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Genetic variance components for alternative definitions of fatty acids in dairy cow milk expressed either as a concentration or yield

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ABSTRACT

The extent of genetic variability in fatty acids in bovine milk has, to date, generally focused on its concentration in either milk or milk fat. Selection for ratio traits, such as fatty acid concentration, is statistically and biologically problematic because it can distort relationships between component traits and lead to unintended genetic responses. The objective of this study was to explore the degree of genetic variability in the total yield of individual fatty acids, including when adjusted to a common fat yield. Animal linear mixed models were used to estimate variance and covariance components for a series of fatty acid phenotypes in part-day milk samples predicted using milk infrared spectroscopy; predictions for a total of 16 individual fatty acids and 13 groups of fatty acids were available for 68,353 test-day samples across 47,904 lactations from 27,023 cows. When expressed as a concentration in fat, the coefficient of genetic variation for the individual fatty acids varied from 2% to 11% with a mean of 5%; when expressed as a concentration in milk, the coefficient of genetic variation for the individual fatty acids varied from 7% to 13% with a mean of 10%. The yield of individual fatty acids per milking had a coefficient of genetic variation varying from 7% to 14% with a mean of 9%; the respective values when phenotypically adjusted to a common fat yield were 2% to 11% with a mean of 5%. The genetic correlation between a given fatty acid expressed as a concentration in fat versus as a concentration in milk varied from −0.03 to 0.82. The genetic correlation between the total yield of a given fatty acid for a milking and that expressed as a concentration in fat varied from 0.19 to 0.74, whereas the genetic correlation varied from 0.35 to 0.75 when expressed as a concentration in milk. The genetic correlations between the total yield of individual fatty acids in a milking and the associated fat yield in that milking varied from 0.48 to 0.96, with the genetic correlations varying from 0.18 to 0.65 between the total yield of individual fatty acids in a milking and the associated milk yield in that milking. Irrespective of definition, exploitable genetic variability was detected for most of the (groups of) fatty acids; the choice of which definition of fatty acid to use is a function of how it will be deployed in a breeding scheme but also its end use.

Key words: heritability, variability, coefficient of genetic variation, mid-infrared

INTRODUCTION

Many dairy cow breeding programs globally actively select for macro-features of milk quality, namely milk fat, milk protein, and SCC (Cole and VanRaden, 2018; Berry et al., 2022). Few, if any, breeding goals for large dairy cow populations include microelements of milk quality; one such microelement includes the fatty acid content. To be considered for inclusion in a breeding goal, Shook (1989) stated that the trait must be important, must demonstrate genetic variability, and there should be a strategy for selection, such as being based on a trait that is well-defined, consistently measurable at a low cost, or indeed, correlated with such a trait. Whereas there is currently no explicit financial incentive to improve the fatty acid of milk in most dairy cow populations, this does not preclude any such incentives in the future, especially if tools (e.g., genetic evaluations) exist to deliver improvements in the quality of the milk.

Extensive research has been undertaken investigating the genetic basis of fatty acids in bovine milk. In a meta-analysis of 20 of such studies in dairy cows, Hossein-Zadeh (2021) summarized the genetic parameters for the different fatty acids in milk. In summary, the heritability estimates for the concentration (in milk or fat) of groups of fatty acids ranged from 0.092 to 0.428, whereas the concentration (in milk or fat) of individual fatty acids ranged from 0.109 to 0.421 (Hossein-Zadeh, 2021). In general, the concentration (in milk or fat) of groups of fatty acids was strongly positively genetically correlated

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with both fat and protein concentration in the milk but strongly negatively genetically correlated with both milk and protein yield (Hossein-Zadeh, 2021); the genetic correlations between the concentration (in milk or fat) of the groups of fatty acids with fat yield were moderately negative (Hossein-Zadeh, 2021).

Research to date on the genetic basis of fatty acids in dairy cow milk has, however, only focused on their concentration in either the milk fat (Soyeurt et al., 2008; Tiplady et al., 2022), the milk itself (Narayana et al., 2017), or as a percentage of the sum of all fatty acids in the milk (Garnsworthy et al., 2010; Lopez-Villalobos et al., 2020); some (Soyeurt et al., 2007) have explored the genetics of the concentration of fatty acids in both milk and fat. This is despite the much publicized (Gunsett, 1984) desire from breeders to avoid, as much as possible, having to select on ratio traits. Concentration is, by definition, a ratio trait. Selecting directly for ratio traits, such as fatty acid concentration, can be problematic because ratios violate statistical assumptions and obscure the underlying biological variation of the numerator and denominator. Such selection may also cause unintended genetic changes in the component traits, leading to unfavorable outcomes. Therefore, it is often more effective to select on the individual traits or a linear index of both, rather than on their ratio itself. The objective, therefore, of the present study was to quantify the genetic variability in the actual yield of different fatty acids but also the extent of that genetic variability independent of milk fat yield. For comparison, the results were evaluated against genetic parameters estimated for fatty acids expressed as a concentration in fat and milk separately.

