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# Factors associated with the concentration of fatty acids in the milk from grazing dairy cows

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## ABSTRACT

Bovine milk fat contains ~400 different fatty acids, with their relative concentration being highly variable and influenced by (or associated with) intrinsic and extrinsic factors such as diet, breed, and lactation stage. Using predicted fatty acids from milk mid-infrared spectra, the objectives of the present study were to (1) quantify the association between cow parity, lactation stage, breed, heterosis, and recombination loss with milk fatty acid composition, and (2) quantify the association between parental average genetic merit for both milk fat concentration and yield with a series of different fatty acids. The dataset used included 644,752 milk test-day records from 303,089 cows across 2,406 commercial dairy farms. The concentration of 16 individual fatty acids as well as 16 fatty acid indices were predicted from spectral analyses of milk samples. Factors associated with all the investigated traits were individually explored using linear mixed models. The fixed effects considered in all models were the interaction between parity and stage of lactation, the calendar month of test, breed composition, heterosis, and recombination; the random effects were contemporary group and a within-lactation repeated effect. In a separate series of analyses, cow genetic merit for fat yield and fat percentage were separately included in the model as linear covariates. The concentration of the different fatty acids changed throughout lactation and across calendar months, coinciding with the seasonal profile in pasture quality, especially during the summer months. Multiparous cows were characterized by milk with a higher concentration of saturated and short-chain fatty acids, along with a lower concentration of unsaturated, medium-chain, and long-chain fatty acids. Jersey bloodline was associated with a higher concentration of milk saturated and short-chain fatty acids, along with a lower concentration of unsaturated, medium-chain, and long-chain fatty acids compared with Holstein-Friesians. Genetic merit for fat yield or fat percentage was associated with a higher concentration of saturated fatty acids and a lower concentration of unsaturated fatty acids.
**Key words:** lactation curve, genetic, mid-infrared, fatty acids predictions, milk

## INTRODUCTION

Bovine milk fat is highly complex, composed of ~400 different fatty acids, typically derived from 2 main sources: those produced within the animal itself through the mammary gland (i.e., de novo synthesis), and preformed fatty acids originating from dietary sources (Månsson, 2008). Fatty acids can be further stratified based on chain length, including short- (C4:0–C6:0), medium- (C8:0–C15:0), and long-chain (≥C16:0) fatty acids, as well as separately based on saturation level, including SFA and UFA. Similarly, UFA can themselves be further stratified into MUFA and PUFA.

Milk fat composition and concentration are both highly variable and can be affected by (or associated with) several intrinsic and extrinsic factors, such as diet, breed, and stage of lactation. Changes in milk fatty acid composition can affect the functional properties of dairy products (MacGibbon, 2020); therefore, it is important to understand the factors contributing to changes in milk fatty acid composition. With this in mind, it is particularly important to quantify how extrinsic factors and current genetic selection practices for milk fat concentration affect or are associated with milk fatty acid profiles. For example, different stages of lactation can have a substantial effect on the quality attributes of commodity and high-value dairy products like butter, such as color, texture, and flavor (Timlin et al., 2024).

One of the widely and routinely used methods to quantify the concentration of various fatty acids in milk is

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

gas chromatography. Nonetheless, gas chromatography can be labor intensive and time-consuming, so high throughput of (milk) samples using this approach can be slow and expensive. Several studies (Soyeurt et al., 2011; Grelet et al., 2014) have demonstrated the potential of mid-infrared (**MIR**) spectroscopy analysis of milk to predict the individual fatty acid content of the milk. Mid-infrared spectroscopy is routinely used to quantify the main milk components of both bulk tank and individual animal samples. Therefore, the usefulness of leveraging these predictions is that they are available for all milk samples that have an associated stored MIR spectrum, contributing to a large dataset across many different herds and years. The accuracy of prediction in milk from the MIR differs by fatty acid (Grelet et al., 2014), with good accuracy (i.e., coefficient of determination in cross-validation >0.90) for fatty acid indices (i.e., total SFA, total UFA, total short-chain fatty acids [**SCFA**], total medium-chain fatty acids [**MCFA**], and total long-chain fatty acids [**LCFA**]), but limited accuracy (i.e., coefficient of determination in cross-validation <0.64) for fatty acids with lower concentrations in the milk (i.e., C14:1 *cis*, C16:1 *cis*, total C18:2, C18:3 *cis*-9,*cis*-12,*cis*-15).

Limited research has been conducted on the associations between cow-level factors like breed, parity, or stage of lactation with milk fatty acids at a national herd or population level using data collected over many years, in particular using data collected from a pasture-based, seasonal calving production system. Therefore, the objectives of the present study were to (1) explore the associations between cow parity, lactation stage, breed, heterosis, and recombination loss with 32 milk fatty acid as well as fat percentage, and (2) investigate the association between genetic merit for both milk fat concentration or yield on the profile of 32 milk fatty acids. Of particular interest in the present study, given it was a large population of grazing dairy cows, was how the fatty acid concentration in milk changed across the calendar year coinciding with differences in the quantity and quality of available pasture.

## MATERIALS AND METHODS

### Data Handling

Animal data, including cow parity, calving dates, animal breed composition, and date of milk testing, along with the respective MIR spectra and milk yield, MIR-predicted milk fat concentration, and MIR-predicted milk protein concentration were available from a sample of Irish dairy cows, all raised in Ireland, between the years 2015 and 2020. The dataset included 644,752 milk test-day records from 303,089 cows across 2,406 commercial farms. Of these records, 289,280 lactations had a single record throughout the study period, with a further 152,799 lactations having between 2 and 15 records. Nonetheless, although lactations had only a single record, the cow may have had multiple lactations. Indeed, a total of 172,200 cows had multiple lactations with milk fatty acid data. Of the total number of cows, 85% were spring-calving cows (those calving between January and May). Parity number varied from 1 to 16 and DIM varied from 5 to 305. The number of records by year, parity, and breed are in Table 1; the number of cows having single or repeated records, as well as the number of lactations having single or repeated records, are in Supplemental Table S1 (see Notes). A histogram of the number of records per stage of lactation is in Supplemental Figure S1 (see Notes).

**Table 1.** Number of records and number of cows by year, by parity group, and breed

| Variable | Records | Cows |
|---|---|---|
| Year | | |
| 2015 | 5,656 | 3,066 |
| 2016 | 13,344 | 4,220 |
| 2017 | 268,835 | 173,769 |
| 2018 | 201,821 | 142,850 |
| 2019 | 142,828 | 112,277 |
| 2020 | 12,268 | 8,070 |
| Parity group | | |
| 1 | 168,067 | 114,210 |
| 2 | 142,077 | 96,688 |
| 3+ | 334,608 | 166,203 |
| Breed | | |
| Holstein-Friesian | 518,109 | 237,078 |
| Jersey | 1,443 | 871 |
| Montbéliarde | 1,732 | 867 |
| Normande | 66 | 24 |
| Others | 123,402 | 64,252 |

The breed composition of each animal was available on a percentage basis for each breed. The breeds considered in the study were Holstein-Friesian, Jersey, Normande, and Montbéliarde, with all other breeds and crossbreds collapsed into a category of "other breeds" (defined as 100 minus the sum of the percentage of breeds of interest). Heterosis and recombination loss coefficients per cow were computed as follows:

$$\text{Heterosis} = 1 - \sum_{i=1}^{n} \text{sire}_i \times \text{dam}_i,$$

and

$$\text{Recombination loss} = 1 - \sum_{i=1}^{n} \frac{\text{sire}_i^2 + \text{dam}_i^2}{2},$$

where $\text{sire}_i$ and $\text{dam}_i$ were the proportion of breed $i$ in the sire and dam, respectively. Heterosis is the increase in performance of crossbred animals relative to the

average of their purebred parents. Recombination loss occurs in subsequent generations when favorable gene combinations are disrupted, potentially reducing performance. Both heterosis and recombination loss were on a scale of 0 to 1.

