The *DGAT1* K232A polymorphism and feeding modify milk fat triacylglycerol composition

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ABSTRACT

In the present study, we aimed to investigate the changes in triacylglycerol (TAG) composition as affected by alterations in the cows' diet due to seasonal variations and genetic factors. For this study, 50 milk fat samples in winter and 50 in summer were used from 25 cows with the *DGAT1* *KK* genotype and 25 cows with the *DGAT1* *AA* genotype. The samples were analyzed for milk fat content (%), fat composition, and TAG composition. We found that the content of TAG species CN54 was higher and that of CN34 and CN36 lower in summer than in winter. This seasonal variation in TAG profile was related to seasonal changes in the fatty acids C14:0, C16:0, C18:0, C18:1 *cis*-9, total unsaturated fatty acids, and total long-chain fatty acids, most likely resulting from dietary differences between seasons. Furthermore, we quantified the effect of *DGAT1* K232A polymorphism on TAG profile and detected a significant effect on TAG species CN36, with higher values for the *DGAT1* *KK* genotype. When adjusting for differences in fat content, we found no significant effects of the *DGAT1* K232A polymorphism on TAG profile. We detected a significant interaction between *DGAT1* K232A polymorphism and season for TAG species CN42 and CN52; in summer, the *KK* genotype was associated with higher levels for CN42 than the *AA* genotype, whereas in winter, the difference between the genotypes was small. For CN52, in summer the *AA* genotype was associated with higher levels than the *KK* genotype. In winter, the difference between the genotypes was also small. We show that,

regardless of preference for *DGAT1* genotype (*AA* or *KK*) and depending on the availability of FA according to season, UFA (C18:1 *cis*-9), short-chain FA (C6:0 and C10:0), and medium-chain FA might be esterified on the glycerol backbone of the TAG, keeping the structure characteristics of each TAG species. To our knowledge, this is the first report on the interaction effect of *DGAT1* K232A polymorphism and season on the TAG composition in milk fat.

Key words: triglyceride, diacylglycerol *O*-acyltransferase 1, seasonal variation, fatty acid enzyme preference

INTRODUCTION

Milk fat (**MF**) is one of the most important components of milk, influencing the technological, sensorial, and nutritional properties of milk and dairy products (Cozma et al., 2013). Milk fat is composed of triacylglycerols (**TAG**; 95% of total lipids) with about 400 fatty acids (**FA**) of different chain lengths, degrees of saturation, and *sn*-positional distribution (Jensen, 2002). The wide range of TAG species with different composition and structure results in a wide range of melting and crystallization temperatures (from 0°C to 40°C), which define many quality specifications such as mouthfeel in chocolate or ice cream and lightness of pastry (Karupaiah and Sundram, 2007; Fredrick et al., 2011). Therefore, a better understanding of factors determining variations in TAG composition and structure is of great interest for product application purposes.

Triacylglycerol composition and structure are influenced by feeding regimen and genetic factors (Capuano et al., 2014; Tzompa-Sosa et al., 2014, 2016). Feeding regimen is an easy-to-implement and effective managerial strategy to modify FA composition and TAG profile. Feeding dairy cows with PUFA present in fresh grass reduces *de novo* synthesized FA and increases long-chain FA (**LCFA**) in MF (Chilliard et al., 2001),

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probably inducing significant changes in TAG composition. Seasonal changes in TAG composition occur mainly due to alterations in the cow's diet, which goes from a silage-based diet (winter) to a fresh grass-based diet (summer) irrespective of the country (Capuano et al., 2014; Liu et al., 2017; Tzompa-Sosa et al., 2018). Capuano et al. (2014) reported that MF of cows on a summer diet rich in unsaturated C18 FA (e.g., linolenic acid) shows relative decreases in concentrations of TAG CN34, CN36, CN42, CN44, and CN46; and relative increases in concentrations of TAG CN24, CN26, CN40, CN50, CN52, and CN54 compared with cows on a winter diet. Liu et al. (2017) found that levels of unsaturated TAG increased in summer compared with winter, indicating a positive correlation between fat content and TAG CN36:1 and CN42:1. Overall, these studies highlighted differences in seasonal MF TAG composition, where the summer MF TAG profile was found to have increased levels of high-carbon-number TAG (>50) and increased unsaturated levels.

Milk TAG composition can also be influenced by genetic factors (Tzompa-Sosa et al., 2016). Milk fat TAG are synthesized via the glycerol-3-phosphate pathway, where the enzyme diacylglycerol O-acyltransferase 1 (DGAT1) is of interest because of its role in the final step of triglyceride synthesis (Coleman and Lee, 2004; Palmquist, 2006) and because it is polymorphic in many dairy cattle populations. The *DGAT1* K232A polymorphism has been shown to affect milk fat content and FA composition, inducing changes in TAG composition. Genotype *KK* has been associated with increased fat content (%), higher concentrations of saturated fat and C16:0, and lower concentrations of C14:0, unsaturated C18, and CLA (Schennink et al., 2007; Tăbăran et al., 2015; Bovenhuis et al., 2016). Few studies have investigated the effect of the *DGAT1* K232A polymorphism on TAG composition. A recent study by Tzompa-Sosa et al. (2016) reported significant effects of the polymorphism on TAG composition in winter MF samples. They suggested that genotype *KK* is associated with increased levels of TAG species CN38 in winter MF. However, the combined influence of season and *DGAT1* K232A polymorphism on MF TAG composition is unknown. Duchemin et al. (2013) reported a significant *DGAT1* K232A $\times$ season interaction for the FA C4:0, C6:0, C8:0, C10:0, 12:0, 14:0, C16:1 *cis*-9, C18:1 *cis*-9, SFA, and UFA in MF, but no information is available on the interaction of both factors on TAG composition.

