4	4.9	0.3	11.0	0.6	1.6	0.2	26.4	0.8	1.3	0.3	8.2	0.4
M8	16	8.3	1.3	6.3	0.4	4.0	0.4	4.5	0.3	4.9	0.4	10.1	0.6	1.7	0.2	23.6	1.5	1.3	0.1	8.6	0.3
M9	10	7.9	1.7	6.2	0.5	3.3	0.4	4.9	0.4	4.3	0.3	10.0	0.6	1.8	0.1	24.8	0.8	1.3	0.1	9.1	0.5
M10+	17	5.8	1.6	6.6	0.3	3.8	0.3	4.8	0.2	4.9	0.2	10.9	0.7	1.9	0.2	25.2	1.0	1.3	0.1	9.0	0.5
Milking interval (I)																					
Short	82	6.6	0.7	6.0	0.4	3.7	0.2	4.9	0.5	4.7	0.2	11.5	0.4	2.0	0.2	26.8	1.3	1.2	0.1	7.7	0.3
Long	47	4.8	0.7	5.8	0.3	3.6	0.2	4.8	0.2	5.2	0.2	11.8	0.4	1.9	0.1	28.6	0.9	1.1	0.1	7.2	0.3
P-value																					
P		0.001		0.599		0.637		0.079		0.192		<0.001		0.843		0.066		0.018		0.045	
M		0.152		<0.001		0.102		0.359		0.504		<0.001		0.111		<0.001		0.015		<0.001	
I		0.066		0.475		0.894		0.94		0.036		0.468		0.484		0.212		0.157		0.166	
P × M		0.132		0.834		0.594		0.922		0.936		0.975		0.847		0.21		0.303		0.262	
P × I		0.175		0.534		0.513		0.279		0.889		0.385		0.713		0.043		0.062		0.671	
M × I		0.408		0.814		0.745		0.905		0.869		0.408		0.478		0.351		0.192		0.465	

Milking interval – Short (< 12 h), Long (> 12 h); Abbreviations: n = number of samples; SEM = standard error of the mean

¹ for names see Nonstandard abbreviations

Table 3

Content of unsaturated fatty acids (UFA, % from total FA) in transition milk collected by automatic milking system (AMS) during first four days postpartum in dependence on cow parity, number of milkings, and between-milking interval.

		Unsaturated fatty acids (% expressed as mean and SEM) ¹													
	<i>n</i>	C14:1	C16:1		C17:1		C18:1n9c		C18:2n6t		C18:2n6c		C18:3n3		
Parity (P)															
P1	55	1.3	0.1	3.1	0.1	0.6	0.0	16.7	0.6	0.5	0.1	2.8	0.1	0.9	0.1
P2	37	1.4	0.2	3.1	0.1	0.5	0.0	14.5	0.5	0.5	0.1	2.7	0.1	0.8	0.1
P3+	38	1.2	0.2	2.7	0.1	0.4	0.1	14.1	0.6	0.3	0.1	3.0	0.2	0.7	0.1
Milking (M)															
M2	18	2.0	0.2	3.2	0.2	0.4	0.1	12.9	1.1	0.3	0.1	3.0	0.2	0.6	0.1
M3	12	1.3	0.2	3.2	0.2	0.6	0.1	14.6	0.9	0.5	0.2	2.4	0.3	0.8	0.1
M4	16	1.4	0.2	2.9	0.2	0.5	0.1	13.8	0.7	0.4	0.2	2.4	0.3	0.8	0.1
M5	13	1.4	0.2	2.9	0.1	0.6	0.1	14.5	1.4	0.6	0.2	2.7	0.2	0.8	0.2
M6	14	1.4	0.2	3.0	0.2	0.7	0.1	16.4	1.3	0.3	0.1	3.3	0.2	1.0	0.1
M7	14	1.2	0.2	3.0	0.2	0.4	0.1	15.8	0.8	0.3	0.1	2.8	0.3	0.6	0.1
M8	16	1.2	0.2	2.8	0.2	0.5	0.1	17.0	0.7	0.4	0.1	2.9	0.2	1.1	0.1
M9	10	0.6	0.2	2.9	0.2	0.6	0.2	17.1	0.7	0.6	0.2	3.3	0.3	0.9	0.2
M10+	17	1.0	0.2	2.9	0.2	0.5	0.1	16.5	0.8	0.4	0.1	3.0	0.2	0.8	0.2
Milking interval (I)															
Short	82	1.2	0.1	2.9	0.1	0.5	0.0	15.2	0.4	0.5	0.1	2.8	0.1	0.8	0.1
Long	47	1.5	0.1	3.1	0.1	0.5	0.1	15.3	0.6	0.3	0.1	2.9	0.1	0.8	0.1
<i>P</i> -value															
P		0.488		0.06		0.001		0.002		0.175		0.363		0.192	
M		<0.001		0.166		0.953		<0.001		0.648		0.085		0.162	
I		0.082		0.161		0.777		0.889		0.018		0.767		0.799	
P × M		0.636		0.546		0.968		0.397		0.597		0.186		0.137	
P × I		0.201		0.596		0.525		0.213		0.913		0.860		0.411	
M × I		0.578		0.004		0.081		0.248		0.685		0.527		0.584	

