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Bovine milk fatty acid and triacylglycerol composition and structure differ between early and late lactation influencing milk fat solid fat content

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ABSTRACT

Milk samples were collected in early lactation (8–14 days in milk; DIM) and late lactation (199–326 DIM) from 11 cows to assess the differences in bovine milk fatty acid (FA) and triacylglycerol (TAG) composition, FA positional distribution in the TAG structure, and milk solid fat content (SFC). Cows in early lactation that were in negative energy balance, mobilised C16:0, C18:0, and C18:1*cis*9 from their body fat stores, increasing the concentrations of these FA in milk fat. These high concentrations of C18:0 and C18:1*cis*9 enhanced the synthesis of high molecular weight TAG and decreased milk fat SFC in early lactation. For both lactation stages, alterations in the total FA concentrations in the TAG structures resulted in changes in the abundances and the proportions of the FA at the *sn*-2 and *sn*-1 (3) positions in the TAG structure, yet without affecting the main esterification preferences of the FA.

1. Introduction

Milk fat, mainly composed of triacylglycerols (TAGs; ~98%), is one of the high value components in milk (Jensen, 2002; Mohan, O'Callaghan, Kelly, & Hogan, 2020). TAGs are composed of three fatty acids (FAs) esterified to a glycerol molecule located at the stereospecific numbering (*sn*)-positions 1, 2, or 3. Many different FAs (~400) are used for TAG synthesis; therefore, changes in the FA composition may influence the formation of specific TAG species in the mammary gland. Several studies analysed the variation in the FA composition in the different lactation stages of dairy cattle (Garnsworthy, Masson, Lock, & Mottram, 2006; Kay et al., 2005; Stoop, Bovenhuis, Heck, & van Arendonk, 2009). The FA differences between lactation stages were related to the energy status of the cows, that triggered changes in the origin of FAs used for TAG synthesis in the mammary gland (Stoop et al., 2009; Van Knegsel, Van den Brand, Dijkstra, Tamminga, & Kemp, 2005). More specifically, the major changes between lactation stages involve the strong FA release from body fat stores in early lactation (<100 days in milk; DIM) compared with predominant de novo FA synthesis in middle (100–200 DIM) and late lactation (>200 DIM) (Kay et al., 2005; Samková, Špička, Pešek, Pelikánová, & Hanuš, 2012; Stoop et al., 2009).

Dairy cows in early lactation are usually in a negative energy balance due to the high energy requirements for milk production after calving (Kay et al., 2005; Stoop et al., 2009; Van Knegsel et al., 2005). To compensate for this energy need, the cow's body starts mobilising FAs from body fat stores, that are mainly composed of C16:0, C18:0, and C18:1*cis*9 (Christie, 1981). As a result, previous studies identified high concentrations of mobilised long-chain FAs (LCFAs) in early lactation milk fat (Garnsworthy et al., 2006; Kay et al., 2005). Moving towards mid and late lactation, the mobilisation of body fat decreases and the synthesis of de novo FAs C6:0 to C16:0 increases (Kay et al., 2005; Palmquist, Denise Beaulieu, & Barbano, 1993; Stoop et al., 2009).

These large differences between lactation stages in the concentrations of LCFAs and de novo FAs in milk fat are likely to influence milk fat TAG composition. High concentrations of LCFAs are expected to increase the formation of high molecular weight

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(HMW) TAG species, whereas high concentrations of de novo FAs are expected to increase the synthesis of low molecular weight (LMW) and medium molecular weight (MMW) TAG species in milk fat (Banks, Clapperton, Muir, & Girdler, 1989; DePeters, German, Taylor, Essex, & Perez-Monti, 2001). To our knowledge, no studies have reported on the effect of lactation stage on the TAG composition in bovine milk fat. In addition, no studies were found on the effect of lactation stage on the FA positional distribution in bovine milk fat TAG structures. The positioning of FAs within TAGs is of importance for digestion and FA absorption after milk consumption. For example, the composition and concentration of FAs at the secondary position (*sn*-2) in the TAG structure are important for infant formula development, because high concentrations of C16:0 at this position were identified to increase free FA (FFA) and calcium absorption in infants (Innis, 2011; Yaron et al., 2013). Moreover, the TAG composition and the stereolocation of FAs in the TAG structures are important for the dairy and food industry because they influence the physical properties of milk fat (e.g., solid fat content (SFC) and crystallisation behaviour). Variations in milk fat physical properties, especially its SFC, are relevant for its suitability and use for food product applications (Mohan et al., 2020).

In this study, analysed the effect of the early versus late lactation stage on bovine milk fat composition by providing insights into its influence on the FA and TAG composition, FA positional distribution in the TAG structure, and SFC. These findings can help the dairy industry to better understand the changes in chemical and physical properties of milk fat that are associated with the cows' lactation stage.

2. Material and methods

2.1. Sample collection

Raw milk samples were collected at Dairy Campus (Leeuwarden, The Netherlands) from 11 multiparous cows twice in the same lactation period: in early (8–14 DIM) and late lactation (199–326 DIM). The cows in early lactation consumed on average approximately 18 kg of dry matter, including a fixed amount of 5.5 kg DM of pelleted concentrates and an unrestrictedly fed roughage-based diet. The main components of this roughage-based diet were 70% maize silage and 20% grass silage mixed with 5–6% soy bean meal, and a premix with minerals and vitamins. During late lactation, the cows consumed approximately 23 kg of dry matter. The amount of pelleted concentrate within total intake varied for each cow, as concentrate allowance was individually determined based on each cow's milk yield. The ranges used were determined as follows: cows with a milk yield of 17 kg of milk d^{-1} were given 1.8 kg dry matter concentrate, and cows with a milk yield of 40 kg of milk d^{-1} were given 7.2 kg dry matter concentrate. The main components of the roughage diet fed unrestrictedly to the cows in late lactation were 60% grass silage and 20% maize silage mixed with 9% soy bean meal, 10% wheat meal, and a premix with minerals and vitamins. The estimation of the energy balance of the 11 cows in early lactation was reported by Keanthao, Goselink, Dijkstra, Bannink, and Schonewille (2021), where the cows used in our study were their control group with normal dietary intake levels of phosphorus (Supplementary material Table S1). Sampling was done during the morning milking (between 5 and 8 a.m.) by taking a representative 100 mL sample from the total milking of each individual cow. Samples were stored frozen (−20 °C) until fat extraction. Before fat extraction, milk samples were thawed in a water bath at 37 °C for approximately 30 min. The fat extraction was done based on the method described by Tzompa-Sosa, van Aken, van Hooijdonk, and van Valenberg (2014). This method only extracts TAG from the fat samples. Milk fat samples were stored at −20 °C until further analysis.