Also available for each cow was the genetic merit for milk fat yield and milk fat percentage. Irish national genetic evaluations for milk fat yield and milk fat percentage are routinely estimated using a test-day animal mixed model, which includes the population mean, cow heterosis, recombination loss, age at calving, days in calf, herd test-day, as well as an interaction between calving year and parity, and random regressions representing the animal genetic effect, the permanent environmental effect, and the within-lactation environmental effect (McCarthy and Veerkamp, 2012). Cow genetic merit values for fat yield and fat concentration used in the present study were calculated as the sum of the cow's parental predicted transmitting ability from the May 2024 Irish national genetic evaluation; this will be referred to hereafter as the pedigree index of the cow for fat yield and fat concentration. The animal's own phenotypic data were therefore not directly included in its estimate of genetic merit in order to avoid shared nongenetic effects between the estimate of genetic merit and phenotypic performance, which could inflate the relationship in the subsequent statistical analyses.

### Fatty Acid Quantification

Each milk sample was analyzed using FOSS MIR spectrometers, and the milk fat percentage, protein percentage, and the generated MIR spectra were stored. The generated MIR spectra were used to estimate the concentration of various fatty acids in the milk using prediction models developed in the OptiMIR project (Grelet et al., 2014). The predicted fatty acids were in grams per deciliter (g/dL) of milk. These equations, created using the MIR spectra from individual cows, had coefficients of determination in external validation ranging from 0.60 for C18:3 *cis*-9,*cis*-12,*cis*-15 to 0.99 for SFA yield (Table 1; Grelet et al., 2014). For the current study, the predicted fatty acids yield was then converted to grams per 100 g of milk fat. Separately for each fatty acid, observations 1.5 times the interquartile range above the third quartile or below the first quartile were considered outliers and excluded from further analyses.

### Statistical Analyses

Factors associated with all 33 traits were individually explored using linear mixed models in R version 4.4.2 (Posit team, 2025); model solutions and predicted marginal means were derived from the fitted models. All available animals in the dataset were included in the analysis, even those with no information on their sire. The reference animal used to estimate the predicted marginal means was a mid-lactation (i.e., fifth stage of lactation) second-parity Holstein-Friesian cow. The fitted model was

$$y_{ijklm} = stage_i + parity_j + stage_i \times parity_j, + month_k + b_1\, Jersey + b_2\, Normande + b_3\, Montbéliarde + b_4\, other\ breeds + b_5\, heterosis + b_6\, recombination + within\_lactation_l + CG_m + e_{ijklm},$$

where $y_{ijklm}$ was the milk phenotype of interest; $stage_i$ was the fixed effect for the stage of lactation (10 classes: $i$ = 5–30, 31–60, 61–90, 91–120, 121–150, 151–180, 181–210, 211–240, 241–270, 271–305); $parity_j$ was the fixed effect for the parity (3 classes: $j$ = 1, 2, and 3+); $stage_i \times parity_j$ was the fixed effect representing the interaction between the stage of lactation class and the parity class; $month_k$ was the fixed effect of the calendar month when the milk sample was collected (10 classes; $k$ = February, March, . . . , November); *Jersey*, *Normande*, *Montbéliarde*, and *other breeds* were the covariates of the percentage of Jersey, Normande, Montbéliarde, and other breeds in the cow multiplied by the regression coefficients $b_1$, $b_2$, $b_3$, and $b_4$, respectively; *heterosis* was the heterosis covariate for the cow multiplied by the regression coefficient $b_5$; *recombination* was the recombination loss covariate for the cow multiplied by the regression coefficient $b_6$; $within\_lactation_l$ was the random environmental effect within parity of the cow lactation where $within\_lactation_l \sim$ iid $N\left(0, \sigma^2_{wl}\right)$, with $\sigma^2_{wl}$ representing the animal variance within parity; contemporary groups ($CG_m$), defined as a combination of herd and date of milk sample collection, was the random effect of the contemporary group where $CG_m \sim$ iid $N\left(0, \sigma^2_{cg}\right)$, with $\sigma^2_{cg}$ representing the contemporary group variance; and $e$ was the residual term, where $e_{ijklm} \sim$ iid $N\left(0, \sigma^2_e\right)$, with $\sigma^2_e$ representing the residual variance. To explore the associations between pedigree index for fat percentage or fat yield and the concentration of the individual and groups of fatty acids in the milk, either the pedigree index for fat percentage or fat yield of the animal were included in the model along with all the other variables.

## RESULTS

The raw mean for the individual fatty acids and the fatty acid indices are shown in Tables 2 and 3, respectively. The SCFA, MCFA, and LCFA represented, on average, 8.9%, 50.9%, and 44.2% of the milk fat concentration,

**Table 2.** Number of records, mean, coefficient of determination in cross-validation of the developed models (Accuracy; Grelet et al., 2014), and contemporary group (CG), within-lactation permanent environmental effect (Within lact), and residual SD for the concentration (g/100 g) in fat for a range of individual fatty acids from the mixed model

| | | | | SD | | |
|---|---|---|---|---|---|---|
| Trait | Records | Mean | Accuracy | CG | Within lact | Residual |
| Fat % | 630,251 | 4.21 | 1.00 | 0.53 | 0.41 | 0.52 |
| C4:0 | 632,133 | 2.89 | 0.90 | 0.17 | 0.13 | 0.16 |
| C6:0 | 632,331 | 1.81 | 0.89 | 0.11 | 0.08 | 0.11 |
| C8:0 | 632,647 | 1.18 | 0.88 | 0.10 | 0.06 | 0.09 |
| C10:0 | 632,998 | 2.58 | 0.89 | 0.32 | 0.20 | 0.28 |
| C12:0 | 630,453 | 3.43 | 0.90 | 0.40 | 0.24 | 0.36 |
| C14:0 | 627,876 | 11.90 | 0.91 | 0.91 | 0.44 | 0.81 |
| C14:1 | 626,471 | 1.05 | 0.63 | 0.11 | 0.09 | 0.10 |
| C16:0 | 633,582 | 29.40 | 0.92 | 2.48 | 1.22 | 1.68 |
| C16:1 *cis* | 626,488 | 1.61 | 0.63 | 0.18 | 0.09 | 0.14 |
| C17:0 | 628,237 | 0.66 | 0.67 | 0.03 | 0.02 | 0.02 |
| C18:0 | 632,599 | 11.59 | 0.84 | 1.01 | 0.43 | 0.75 |
| C18:1 *cis*-9 | 633,394 | 21.43 | 0.95 | 2.58 | 1.39 | 1.93 |
| C18:2 | 633,923 | 2.52 | 0.63 | 0.19 | 0.12 | 0.14 |
| C18:2 *cis*-9,*cis*-12 | 635,725 | 1.45 | 0.65 | 0.14 | 0.09 | 0.12 |
| C18:2 *cis*-9,*trans*-11 | 635,227 | 0.81 | 0.69 | 0.24 | 0.11 | 0.15 |
| C18:3 *cis*-9,*cis*-12,*cis*-15 | 633,246 | 0.56 | 0.60 | 0.08 | 0.03 | 0.05 |