Milking interval – Short (< 12 h), Long (> 12 h);

Abbreviations: *n* = number of samples; SEM = standard error of the mean

¹ for names see Nonstandard abbreviations

et al., 2022) in comparison to our results. There were also differences in the effect of parity on individual FA. Different trends were noted, for example, for butyric acid (a decrease by 52% and 16% in P2 and P3+, respectively), myristic acid (an increase by approximately 20% in both P2 and P3+), or oleic acid (a decrease by approximately 15% in both P2 and P3+), which were affected by parity in our study but not in the above-mentioned studies. On the other hand, the findings were the opposite for palmitic acid: there was no parity effect in our study, whereas the above-mentioned studies reported parity-dependent changes. This can be explained by the differences in de novo synthesis of FA between primiparous and multiparous cows and in the factors that influence it. In our study, the sum of de novo FA and mixed FA in P1 cows was lower compared to P3+ cows, while the sum of preformed FA was not affected by parity. This is in line with the theory of Miller et al. (2006), which states that primiparous cows at the onset of lactation exhibit lower mammary gland metabolic activity than multiparous cows at the same stage of lactation. Further, it is known that de novo synthesis can be negatively influenced by increasing amounts of supplemented UFA, particularly through certain rumen biohydrogenation isomers (Chilliard et al., 2000). As the exact mechanism of this inhibition is still unknown, Glasser et al. (2008) considered all identified C18 FA in their meta-analysis and found that milk yield of C4 to C16 decreased linearly with increasing C18 duodenal flow. Although their calculations were based on mid- and late lactation cows, they suggested that UFA released during lipomobilization may have a similar effect on de novo FA synthesis. Lipomobilization, which typically occurs in the early lactation during negative energy balance, is connected with increased levels of preformed FA in milk, especially oleic acid (Gross et al., 2011). In primiparous cows, the impact of negative energy balance on the cow's organism can be more severe because they require additional nutrients to support their own growth as they are not physically mature (Wathes et al., 2007). Another factor affecting de novo synthesis may be the availability of precursors produced during microbial fermentation in the rumen (Woolpert et al., 2017) that can be influenced by the dry matter intake, which is lower in primiparous cows compared to multiparous ones (Neave et al., 2017) and by the differences in the bacterial

community between primiparous and multiparous cows (Kumar et al., 2015). However, it should be noted that in our study, body condition score and dry matter intake were not measured; therefore, it is not possible to assess the extent of mobilization of body reserves in the experimental dairy cows, nor to detect differences in mobilization between P1 and multiparous cows and their impact on changes in FA profile.

In our study, desaturation indices, which reflect stearyl-CoA desaturase activity, were higher in P1 cows compared to multiparous cows. This disagrees with O'Callaghan et al. (2020) and Wilms et al. (2022), but it reflects the higher concentrations of the respective MUFA and lower concentrations of their SFA counterparts found in P1 cows observed in our study.

Effect of milking

During the first days postpartum, the FA profile of milk fat gradually changes to a profile of mature milk (Silva et al., 2024). However, the description of these changes is inconsistent among studies, mainly due to differences in sampling that reflect the variability in the definition of transition milk production. While some studies described changes in FA profile between days postpartum using morning milking for sampling (O'Callaghan et al., 2020), others collected samples at each milking during regular milking twice a day (Wilms et al., 2022) or in 24h intervals from calving (Contarini et al., 2014). Using AMS for the collection of transition milk with variability in milking frequency and intervals can cause another source of variability in the FA profile. The trends in the concentrations of total as well as the most abundant individual SFA (palmitic, myristic, stearic, butyric, and lauric acids) in dependence on milkings are similar to those summarized by Silva et al. (2024) in their review, except for lauric and stearic acid, for which the reviewed results were inconclusive.

The proportions of UFA and MUFA showed a slight increase over successive milkings, while PUFA proportions were not affected by the milkings. Regarding MUFA changes across milkings, our findings are consistent with those of Contarini et al. (2014), O'Callaghan et al.

Table 4

Sums of FA (% from total FA) and calculated ratios and indexes* of transition milk collected by automatic milking system (AMS) during first four days postpartum in dependence on cow parity, number of milkings, and between-milking interval.