2.2. Fatty acid composition analysis

Fatty acid methyl esters (FAME) were analysed for all milk fat samples (n = 22) as described by Pacheco-Pappenheim, Yener, van Valenberg, Tzompa-Sosa, and Bovenhuis (2019). Fatty acid methyl esters were prepared according to the ISO Standard 15884 (ISO, 2002) and the FAME composition was determined by the ISO Standard 16958 (ISO, 2015) using gas chromatography (GC) with a flame-ionisation detector (FID; GC-FID; Thermo Focus, Thermo Fisher, Rodano, Italy) and an Agilent CPSil 88 FAME column (100 m × 0.25 mm i.d. × 0.2 μm film thickness).

2.3. Triacylglycerol composition analysis

Two methods were used to analyse the TAG composition: GC-FID and Matrix-assisted laser desorption/ionisation-time of flight-mass spectrometry (MALDI-TOF-MS). The same approach was previously described by Pacheco-Pappenheim, Yener, Heck, Dijkstra, and van Valenberg (2021), where combining the two methods provided a complete overview of the TAG composition in milk fat. On the one hand, with GC-FID the TAG composition of even chain TAG groups composed of TAG species with the same number of total carbon atoms was determined. On the other hand, to obtain information on TAG species level, MALDI-TOF-MS was used to determine the molecular weight of individual TAG species expressed in total carbon number (CN) and number of double bonds (DB) (CN:DB).

2.3.1. GC-FID

TAG compositions of all milk fat samples (n = 22) were analysed according to ISO Standard 17678 (ISO, 2010). Even-chain TAG groups with CN from 24 to 54 and cholesterol were determined with GC-FID, with a column injector port (Thermo Trace GC ultra; Thermo Scientific, Rodano, Italy) and a UltiMetal CP7532 column (5 m × 0.53 mm i.d. × 0.17 μm film thickness; Varian, Houten, The Netherlands). Anhydrous milk fat standard (BCR519; Sigma–Aldrich Chemie GmbH, Buchs, Switzerland) was included in the analysis as a reference for the identification of the TAG groups. Triacylglycerol composition was expressed as g 100 g^{-1} TAG based on the normalised peak areas of each TAG group.

2.3.2. MALDI-TOF-MS

TAG composition of all milk fat samples (n = 22) were also analysed with MALDI-TOF-MS (UltrafleXtreme, Bruker Corporation, Germany) according to Tzompa-Sosa, Meurs, and van Valenberg (2018) and Yener and van Valenberg (2019). Each milk fat sample was measured 5 times in automatic mode, which allowed acquisition of mass peaks in a random manner. The obtained mass spectra were analysed with the MALDIquant package using R (Gibb & Strimmer, 2012; Yener & van Valenberg, 2019). Signal-to-noise ratio >6 was used for the selection of the mass peaks. Each mass peak intensity was divided by the sum of the intensities of all mass peaks to express the results as relative intensities, which were reported as percentages (%). To assign the mass peaks to TAG species, the LIPID MAPS Online Tools library was used (https://www.lipidmaps.org/resources/tools/bulk-structure-search/create?database = LMSD; Fahy et al., 2005). TAG species were defined according to their CN:DB and reported as relative intensities of each TAG species, based on total TAG intensities.

2.4. Regiospecific distribution analysis of fatty acids

The composition of FAs at the *sn*-2 position of TAG structures was analysed for all milk fat samples (n = 22) based on the JOCS/AOCS Joint Method Ch3a-19 (AOCS, 2019). In this method, enzymatic transesterification using *Candida antarctica* Lipase (fraction B; Novozym 435 (10,000 PLU g^{-1}), Novozymes A/S, Frederiksberg, Denmark) was performed to determine the FA composition at the *sn*-2 position in the TAG structures. After the enzymatic transesterification, the 2-monoacylglycerol fractions of the 22 samples were collected through solid-phase extraction and analysed for FAME composition. The FAMEs were prepared and analysed as described in the FA composition analysis section. The FA composition at the *sn*-2 position was calculated and expressed in molar percentages (%). The FA composition at the *sn*-1 (3) (the sum of the FAs at the *sn*-1 and *sn*-3 positions) was calculated based on the analysed composition at the *sn*-2 position. As described by Tzompa-Sosa et al. (2014), intra- and interpositional distribution approaches were used to describe the FA positional distribution in the TAG structure. The intrapositional distribution assesses the FA relative concentrations (mol %) at the *sn*-2 and *sn*-1 (3) positions, whereas the interpositional distribution assesses the proportions (%) of FAs over the 3 positions in the TAG structures. Intrapositional and interpositional distributions were calculated based on Tzompa-Sosa et al. (2014):

Intrapositional distribution calculations [sn-1 (3)]:

$$sn\text{-}1(3)_{FAi} = (TAG_i \times 3 - sn\text{-}2_{FAi})/\ 2 \quad \text{(Eq. 1)}$$

where $sn\text{-}1\ (3)_{FAi}$ is the molar percentage of the FA at *sn*-1 (3) positions, TAG_i is the molar percentage of the FAs in TAGs and $sn\text{-}2_{FAi}$ is the molar percentage of the FA measured at the *sn*-2 position.

Interpositional distribution calculations:

sn-2:

$$\%\ sn\text{-}2_{FAi} = [sn\text{-}2_{FAi}/(sn\text{-}2_{FAi} + (sn\text{-}1(3)_{FAi}\ \text{x}\ 2)]\times 100 \quad \text{(Eq. 2)}$$

sn-1 (3):

$$\%\ sn\text{-}1(3)_{FAi} = sn\text{-}1(3)_{FAi}\ \text{x}\ 2)/\ [sn\text{-}2_{FAi} + (sn\text{-}1(3)_{FAi}\ \text{x}\ 2)]\times 100 \quad \text{(Eq. 3)}$$

where $\%\ sn\text{-}2_{FAi}$ is the proportion of the FA at the *sn*-2 position, $\%\ sn\text{-}1\ (3)_{FAi}$ is the proportion of the FA at the sn-1 (3) positions, $sn\text{-}2_{FAi}$ is the molar percentage of the FA measured at the *sn*-2 position and $sn\text{-}1\ (3)_{FAi}$ is the molar percentage of the FA at *sn*-1 (3) positions calculated with equation (1).

2.5. Solid fat content analysis

The SFC analysis was carried out by nuclear magnetic resonance (NMR; Bruker mq20 minispec NMR analyser, Bruker, Mississauga, ON, Canada) according to the AOCS Official Method Cd 16b-93 (AOCS, 2017). The SFC was determined at 0, 10, 20, 25, 30, and 40 °C. The parameters for the NMR were a frequency of 19.95 MHz, a dead time of 0.0073 ms and a cell temperature of 40 °C. Milk fat samples were totally melted using a water bath at 40 °C and once melted 1 g of sample was transferred into an NMR glass tube (diameter 10 mm, length 150 mm, wall thickness 0.6 mm) (Bruker Nederland B.V., Leiderdorp, Netherlands). Before analysis of the SFC, the tubes containing the milk fat samples were heated to 100 °C for 15 min followed by 15 min at 60 °C and then cooled to 0 °C for 60 min. At 0 °C, the SFC was measured and, after that, the samples were kept for 30 ± 1 min at each temperature (10, 20, 25, 30, and 40 °C) before subsequent SFC measurements.