In the present study, we aimed to investigate changes in TAG composition affected by changes in the cows' diet due to season, variations in the *DGAT1* K232A polymorphism, and the season $\times$ *DGAT1* interaction.

MATERIALS AND METHODS

Sample Selection

For this study, 50 milk fat samples in winter and 50 milk fat samples in summer were used from 25 cows with the *DGAT1* *KK* genotype and 25 cows with the *DGAT1* *AA* genotype. These samples were randomly selected from the total samples collected as part of the Dutch Milk Genomics Initiative project. Within this project, 1,918 morning milk samples were collected from individual cows (Duchemin et al., 2013). The cows that were included were in 37 different herds and originated from 22 different sires. For the winter milk samples, the cows were between 79 and 249 DIM; for the summer milk samples, cows were between 163 and 332 DIM. Sodium azide (0.03%) was added to the milk to prevent microbiological growth. Milk fat was extracted from the milk samples as described by Tzompa-Sosa et al. (2014). This fat extraction method ensures that only nonpolar lipids will be present in the extract. After extraction, the fat was stored at -80°C until further analysis.

FA Analysis

Fatty acid methyl esters were prepared from MF as described in ISO standard 15884 (ISO-IDF, 2002). Methyl esters were analyzed following ISO standard 16958 (ISO-IDF, 2015) by GC (Thermo Focus, Thermo Fisher Scientific, Rodano, Italy) using an Agilent CPSil 88 FAME column (100 m $\times$ 0.25 mm i.d.; film 0.2 μm). The standard used for identification and quantification of the FA were pure FAME (Sigma-Aldrich; Larodan, Solna, Sweden). This analysis was done at the COKZ laboratory (Qlip NV, Leusden, the Netherlands).

TAG Profiling Using GC-Flame-Ionization Detection

The identification and quantification of TAG composition was carried out according to ISO Standard 17678/IDF 202 (ISO-IDF, 2010). The standard mix used for identification of the TAG was BCR 519 (anhydrous MF) mix (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). The TAG were separated by carbon number using GC fitted with a flame-ionization detector and a column injector port (Thermo Trace GC ultra, Thermo Fisher Scientific). The GC column was UltiMetal CP7532 (5 m $\times$ 0.53 mm $\times$ 0.17 μm ; Varian, Houten, the Netherlands). The TAG were identified by comparing their retention times to those found in the TAG standard mixture with certified reference materials BCR632a and BCR632b (Institute for Refer-

ence Materials and Measurements, Geel, Belgium). The results were expressed as percentage of the normalized peak areas.

Statistical Analysis

The effects of *DGAT1* K232A polymorphism and seasonal changes on the TAG and FA composition in MF were examined using the following linear mixed model:

$$Y_{ijklm} = \mu + \text{Season}_j + \text{DGAT1}_k + \text{Cow}_l + \varepsilon_{ijklm}, \quad [1]$$

where Y_{ijklm} are observations for individual carbon number (TAG species from CN26 to CN54), fixed effects are the overall mean μ ; Season is the effect of summer or winter; DGAT1 is the effect of AA and KK genotypes; Cow_l is the random effect accounting for repeated observations of cows in winter and summer; and ε_{ijklm} is the random residual error. Cow was assumed to be distributed as $N(\mathbf{0}, \mathbf{M}\sigma_{cow}^2)$, where $\mathbf{M}$ is the unstructured variance-covariance matrix.

It is known that *DGAT1* K232A polymorphism has a major effect on milk fat content. To investigate whether potential effects of the *DGAT1* K232A polymorphism on TAG can be explained based on its effect on fat content, we also performed an analysis in which we adjusted for differences in fat content using the following model:

$$Y_{ijklm} = \mu + \text{Season}_j + \text{DGAT1}_k + \beta \text{Fat}_{ijklm} + \text{Cow}_l + \varepsilon_{ijklm}, \quad [2]$$

where the effects are as described previously, β is a regression coefficient, and Fat_{ijklm} is a covariable accounting for differences in fat content.

Possible season and *DGAT1* interactions were analyzed using the following models:

$$Y_{ijklm} = \mu + \text{Season}_j + \text{DGAT1}_k + (\text{Season} \times \text{DGAT1})_{jk} + \text{Cow}_l + \varepsilon_{ijklm}, \quad [3]$$

$$Y_{ijklm} = \mu + \text{Season}_j + \text{DGAT1}_k + \beta \text{Fat}_{ijklm} + (\text{Season} \times \text{DGAT1})_{jk} + \text{Cow}_l + \varepsilon_{ijklm}, \quad [4]$$

where the effects are as described previously and $(\text{Season} \times \text{DGAT1})_{jk}$ is the interaction between season and *DGAT1* genotype.

The significance of fixed effects on the different traits (TAG and FA) was tested using the Proc Mixed method in the SAS/STAT statistic software. The Kenward-

Roger approximation (Kenward and Roger, 1997) was used to calculate degrees of freedom.

The Bonferroni correction was used to determine the appropriate significance level while accounting for multiple testing. The number of independent traits was determined based on a principal component analysis. Five principal components were identified that represented more than 90% of the variation in the data, and the corrected *P*-value was calculated as $P = \alpha/n = 0.05/5$, defining as cut-off a significance value of $P < 0.01$. Finally, the correlation between TAG and FA was calculated using the Pearson correlation coefficient.