Item	Fatty acids sums / ratios / indexes ¹ (mean ± SEM)																				
<i>n</i>	SCFA	MCFA		LCFA		SFA		MUFA		PUFA		UFA		Trans-FA		n-6 PUFA		n-3 PUFA			
Parity (P)																					
P1	55	21.4	0.9	49.6	1.2	29.0	0.8	73.1	0.7	21.8	0.7	5.1	0.2	26.9	0.7	0.5	0.1	4.1	0.1	1.0	0.1
P2	37	18.0	0.8	55.3	1.3	26.7	0.9	75.8	0.6	19.5	0.6	4.7	0.2	24.2	0.6	0.5	0.1	3.8	0.2	0.9	0.1
P3+	38	21.3	1.0	51.6	1.4	27.1	0.9	76.7	0.7	18.4	0.6	4.9	0.3	23.3	0.7	0.3	0.1	4.0	0.2	0.8	0.1
Milking (M)																					
M2	18	16.0	1.4	60.5	2.8	23.5	1.7	76.4	1.2	18.5	1.1	5.1	0.4	23.6	1.2	0.3	0.1	4.4	0.3	0.7	0.1
M3	12	18.3	1.5	55.6	1.6	26.1	1.1	75.3	1.2	19.8	1.0	4.9	0.4	24.7	1.2	0.5	0.2	4.0	0.4	0.9	0.2
M4	16	20.0	1.1	55.2	1.3	24.8	0.9	76.9	1.0	18.6	0.8	4.5	0.4	23.1	1.0	0.4	0.2	3.5	0.3	1.0	0.1
M5	13	22.5	1.8	50.6	2.0	26.8	1.6	75.4	1.4	19.5	1.4	5.1	0.4	24.6	1.4	0.6	0.2	4.1	0.3	0.9	0.2
M6	14	19.4	1.6	51.4	3.2	29.2	2.1	73.2	1.9	21.6	1.6	5.3	0.4	26.8	1.9	0.3	0.1	4.1	0.3	1.1	0.1
M7	14	22.1	1.5	49.7	1.2	28.2	1.2	75.5	1.2	20.4	1.1	4.1	0.4	24.5	1.2	0.3	0.1	3.5	0.3	0.6	0.1
M8	16	23.1	1.7	46.1	1.4	30.8	0.9	73.3	1.0	21.5	1.0	5.2	0.3	26.7	1.0	0.4	0.1	4.0	0.2	1.2	0.2
M9	10	22.3	1.5	46.3	1.3	31.5	1.0	73.5	1.0	21.3	1.0	5.2	0.3	26.5	1.0	0.6	0.2	4.2	0.3	0.9	0.2
M10+	17	20.9	1.5	48.5	1.8	30.6	1.2	74.0	1.1	20.9	0.9	5.1	0.4	26.0	1.1	0.4	0.1	4.2	0.3	0.9	0.2
Milking interval (I)																					
Short	82	21.1	0.7	50.9	1.0	28.0	0.6	75.0	0.5	19.9	0.5	5.0	0.2	25.0	0.5	0.5	0.1	4.1	0.1	0.9	0.1
Long	47	19.0	0.8	53.7	1.2	27.3	0.8	74.8	0.7	20.5	0.6	4.8	0.2	25.2	0.7	0.3	0.1	3.9	0.2	0.9	0.1
P-value																					
P		0.024		0.003		0.172		<0.001		<0.001		0.400		<0.001		0.106		0.464		0.240	
M		0.002		<0.001		<0.001		0.017		0.013		0.682		0.018		0.655		0.987		0.410	
I				0.013		0.273		0.955		0.757		0.260		0.955		0.041		0.302		0.917	

Table 4 (continued)

		Fatty acids sums / ratios / indexes ¹ (mean ± SEM)																	
Item	n	n-6: n-3 ²		EFA		LA: ALA		De novo FA		Mixed FA		Preformed FA		DI14		DI16		DI18	
Parity (P)																			
P1	55	4.1	0.3	3.7	0.1	2.9	0.2	37.8	0.9	29.2	1.1	32.0	0.8	11.4	0.7	11.8	1.1	69.7	1.1
P2	37	4.3	0.4	3.5	0.2	3.1	0.3	36.7	0.6	32.9	0.9	29.6	1.0	10.0	1.0	9.4	0.3	66.3	0.6
P3+	38	5.1	0.6	3.7	0.2	3.9	0.4	40.0	1.0	29.6	1.3	29.8	1.0	8.2	1.0	11.4	1.7	63.3	0.5
Milking (M)																			
M2	18	5.9	0.7	3.6	1.0	3.8	1.3	37.3	0.8	35.7	2.3	26.0	1.8	11.6	1.0	10.3	2.0	69.7	1.3
M3	12	4.8	0.9	3.2	0.9	3.2	1.8	36.9	1.0	33.1	0.9	29.1	1.2	9.5	1.4	9.7	0.7	69.0	1.7
M4	16	3.1	0.3	3.2	1.2	2.5	1.0	38.9	0.9	32.7	0.9	27.5	1.1	9.9	1.3	8.9	0.5	67.8	1.1
M5	13	4.1	0.6	3.5	1.1	2.7	1.1	40.6	1.9	29.1	2.0	29.3	1.7	11.1	1.8	12.1	2.5	64.5	4.1
M6	14	3.8	0.3	4.3	1.2	3.0	0.6	36.2	2.7	30.0	4.1	33.0	1.8	12.1	1.3	15.7	4.4	68.5	1.1
M7	14	5.6	1.1	3.3	1.1	4.0	2.6	39.2	1.7	29.3	0.9	31.0	1.3	10.2	1.9	10.1	0.7	65.6	1.1
M8	16	3.7	0.4	3.9	0.6	3.0	1.3	39.3	1.8	26.4	1.6	33.5	1.0	11.2	1.6	11.9	1.8	66.1	1.2
M9	10	3.7	0.4	4.2	0.7	3.0	1.0	37.2	1.5	27.7	0.9	34.5	1.0	5.9	2.0	10.5	0.7	65.4	1.2
M10+	17	5.6	1.2	3.8	0.8	4.1	3.2	37.7	1.1	28.1	1.0	33.4	1.2	7.8	1.5	10.4	0.6	64.7	1.2
Milking interval (I)																			
Short	82	4.7	0.3	3.6	0.1	3.3	0.2	38.5	0.7	29.7	0.9	30.9	0.7	9.5	0.6	11.3	0.9	66.3	0.8
Long	47	4.0	0.4	3.7	0.2	3.1	0.3	37.5	0.7	31.7	0.9	30.1	0.9	11.2	1.0	10.6	1.0	67.8	0.7
P-value																			
P		0.267		0.771		0.010		0.028		0.003		0.177		0.022		0.004		<0.001	
M		0.780		0.023		0.406		0.824		<0.001		<0.001		0.068		0.652		0.007	
I		0.138		0.955		0.407		0.117		0.012		0.262		0.117		0.859		0.185	