2.6. Statistical analysis

The effect of lactation stage on milk FA composition, TAG composition, FA regiospecific distribution in the TAG structures, and SFC was tested with one-way ANOVA with repeated measures. The cut-off value defined for significant differences was $P < 0.05$. All statistical analyses were performed with IBM SPSS Statistics 25.0 software (2017). Pearson correlation analysis was performed between FA, TAG, and SFC using R version 3.6.1 (2019).

3. Results and discussion

3.1. Fatty acid composition

The average milk fat content was not different between both lactation stages (4.50 g 100 g^{-1} milk; Table 1). Most FAs were significantly affected by the stage of lactation ($P < 0.05$), with the exceptions of C6:0, C8:0, C16:1*cis*9, C17:0*anteiso*, C18:0:1*trans*9, C18:1*trans*10, C18:1*cis*12, C18:1*cis*14, C18:2*cis*9,12 (linoleic acid; LA), C19:0, and C20:0 (Table 1). Increased concentrations of C4:0, LCFAs ≥C17:0 and most of the unsaturated FAs (UFAs) were found in early lactation, whereas increased concentrations of medium-chain FAs (MCFAs; C10:0 to C16:0), C18:1*cis*15, C18:3*cis*9,12,15 (linolenic acid; ALA), C18:2*cis*9,*trans*11 (CLA), C20:3*cis*8,11,14 (DGLA), and C22:5*cis*7,10,13,16,19 (DPA) were found in late lactation. These results are in agreement with previous studies, where similar changes in bovine milk FA composition were reported between early and late lactation (Garnsworthy et al., 2006; Kay et al., 2005; Stoop et al., 2009).

As expected, cows in early lactation were in negative energy balance due to the high energy requirements after calving for milk production (Supplementary material Table S1; Garnsworthy et al., 2006; Keanthao et al., 2021; Stoop et al., 2009; Van Knegsel et al., 2005). To fulfil their energy needs for milk production, cows start mobilising adipose tissue. This explains the increased concentrations of C18:0 and C18:1*cis*9 in early lactation (Table 1). Bovine adipose tissue is mainly composed of C16:0, C18:0, and C18:1*cis*9 (Christie, 1981), so large proportions of these FAs are expected to be used by the mammary gland for milk fat synthesis. Moreover, in early lactation, we identified lower concentrations of de novo FAs from C10:0 to C15:0 and C16:0 (50% de novo formed; Table 1). This may be the result of the limited energy availability for the synthesis of de novo FAs in the mammary gland due to the negative energy balance of cows in early lactation (Van Knegsel et al., 2007). In contrast to the de novo FAs, C4:0 concentrations were higher in early lactation compared with late lactation. This result is in line with previous studies, which suggested that the C4:0 increase in early lactation was related to the high concentrations of β-hydroxybutyrate found in the blood after calving (Garnsworthy et al., 2006; Palmquist et al., 1993). Possibly, the high concentrations of β-hydroxybutyrate in the blood may have enhanced the formation and esterification of C4:0, which can be directly formed in the mammary epithelial cells from β-hydroxybutyrate taken up from the blood.

The results of this study showed a characteristic shift in the FA composition between early and late lactation (Garnsworthy et al., 2006; Kay et al., 2005; Stoop et al., 2009). However, the differences between the contents of maize and grass silage in the diets fed to the cows in early and late lactation might also be partly responsible for the variation found in FA composition. The diet fed to the cows in early lactation was relatively high in maize silage (~70%) and low in grass silage (~20%) compared with the diet in late lactation (maize ~20%, grass ~60%). Maize silage is rich in LA, whereas grass silage is rich in ALA. Based on these differences in FA composition between maize and grass silage, increased

Table 1
Milk fatty acid (FA) composition (g 100 g^{-1} total FAs) of cows in early and late lactation stages.[a]

Item	Early		Late		Lactation stage effect
	Mean	SD	Mean	SD	*P* value
Fat content (%)	4.51	0.37	4.49	0.52	0.917
C4:0	4.14	0.45	3.39	0.23	<0.001
C6:0	2.34	0.39	2.36	0.14	0.868
C8:0	1.16	0.30	1.34	0.11	0.091
C10:0	2.42	0.85	3.23	0.41	0.014
C10:1*cis*9	0.16	0.07	0.34	0.08	0.001
C12:0	2.62	0.98	4.16	0.61	0.002
C14:0*iso*	0.05	0.02	0.08	0.02	0.003
C14:0	9.36	2.14	12.13	0.71	0.001
C14:1*cis*9	0.62	0.22	1.19	0.31	0.001
C15:0*iso*	0.18	0.04	0.25	0.04	0.005
C15:0*anteiso*	0.32	0.07	0.49	0.07	<0.001
C15:0	0.74	0.19	1.17	0.18	0.001
C16:0*iso*	0.16	0.03	0.20	0.03	0.014
C16:0	28.63	1.82	32.10	2.08	0.002
C16:1*trans*9	0.20	0.03	0.16	0.03	0.011
C16:1*cis*9	2.06	0.44	1.92	0.41	0.344
C17:0*iso*	0.39	0.04	0.36	0.02	0.039
C17:0*anteiso*	0.39	0.07	0.40	0.06	0.505
C17:0	0.61	0.09	0.52	0.06	0.007
C17:1*cis*9	0.33	0.09	0.22	0.05	0.003
C18:0	11.34	1.25	8.34	1.64	0.001
C18:1*trans*6	0.25	0.05	0.19	0.02	0.004
C18:1*trans*9	0.16	0.01	0.15	0.01	0.304
C18:1*trans*10	0.21	0.03	0.23	0.06	0.169
C18:1*trans*11	0.86	0.10	0.75	0.13	0.022
C18:1*cis*9	24.88	5.13	18.48	2.09	0.005
C18:1*cis*11	1.04	0.16	0.66	0.15	0.001
C18:1*cis*12	0.19	0.04	0.19	0.03	0.641
C18:1*cis*13	0.16	0.04	0.10	0.02	0.001
C18:1*cis*14	0.31	0.04	0.34	0.02	0.087
C18:1*cis*15	0.18	0.03	0.26	0.06	0.002
C18:2*cis*9,12 (LA)	1.73	0.20	1.69	0.16	0.561
C18:3*cis*9,12,15 (ALA)	0.34	0.05	0.41	0.06	0.032
C18:2*cis*9,*trans*11 (CLA)	0.30	0.06	0.40	0.06	0.001
C19:0	0.14	0.01	0.13	0.01	0.052
C20:0	0.11	0.02	0.11	0.03	0.812
C20:3*cis*8,11,14 (DGLA)	0.05	0.02	0.08	0.02	<0.001
C20:4*cis*5,8,11,14 (AA)	0.13	0.02	0.12	0.02	<0.001
C22:5*cis*7,10,13,16,19 (DPA)	0.06	0.01	0.08	0.02	0.008
FA groups					
SCFAs	10.22	1.51	10.71	0.77	0.348
MCFAs	46.82	4.62	55.68	3.05	0.001
LCFAs	42.48	5.79	32.77	3.57	0.002
SFAs	65.27	5.62	71.10	2.59	0.010
UFAs	34.39	5.61	28.29	2.51	0.008