RESULTS AND DISCUSSION

Effect of Season and *DGAT1* Genotypes on Fat Content

Differences in fat content between seasons and *DGAT1* K232A genotypes are shown in Table 1 and Table 2 (model [1]). No significant differences (Table 2, model [1]) were observed between winter and summer MF content ($P = 0.11$). However, we detected a tendency for higher MF content in winter (-0.14), similar to that reported in previous studies (Couvreur et al., 2006; Heck et al., 2009; Duchemin et al., 2013). The intake of fresh grass in summer increases the content of C18:3 *cis*-9,12,15 (linolenic acid), which is related to high concentrations of long-chain PUFA in the blood that reduces MF content because of impaired rumen biohydrogenation. The increased concentration of PUFA (intermediate rumen biohydrogenation products) affects fermentation and fiber digestibility in ruminants, resulting in lower acetate production and then lower milk fat synthesis (Harvatine et al., 2009). The *DGAT1* genotype KK cows had a higher fat content than the AA cows (-1.18 ; $P < 0.001$, Table 2, model [1]). The association of KK genotype with increased fat content was previously reported (Schennink et al., 2007; Barbosa da Silva et al., 2010; Tăbăran et al., 2015), where Schennink et al. (2007) reported a smaller difference between AA and KK genotypes based on a larger sample size and considering only winter milk samples.

Season

Descriptive statistics for the MF TAG profiles are shown in Table 1. The most abundant TAG species present in both winter and summer were CN36, CN38, CN40, CN48, and CN50. These TAG composition profiles were similar to those in previous studies using MF

Table 1. Descriptive statistics for season (winter/summer) and *DGAT1* K232A genotypes (*AA/KK*) for fat content (%) and relative triacylglycerol (TAG) composition (mean $\pm$ SD) based on milk fat samples from 50 individual cows

Trait	Season		<i>DGAT1</i> K232A	
	Winter	Summer	<i>AA</i>	<i>KK</i>
Fat content (%)	4.49 $\pm$ 0.84	4.28 $\pm$ 1.07	3.79 $\pm$ 0.83	5.08 $\pm$ 0.57
TAG				
CN26	0.28 $\pm$ 0.03	0.29 $\pm$ 0.04	0.28 $\pm$ 0.04	0.29 $\pm$ 0.04
CN28	0.86 $\pm$ 0.13	0.87 $\pm$ 0.14	0.87 $\pm$ 0.15	0.86 $\pm$ 0.12
CN30	1.51 $\pm$ 0.23	1.49 $\pm$ 0.25	1.50 $\pm$ 0.26	1.50 $\pm$ 0.22
CN32	2.91 $\pm$ 0.43	2.80 $\pm$ 0.48	2.86 $\pm$ 0.49	2.84 $\pm$ 0.42
CN34	6.51 $\pm$ 0.68	6.16 $\pm$ 0.89	6.31 $\pm$ 0.84	6.36 $\pm$ 0.78
CN36	11.91 $\pm$ 1.31	11.23 $\pm$ 1.53	11.16 $\pm$ 1.49	12.04 $\pm$ 1.28
CN38	13.16 $\pm$ 0.55	12.87 $\pm$ 1.10	12.77 $\pm$ 0.86	13.29 $\pm$ 0.83
CN40	10.27 $\pm$ 0.64	10.64 $\pm$ 0.74	10.43 $\pm$ 0.67	10.48 $\pm$ 0.77
CN42	8.22 $\pm$ 0.74	8.18 $\pm$ 0.83	8.12 $\pm$ 0.78	8.29 $\pm$ 0.78
CN44	8.00 $\pm$ 0.73	7.69 $\pm$ 0.85	7.81 $\pm$ 0.85	7.88 $\pm$ 0.75
CN46	8.52 $\pm$ 0.56	8.28 $\pm$ 0.82	8.40 $\pm$ 0.74	8.40 $\pm$ 0.68
CN48	9.59 $\pm$ 0.63	9.47 $\pm$ 0.81	9.63 $\pm$ 0.74	9.42 $\pm$ 0.70
CN50	9.95 $\pm$ 1.08	10.18 $\pm$ 1.36	10.29 $\pm$ 1.33	9.81 $\pm$ 1.06
CN52	6.57 $\pm$ 1.41	7.41 $\pm$ 2.48	7.52 $\pm$ 1.95	6.39 $\pm$ 2.03
CN54	1.64 $\pm$ 0.93	1.92 $\pm$ 1.55	1.75 $\pm$ 1.22	1.81 $\pm$ 1.37

from individual cows (Tzompa-Sosa et al., 2016; Liu et al., 2017) and bulk MF samples (Capuano et al., 2014; Larsen et al., 2014; Tzompa-Sosa et al., 2018). The relative concentrations of each identified TAG species (CN26 to CN54) were within the ranges reported in the review by Jensen (2002).

The TAG species CN36 and CN54 were significantly different ($P < 0.01$) between winter and summer when not accounting for fat content (Table 2, model [1]). Adjusting for differences in fat content, CN34 was also significantly different between seasons (Table 2, model [2]). These results indicate that there is an effect of

Table 2. Effects of season (winter/summer) and *DGAT1* K232A genotypes (*AA/KK*) on fat content (%) and triacylglycerol (TAG) composition (model estimates and *P*-values) based on 50 individual cow samples¹