Milkings interval (h) – Short (<12 h), Long (>12 h)

Abbreviations: n = number of samples; SEM = standard error of the mean; SCFA – short-chain fatty acids (sum of FA with chain length < 12); MCFA – medium-chain fatty acids (sum of FA with chain length ≥ 12 and < 18); LCFA – long-chain fatty acids (sum of FA with chain length ≥ 18); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; UFA – unsaturated fatty acids; EFA – essential fatty acids; de novo FA – a sum of C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, and C14:1; Mixed FA – a sum of C16:0 and C16:1; Preformed FA – a sum of C15:0, C17:0, C18:0, C18:1, C18:2, C18:3, C20:0, C20:2, C20:4, C22:0, and C24:0; DI14, DI16, DI18 – desaturase indexes calculated for C14, C16 and C18 FA (for formulas see Material and Methods) for FA names see Nonstandard abbreviations

¹ also including minor FA (C11, C13, C15:1, C18:3n6, C20:1n9, C20:2, C20:3n6, C20:3n3, C20:5n3, C22:6n3, for their names see Nonstandard abbreviations);

² for statistics calculations, zero values of n-3 were replaced with 0.1;

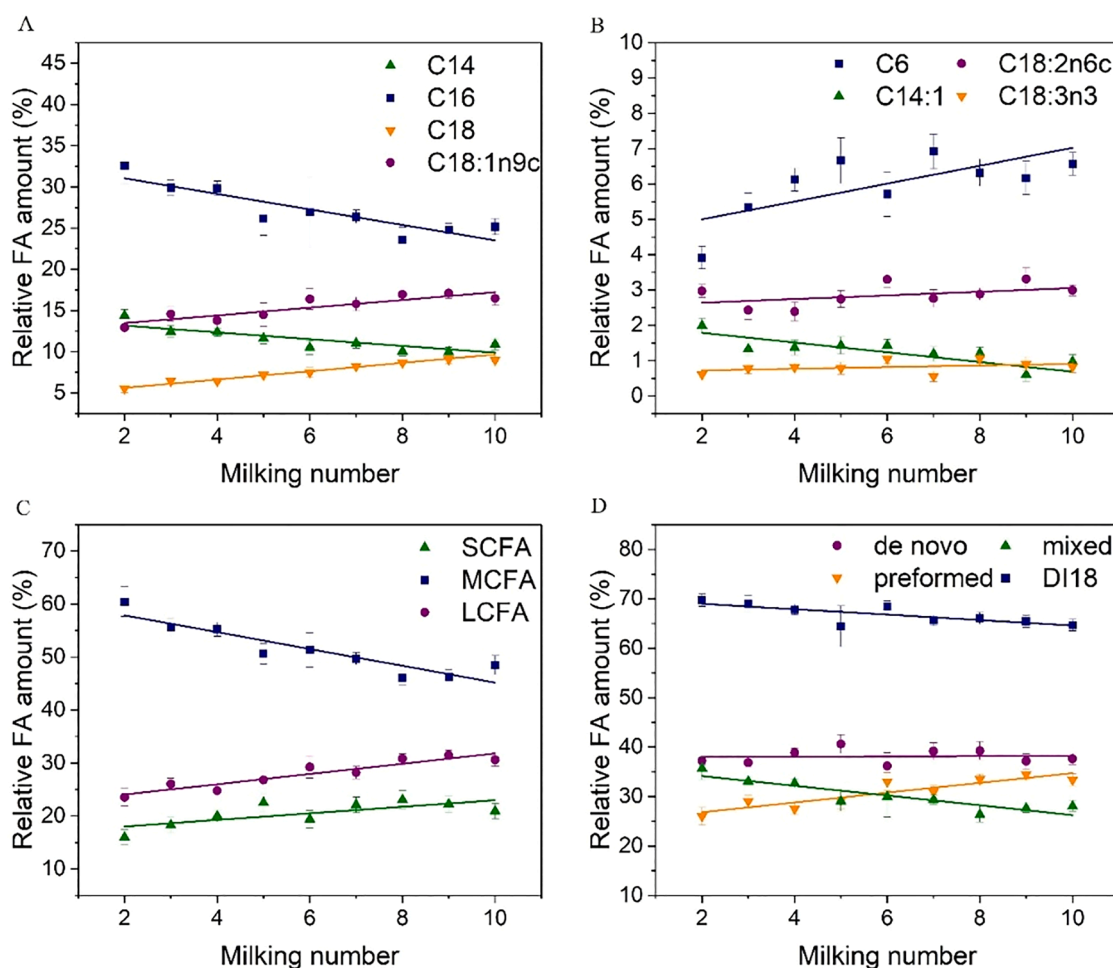


Fig. 1. Time-related changes in the fatty acid composition and indexes in milk from the second to the tenth milking (expressed as means $\pm$ SEM). C6 – caproic acid; C14 – myristic acid; C14:1 – myristoleic acid; C16 – palmitic acid; C18 – stearic acid; C18:1n9c – oleic acid; C18:2n6c – linoleic acid; C18:3n3 – α -linoleic acid; SCFA – short-chain fatty acids (sum of FA with chain length < 12); MCFA – medium-chain fatty acids (sum of FA with chain length ≥ 12 and < 18); LCFA – long-chain fatty acids (sum of FA with chain length ≥ 18).