respectively; the sum of these percentages is greater than 100%, indicating an over prediction of some (or all) the fatty acid indices. Of the concentration of milk fatty acids, 69.76% were predicted to be SFA and 34.06% were predicted to be UFA. As for the fatty acids chain length, the sum of these percentages also exceeds 100%, suggesting that the predicted content of some (or all) fatty acid indices may be overestimated. Of the concentration of UFA, 88% were predicted to be MUFA with the remainder being PUFA. The correlations between all the individual fatty acids, as well the correlations between all the fatty acids indices, are in Supplemental Figures S2 and S3, respectively (see Notes).

The SD of the contemporary group and within-lactation effect from the mixed model analysis of each of the individual and fatty acid indices are depicted in Tables 2 and 3, respectively. The SD attributable to contemporary group and within-lactation effect is useful to know how much of the variability in a trait is attributable to these features. The proportion of the total variance explained by the within-lactation effect ranged from 9% to 25% (for n-3 and C4:0, respectively), with 39% and 67% of the variance in the individual fatty acids attributable to contemporary group (for total fat percentage and n-3, respectively).

## Parity and Stage Effects

The association between parity and each of the individual fatty acids and fatty acid indices varied by stage of lactation. The concentration in fat of the fatty acid indices across stages of lactation by parity is illustrated in Figure 1 for SCFA, MCFA, and LCFA. The individual parity lactation profiles for SFA, MUFA, and PUFA are in Figure 2. Likewise, the lactation profiles for n-3 and n-6 concentrations are in Figure 3. For most of the fatty acid traits examined, the interaction between parity and stage of lactation was primarily reflected in a difference in the magnitude, rather than the pattern, of fatty acid concentration across parities, with the most notable contrasts being between primiparous and multiparous cows. Irrespective of stage of lactation, fatty acid concentration of C4:0 to C16:0 in the fat generally increased with parity, whereas the fatty acid concentration of C16:1 *cis* to C18:2 reduced with parity. Of the main fatty acid indices, multiparous cows generally had a higher concentration of SCFA and MCFA and a lower concentration of LCFA compared with primiparous cows, with the trend persisting across stages of lactation. First-parity cows had a lower concentration of SFA and a higher concentration of MUFA and PUFA compared with cows in their third or greater parity, and this effect persisted across lactations, although the differences between parities were greatest in early lactation.

Irrespective of parity, the lactation profile for the concentration in fat of the individual fatty acids of C6:0 to C16:0 resembled a milk yield lactation profile, increasing to a peak between 90 and 150 DIM, followed by a linear decline thereafter. The lactation profile of C16:1, C17, and C18:1 *cis*-9 was a mirror image of the C6:0 to C16:0 fatty acids, reducing after calving to early-mid lactation and increasing linearly thereafter. For the fatty acid indices, SCFA and SFA resembled a milk yield lactation profile, whereas LCFA and MUFA

**Table 3.** Number of records, mean, coefficient of determination in cross-validation of the developed models (Accuracy; Grelet et al., 2014), and contemporary group (CG), within-lactation permanent environmental effect (Within lact), and residual SD for the concentration (g/100 g) in fat for a range of groups of fatty acids from the mixed model

| | | | | SD | | |
|---|---|---|---|---|---|---|
| Trait[1] | Records | Mean | Accuracy[2] | CG | Within lact | Residual |
| Total C18:1 | 635,500 | 26.36 | 0.96 | 2.87 | 1.60 | 2.21 |
| Total C18:1 *cis* | 633,599 | 23.09 | 0.95 | 2.40 | 1.49 | 2.05 |
| Total C18:1 *trans* | 636,595 | 3.78 | 0.73 | 0.70 | 0.28 | 0.46 |
| SCFA | 631,509 | 8.87 | 0.92 | 0.58 | 0.38 | 0.55 |
| MCFA | 633,230 | 50.91 | 0.95 | 3.49 | 1.62 | 2.68 |
| LCFA | 634,575 | 44.22 | 0.95 | 4.03 | 1.98 | 2.98 |
| Saturated | 630,567 | 69.76 | 0.99 | 2.99 | 1.65 | 2.36 |
| Unsaturated | 636,254 | 34.06 | 0.96 | 3.27 | 1.78 | 2.40 |
| MUFA | 635,402 | 30.02 | 0.97 | 2.90 | 1.61 | 2.21 |
| PUFA | 636,310 | 4.07 | 0.75 | 0.49 | 0.23 | 0.30 |
| OCFA | 628,970 | 3.87 | 0.76 | 0.22 | 0.12 | 0.16 |
| n-3 | 632,873 | 0.67 | 0.64 | 0.09 | 0.03 | 0.06 |
| n-6 | 634,505 | 2.62 | 0.66 | 0.23 | 0.12 | 0.15 |
| *Trans* FA | 636,935 | 4.78 | 0.76 | 0.89 | 0.34 | 0.56 |
| De novo | 601,249 | 25.02 | — | 1.79 | 0.96 | 1.55 |
| Total branched FA | 627,404 | 2.31 | 0.64 | 0.15 | 0.08 | 0.11 |

[1]SCFA = short-chain fatty acid; MCFA = medium-chain fatty acid; LCFA = long-chain fatty acid; OCFA = odd-chain fatty acid; FA = fatty acid.

[2]No accuracy for the novo fatty acids was reported because no equations for the novo fatty acids exist. In the present study it was quantified as the sum of all the individual fatty acids from C4:0 to C14:1.

resembled a fat concentration lactation profile (Figures 1 and 2). The MCFA concentration increased up to 150 DIM and then decreased, albeit at a slower rate compared with SCFA.

## Breed and Heterosis Effects

The model regression coefficients on proportion of Jersey, Normande, and Montbéliarde for the concentration of each individual fatty acid and fatty acid index are in Tables 4 and 5, respectively. Although the concentration of 31 of the 32 fatty acids and fatty acid indices differed between the Jersey and Holstein-Friesian (the exception was C18:0), the concentration in milk fat for Normande and Montbéliarde cows differed from the concentration in the fat of Holstein-Friesians for 25 and 21 traits (spread across all individual fatty acids and fatty acid indices), respectively.

Regression coefficients for Jersey percentage were all positive from C4:0 to C16:0 but were all negative from C16:1 *cis* to C18:3 *cis*-9,*cis*-12,*cis*-15; this implies that the concentration of C4:0 to C16:0 fatty acids in the fat of Jersey cows was, on average, higher than that of Holstein-Friesians, with the opposite being true for C16:1 *cis* to C18:3 *cis*-9,*cis*-12,*cis*-15. For the fatty acid indices, the regression coefficients for Jersey percentage were positive for SCFA, MCFA, and SFA, but negative for LCFA, UFA, MUFA, and PUFA.