Item	Season				<i>DGAT1</i> K232A			
	Model [1]		Model [2]		Model [1]		Model [2]	
	Estimate summer	<i>P</i> -value	Estimate summer	<i>P</i> -value	Estimate <i>AA</i>	<i>P</i> -value	Estimate <i>AA</i>	<i>P</i> -value
Fat content (%)	-0.14	0.11	—	—	-1.18	0.00**	—	—
TAG								
CN26	0.01	0.40	0.01	0.38	-0.01	0.36	-0.01	0.58
CN28	0.00	0.90	0.00	0.99	0.01	0.73	0.00	0.96
CN30	-0.03	0.60	-0.03	0.56	0.00	0.97	0.01	0.94
CN32	-0.11	0.21	-0.13	0.16	0.02	0.89	-0.07	0.71
CN34	-0.36	0.03	-0.40	0.01*	-0.07	0.75	-0.34	0.26
CN36	-0.76	0.01*	-0.76	0.01*	-0.88	0.01*	-0.86	0.06
CN38	-0.30	0.13	-0.25	0.20	-0.53	0.02	-0.19	0.55
CN40	0.37	0.02	0.38	0.02	-0.04	0.81	0.00	1.00
CN42	-0.06	0.65	-0.07	0.63	-0.18	0.43	-0.16	0.61
CN44	-0.33	0.06	-0.36	0.04	-0.09	0.67	-0.21	0.51
CN46	-0.25	0.12	-0.28	0.08	-0.01	0.97	-0.17	0.55
CN48	-0.13	0.43	-0.17	0.32	0.22	0.24	-0.11	0.68
CN50	0.25	0.30	0.23	0.36	0.45	0.18	0.10	0.83
CN52	0.90	0.03	0.95	0.02	1.13	0.04	1.19	0.13
CN54	0.81	0.00**	0.80	0.00**	-0.37	0.26	-0.58	0.24

¹Estimates and *P*-values calculated from models 1 and 2. Results are from the solution for fixed effects where genotype *KK* and winter were set to zero.

* $P < 0.01$, ** $P < 0.001$: significantly different TAG among seasons (summer vs. winter).

season on TAG composition in MF. The effect of season is most likely due to diet changes between seasons. Winter MF samples showed a higher relative concentration of TAG species CN34 (-0.40 ; Table 2, model [2]) and CN36 (-0.76 ; Table 2, model [1]). The summer MF samples, in contrast, showed higher relative concentrations of TAG species CN40 (0.37), CN52 (0.90), and CN54 (0.81) (Table 2, model [1]).

To further explore the differences between summer and winter TAG profiles and FA composition, we calculated Pearson correlation coefficients between TAG and FA (Table 3). Tzompa-Sosa et al. (2016) showed that FA with common synthesis pathways have similar effects on TAG composition. This trend was also observed in our results; the correlations were similar between TAG and FA for TAG species CN26 to CN34 and CN48 to CN52 with de novo FA (C6:0 to C14:0). The de novo FA (C6:0 to C14:0) were positively correlated with TAG species CN26 to CN34 and negatively correlated with TAG species CN48 to CN52. Most FA with chain lengths >18 carbons were positively correlated with TAG species CN50, CN52, and CN54, which indicates that these FA are most likely in the structure of these TAG species. These findings are consistent with previous studies (Capuano et al., 2014; Tzompa-Sosa et al., 2016; Liu et al., 2017) and are associated with the fact that feeding dairy cows PUFA present in pastures (C18:3 *cis*-9,12,15) reduces de novo synthesized FA and increases LCFA in MF (Chilliard et al., 2001); hence, inducing a significant increase in the high-carbon-chain TAG species CN52 and CN54 in summer. Accordingly, it has been reported that the high-carbon-number TAG species (CN50 to CN54) are mainly composed of C18:0, C18:1 *cis*-9 and C16:0 (Gresti et al., 1993; Jensen, 2002). The FA composition is reported in Supplemental Table S1 (<https://doi.org/10.3168/jds.2019-16554>). These FA differed significantly ($P < 0.001$) between seasons in both models (Supplemental Table S2; <https://doi.org/10.3168/jds.2019-16554>).

The *cis*-9 FA, resulting from Δ^9 -desaturase activity in the mammary gland, had similar significant correlation trends between TAG species CN28 to CN46 and CN48 to CN52. Both C17:1 *cis*-9 and C18:1 *cis*-9 were negatively correlated with TAG species CN28 to CN38 and CN42 to CN46 and positively correlated with CN48 to CN52. The FA C16:1 *cis*-9 was negatively correlated with CN28 to CN32 and, along with C14:1 *cis*-9, was negatively correlated with CN40 and positively correlated with CN44 and CN48. The FA C18:1 *cis*-9 was found to differ significantly among seasons ($P < 0.001$), with increased concentrations in summer. According to previous studies (Jensen, 2002), C18:1 *cis*-9 is incorporated in the structure of high-carbon-chain TAG spe-

cies CN48 to CN52. The increase of high-carbon TAG (CN48–CN54) in summer can be explained by the increased levels of C18:1 *cis*-9, which can be attributed to the intake of fresh grass. It is known that C18:1 *cis*-9 is mainly formed from the biohydrogenation of PUFA and the subsequent desaturation of C18:0. Finally, C14:1 *cis*-9, which is mainly de novo synthesized, showed significantly ($P < 0.001$) higher levels in winter than in summer. It was negatively correlated with low-carbon-chain TAG (CN28 to CN32) and positively correlated with middle-chain-carbon TAG (CN44 to CN48). These findings suggest that there is a preference for C14:1 *cis*-9 to be included in the structure of the TAG species (CN44 to CN48). In the literature reviewed for this study, we found evidence only for C18:1 *cis*-9 (Tzompa-Sosa et al., 2014) and its relations with TAG composition and structure. The samples used for Tzompa-Sosa et al. (2014) were also collected and selected from the Dutch Milk Genomics project.