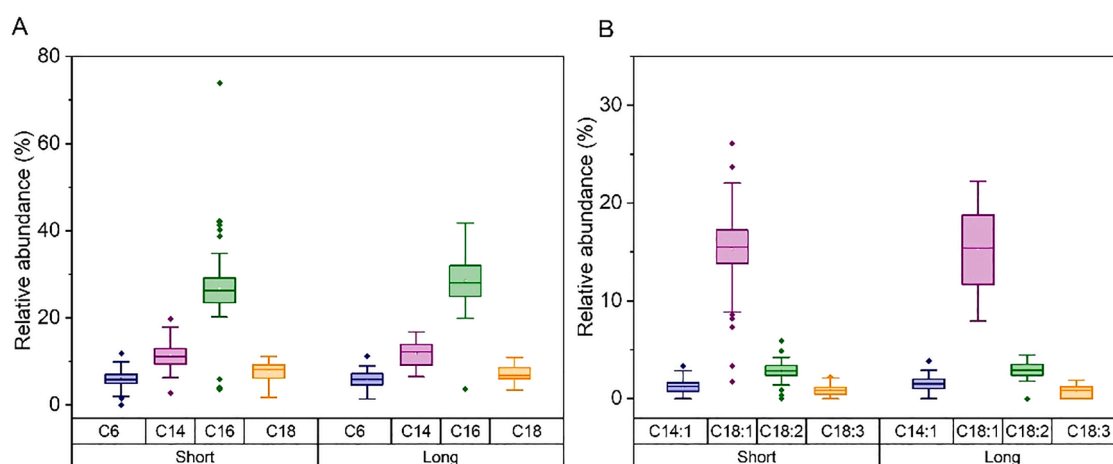


Fig. 2. Percentages of selected saturated (A) and unsaturated fatty acids (B) in milk were obtained after Short or Long intervals between milkings. The data is visualized using box plots that depict the interquartile range (IQR). The median values are indicated by horizontal lines within the boxes, while the mean values are represented as empty squares. Error bars extend to 1.5 times the IQR. Any outliers beyond these limits are marked with diamond symbols. (C6 – caproic acid; C14 – myristic acid; C16 – palmitic acid; C18 – stearic acid; C14:1 – myristoleic acid; C18:1 – oleic acid; C18:2 – linoleic acid; C18:3 – α -linoleic acid; Short – milking interval < 12 h; Long – milking interval > 12 h).

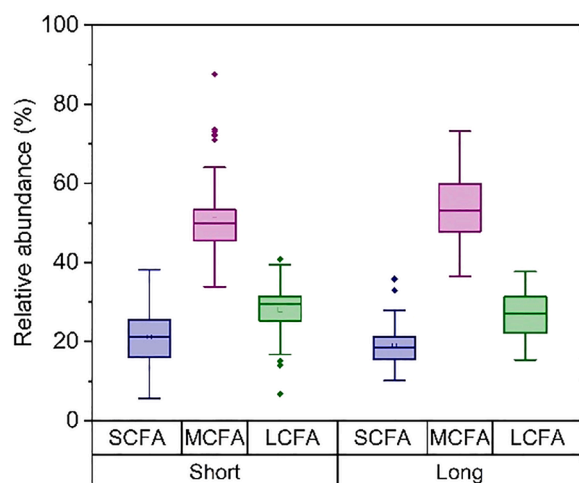


Fig. 3. The percentages of SCFA (blue), MCFA (violet), and LCFA (green) in milk were obtained after Short or Long intervals between milkings. The data is visualized using box plots that depict the interquartile range (IQR). The median values are indicated by horizontal lines within the boxes, while the mean values are represented as empty squares. Error bars extend to 1.5 times the IQR. Any outliers beyond these limits are marked with diamond symbols. (SCFA – short-chain fatty acids (sum of FA with chain length < 12); MCFA – medium-chain fatty acids (sum of FA with chain length ≥ 12 and < 18); LCFA – long-chain fatty acids (sum of FA with chain length ≥ 18); Short – milking interval < 12 h; Long – milking interval > 12 h).