Regression coefficients for the Normande percentage were all positive from C4:0 to C17:0, and negative from C18:0 to C18:2, with no difference relative to Holstein-Friesian for C16:1 *cis* and C18:3 *cis*-9,*cis*-12,*cis*-15. Lastly, for the Montbéliarde percentage, the regression coefficients were all positive, with the exception of C10:0, C12:0, C16:1 *cis*, C17:0, and C18:0 which were all negative.

The regression coefficients on heterosis and recombination loss coefficients for the concentration of both individual fatty acids and fatty acid indices are in Tables 6 and 7, respectively. Regression coefficients for heterosis were always different from 0, with the 2 exceptions of C18:2 *cis*-9,*cis*-12 and MCFA. Regression coefficients were all positive from C6:0 to C14:0 and negative from C14:1 to C18:1 *cis*-9 (with the exception of C17:0, which was positive). For the fatty acid indices, the regression coefficients on heterosis coefficient were positive for SCFA, MCFA, SFA, PUFA, n-3 fatty acids, and n-6 fatty acids. The regression coefficients on recombination loss coefficient were all different from 0, with the exception of C18:0, C18:2, MCFA, and odd-chain fatty acids. The regression coefficients on recombination loss were all positive from C6:0 to C14:0, as well as for C17:0, C18:2 *cis*-9,*trans*-11, and C18:3 *cis*-9,*cis*-12,*cis*-15.

## Genetic Merit

The regression coefficients of the concentration in fat of individual fatty acids or fatty acid indices on cow milk fat yield pedigree index or milk fat percentage pedigree index are in Tables 6 and 7, respectively. An increase in

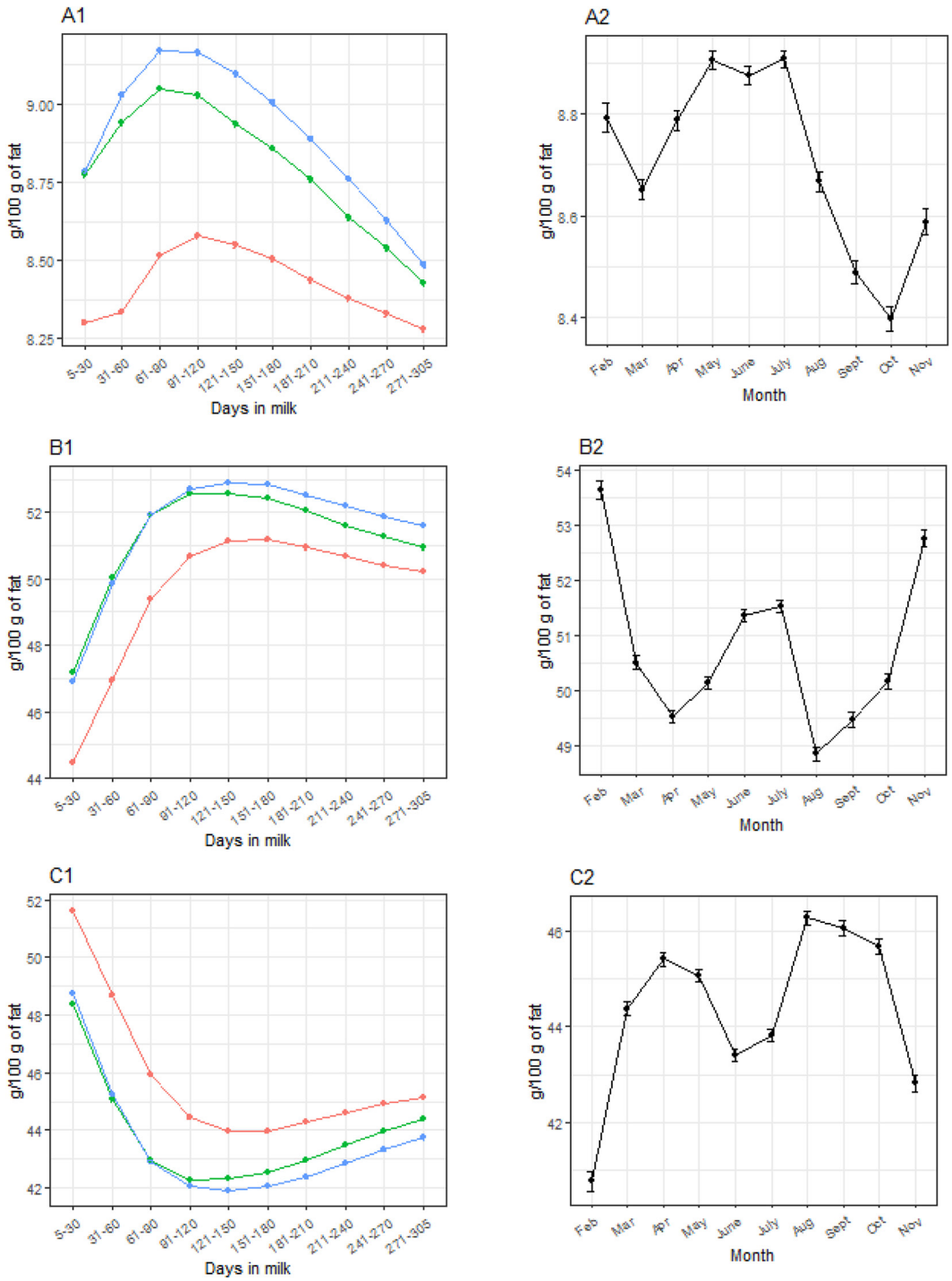


**Figure 1.** Profile of (A) short-chain fatty acids, (B) medium-chain fatty acids, and (C) long-chain fatty acids across (1, left) stage of lactation for parity 1 (red line), parity 2 (green line), and parity 3 or greater (blue line); and (2, right) across calendar months of the years. The SE for each of the presented values is reported within the whiskers.

genetic merit for both fat measures was associated with a concomitant within-breed increase in the concentration of all fatty acids in the fat from C6:0 to C14:0, as well as C16:0, but was associated with a within-breed reduction in the concentration in fat of C4:0, C16:1, and of all fatty acids containing 17 or more carbons. Consequently, an increase in fat yield pedigree index or fat percentage pedigree index was associated with an