The relative concentrations of long-chain odd- and branched-chain FA (OBCFA; $>C14$), specifically C15:0 *anteiso*, C17:0 *iso*, and C17:0 *anteiso*, were significantly higher ($P < 0.001$) in summer than in winter in both models (Supplemental Table S2; <https://doi.org/10.3168/jds.2019-16554>). These findings are consistent with that of Heck et al. (2009). Furthermore, this group of FA was positively correlated with TAG species CN50 ($P < 0.05$), CN52 ($P < 0.05$), and CN54 ($P < 0.001$), which might be explained by the increase in the forage:concentrate ratio in the feed that leads to higher relative concentrations of *iso* FA and lower concentrations of *anteiso* and linear odd-chain FA (Fievez et al., 2012) available for TAG synthesis. There is limited information on the relation between OBCFA and TAG composition in MF. Duncan and Garton (1978) reported differences in proportions of branched-chain FA in subcutaneous TAG of barley-fed ruminants but no studies were found on the relation between OBCFA and TAG composition in MF. Therefore, this is important to consider when relating TAG composition to crystallization behavior in MF because of their low melting points relative to chain length. It is known that *anteiso* FA have particularly low melting points (-14°C to 7°C ; Knothe and Dunn, 2009) and are used by some bacteria to maintain membrane fluidity when exposed to low temperatures (Annous et al., 1997; Vlaeminck et al., 2006).

In winter, as expected, more middle-carbon-chain TAG species (CN34 to CN38 and CN44 to CN48) are present that incorporate mainly short- and medium-chain FA (Capuano et al., 2014). According to previous studies (Gresti et al., 1993; Jensen, 2002; Tzompa-Sosa et al., 2014), the FA composition suggested for these

Table 3. Pearson correlation coefficients between triacylglycerols (TAG) and fatty acids (FA) in milk fat