(2020), or Wilms et al. (2022). For PUFA, the results are inconsistent. While no effect of milking was noted in our study and by O’Callaghan et al. (2020), Contarini et al. (2014) found a decreasing trend, and Wilms et al. (2022) found significant changes in PUFA concentration between M3 and the other milkings. The most abundant UFA in transition milk across milkings was oleic acid, followed by palmitoleic acid, LA, myristoleic acid, and ALA, of which oleic acid had the greatest influence on the aforementioned trends in UFA and MUFA.

The milkings had a significant effect only on DI18 which showed a decreasing trend ($P = 0.007$); DI14 and DI16 were not affected by the milkings. Although O’Callaghan et al. (2020) or Wilms et al. (2022) calculated cumulative DI for C14, C16, and C18, the trends in their DI were similar to those found in our study.

Effect of milking interval

In our study, only subtle changes in individual FA were noted, resulting in a significant increase in SCFA and a decrease in MCFA and mixed FA in shorter milking interval (< 12 h) compared to longer one (> 12 h). To our knowledge, there are no studies describing the effect of milking interval or increased milking frequency on FA profiles of transition milk. However, the majority of studies agreed that milking interval or milking frequency has a minor effect on the FA profile of mature milk, but the findings are inconsistent. While Wiking et al. (2006) found a decreased proportion of PUFA from the udder half milked 4 times compared with the other udder half milked twice daily, others found no effect of increased milking frequency on the FA composition (Shields et al., 2011). On the other hand, Klei et al. (1997) suggested that increased milking frequency may increase the ratio of de novo-synthesized to preformed FA in milk fat, particularly during early and mid-lactation, and Ferneborg et al. (2017) found decreased proportion of lignoceric acid associated with more frequent milkings. Further research in this area is needed to clarify the effects of milking interval/milking frequency on the FA profile during the period of transition milk production.

Effect of changes in FA profile in transition milk on calves' health and development

Short-chain FA

Prior to the rumen development, milk is the main source of butyric and caproic acids for the newborn calf (Górka et al., 2018). Beyond its role as a nutrient, butyric acid contributes to gut tissue development, immune modulation, and oxidative stress reduction (Bedford and Gong, 2018). In milk fat, butyrate (or other SCFA and MCFA) is mainly located at the sn-3 position of triacylglycerols, and it is released by the action of pre-gastric lipase that is active only on that third position of the molecule (Drackley, 2008). The released butyrate is absorbed in the small intestine and is mostly oxidized for energy by the liver before reaching the peripheral circulation (Drackley, 2008). Positive role of butyrate on gastrointestinal tract development was documented mainly after the supplementation of milk replacers that typically do not contain butyric acid with butyrate (Górka et al., 2014, 2011). Furthermore, the positive effect of butyrate on the abundance of SCFA producing and health-associated bacteria in the intestine was reported (O’Hara et al., 2018). On the other hand, butyrate supplementation to colostrum negatively affected IgG absorption (Hiltz and Laarman, 2019). Absorption of colostrum IgG is mediated by pinocytotic activity of the intestine that is depleted within 24 h after birth (Stott et al., 1979), and it is also influenced by the replacement of enterocytes with mature epithelial cells (Smeaton and Simpson-Morgan, 1985). According to Górka et al. (2014), butyrate stimulates cell differentiation and proliferation, thereby accelerating cell maturation. Thus, lower levels of butyric acid in colostrum compared to transition or mature milk (O’Callaghan et al., 2020; Wilms et al., 2022), with subsequent increase within the first one or two days of lactation, followed by stable values, seem to meet the physiological requirements of newborn calves. Similar trends were also observed in our study.

Compared to butyric acid, information about the physiological role of caproic acid in newborn calves is limited because caproic acid is often supplemented together with butyric acid, which does not allow for isolating its individual effects. Caproic acid serves as a substrate in mammalian energy metabolism and anabolic processes (Schönfeld and Wojtczak, 2016). Supplementing milk replacers with different inclusion levels of a tributyrin and tricaproin mixture to newborn calves for 21 days had no effect on feed intake, growth, or faecal scores (Castro et al., 2025a). On the other hand, supplementing milk replacers with milk fat that is natural source of SCFA for newborns revealed no effect on the hepatic metabolome profiles but shifts in lipid metabolism as calves had higher concentrations of key liver metabolites, including phosphatidylcholine, sphingomyelin, lysophosphatidylcholine, and eicosa-pentaenoic acid in comparison to calves fed milk replacers with vegetable fats rich in butyric and caproic acids (Castro et al., 2025b). Further, positive effects on gut permeability and the FA composition of the gastrointestinal tract were observed after feeding milk fat (Mellors et al., 2023), as well as a shift in the plasma metabolic profile (Ghaffari et al., 2025). The moderate concentrations of caproic acid observed in our study are consistent with the recommendations of Wilms et al. (2022). The increasing trend in caproic acid concentrations across the milkings, regardless of parity and milking interval, seems to correspond with newborn calf needs.