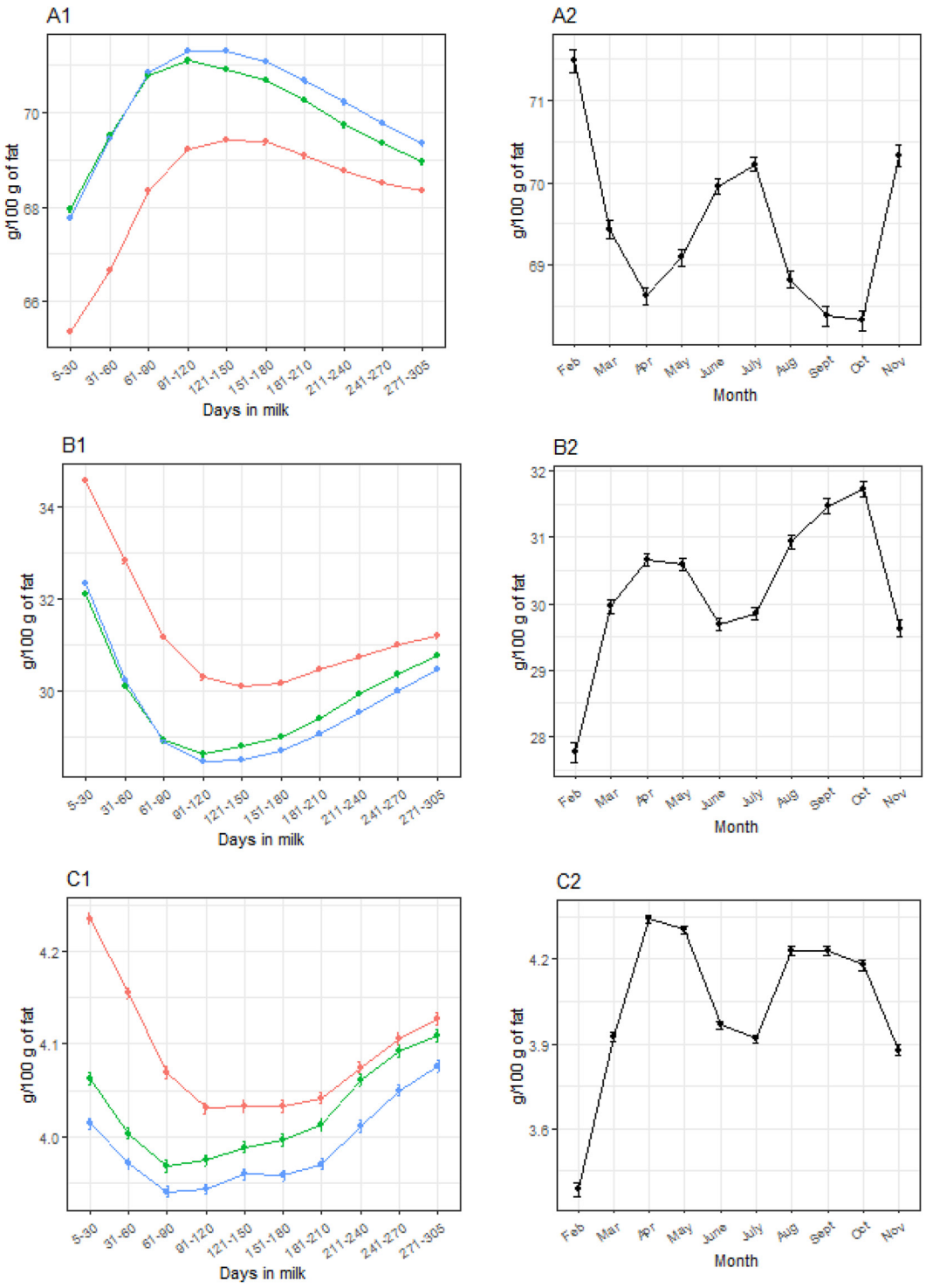


**Figure 2.** Profile of (A) SFA, (B) MUFA, and (C) PUFA across (1, left) stage of lactation for parity 1 (red line), parity 2 (green line), and parity 3 or greater (blue line) and (2, right) across calendar months of the years. The SE for each of the presented values is reported within the whiskers.

increase, within breed, in the sum of all SFA, as well as both SCFA and MCFA concentrations, but a decrease in the concentration of the sum of all UFA, as well as LCFA, n-3, and n-6 fatty acid concentrations.

## DISCUSSION

Fat is a valuable component of milk, affecting the quality and underlying properties of several premium

**Table 4.** Regression coefficients (×1,000; SE in parentheses ×1,000) on Jersey percentage, Normande percentage, and Montbéliarde percentage for the different fatty acids

| Trait | Jersey % (SE) | Normande % (SE) | Montbéliarde % (SE) |
|---|---|---|---|
| Fat % | 9.46* (0.12) | −0.04 (0.25) | −2.77* (0.18) |
| C4:0 | 0.12* (0.04) | 0.14* (0.08) | 1.16* (0.06) |
| C6:0 | 1.24* (0.02) | 0.11* (0.05) | 0.28* (0.04) |
| C8:0 | 1.10* (0.02) | 0.16* (0.04) | 0.17* (0.03) |
| C10:0 | 2.88* (0.06) | 0.64* (0.13) | −0.60* (0.10) |
| C12:0 | 2.78* (0.07) | 0.98* (0.16) | −0.79* (0.12) |
| C14:0 | 5.12* (0.16) | 2.17* (0.34) | 0.88* (0.26) |
| C14:1 | 0.06* (0.02) | 0.31* (0.04) | 0.07* (0.03) |
| C16:0 | 23.59* (0.36) | 1.56* (0.77) | 0.60 (0.59) |
| C16:1 *cis* | −0.80* (0.03) | 0.03 (0.06) | −0.29* (0.05) |
| C17:0 | −0.28* (0.00) | 0.06* (0.01) | −0.05* (0.01) |
| C18:0 | 0.03 (0.15) | −1.92* (0.32) | −0.45* (0.24) |
| C18:1 *cis*-9 | −30.01* (0.40) | −5.08* (0.89) | 1.09* (0.67) |
| C18:2 | −2.33* (0.03) | −0.38* (0.06) | 0.04 (0.05) |
| C18:2 *cis*-9, *cis*-12 | −1.55* (0.02) | −0.40* (0.05) | 0.00 (0.04) |
| C18:2 *cis*-9,*trans*-11 | −1.42* (0.03) | 0.36* (0.07) | 0.08* (0.05) |
| C18:3 *cis*-9,*cis*-12,*cis*-15 | −0.20* (0.01) | 0.01 (0.02) | 0.03 (0.02) |

*Slope differed ($P < 0.05$) from 0.

products, such as butter, cheese, and whole milk powder. Compared with milk protein fractions, the composition of fat (i.e., its fatty acid profile) is highly variable and is dependent and responsive to factors such as diet, breed, stage of lactation, and animal health (Bilal et al., 2014; Morales-Almaráz et al., 2017). As such, a greater understanding of how fatty acid concentration associates with key selection decision factors in dairy herds, such as breed and genotype choice, as well as factors such as parity and stage of lactation (including their interaction), can be highly insightful.