FA	TAG																
	Fat (%)	CN26	CN28	CN30 ^c	CN32 ^c	CN34 ^{a,b,c}	CN36 ^{a,b}	CN38 ^b	CN40 ^{a,c}	CN42 ^c	CN44	CN46	CN48	CN50 ^{b,c}	CN52 ^{a,b,c}	CN54 ^a	
C4:0	-0.05	-0.02	-0.15	-0.14	-0.11	0.08	0.33*	0.47**	0.08	-0.32*	-0.46**	-0.51**	-0.28*	0.11	0.17	0.02	
C6:0	0.32*	0.24*	0.50**	0.62**	0.66**	0.66**	0.55**	0.62**	0.40**	0.59**	0.29*	-0.11	-0.69**	-0.76**	-0.64**	-0.07	
C8:0	0.22	0.26*	0.69**	0.78**	0.78**	0.58**	0.26*	0.26*	0.50**	0.80**	0.53**	0.12	-0.58**	-0.82**	-0.66**	-0.15	
C10:0	0.21	0.24*	0.73**	0.83**	0.84**	0.61**	0.21	0.15	0.45**	0.89**	0.66**	0.26*	-0.50**	-0.85**	-0.71**	-0.17	
C10:1	0.05	0.24*	0.43**	0.45**	0.41**	0.33*	-0.02	-0.12	0.15	0.46**	0.50**	0.40**	0.01	-0.42**	-0.44**	-0.07	
C11:0	0.30**	0.13	0.61**	0.61**	0.55**	0.31*	0.07	-0.05	0.30*	0.63**	0.48**	0.25*	-0.23	-0.53**	-0.52**	-0.07	
C12:0	-0.10	0.08*	0.61**	0.68**	0.79**	0.57**	-0.02	-0.13	0.20	0.73**	0.73**	0.54**	-0.14	-0.70**	-0.57**	-0.27*	
C12:1	-0.13	0.09	0.35*	0.35*	0.38**	0.24*	-0.24*	-0.36*	-0.01	0.40**	0.58**	0.61**	0.24*	-0.33*	-0.32*	-0.12	
C14:0 <i>iso</i>	0.09	0.02	-0.29*	-0.30*	-0.37*	-0.25*	-0.06	0.03	-0.11	-0.25*	-0.26*	-0.15	0.10	0.27*	0.20	0.37*	
C14:0 <i>iso</i>	-0.23	0.12	0.56**	0.60**	0.71**	0.62**	0.10	-0.10	0.04	0.62**	0.62**	0.49**	-0.05	-0.58**	-0.55**	0.37*	
C15:0 <i>iso</i>	-0.03	-0.04	-0.16	-0.20	-0.23	-0.22	-0.08	0.04	0.06	-0.20	-0.32*	-0.33*	-0.16	0.15	0.19	0.42*	
C14:1 <i>cis</i> -9	-0.01	0.08	0.04	0.02	0.01	0.03	-0.19	-0.32*	-0.25*	0.07	0.33*	0.52**	0.46**	0.00	-0.14	-0.06	
C15:0 <i>anteiso</i>	-0.30*	-0.07	-0.10	-0.24*	-0.32*	-0.36*	-0.40**	-0.34*	0.02	-0.29*	-0.23*	-0.12	0.09	0.26*	0.32*	0.55**	
C15:0	0.25*	0.08	0.00	0.00	-0.08	-0.02	0.12	0.06	0.00	0.00	-0.07	-0.09	-0.02	0.04	-0.13	0.23*	
C16:0 <i>iso</i>	0.05	0.01	-0.18	-0.19	-0.28	-0.22	-0.09	0.05	0.04	-0.18	-0.29*	-0.26*	-0.07	0.17	0.21	0.34*	
C16:0	0.42**	-0.07	-0.28*	-0.15	-0.02	0.37**	0.71**	0.43**	-0.55**	-0.05	0.13	0.22	0.21	-0.06	-0.43**	-0.23	
C16:1 <i>trans</i> -9	-0.29*	0.00	0.01	-0.13	-0.27*	-0.41**	-0.42**	-0.34**	0.23*	-0.23*	-0.33*	-0.31*	-0.06	0.28*	0.41**	0.46**	
C16:1 <i>cis</i> -9	0.37**	0.09	-0.35*	-0.33*	-0.34*	-0.21	0.09	-0.01	-0.35*	-0.18	0.06	0.28*	0.42**	0.20	-0.01	-0.06	
C17:0 <i>iso</i>	-0.42**	-0.10	-0.20	-0.34*	-0.46**	-0.52**	-0.54**	-0.40**	0.00	-0.40*	-0.36**	-0.17	0.21	0.46**	0.55**	0.49**	
C17:0 <i>anteiso</i>	-0.26*	0.02	-0.09	-0.19	-0.29*	-0.37*	-0.32*	-0.31*	0.02	-0.26*	-0.25*	-0.11	0.14	0.23*	0.32*	0.42**	
C17:0	0.01	-0.01	-0.34*	-0.39**	-0.53**	-0.46**	-0.11	0.03	0.03	-0.43**	-0.55**	-0.50**	-0.04	0.48**	0.47**	0.29*	
C17:1 <i>cis</i> -9	-0.03	0.00	-0.55**	-0.61**	-0.71**	-0.65**	-0.32*	-0.13	-0.11	-0.53**	-0.49**	-0.28*	0.26*	0.63**	0.63**	0.21	
C18:0	0.07	-0.11	-0.21	-0.25*	-0.30*	-0.39**	-0.21	0.14	0.37*	-0.20	-0.46**	-0.55**	-0.37**	0.21	0.41**	0.34*	
C18:1 <i>trans</i> -6	-0.31**	-0.17	0.15	0.03	-0.10	-0.41**	-0.55**	-0.42**	0.28*	-0.15	-0.24*	-0.22	-0.02	0.21	0.45**	0.04	
C18:1 <i>trans</i> -9	-0.39**	-0.08	0.24*	0.12	-0.03	-0.41**	-0.58**	-0.51**	0.21	-0.09	-0.06	-0.01	0.12	0.18	0.38**	0.05	
C18:1 <i>trans</i> -10	-0.23*	-0.10	0.13	0.05	-0.07	-0.38**	-0.53**	-0.54**	0.18	-0.07	-0.08	-0.02	0.08	0.14	0.33*	-0.15	
C18:1 <i>trans</i> -11	-0.24*	0.00	0.06	-0.07	-0.22	-0.39**	-0.39**	-0.29*	0.24*	-0.18	-0.29*	-0.33*	-0.10	0.23*	0.37**	0.45**	
C18:1 <i>cis</i> -9	-0.40**	-0.08	-0.29*	-0.45**	-0.56**	-0.71**	-0.67**	-0.41**	0.13	-0.53**	-0.52**	-0.33*	0.19	0.63**	0.82**	0.19	
C18:1 <i>cis</i> -11	-0.17	0.09	0.11	0.04	-0.05	-0.29*	-0.49**	-0.29*	0.23*	-0.01	-0.07	-0.09	-0.02	0.12	0.37**	0.01	
C18:1 <i>cis</i> -12	-0.20	0.07	0.39**	0.38**	0.31*	0.00	-0.30*	-0.29*	0.17	0.22	0.22	0.17	0.00	-0.17	0.01	-0.21	
C18:1 <i>cis</i> -13	-0.29*	0.09	0.08	-0.01	-0.13	-0.39**	-0.60**	-0.45**	0.24*	-0.08	-0.10	-0.08	0.06	0.23	0.41*	0.28*	
C19:0	-0.12	0.00	-0.15	-0.16	-0.24	-0.20	-0.11	0.02	0.12	-0.18	-0.29*	-0.33*	-0.12	0.21	0.26*	0.10	
C18:2 <i>cis</i> -9,12	-0.37**	0.14	0.33*	0.26*	0.17	-0.15	-0.40*	-0.33*	0.20	0.12	0.08	0.05	0.02	-0.01	0.20	-0.22	
C18:3 <i>cis</i> -9,12,15	-0.25*	0.02	-0.12	-0.23*	-0.34*	-0.42**	-0.48**	-0.21	0.17	-0.27*	-0.35*	-0.31*	-0.04	0.32*	0.46**	0.35*	
FA groups																	
SFA	0.40**	0.04	0.17	0.34*	0.49**	0.72**	0.75**	0.54**	-0.16	0.44**	0.40**	0.23*	-0.20	-0.55**	-0.74**	-0.27*	
UFA	-0.40**	-0.04	-0.19	-0.36*	-0.51**	-0.72**	-0.74**	-0.52**	0.15	-0.45**	-0.43**	-0.24*	0.22	0.58**	0.76**	0.25*	
Short-chain FA	0.12	0.21	0.73**	0.84**	0.91**	0.74**	0.31*	0.29*	0.44**	0.82**	0.60**	0.21	-0.55**	-0.89**	-0.72**	-0.24	
Medium-chain FA	0.36**	-0.01	-0.16	-0.03	0.11	0.47**	0.67**	0.33*	-0.56**	0.09	0.30*	0.40*	0.28*	-0.17	-0.55**	-0.26*	
Long-chain FA	-0.35**	-0.08	-0.21	-0.37**	-0.51**	-0.73**	-0.69**	-0.37**	0.28*	-0.45**	-0.55**	-0.46**	0.00	0.55**	0.81**	0.34*	
Odd- and branched-chain FA	-0.09	0.03	-0.21	-0.31*	-0.44**	-0.45**	-0.29*	-0.20	0.06	-0.32*	-0.39**	-0.28*	0.06	0.37*	0.37*	0.52**	

*FA and TAG significantly different ($P < 0.05$) between seasons (winter and summer).^bFA and TAG significantly different ($P < 0.05$) between *DGAT1* genotypes AA and KK.^cFA and TAG with significant difference ($P < 0.05$) for the interaction effect *DGAT1* genotypes $\times$ season.* $P < 0.05$, ** $P < 0.001$; correlations with significant difference.