[a] Collection of the early lactation milk samples was done between 8 and 14 days in milk (DIM) of the 11 cows; collection of the late lactation milk samples was done between 199 and 326 DIM of the 11 cows. The lactation stage effect was considered significant at $P < 0.05$. The total sum of the FA composition is 99.6%, 0.4% of the total FA composition is not included in the table due to low concentrations (<0.05%) in the GC-FID analysis. Abbreviations are: LA, linoleic acid; ALA, linolenic acid; CLA, conjugated linoleic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; DPA, docosapentaenoic acid; SCFAs, short-chain FAs (sum of FAs with chain length <12); MCFAs, medium-chain FAs (sum of FAs with chain length ≥12 and < 18); LCFAs, long-chain FAs (sum of FAs with chain length ≥18); SFAs, saturated FAs; UFAs, unsaturated FAs.

concentrations of LA were expected in early lactation and increased concentrations of ALA were expected in late lactation, assuming that feed plays a major role in milk fat FA composition.

In a previous study, high concentrations of ALA lead to increased formation of intermediate biohydrogenation products such as CLA, C18:1*trans* FA, C18:1*cis*14, and C18:1*cis*15 (Pacheco-Pappenheim et al., 2021). This study indicates that the high concentration of grass silage in late lactation indeed resulted in increased concentrations of ALA, CLA and C18:1*cis*15. Overall, the major differences in the FA composition, being the increase in mobilised FAs versus de novo FAs, could be related to lactation stage, yet some small additional differences between lactation stages in the concentrations of ALA, LA, CLA, and C18:1*cis*15 suggest that feed played a role as well. Moreover, the FA composition of our study matched with the differences in FA composition between early and late lactation of previous studies that assessed this effect (Garnsworthy et al., 2006; Stoop et al., 2009; Van Knegsel et al., 2007). However, in contrast to our study, these studies had similar feeding regimes at the different lactation stages. One of the few studies that evaluated the effect of lactation stage based on milk fat samples of dairy cattle fed different diets between lactation stages was that of Bilal, Cue, Mustafa, and Hayes (2014). In that study, regardless of the changes in the specific diet of each season (summer versus winter), lactation stage was the largest contributor to the variations in milk FA composition, showing the same shift of mobilised FAs versus de novo FAs between early and late lactation as in ours and previous studies (Garnsworthy et al., 2006; Stoop et al., 2009; Van Knegsel et al., 2007). This supports that in our study lactation stage seems to be the main driving factor responsible for the milk FA compositional differences. However, it should be acknowledged that milk FA composition may also be influenced by differences in the feeding regimes.

3.2. Triacylglycerol composition

TAG composition between early and late lactation changed significantly. Table 2 presents the TAG composition in early and late lactation as analysed by GC-FID. The MMW TAGs CN38 and CN40 and the HMW TAGs CN52 and CN54 increased ($P < 0.05$) in early lactation, whereas in late lactation the LMW TAGs CN32 and CN34 and the MMW TAGs CN42 to CN48 increased ($P < 0.05$). Similar variations in TAG composition were also identified by MALDI-TOF-MS between early and late lactation (Supplementary material Table S2).

Table 2
Milk fat triacylglycerol (TAG) composition (g 100 g^{-1} total TAGs) from cows in early and late lactation stages analysed by GC-flame ionisation detector.[a]

Item	TAG group	Early		Late		Lactation stage effect
		Mean	SD	Mean	SD	*P* value
Cholesterol		0.26	0.03	0.33	0.04	0.001
TAG						
CN24	LMW	0.03	0.01	0.03	0.01	0.910
CN26	LMW	0.24	0.07	0.26	0.04	0.515
CN28	LMW	0.53	0.17	0.60	0.08	0.218
CN30	LMW	0.98	0.32	1.24	0.17	0.059
CN32	LMW	2.03	0.67	2.76	0.36	0.023
CN34	LMW	4.96	1.22	6.36	0.67	0.019
CN36	LMW	10.78	1.23	11.15	0.87	0.465
CN38	MMW	14.67	1.23	12.23	0.57	<0.001
CN40	MMW	10.95	0.69	9.70	0.34	<0.001
CN42	MMW	6.34	1.30	7.87	0.61	0.016
CN44	MMW	5.33	1.50	7.81	0.72	0.002
CN46	MMW	5.68	1.44	8.53	0.68	0.001
CN48	MMW	7.69	1.08	9.89	0.44	<0.001
CN50	HMW	11.71	1.68	10.67	0.97	0.166
CN52	MMW	12.47	4.26	7.61	1.71	0.015
CN54	MMW	5.36	2.00	2.96	0.99	0.017
TAG groups						
	LMW	19.54	3.61	22.40	2.04	0.077
	MMW	50.66	4.38	56.02	1.93	0.013
	HMW	29.54	7.78	21.24	3.60	0.025

[a] Collection of the early lactation milk samples was done between 8 and 14 DIM of the 11 cows; collection of the late lactation milk samples was done between 199 and 326 DIM of the 11 cows. The lactation stage effect was considered significant at $P < 0.05$. Abbreviations are: LMW, low molecular weight TAG (sum of CN24 to CN36); MMW, medium molecular weight TAG (sum of CN38 to CN48); HMW, high molecular weight TAG (sum of CN50 to CN54).

These variations in the TAG composition can be explained by the changes in the FA composition in each lactation stage (Table 1). The LMW and MMW TAG groups are mainly composed of de novo FAs, whereas HMW TAG group are mainly composed of LCFAs (Liu, Li, Pryce, & Rochfort, 2020). This was supported by Pearson correlation analyses between the TAG and FA composition as analysed by GC-FID (Supplementary material Table S3), where the HMW TAGs CN52 and CN54 showed high positive correlations ($r > 0.6$; $P < 0.05$) with C18:0, C18:1*cis*9, C18:1*cis*11, and C18:1*cis*13, and high negative correlations ($r < -0.6$; $P < 0.05$) with de novo FAs. The LMW TAGs CN32 and CN34 and the MMW TAGs CN42 to CN48 were positively correlated ($r > 0.6$; $P < 0.05$) with mainly SCFAs and MCFAs (C8:0 to C16:0) and negatively correlated ($r < -0.6$; $P < 0.05$) with LCFAs C17:0 to C18:1*cis*13 (Supplementary material Table S3). We, therefore, suggest that the high availability of the LCFAs and UFAs (mainly C18:0 and C18:1*cis*9, respectively) mobilised from body fat in early lactation underlies the increased synthesis of HMW TAG species (especially CN52 and CN54) in the mammary gland. Supporting this hypothesis, MALDI-TOF-MS data showed that in early lactation higher amounts of unsaturated HMW species were formed (Fig. 1, Supplementary material Table S2).