### Comparison with Other Studies

The mean predicted concentration of the majority of the fatty acids in the milk of the cows in the present study agree with the mean fatty acid concentrations reported previously by Timlin et al. (2023) and Soyeurt et al. (2011) also in dairy cows, but where the fatty acid concentrations were quantified using gas chromatography. Nonetheless, it is important to note that comparing results across studies can be challenging, especially for fatty acids with low prediction accuracy from MIR. This is particularly so when comparing MIR-predicted fatty acid concentrations with the concentration of fatty acids quantified using gas chromatography. Timlin et al. (2023) used milk samples collected from 54 spring-calving Irish Holstein-Friesian cows, whereas Soyeurt et al. (2011) used 538 milk samples collected from 475 cows located in Belgium, Ireland, or Scotland; the study of Timlin et al. (2023) included a cohort of cows fed pasture as well as a cohort fed TMR or partial mixed ration. In the study by Soyeurt et al. (2011), milk samples were collected from various breeds across 3 countries: in Belgium, from dual-purpose Belgian Blue, Holstein, Jersey, Normande, Montbéliarde, and Red and White cows; in Ireland, from cows of multiple breeds; and in Scotland, from 2 genetically divergent lines. Nonetheless, the mean fatty acids values in the present study for C18:2 *cis*-9,*trans*-11 and C18:3 *cis*-9,*cis*-12,*cis*-15, which have been proposed as biomarkers to identify milk produced by pasture-fed cows, were lower than those reported by Timlin et al. (2023) for the cohort of pasture-based cows but similar to the cohort of partial mixed ration cows. One of the reasons for the lower concentration of these fatty acids compared with Timlin et al. (2023) could be due the low accuracy of prediction for these individual fatty acids (Soyeurt et al., 2011; the coefficient of determination in cross-validation of C18:2 *cis*-9,*trans*-11 and C18:3 *cis*-9,*cis*-12,*cis*-15 was 0.63 and 0.60, respectively). Despite this, the mean values in the present study for n-3 and n-6 fatty acid concentration in the milk, which are also indicators of milk produced by pasture-based cows, agree with those of Timlin et al. (2023) for the pasture-fed cohort of cows.

Compared with the mean concentration of fatty acids in the milk of indoor-fed cows (i.e., samples collected from 3,185 lactating dairy cows from 52 commercial herds in Canada), the concentration of predicted fatty acids in the present study were higher for the C4:0 to C8:0 fatty acids, as well as for C18:0 to C18:3 *cis*-9,*cis*-12,*cis*-15; the concentration of the C10:0 to C16:0 fatty acids in the milk were lower for the predominantly grass-fed cows in the present study compared with the indoor-fed cows in Canada (Bilal et al., 2014). These differences are probably due to the different diets in Canada and Ireland, as pasture-based diets are known to result in an increase in the proportion of LCFA in milk

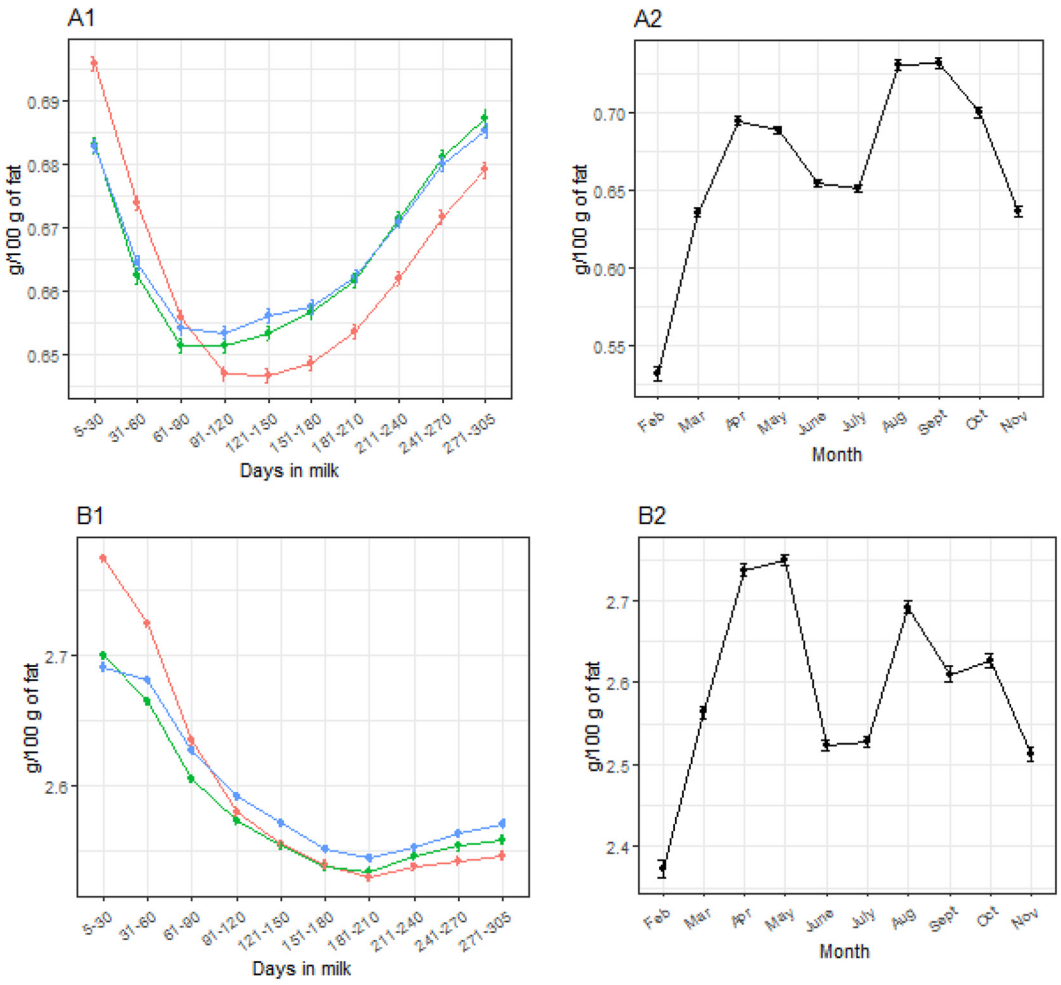


**Figure 3.** Concentrations of (A) n-3 and (B) n-6 fatty acids across (1, left) stage of lactation for parity 1 (red line), parity 2 (green line), and parity 3 or greater (blue line); and (2, right) across calendar months of the years. The SE for each of the presented values is reported within the whiskers.

fat of dairy cows (Timlin et al., 2023). Mean differences in fatty acid concentration by parity, which were evident in the present study, have also been reported elsewhere in indoor-fed cows (Bastin et al., 2013; Samková et al., 2018); older parity cows have, on average, a higher concentration in fat of SFA and SCFA, but a lower concentration of UFA and LCFA, and this observation seems to be largely unaffected by the diet fed.

Breed-related differences in milk fatty acid profiles observed in the present study are likely due to physiological differences that have emerged through divergent breeding programs across breeds. Indeed, given that all cows in this study were managed under similar pasture-based feeding systems, the observed differences are unlikely to be due to nutrition, and instead reflect genetic and physiological variation among breeds shaped by breed-specific selection objectives. Similar results were also observed in other studies. For example, Soyeurt et al. (2006) documented inter-breed variability in milk fatty acid composition across Holstein, Jersey, Montbéliarde, and Belgian Blue dairy cattle. Similarly, Sanjayaranj et al. (2022) reported that grazing Holstein-Friesian cows in New Zealand produced a lower proportion of long-chain SFA concentrations in fat (i.e., C16:0 and C18:0) compared with their Jersey and crossbred contemporaries, even when managed similarly.