TAG species are SCFA, C14:0, C16:0, C18:0, and C18:1 *cis*-9. In our study, all FA besides SCFA were found to be different ($P < 0.010$) between seasons for both models (Supplemental Table S2; <https://doi.org/10.3168/jds.2019-16554>); C14:0 was no longer significantly different between seasons when differences in fat content were not accounted for in the model.

In general, we concluded that the main changes between winter and summer MF TAG composition were the higher levels of high-carbon-chain TAG species CN54 in summer and higher levels of middle-carbon-chain TAG (CN34 and CN36) species in winter. These differences can be explained by the changes in FA composition (C14:0, C16:0, C18:0 C18:1 *cis*-9, UFA, and LCFA), which are most likely due to differences in diet between seasons.

DGAT1 K232A Polymorphism

The effects of *DGAT1* genotype *AA* and *KK* on MF TAG composition are presented in Table 2. Milk fat samples from genotype *AA* had higher relative concentrations of TAG species CN28 (0.01), CN32 (0.02), CN48 (0.22), CN50 (0.45), and CN52 (1.13) (Table 2, model [1]). The TAG species CN34 (−0.07), CN36 (−0.88), CN38 (−0.53), CN42 (−0.18), CN44 (−0.09), and CN54 (−0.37) were higher for genotype *KK* (Table 2, model [1]). No TAG species were significantly different ($P < 0.01$) between genotypes when accounting for differences in fat content (model [2]). If differences in fat content were not considered in the model (Table 2, model [1]), significant differences ($P < 0.01$) were observed for TAG species CN36. Therefore, the *DGAT1* K232A genotype did affect TAG composition in MF but these effects can be explained by the effects of the *DGAT1* K232A polymorphism on fat content (Table 2, model [2]). The TAG species CN36 is positively correlated with short- and medium-chain FA (mainly SFA). Schennink et al. (2007) showed that the *DGAT1* K232A *KK* genotype is associated with higher fat content and higher levels of de novo synthesized short- and medium-chain FA, explaining the higher levels for CN36. Tzompa-Sosa et al. (2016) reported a significant difference for TAG species CN38 between *AA* and *KK* genotypes in winter samples, which was positively associated with the effect of genotype *KK* on the TAG composition in MF; this difference was not found in our study but it was in the same direction ($P = 0.02$). Tzompa-Sosa et al. (2016) selected cows with similar fat content in their study and therefore differences in fat content were relatively small. In the current study, differences in fat content between cows are taken into account using model [2]. Hence, a model that does not

account for differences in fat content shows an effect of *DGAT1* K232A genotype on TAG composition (in our study, on TAG CN36). Model [2] shows that *DGAT1* K232A genotype has no effect on TAG composition after accounting for differences in fat content in MF. These results should not be influenced by the different lactation stages (DIM) in summer and winter in the cows selected for this study. Bovenhuis et al. (2016) showed that the effect of *DGAT1* is not constant but changes during lactation. However, these changes occur rarely during early lactation and therefore are not expected to affect the results reported.

Interaction of DGAT1 K232A Polymorphism and Season

The significance of the interaction of *DGAT1* genotype (*AA* or *KK*) and season (winter/summer) on MF TAG composition is shown in Table 4. The TAG species CN32 (model [3]), CN42 and CN52 (models [3] and [4]) were found to be significant ($P < 0.01$).

Figure 1 shows the estimated effects between *AA* and *KK* genotypes and season for TAG species CN42 and CN52, which showed a significant ($P < 0.01$) *DGAT1* × season interaction but were not significantly affected

Table 4. Interaction of season × *DGAT1* K232A genotypes effect on fat content (%) and triacylglycerol (TAG) composition (P -value) based on 25 individual cow samples for each genotype¹

Item	P -value	
	Model [3]	Model [4]
Fat content (%)	0.63	
TAG		
CN26	0.23	0.24
CN28	0.16	0.13
CN30	0.03	0.03
CN32	0.02	0.01*
CN34	0.03	0.02
CN36	0.14	0.13
CN38	0.16	0.19
CN40	0.05	0.05
CN42	0.00**	0.00**
CN44	0.24	0.18
CN46	0.98	0.93
CN48	0.08	0.11
CN50	0.0*	0.03
CN52	0.01*	0.01*
CN54	0.16	0.15

¹Estimates and P -values calculated from models [3] and [4]. Results are from the solution for fixed effects where genotype *KK* and winter were set to zero.

* $P < 0.05$, ** $P < 0.001$: significantly different TAG; TAG species found significantly different for both models [3] and [4] show that regardless of whether differences in fat content are considered, there is an effect of the interaction of season × *DGAT1* K232A genotypes on that TAG species.

Figure 1. Estimated effects of *DGAT1* K232A AA versus KK in winter and summer based on 25 individual cow samples for triacylglycerol (TAG) species CN42 (a) and CN52 (b). Both TAG species were significantly different with and without accounting for differences in fat content for the interaction effect season $\times$ *DGAT1* K232A AA versus KK. Genotype KK was set to zero in winter for both TAG species. The error bars indicate the SE of the solution of the model for fixed effects.

by *DGAT1* or season (Table 2). The estimated effects were obtained from model [3] without the main effects (season and *DGAT1*). Effects of *DGAT1* genotypes AA and KK on TAG species CN42 were reversed in winter and summer: in summer, KK cows had higher levels of TAG CN42 than AA cows, whereas in winter, AA cows tended to have higher levels than KK cows, although this difference was not significant. In contrast, *DGAT1* AA cows had higher levels of CN52 than KK cows in both seasons but in winter the difference was not significant. In summer, UFA and LCFA were higher in AA cows than in KK cows and SFA were higher in KK cows than in AA cows (Figure 2). This is in agreement with previous studies (Duchemin et al., 2013; Tăbăran

et al., 2015). The SCFA showed a different trend, where the KK genotype had higher relative concentrations in summer and lower relative concentrations in winter than the AA genotype (Figure 2d).