Medium-chain FA

In addition to its most important role, nutrient absorption, the digestive tract protects the host from luminal contents, including pathogens and toxins (Steele et al., 2016). In this role, it is also assisted by MCFA, which exhibits antibacterial and antiviral properties (Hristov et al., 2004), with the capric and lauric acids showing the broadest spectra of antimicrobial activities (Desbois, 2012). Furthermore, Sato et al. (2004) also found anticoccidial effects of a mixture of caprylic, capric and lauric acids on calves.

Concentrations of capric and lauric acids in our study remained

steady across milkings and were not affected by cows' parity. This underscores the importance of these FAs in protecting newborns against environmental pathogens.

The declining trends in myristic acid concentrations across milkings are consistent with O'Callaghan et al. (2020) and Wilms et al. (2022). In addition to its energetic and antimicrobial roles, myristic acid has been identified as an olfactory stimulant in humans, eliciting an appetitive response in neonates (Gutiérrez-García et al., 2017). It could have a similar mode of action in calves, thus potentially allowing for greater colostrum and milk intake. The association between myristic acid and dietary intake was recently described in Nelore bulls (Malheiros et al., 2023). Further, characterization of the faecal lipidome of newborn and preweaning heifers revealed a high percentage of myristic acid in faecal lipids that reflects high intake of this FA from colostrum and milk replacer that contained coconut and palm oil, both rich in C14:0 (Ghaffari et al., 2026). They suggested that luminal myristic acid may play an important role in protein lipidation, potentially influencing intestinal homeostasis. This remains unclear and requires further investigation.

From the perspective of newborn calf nutrition, palmitic acid represents an important energy source. However, for newborns with an immature digestive tract, its position on the glycerol backbone is crucial. Two enzymes are involved in fat digestion in newborns: gastric lipase, which is already well developed but contributes to fat digestion by only 10%, and pancreatic lipase, in which a certain immaturity of the exocrine function was observed in neonates (Havlicekova et al., 2016). Pancreatic lipase selectively hydrolyses fatty acids bound to the sn-1 and sn-3 positions, resulting in free FA and 2-monoglycerides that create micelles with bile acids and are absorbed rapidly. Furthermore, when calcium is present in digesta in sufficient amounts, free FA with a long chain can form insoluble calcium soaps that are poorly absorbed (Havlicekova et al., 2016). In bovine milk, 43% of palmitic acid is located at the sn-2 position (β -palmitate, Bracco, 1994), which prevents its release from the glycerol backbone and allows for rapid absorption. Moreover, the positive effect of β -palmitate on the intestinal microflora and intestine maturation was observed in humans (Havlicekova et al., 2016). From the viewpoint of the healthy development of a newborn calf, palmitic acid plays an important role as a precursor for palmitoyl-CoA, which is needed for the start of de novo synthesis of all ceramides that can subsequently be used in sphingomyelin synthesis (Dei Cas et al., 2020). Both, ceramides and sphingomyelin, are implicated in multiple cellular functions, such as proliferation, differentiation, apoptosis, and cell signalling, and play a major role in the immune system development. Furthermore, sphingomyelin is essential for neuronal myelination, which is closely related to brain maturation (Dei Cas et al., 2020). In this connection, Rico et al. (2016) found that increased palmitic acid intake increased hepatic ceramide synthesis. Such metabolic functions, as well as the nutritive properties, could explain why a higher proportion of palmitic acid is found in bovine colostrum compared with mature milk (O'Callaghan et al., 2020) and why higher values are also found in transition milk.

Long-chain FA

Similar to O'Callaghan et al. (2020) and Lashkari et al. (2025), the content of stearic acid gradually increased over the whole period of the transition milk production. This increase reflects a need for stearic and other long-chain FA for the synthesis of phosphatidylethanolamine and phosphatidylcholine, which are vital for neurogenesis and cell membranes (Bernhard et al., 2016).

Ruminants are born with low plasma levels of essential FA (Noble et al., 1971) and are dependent on dietary intake. Generally, these essential FA are substrates for the synthesis of other long-chain PUFA of n3 and n6 series, such as eicosapentaenoic, docosahexaenoic, γ -linolenic, eicosatrienoic, and arachidonic acids, respectively, that are important for neural development and the production of hormones in newborns (Klein, 2002). Both n3 and n6 fatty acids are precursors of

signalling molecules with opposing effects that modulate the membrane microdomain composition, receptor signalling, and gene expression (Schmitz and Ecker, 2008). Positive effect of increased essential FA intake either via colostrum from supplemented cows (Uken et al., 2021) or via supplemented milk replacer or starter (Garcia et al., 2015) on calf health and development was reported. The increasing trend in EFA concentrations across milking observed in our study corresponds with the calves' needs for these FA.