### *Fatty Acid Concentration Across Lactation and Year*

It is not always obvious in seasonal calving production systems, like those that exist in Ireland, if the changes in the milk fatty acid composition during the year are a function of stage of lactation or time of the calendar year. Nevertheless, when the relationship between lactation stage and month of year with fatty acid concentration is examined separately, as in the present study, distinct and additive stage of lactation and month of year associations with fatty acid concentration are evident (FigureS 1, 2,

**Table 5.** Regression coefficients (×1,000; SE in parentheses ×1,000) on Jersey percentage, Normande percentage, and Montbéliarde percentage for the different groups of fatty acids

| Trait[1] | Jersey % (SE) | Normande % (SE) | Montbéliarde % (SE) |
|---|---|---|---|
| Total C18:1 | −33.42* (0.47) | −4.48* (1.02) | 2.34* (0.77) |
| Total C18:1 *cis* | −31.88* (0.43) | −5.60* (0.95) | 1.24 (0.71) |
| Total C18:1 *trans* | −3.95* (0.09) | 0.04 (0.20) | 0.01 (0.15) |
| SCFA | 6.33* (0.11) | 1.09* (0.25) | 1.39* (0.19) |
| MCFA | 29.71* (0.53) | 5.60* (1.16) | −1.01 (0.87) |
| LCFA | −39.31* (0.61) | −6.70* (1.33) | 1.41* (1.01) |
| Saturated | 36.79* (0.49) | 4.70* (1.08) | 0.27 (0.81) |
| Unsaturated | −38.50* (0.51) | −4.36* (1.12) | 1.80* (0.85) |
| MUFA | −34.50* (0.47) | −4.38* (1.03) | 1.71* (0.77) |
| PUFA | −4.20* (0.06) | −0.24 (0.14) | −0.29* (0.10) |
| OCFA | −1.87* (0.04) | 0.00 (0.07) | −0.23* (0.06) |
| n-3 | −0.32* (0.01) | 0.02 (0.02) | 0.03 (0.02) |
| n-6 | −2.00* (0.03) | −0.38* (0.07) | −0.09 (0.05) |
| *Trans* FA | −4.31* (0.11) | −0.32 (0.24) | −0.26 (0.18) |
| De novo | 13.36* (0.32) | 4.29* (0.69) | 1.00* (0.52) |
| Total branched FA | −1.24* (0.02) | 0.11* (0.05) | 0.28* (0.04) |

[1]SCFA = short-chain fatty acid; MCFA = medium-chain fatty acid; LCFA = long-chain fatty acid; OCFA = odd-chain fatty acid; FA = fatty acid.

*Slope differed ($P < 0.05$) from 0.

and 3). For example, although MCFA and SFA concentrations in fat increased from early to mid lactation, their concentration decreased from February to April. The opposite was true for LCFA, MUFA, and PUFA concentrations, with a decrease in concentration in the fat from early to mid lactation, but with an increase in concentration from February to April. During the summer months, the concentration of SCFA, MCFA, LCFA, SFA, MUFA, and PUFA changed rapidly, with a reduction in concentration of LCFA, MUFA, and PUFA, and an increase in the concentration of SCFA, MCFA, and SFA.

The profile of the different fatty acids across stages of lactation in the present study (which had the association with month of the calendar year removed) was similar in shape to the concentration of various fatty acids in fat across the lactation reported for indoor-fed cows (Stoop et al., 2009; Bilal et al., 2014). Such changes in milk fat composition across lactation are likely influenced by factors such as energy balance, nutrient availability, and milk yield (Bauman and Griinari, 2003), and these are expected to be largely similar in both pasture-fed and indoor-fed cows. The real novelty in the present study was how the fatty acid concentration in fat changes across the calendar year, reflecting the natural changes in the quality and quantity of the cow's diet, which may not be experienced to such an extent in indoor-fed cows. The effect of the diet on fatty acid concentration was particularly evident during the summer, with a rapid increase in the concentration of the some fatty acids (i.e., SCFA, MCFA, and SFA), while others decreased in concentration (MUFA and PUFA); such changes possibly reflect changes in grass quality (Roche et al., 2009). This could be related to variability in the NDF content of the pasture across the season, being highest in the summer (Hearn et al., 2022), resulting in an elevated production of acetate in the rumen, causing an increased level of de novo synthesized fatty acids which would coincide with higher levels of SCFA and SFA. Variation across the season in both the concentration of fatty acids and the ratios between them (i.e., SFA to UFA) can have important implications on final product quality, most notably high-fat products such as butter (Timlin et al., 2021). This is particularly prevalent in pasture- and seasonal-based systems, where these trending differences are more prevalent than in nonseasonal, nonpasture production systems. The textural properties like softness and hardness and melt-in-the-mouth characteristics are highly dependent on the ratio of SFA to UFA, as the varying melting points of these fatty acids result in more or less crystalline fat at room temperature. As such, increased SFA (in particular C16:0) has been linked to harder butter properties. Consistency of final product quality and characteristics are important goals for manufacturers and, as such, manufacturers aim to limit inter-batch variability in these properties through processes like cream aging and temperature cycling in response to evolving fatty acid composition. A greater understanding of the intrinsic changes to the fatty acid profile, and indeed the ability to rapidly predict fatty acid profile of milk at intake, enables greater control and decision making for final product quality.

### Genetic Merit for Milk Fat Yield or Milk Fat Percentage

Most, if not all, dairy cow breeding objectives globally have a positive emphasis on fat yield, with many,

**Table 6.** Regression coefficients (×100; SE in parentheses ×100) of heterosis, recombination loss, cow fat yield genetic merit, and cow fat percentage genetic merit for the different fatty acids

| | | | Fat | |
|---|---|---|---|---|
| Trait | Heterosis (SE) | Recombination (SE) | Yield (SE) | Percentage (SE) |
| Fat | 6.44* (0.43) | 13.21* (0.67) | 1.37* (0.01) | 150.91* (5.78) |
| C4:0 | −0.20 (0.14) | −1.70* (0.21) | −0.10* (0.00) | −18.47* (0.19) |
| C6:0 | 1.30* (0.09) | 0.80* (0.14) | 0.09* (0.00) | 7.50* (0.13) |
| C8:0 | 1.40* (0.07) | 0.80* (0.11) | 0.07* (0.00) | 7.79* (0.10) |
| C10:0 | 5.90* (0.22) | 6.00* (0.35) | 0.25* (0.01) | 34.87* (0.32) |
| C12:0 | 6.30* (0.28) | 6.00* (0.44) | 0.21* (0.01) | 34.90* (0.40) |
| C14:0 | 4.90* (0.58) | 2.00* (0.91) | 0.20* (0.01) | 27.41* (0.84) |
| C14:1 | −0.50* (0.07) | −0.80* (0.11) | −0.01* (0.00) | −0.15* (0.10) |
| C16:0 | −9.00* (1.34) | −5.70* (2.09) | 2.37* (0.03) | 216.43* (1.91) |
| C16:1 *cis* | −0.50* (0.10) | 0.90* (0.17) | −0.01* (0.00) | 3.52* (0.16) |
| C17:0 | 0.08* (0.02) | 0.22* (0.03) | −0.03* (0.00) | −0.85* (0.03) |
| C18:0 | −2.02* (0.55) | −1.10 (0.86) | −0.24* (0.01) | −39.68* (0.78) |
| C18:1 *cis*-9 | −13.00* (1.54) | −14.60* (2.40) | −2.80* (0.04) | −277.51* (2.18) |
| C18:2 | 0.43* (0.12) | −0.17 (0.19) | −0.17* (0.00) | −21.41* (0.17) |
| C18:2 *cis*-9, *cis*-12 | −0.27* (0.09) | −0.86* (0.15) | −0.19* (0.01) | −15.20* (0.13) |
| C18:2 *cis*-9,*trans*-11 | 0.58* (0.12) | 0.56* (0.18) | −0.11* (0.00) | −10.35* (0.17) |
| C18:3 *cis*-9,*cis*-12,*cis*-15 | 0.35* (0.04) | 0.53* (0.06) | −0.01* (0.00) | −0.99* (0.05) |