Next to the HMW TAG species, the MMW TAG species CN38 and CN40 were reported to also include C16:0, C18:0, and C18:1*cis*9, together with de novo short chain FAs, in their structures (Gresti, Bugaut, Maniongui, & Bezard, 1993; Liu et al., 2020). The TAGs CN38 and CN40 presented high positive correlations ($r > 0.6$; $P < 0.05$) with C18:0, C18:1*cis*9, C18:1*cis*11 and C18:1*cis*13 similar to the correlations observed for the HMW TAG (Supplementary material Table S3). For this reason, it is likely that the high availability of C18:0 and C18:1*cis*9 in early lactation also led to the increased synthesis of TAG species CN38 and CN40. In late lactation, on the other hand, the high concentrations of de novo FAs C8:0 to C16:0 may have increased the formation of the LMW TAG species CN32 and CN34 and the MMW TAG species CN42–CN48.

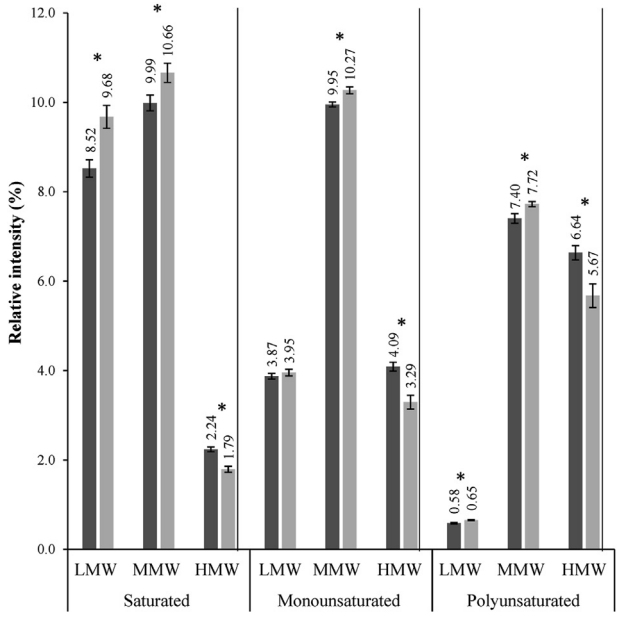

Fig. 1. Milk fat saturated, mono- and polyunsaturated triacylglycerol (TAG) profiles (relative intensity; %) from cows in early and late lactation stages analysed by MALDI-TOF-MS. Differences between low, medium, and high molecular weight (LMW, MMW, and HMW) TAG species are presented separately in each TAG profile. Early lactation LMW, MMW and HMW TAG profiles are presented in dark grey (■) and late lactation LMW, MMW and HMW TAG profiles are presented in light grey (■). Asterisks (*) indicate significant differences ($P < 0.05$) between early and late lactation LMW, MMW, and HMW TAG species.

Looking more in-depth at the effect of lactation stage on the saturation degree of TAG species, Fig. 1 presents the variations of the saturated, mono-, and polyunsaturated TAG species between early and late lactation, assessed separately for the LMW, MMW, and HMW TAG groups. Regardless of the saturation degree, the HMW TAG species increased ($P < 0.05$) in early lactation and most LMW and the MMW TAG species increased ($P < 0.05$) in late lactation (Fig. 1; Supplementary material Table S2). Based on the FAs identified in each TAG group structure, combined with the Pearson correlation analysis (Supplementary material Table S3; Liu et al., 2020), we suggest that the high abundance of C18:0 and C18:1 FA isomers (mainly C18:1*cis*9) enhanced the formation of saturated, mono- and polyunsaturated HMW TAG species in early lactation. On the other hand, in late lactation, the high concentrations of de novo FAs and C16:0 increased the formation of saturated and polyunsaturated LMW TAG species and all MMW TAGs (saturated, mono-, and polyunsaturated).

3.3. Regiospecific positional distribution of FAs in the TAGs

Higher concentrations of C18:0 and C18:1*cis*9 in early lactation and higher concentrations of C16:0 in late lactation may have affected the FA positional distribution in the TAG structures. These three major FAs in milk fat are esterified in most TAG species; hence, changes in their total abundance, and thereby their availability for TAG synthesis in the mammary gland, can be expected to affect FA positional distribution in the TAG structures. To examine this effect, first we analysed the FA composition at the *sn*-2 and *sn*-1 (3) positions using the intrapositional distribution. The intrapositional distribution provides information on the FA relative concentrations (mol %) at the *sn*-2 and *sn*-1 (3) positions in the TAG structures. Second, based on these results, the interpositional distribution was determined, where the proportions of a specific FA over the three positions (*sn*-2 and *sn*-1 (3)) in early and late lactation was calculated. Knowing the proportions of a FA at the *sn*-2 and *sn*-1 (3) positions is a suitable approach to analyse the esterification preferences of FAs in the TAG structures and to understand the mechanisms for TAG synthesis in the mammary gland (Blasi et al., 2008; Tzompa-Sosa et al., 2014). With the interpositional distribution, a FA distributed over the three *sn*-positions in TAG structure represents 100%, meaning that based on equal FA distribution the proportion of each individual *sn*-position corresponds to 33.3%. Combining intra- and interpositional distributions, we were able to determine the influence of lactation stage on the FA abundance variation and the FA esterification preferences at the *sn*-2 and *sn*-1 (3) positions in the TAG structures.