Conclusions

Our study examined the changes in the fatty acid profile of transition milk in cows milked in an automated milking system, considering the cows' parity and the number of milkings. Cows' parity had a significant effect on the fatty acid profile. Primiparous cows produced lower concentrations of saturated fatty acids, mainly myristic and stearic, and higher concentrations of monounsaturated fatty acids, mainly oleic, than multiparous cows (parity 2 and 3 and higher). These observed changes are likely related to the different physiological responses of cows to the increased energy requirements associated with the onset of lactation. The milking number significantly influenced the fatty acid profile. The relative contents of caproic, margaric, stearic, and oleic acids increased and the relative contents of palmitic, myristic, and myristoleic acids decreased with an increasing number of milkings. The different length of the between milking interval that may occur in individual cows when using automated milking system since the second milking postpartum had only a minor effect on the fatty acid profile of transition milk.

It should be noted that, although the present study provides valuable insights into changes in the fatty acid profile of transition milk in cows milked using an automated milking system, several aspects warrant further investigation. First, the number of cows included in the experiment was relatively small, and future studies involving a larger number of animals could provide additional insights and further strengthen the conclusions of the research. Second, measuring feed and fatty acid intake during the close-up and fresh periods would enable a more precise interpretation of the metabolic processes associated with milk fatty acid production. Finally, monitoring additional parameters, such as changes in body condition score, could improve the assessment of body reserve mobilization during the transition period and its relationship with changes in the milk fatty acid profile.

Overall, the findings contribute to a better understanding of the physiological changes occurring during early lactation and provide a valuable basis for future research aimed at optimizing dairy cow management and milk quality.

Abbreviations

ALA, α -linolenic acid; AMS, automated milking system; CLA, conjugated linoleic acid; DI, desaturation index; FA, fatty acid; FAME, fatty acid methyl ester; LA, linoleic acid; LCFA, long-chain fatty acids; MCFA, medium-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCFA, short-chain fatty acids; SFA, saturated FA; C4, butyric acid; C6, caproic acid; C8, caprylic acid; C10, capric acid; C11, undecanoic acid; C12, lauric acid; C13, tridecanoic acid; C14, myristic acid; C14:1, myristoleic acid; C15, pentadecanoic acid; C15:1, pentadecenoic acid; C16, palmitic acid; C16:1, palmitoleic acid; C17, margaric acid; C17:1, heptadecenoic acid; C18, stearic acid; C18:1n9c, oleic acid; C18:2n6t, linolelaidic acid; C18:2n6c, linoleic acid; C18:3n3, α -linolenic acid; C18:3n6, γ -linolenic acid; C20:1n9, eicosenoic acid; C20:2, eicosadienoic acid; C20:3n6, eicosatrienoic acid; C20:3n3, eicosatrienoic acid; C20:5n3, eicosapentaenoic acid; C22, behenic acid; C22:6n3, docosahexaenoic acid; C24, lignoceric acid

Data and model availability statement

All raw data generated and analysed during this study are available from the corresponding author upon reasonable request. Supplementary data to this article can be found online in the Supplementary File.

Declaration of generative AI and AI-assisted technologies in the writing process

AI-assisted tools were used only to improve the grammar and readability of the English language in the manuscript.

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Implications

Worldwide, the adoption of automated milking systems (AMS) has increased significantly. In addition to its regular use for the collection of milk for consumption, AMS can also be used early postpartum to collect transition milk. The composition of transition milk, including the fatty acid profile, undergoes gradual changes that reflect the biological needs of newborn calves. When using AMS, where cows determine their own milking frequency and milking interval, our study revealed that the fatty acid profile was affected by cows' parity and the number of milkings, but not by the interval between milkings.

Ethical statement

Authors declare that

- The work described has not been published previously except in the form of a preprint, an abstract, a published lecture, an academic thesis, or a registered report.
- The article is not under consideration for publication elsewhere.
- The article's publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out.
- If accepted, the article will not be published elsewhere in the same form, in English or in any other language, including electronically, without the written consent of the copyright-holder.
- All authors of this research paper have directly participated in the planning, execution, or analysis of this study.

CRedit authorship contribution statement

Veronika Farková: Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Katerina Dadáková:** Writing – review & editing, Writing – original draft, Visualization, Data curation. **Zdeněk Farka:** Writing – review & editing, Visualization, Data curation. **Steven Mascres:** Writing – review & editing, Methodology, Data curation. **Damien Eggermont:** Validation, Data curation. **Giorgia Purcaro:** Writing – review & editing, Supervision, Data curation. **Tomáš Kašparovský:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Ludmila Krížová:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.vas.2026.100850](https://doi.org/10.1016/j.vas.2026.100850).

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