*Slope differed ($P < 0.05$) from 0.

but not all, also placing a negative emphasis on milk yield; combined, such a strategy is also expected to increase the milk fat percentage in the milk. There is a paucity of information in dairy cow populations on how genetic selection for either milk fat yield or milk fat percentage, or both, may alter the relative concentrations of different individual fatty acids or fatty acid indices in the milk. Using a population of 1,918 Dutch Holstein-Friesian dairy cows from 398 commercial herds, Stoop et al. (2009) documented positive genetic correlations between both fat percentage and fat yield with the concentration of individual SFA in milk fat (with the exception of C14:0, which was negatively genetically correlated with both fat percentage and fat yield), and a negative correlation between both fat percentage and fat yield with the concentration of all the individual UFA. Similar associations have also been reported from genetic analyses of dairy cows in Belgium (Soyeurt et al., 2007) and in New Zealand (Lopez-Villalobos et al., 2020). These reported genetic correlations between fat percentage or fat yield and concentration of individual fatty acids from other dairy cow studies (Stoop et al., 2009; Soyeurt et al., 2007; Lopez-Villalobos et al., 2020) support the direction of the model coefficients estimated in this study, which were obtained by regressing phenotypic fatty acid concentrations on pedigree index for fat yield or fat percentage (Tables 6 and 7). These regression coefficients can be used to infer genetic correlations.

Based on the estimated regression coefficients, increased genetic merit for fat percentage is associated with a greater concentration in the fat of SCFA, MCFA, and SFA, along with a reduction in LCFA, UFA, n-3 fatty acid, and n-6 fatty acid concentration. More specifically, a 1-unit increase in genetic SD for fat percentage is expected to result in, on average, 0.09, 0.76, and 0.82 g/100 g of fat more SCFA, MCFA, and SFA, respectively, in the milk fat, and 1.02, 0.92, 0.004, and 0.05 g/100 g of fat lower LCFA, UFA, n-3 fatty acid, and n-6 fatty acid, respectively. An important indicator of processability, especially for butter production, is the spreadability index, which is reflected by the ratio of C16:0 to C18:1 *cis*-9. Based on the estimated regression coefficients in the present study, genetic merit for greater fat percentage is associated with greater C16:0 concentration in the milk fat with, on average, a 0.58-g increase in C16:0 per 100 g of fat per 1 SD increase in genetic merit for milk fat percentage, whereas the concentration of C18:1 *cis*-9 in the milk fat is expected to decrease, on average, by 0.75 g/100 g of fat per 1 SD increase in genetic merit for milk fat percentage. Such a trend would lead to an increase in the spreadability index, with a consequential reduction in butter spreadability. From a final product quality perspective, strategies to counteract this trend should be explored, including greater consideration in the future of potential changes to milk composition beyond traditional fat and protein concentration. This could include monitoring changes of fatty acid profile in response to genetic merit and design of appropriate in-process modification of fat structure through cream aging programs. Finally, opportunities for incorporation of feed components in tandem with supplementation to increase UFA in milk without negative consequences on animal productivity could be explored.

**Table 7.** Regression coefficients (×100; SE in parentheses ×100) of heterosis, recombination loss, cow fat yield genetic merit, and cow fat percentage genetic merit for the different groups of fatty acids

| | | | Fat | |
|---|---|---|---|---|
| Trait[1] | Heterosis (SE) | Recombination (SE) | Yield (SE) | Percentage (SE) |
| Total C18:1 | −12.47* (1.76) | −15.42* (2.74) | −3.36* (0.04) | −323.80* (2.49) |
| Total C18:1 *cis* | −15.36* (1.64) | −17.50* (2.55) | −3.01* (0.04) | −301.58* (2.31) |
| Total C18:1 *trans* | 3.61* (0.34) | 5.20* (0.53) | −0.27* (0.01) | −33.17* (0.49) |
| SCFA | 8.09* (0.43) | 4.22* (0.68) | 0.35* (0.01) | 35.29* (0.62) |
| MCFA | 2.41 (2.00) | 2.04 (3.12) | 2.77* (0.05) | 280.16* (2.84) |
| LCFA | −7.12* (2.31) | −4.71 (3.59) | −3.76* (0.06) | −379.47* (3.26) |
| Saturated | 14.44* (1.86) | 14.07* (2.90) | 3.16* (0.05) | 302.08* (2.64) |
| Unsaturated | −12.92* (1.94) | −15.39* (3.01) | −3.65* (0.05) | −342.28* (2.73) |
| MUFA | −13.94* (1.78) | −16.35* (2.76) | −3.32* (0.04) | −314.09* (2.51) |
| PUFA | 2.90* (0.24) | 3.57* (0.38) | −0.34* (0.01) | −25.41* (0.35) |
| OCFA | 0.55* (0.13) | −0.07 (0.20) | −0.09* (0.00) | −3.21* (1.93) |
| n-3 | 0.34* (0.04) | 0.54* (0.07) | −0.02* (0.00) | −1.46* (0.06) |
| n-6 | 0.93* (0.12) | 1.16* (0.19) | −0.22* (0.01) | −16.73* (0.18) |
| *Trans* FA | 5.40* (0.41) | 8.35* (0.65) | −0.31* (0.01) | −33.92* (0.60) |
| De novo | 19.17* (1.19) | 11.64* (1.86) | 0.70* (0.03) | 91.52* (1.71) |
| Total branched FA | −0.47* (0.08) | −1.71* (0.13) | −0.07* (0.00) | −9.91* (0.12) |

[1]SCFA = short-chain fatty acid; MCFA = medium-chain fatty acid; LCFA = long-chain fatty acid; OCFA = odd-chain fatty acid; FA = fatty acid.

*Slope differed ($P < 0.05$) from 0.

## CONCLUSIONS

Clear differences across lactation stages were obvious for all the investigated fatty acids. In general, SFA and SCFA concentrations were low in early lactation, increased in mid lactation, and then linearly decreased to the end of lactation. The opposite was evident for LCFA and UFA. Results from the present study also demonstrate the association between fatty acid concentration with genetic merit for fat yield or fat concentration, with selection for both traits being common in most dairy cow breeding objectives.

## NOTES

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**Nonstandard abbreviations used:** CG = contemporary group; FA = fatty acid; LCFA = long-chain fatty acid; MCFA = medium-chain fatty acid; MIR = mid-infrared; OCFA = odd-chain fatty acid; SCFA = short-chain fatty acid.

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