Table 3 presents the results of the FA interpositional distributions in early and late lactation stages. At the *sn*-2 position, FA proportions larger than 33.3% indicate an esterification preference, whereas proportions larger than 66.7% at the *sn*-1 (3) positions indicate an esterification preference for these positions. We identified that, regardless of the lactation stage, the FAs that were preferentially esterified at the *sn*-2 position were the MCFAs from C12:0 to C16:1*cis*9, C17:0, C17:1*cis*9, C18:1*cis*9, LA, and ALA. Moreover, the FAs that were preferentially esterified at the *sn*-1 (3) positions were the SCFAs from C4:0 to C10:0, C18:0, C18:1*cis*9, C18:1*cis*11, and C20:0. These esterification preferences of the FAs are in accordance with previous studies (Blasi et al., 2008; Parodi, 1983; Tzompa-Sosa et al., 2014). In addition, we also found differences in the FA interpositional distributions at both positions between lactation stages. At the *sn*-2 position, early lactation milk fat samples had higher proportions of C4:0 to C15:0, C16:0, C16:1*cis*9, C17:0, C17:1*cis*9, C18:1*cis*11, C20:0, and C18:3*cis*9,12,15 ($P < 0.05$). Late lactation milk fat samples had higher proportions of C17:0*iso*, C16:1*trans*9, and C18:1*cis*9 at the *sn*-2 position ($P < 0.05$). The

Table 3
Interpositional distribution of fatty acids (FAs) in milk fat triacylglycerol structures of cows in early and late lactation stages.[a]

Fatty acid	*sn*-2					*sn*-1 (3)				
	Early		Late		Lactation stage effect	Early		Late		Lactation stage effect
	Mean	SD	Mean	SD	*P* value	Mean	SD	Mean	SD	*P* value
C4:0	0.84	0.13	0.70	0.11	0.040	99.16	0.13	99.30	0.11	0.040
C6:0	3.98	0.59	3.87	0.81	0.592	96.02	0.59	96.13	0.81	0.592
C8:0	28.66	2.78	26.59	1.93	0.001	71.34	2.78	73.41	1.93	0.001
C10:0	29.15	2.21	26.34	1.57	<0.001	70.85	2.21	73.66	1.57	<0.001
C12:0	52.78	4.74	44.89	1.17	<0.001	47.22	4.74	55.11	1.17	<0.001
C14:0	65.65	4.14	56.97	2.15	<0.001	34.35	4.14	43.03	2.15	<0.001
C14:1*cis*9	55.06	4.22	49.09	1.23	0.001	44.94	4.22	50.91	1.23	0.001
C15:0*iso*	60.01	4.98	55.51	3.35	0.017	39.99	4.98	44.49	3.35	0.017
C15:0*anteiso*	73.10	3.63	67.05	1.98	0.001	26.90	3.63	32.95	1.98	0.001
C15:0	57.85	4.30	48.58	2.74	<0.001	42.15	4.30	51.42	2.74	<0.001
C16:0*iso*	64.53	8.90	61.47	5.99	0.354	35.47	8.90	38.53	5.99	0.354
C16:0	51.26	2.95	44.21	2.68	<0.001	48.74	2.95	55.79	2.68	<0.001
C16:1*trans*9	13.55	2.48	15.74	2.28	0.020	86.45	2.48	84.26	2.28	0.020
C16:1*cis*9	66.67	4.43	60.99	1.40	0.002	33.33	4.43	39.01	1.40	0.002
C17:0*iso*	40.63	3.50	44.45	3.14	0.008	59.37	3.50	55.55	3.14	0.008
C17:0 *anteiso*	52.25	3.65	53.62	4.13	0.379	47.75	3.65	46.38	4.13	0.379
C17:0	43.78	3.60	38.09	3.39	0.006	56.22	3.60	61.91	3.39	0.006
C17:1*cis*9	63.94	4.43	57.54	2.98	0.002	36.06	4.43	42.46	2.98	0.002
C18:0	20.01	1.61	20.91	1.91	0.129	79.99	1.61	79.09	1.91	0.129
C18:1*trans*	13.54	3.17	15.51	5.89	0.368	86.46	3.17	84.49	5.89	0.368
C18:1*cis*9	32.94	1.61	35.53	2.35	0.002	67.06	1.61	64.47	2.35	0.002
C18:1*cis*11	13.20	1.99	10.15	1.26	0.003	86.80	1.99	89.85	1.26	0.003
C18:2*cis*9,12 (LA)	49.03	3.12	48.56	2.67	0.708	50.97	3.12	51.44	2.67	0.708
C18:3*cis*9,12,15 (ALA)	41.55	2.91	38.79	1.68	0.006	58.45	2.91	61.21	1.68	0.006
C18:2*cis*9,*trans*11(CLA)	31.35	2.56	31.46	2.01	0.883	68.65	2.56	68.54	2.01	0.883
C20:0	14.77	6.61	10.24	2.06	0.017	85.23	6.61	89.76	2.06	0.017
C20:4*cis*5,8,11,14 (AA)	27.48	3.45	26.48	2.52	0.449	72.52	3.45	73.52	2.52	0.449

[a] The interpositional distribution at the *sn*-2 and *sn*-1 (3) positions are expressed in mol (%). Collection of the early lactation milk samples was done between 8 and 14 DIM of the 11 cows; collection of the late lactation milk samples was done between 199 and 326 DIM of the 11 cows. The lactation stage effect was considered significant at $P < 0.05$. Abbreviations are: LA, linoleic acid; ALA, linolenic acid; CLA, conjugated linoleic acid; AA, arachidonic acid.

opposite trends were observed at the *sn*-1 (3) position. These shifts from *sn*-2 to *sn*-1 (3) positions in the TAG structure between lactation stages may be related to the differences in the availability of C16:0, 18:0, and C18:1*cis*9 for TAG synthesis in each lactation stage. In early lactation, high concentrations of C18:0 and C18:1*cis*9 may have enhanced the proportions of most FAs (mainly de novo FAs and C16:0) at the *sn*-2 position, and resulted in decreased proportions of the same FA at the *sn*-1 (3) position in the TAG structures. On the other hand, high concentrations of de novo FAs and C16:0 in late lactation may have enhanced the proportions of most de novo FAs at the *sn*-1 (3) positions, which in turn decreased the proportions of these FAs at the *sn*-2 position.

It was previously suggested by Tzompa-Sosa et al. (2014) that high abundance of C16:0 may increase the activity of the glycerol-3-phospate acyltransferase (GPAT) enzyme towards C16:0 that is responsible for the esterification of FAs at the *sn*-1 position during TAG synthesis in the mammary gland. The high availability of C16:0 in the TAGs in late lactation may have increased the activity of the GPAT enzyme towards different FAs, and thereby increased the proportions of mainly de novo FAs at the *sn*-1 position and decreased the proportions of these FAs at the *sn*-2 position in the TAG structure. To balance the higher proportions of C16:0 at the *sn*-1 (3) positions in late lactation, the proportions of C18:1*cis*9 were increased at the *sn*-2 position in the TAG structures. These results were similar to those of Tzompa-Sosa et al. (2014), who showed the same trends for interpositional distribution of C16:0 and C18:1*cis*9.