Our findings suggest that dietary FA variations due to seasonal changes have affected the FA esterified by the different *DGAT1* genotypes. According to previous studies, the structure of the TAG species CN42 and CN52 have the following FA in the *sn*-3 position: CN42—C10:0, C12:0, C8:0, C6:0, C16:1, C14:1 *cis*-9, and CN52—C16:0, C16:1 *cis*-9, and C18:1 *cis*-9 (Gresti et al., 1993; Jensen, 2002). Consequently, in our study, significant differences in both models ($P < 0.01$) were found for FA C6:0, C10:0, and C18:1 *cis*-9 levels under the interaction effect of *DGAT1* genotype and season (Supplemental Table S3; <https://doi.org/10.3168/jds.2019-16554>). Considering the role of the *DGAT1* enzyme as catalyzing agent in the last step of TAG synthesis (Lehner and Kuksis, 1996; Yen et al., 2008) and the association of the KK genotype with increased preference for esterifying SCFA and C16:0 in the *sn*-3 position of a diacylglycerol (Tăbăran et al., 2015; Tzompa-Sosa et al., 2016), we speculate that these FA are most likely to be found at the *sn*-3 position and preferred by *DGAT1*. In addition, we suggest that, according to the pool of FA available in each season, the preferences of the genotypes for a specific type of FA might change during TAG synthesis, always keeping the structural characteristics of each TAG species. This could be a mechanism regulated by the cow to lower the melting point of TAG during MF synthesis, as previously suggested (Tzompa-Sosa et al., 2016). To achieve this, the mammary gland increases the degree of unsaturation of the LCFA during lactation in a seasonal system, increases the proportion of SCFA and MCFA, and uses asymmetric positioning of SCFA on the glycerol backbone of the TAG (Dils, 1986). Hence, considering the FA in the *sn*-3 position, in summer, when the available pool of FA is mainly UFA and LCFA, for TAG species CN42 the KK genotype will most likely esterify UFA as well as SFA. In winter, when the pool of FA is mainly SCFA, MCFA, and SFA, the AA genotype will favor esterification of SCFA at first (Figure 2d) in these TAG structures; therefore, the preference of the genotype will be altered. In summer, the AA genotype will favor esterification of UFA and LCFA in TAG CN52, whereas the KK genotype will favor esterification of SFA. This is probably because of the positioning of C18:1 *cis*-9 in CN52 (Gresti et al., 1993; Jensen, 2002). In winter, the AA genotype will likely favor SFA (C16:0) and UFA (C18:1 *cis*-9) for esterification of CN52. Tzompa-Sosa et al. (2016) identified an increase in CN38 in winter MF TAG composition; however, in our study, this TAG

Figure 2. Estimated effect of *DGAT1* K232A AA versus KK in winter and summer for fatty acid (FA) groups UFA (a), SFA (b), long-chain FA (c) and short-chain FA (d) based on 25 individual cow samples. Genotype KK was set to zero in winter for all FA groups. The UFA include C16:1 *trans*-9, C16:1 *cis*-9, C18:1 *trans*-6, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, C18:1 *cis*-9, C18:1 *trans*-15, C18:1 *cis*-11, C18:1 *cis*-12, C18:1 *cis*-13, C18:2 *cis*-9,12, C18:3 *cis*-9,12,15, C18:2 *cis*-9,*trans*-11, C20:1 *cis*-11, C20:3 *cis*-8,11,14, and other UFA with lower relative concentrations; SFA include C4:0, C5:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0, C19:0, C20:0, and C22:0; long-chain FA include from C18:0 to docosahexaenoic acid; short-chain FA include from C4:0 to C12:0. The error bars indicate the SE of the solution of the model for fixed effects.

species was not significantly different when the interaction effect of *DGAT1* genotype and season was taken into account. Nevertheless, our results show the same trend in AA and KK genotypes on the esterification of middle-carbon-chain TAG species (i.e., CN42) in winter.

CONCLUSIONS

The main goal of the current study was to investigate changes in TAG composition as affected by the changes in cows' diet due to the seasonal variations and genetic traits. The main changes between winter and summer in MF TAG composition, with and without considering

the differences in fat content, were the increase of TAG species CN54 in summer and of CN34 and CN36 in winter. Our results suggest that diet changes among seasons have a great effect on MF TAG composition. We showed that FA C14:0, C16:0, C18:0, C18:1 *cis*-9, UFA, and LCFA are the main FA involved in changes in MF TAG composition. The *DGAT1* K232A polymorphism has an individual effect on TAG composition when differences in fat content are not accounted for, such that TAG species CN36 showed a significant increase with the KK genotype. Furthermore, the interaction effect of *DGAT1* K232A genotype and diet according to seasonal variation was found to be significant, with and without accounting for differences in fat

content for TAG species CN42 and CN52. Thus, there is an interaction effect of DGAT1 and season on TAG composition in MF. Regardless of the preference of the genotype (AA/KK) and depending on the availability of FA according to season, the mammary gland will most likely esterify UFA (C18:1 *cis*-9), SCFA (C6:0 and C10:0) to the *sn*-3 position, and MCFA on the glycerol backbone of the TAG, keeping the structural characteristics of each TAG. We propose that this is a mechanism of the mammary gland to maintain the melting point of TAG during MF synthesis. Further compositional and structural analyses should be done to confirm our proposed mechanism of the *DGAT1* K232A polymorphism during MF TAG synthesis. To our knowledge, this is the first report where the combined effect of *DGAT1* K232A polymorphism was analyzed in MF.

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