Table 4 presents the intrapositional distribution at the *sn*-2 and *sn*-1 (3) positions in the TAG structures at each lactation stage. Regardless of the lactation stage, we identified that the most abundant FAs at the *sn*-2 position were C14:0, C16:0, 18:0, and C18:1*cis*9 and at the *sn*-1 (3) were FAs C4:0, C16:0, C18:0, and C18:1*cis*9. These results are in agreement with previous studies (Blasi et al., 2008; Jensen, 2002; Tzompa-Sosa et al., 2014). In early lactation, higher concentrations of C4:0, C16:0, C17:0, C17:1*cis*9, C18:0, C18:1*cis*9, and C20:0 were identified at the *sn*-2 position. At this same position in late lactation, concentrations of de novo FAs C12:0 to C15:0, ALA and DGLA were higher. Similar to the *sn*-2 position, at the *sn*-1 (3) positions increased concentrations of C4:0, C17:0*iso*, C16:1*trans*9, C17:1*cis*9, C18:0, C18:1*trans* FAs, C18:1*cis*9, and C18:1*cis*11 were found in early lactation, and in late lactation increased concentrations of de novo FAs from C8:0 to C16:0, ALA and DGLA were found. Except for C16:0, all these described changes in FA esterification at the *sn*-2 and *sn*-1 (3) positions are in line with the overall FA composition of each lactation stage (Table 1). These results suggest that, with the exception of C16:0, increased total abundance of a FAs in the TAGs leads to increases at both the *sn*-2 and *sn*-1 (3) positions. Similar trends were previously reported by Parodi (1983). At the *sn*-2 position, the C16:0 concentration was higher in early lactation, whereas at the *sn*-1 (3) position, the C16:0 concentration was higher in late lactation. Thus, it can be suggested that the C16:0 concentration may play an important role in the positioning of FAs. It is possible that the high concentrations of C16:0 in the TAGs in late lactation may have increased GPAT activity towards C16:0 and therefore resulted in higher concentrations of C16:0 at the *sn*-1 (3) positions and lower concentrations of C16:0 at the *sn*-2 position (Parodi, 1983; Tzompa-Sosa et al., 2014). All in all, the results indicate that the differences between lactation stages in the FA distribution in the TAG structures were due to the changes in the overall concentrations of FAs at each lactation stage (Table 1). Alterations in the total FA concentrations in the TAG structures resulted in changes in the abundance and the proportions of the FAs at the *sn*-2 and *sn*-1 (3) positions in the TAG structures, yet without affecting the main esterification preferences of the FAs (Table 3).

Table 4
Intrapositional distribution of fatty acids (FA) in milk fat triacylglycerol structures of cows in their early and late lactation stages. The intrapositional distribution at the *sn*-2 and *sn*-1 (3) positions are expressed in mol (%).[a]

Fatty acid	*sn*-2					*sn*-1 (3)				
	Early		Late		Lactation stage effect	Early		Late		Lactation stage effect
	Mean	SD	Mean	SD	*P* value	Mean	SD	Mean	SD	*P* value
C4:0	0.43	0.08	0.30	0.06	0.001	25.36	2.65	20.83	1.17	<0.001
C6:0	0.69	0.14	0.68	0.14	0.843	8.30	1.20	8.41	0.41	0.770
C8:0	1.74	0.37	1.89	0.18	0.265	2.20	0.57	2.61	0.19	0.036
C10:0	2.86	0.89	3.50	0.40	0.057	3.54	1.26	4.91	0.62	0.005
C12:0	4.53	1.32	6.28	0.83	0.005	2.13	0.96	3.87	0.55	0.001
C14:0*iso*	0.13	0.03	0.17	0.03	0.007	0.00	0.02	0.03	0.02	0.005
C14:0	17.32	2.96	19.72	0.99	0.015	4.66	1.46	7.46	0.63	<0.001
C14:1*cis*9	0.96	0.27	1.67	0.45	0.001	0.41	0.18	0.86	0.21	0.001
C15:0*iso*	0.29	0.05	0.36	0.05	0.002	0.10	0.04	0.15	0.04	0.010
C15:0*anteiso*	0.62	0.12	0.86	0.12	<0.001	0.12	0.04	0.21	0.03	<0.001
C15:0	1.13	0.24	1.50	0.16	0.002	0.42	0.14	0.80	0.15	<0.001
C16:0*iso*	0.25	0.04	0.30	0.06	0.050	0.07	0.02	0.10	0.03	0.043
C16:0	36.27	2.35	35.10	2.47	0.020	17.29	1.85	22.18	1.79	<0.001
C16:1*trans*9	0.07	0.01	0.06	0.01	0.336	0.21	0.04	0.17	0.03	0.011
C16:1*cis*9	3.42	0.82	2.90	0.59	0.052	0.85	0.23	0.93	0.21	0.311
C17:0*iso*	0.36	0.04	0.37	0.03	0.433	0.27	0.04	0.23	0.02	0.016
C17:0*anteiso*	0.47	0.07	0.50	0.07	0.183	0.22	0.05	0.22	0.05	0.990
C17:0	0.63	0.13	0.46	0.08	0.005	0.40	0.06	0.37	0.05	0.128
C17:1*cis*9	0.49	0.16	0.30	0.07	0.002	0.14	0.04	0.11	0.02	0.025
C18:0	4.96	0.66	3.78	0.69	0.001	9.90	1.05	7.24	1.60	0.002
C18:1*trans*	0.43	0.11	0.45	0.18	0.871	1.39	0.16	1.22	0.18	0.023
C18:1*cis*9	17.93	3.91	14.35	1.88	0.021	18.30	4.28	13.05	1.86	0.006
C18:1*cis*11	0.31	0.08	0.15	0.05	0.001	0.99	0.15	0.65	0.15	0.002
C18:2*cis*9,12 (LA)	1.86	0.29	1.79	0.23	0.542	0.96	0.14	0.95	0.10	0.750
C18:3*cis*9,12,15 (ALA)	0.31	0.06	0.35	0.06	0.231	0.22	0.03	0.27	0.04	0.013
C18:2*cis*9,*trans*11 (CLA)	0.20	0.04	0.28	0.05	0.001	0.22	0.05	0.30	0.05	0.002
C20:0	0.03	0.01	0.02	0.00	0.017	0.09	0.02	0.10	0.03	0.729
C20:3*cis*8,11,14 (DGLA)	0.06	0.02	0.09	0.03	0.023	0.02	0.02	0.04	0.01	0.030
C20:4*cis*5,8,11,14 (AA)	0.07	0.01	0.06	0.01	0.055	0.09	0.01	0.09	0.01	0.118

[a] The intrapositional distribution at the *sn*-2 and *sn*-1 (3) positions are expressed in mol (%). Collection of the early lactation milk samples was done between 8 and 14 DIM of the 11 cows; collection of the late lactation milk samples was done between 199 and 326 DIM of the 11 cows; The lactation stage effect was considered significant at $P < 0.05$. Abbreviations are: LA, linoleic acid; ALA, linolenic acid; CLA, conjugated linoleic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid.

Information of the FA positional distribution in the TAG structures is important for infant formula producers (Kloek, Vonk, Feitsma, & Timmer, 2020; Yong-Hua et al., 2010). The stereolocation of C16:0 at the *sn*-2 position in the form of palmitate enhances the absorption of FFAs and minerals in infants. In contrast, C16:0 at the *sn*-1 and *sn*-3 positions in the TAG structures are hydrolysed into free C16:0. Free C16:0 can bind to dietary minerals (e.g., calcium and magnesium) forming indigestible complexes. These complexes are difficult to absorb and result in a lower availability of both FAs and minerals in infants (Innis, 2011; Mu & Høy, 2004). In early lactation, C16:0 has a greater abundance and proportion at the *sn*-2 position than late lactation milk fat. Therefore, to increase the abundance of C16:0 at the *sn*-2 position in the TAG structures and improve FA and mineral absorption in infants, milk fat from early lactation may be suggested as a more suitable lipid source for infant formula developers compared with late lactation or bulk milk fat. Such a strategy should be further studied and considered with caution keeping in mind, amongst others, the welfare of dairy cattle.

3.4. Solid fat content

Variations in the FA and TAG composition can influence the milk fat SFC. Table 5 presents the milk fat SFC in early and late lactation stages measured at 0, 10, 20, 25, 30 and 40 °C. The SFC was higher ($P < 0.05$) in late lactation for all measured temperatures, except for 40 °C. Because milk fat is almost completely liquid at 40 °C, no difference in milk fat SFC between early and late lactation at this temperature is to be expected. The SFC differences between 0 and 30 °C can be explained by the changes in milk FA and TAG composition in early and late lactation (Tables 1 and 2; Fig. 1; Supplementary material Table S2). As mentioned previously, the high concentrations of C18:0 and different C18:1 FA isomers in early lactation may have increased the formation of HMW TAG species and decreased the formation of LMW and MMW TAG species (Tables 1 and 2; Supplementary material Tables S2 and S3). The TAGs with the largest differences between early and late lactation were the saturated LMW and MMW TAG species (1.16% and 0.68%), and the mono- and polyunsaturated HMW TAG species (0.79% and 0.96%) (Fig. 1; Supplementary material Table S2). C18:1 FA isomers (especially C18:1*cis*9), the FAs that are most likely present in unsaturated HMW TAG species (Fig. 1; Supplementary material Table S3; Liu et al., 2020), are characterised by low

Table 5
Milk fat solid fat content (%) from cows in early and late lactation stages.[a]

Temperature (°C)	Early		Late		Lactation stage effect
	Mean	SD	Mean	SD	*P* value
0	52.78	7.91	62.33	2.91	0.002
10	40.50	8.04	50.35	3.55	<0.001
20	14.46	4.56	20.47	2.46	0.001
25	8.28	2.52	11.94	1.50	0.001
30	4.18	1.50	6.08	1.04	0.004
40	0.98	0.16	1.00	0.25	0.842

[a] Collection of the early lactation milk samples was done between 8 and 14 DIM of the 11 cows; collection of the late lactation milk samples was done between 199 and 326 DIM of the 11 cows. The lactation stage effect was considered significant at $P < 0.05$. Temperature is the measuring temperature selected to analyse the milk fat solid fat content.

melting points (Knothe & Dunn, 2009). In turn, mono- and poly-unsaturated HMW TAG species are thus expected to decrease milk fat SFC. This was confirmed by the Pearson correlation analysis between the FA and TAG compositions and milk fat SFC, which showed negative correlations between C18:1*cis*9, C18:1*cis*11, C18:1*cis*13, CN52, CN54 and the SFC measured between 0 and 30 °C ($r < -0.6$; $P < 0.05$; Supplementary material Table S4). Therefore, it can be suggested that the high availability of C18:1 isomers mobilised from body fat in early lactation enhanced the formation of unsaturated HMW TAG species in the mammary gland, resulting in a decreased milk fat SFC.

Compared with early lactation, the high SFC in late lactation can be explained by the high concentrations of saturated LMW and MMW TAG species (except CN38 and CN40) and by the lower concentrations of unsaturated HMW TAG (Table 2; Fig. 1; Supplementary material Table S2). The MCFAs C14:0 and C16:0, mainly found in saturated LMW and MMW TAG species, have a higher melting point compared with C18:1 FA isomers (Knothe & Dunn, 2009). This may have increased SFC in late lactations, as was supported by the Pearson correlation analysis showing high positive correlations ($r > 0.6$; $P < 0.05$) between de novo FAs C8:0 to C16:0, LMW TAGs CN32 and CN34, MMW TAGs CN42 to CN48, and the SFC measured between 0 and 30 °C (Supplementary material Table S4). All in all, milk fat SFC differences between early and late lactation were mainly related to the concentrations of saturated LMW and MMW TAG species and unsaturated HMW TAG species. Similar to our results, previous studies reported a decrease in milk fat SFC as a result of high concentrations of unsaturated LCFAs in milk fat (Couvreur, Hurtaud, Lopez, Delaby, & Peyraud, 2006; Precht & Frede, 1994; Smet et al., 2010), in our case mainly C18:1*cis*9.

To summarise, the SFC in early lactation was lower than in late lactation, mainly due to the higher concentrations of unsaturated HMW TAG species in early lactation and higher concentration of saturated LMW and MMW TAG species in late lactation. Variations in milk SFC are important for product development of dairy and dairy-based food products because it determines products texture characteristics at refrigerator (4 °C < T < 10 °C) and room temperatures (20–30 °C) (e.g., spreadability of butter and spreads). Variations in milk fat SFC, driven by the characteristic FA and TAG compositions in each lactation stage, may result in unexpected changes in milk fat processing conditions (e.g., fractionation processes, cream ripening for butter production) and texture characteristics of such products (e.g., hard, smooth, sandy mouthfeel; Mohan et al., 2020). This might be the case in seasonal milking systems where cows calve at the same time, and thus a high impact of lactation stage can be especially expected on the seasonal milk fat composition and physical properties (Auldist, Walsh, & Thomson, 1998; Li, Ye, & Singh, 2019).

4. Conclusions

This study showed that milk fat FAs in early lactation showed higher concentrations of C18:0 and C18:1*cis*9, most likely mobilised from body fat reserves. Late lactation milk fat FAs showed higher concentrations of de novo FAs, including C16:0. These FA differences between lactation stages resulted in higher concentrations of unsaturated HMW TAGs in early lactation milk fat and higher concentrations of saturated LMW and MMW TAGs in late lactation milk fat. Variations in the total FA concentrations in the TAG structure resulted in changes in the abundance and the proportions of the FAs at the *sn*-2 and *sn*-1 (3) positions in the TAG structure, yet without changing the main esterification preferences of the FAs. The SFC in early lactation was lower than in late lactation, which may be the result of higher concentrations of unsaturated HMW TAGs in early lactation and higher concentration of saturated LMW and MMW TAGs in late lactation.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2022.105370.

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