MATERIALS AND METHODS

Data

Data on Irish dairy cows from the years 2015 to 2020 inclusive were available, which included information on cow parity, calving dates, breed composition, and milk test dates, alongside corresponding milk mid-infrared (**MIR**) spectra, milk yield, fat concentration, and protein concentration. The dataset comprised 644,752 milk-test day records from 303,089 cows producing on 2,406 commercial farms. These milk yield and composition samples all originated from part-day samples where all cows were milked twice daily. More detailed information on the dataset used is given in Frizzarin et al. (2025).

Spring-calving cows (calving between January and May) accounted for 85% of all records in the dataset. Parity ranged from 1 to 16, and DIM ranged from 5 to 305. Breed composition, recorded in the national database in 1/32 fractions, was converted to percentages for this study. Breeds considered were Holstein-Friesian, Jersey, Normande, and Montbéliarde, with other breeds grouped as "other breeds" (calculated as 100% minus the sum of the specified breeds); the mean percentage of "other breeds" in the cows was 4.01%. Heterosis and recombination loss coefficients were calculated per cow using the equations presented by Frizzarin et al. (2025). Contemporary groups for each record were defined as herd-date of milk recording.

Prediction of Fatty Acids

Milk samples were analyzed using FOSS mid-infrared spectrometers, with the milk fat percentage, protein percentage, and corresponding MIR spectra recorded. The MIR spectra were used to estimate the concentration, in milk, of various milk fatty acids using the prediction models developed in the OptiMIR project (Grelet et al., 2014). Concentrations of fatty acids and groups of fatty acids were predicted in grams per 100 mL of milk. These MIR-based prediction equations, developed from individual cow milk samples, reported coefficients of determination from external validation ranging from 0.60 for C18:3 *cis*-9,*cis*-12, and *cis*-15 to 0.99 for SFA yield (Grelet et al., 2014). For use in the present study, the predicted fatty acid yields were also converted to grams per 100 g of milk fat as well as being expressed in total grams of the fatty acid (i.e., concentration of fatty acid in the milk times the part-day milk yield).

Outliers for each fatty acid and group of fatty acids were identified as values exceeding 1.5 times the interquartile range above the third quartile or below the first quartile. Only milk-test day records with no outliers for any of the investigated individual fatty acids or groups of fatty acids were retained for analysis. Of the dataset, only cows with a known sire and dam were retained, and only records belonging to contemporary groups with at least 10 records were retained. Moreover, only farms with milk fatty acid predictions recorded across at least 3 years of the study period and with >100 cows recorded across those years were retained. A total of 214,620 test-day records from 82,301 cows in 410 herds remained. A random sample of herds was chosen to reduce the dataset size for variance component estimation. The final dataset for the analyses consisted of 68,353 records from 27,023 cows collected across 137 farms.

(Co)variance Component Estimation

Univariate animal linear mixed models in ASReml (Gilmour et al., 2009) were used to estimate the variance components for milk yield, fat, and protein concentration, as well as each of the 3 definitions (i.e., concentration in fat, concentration in milk, and yield) of the 29 fatty acid traits. The following model was used:

$$y_{ijklmn} = Stage_j + Parity_k + Stage_j \times Parity_k + Je + No + Mo + other\ breeds + heterosis + recombination + CG_l + a_i + pe\_within_m + pe\_across_n + e_{ijklmn},$$

where y_{ijklmn} was the observed value for cow i; $Stage_j$ was the fixed effect for the stage of lactation of the cow i (10 classes: 5–30, 31–60, . . ., 271–305); $Parity_k$ was the fixed effect for the parity of the cow i (3 classes: 1, 2, and 3+); *Je*, *No*, *Mo*, and *other breeds* were the covariates of the percentage of Jersey, Normande, Montbéliarde, and other breeds in the cow i, respectively; *heterosis* was the heterosis covariate for cow i; *recombination* was the recombination covariate for cow i; CG_l was the fixed effect for contemporary group; a_i was the additive random effect of cow i, where $a \sim N\left(0, \mathbf{A}\sigma_a^2\right)$ and σ_a^2 represent the direct genetic variance and **A** the numerator relationship matrix; the pedigree of all cows was traced back at least 4 generations; pe_within_m was the random cow by lactation permanent environmental effect, where $pe\_within_m \sim N\left(0, \mathbf{I}\sigma^2_{pe\_within}\right)$ and $\sigma^2_{pe\_within}$ represent the within-lactation permanent environmental variance and **I** the identity matrix; pe_across_n was the random cow across-lactation permanent environmental effect, where $pe\_across_n \sim N\left(0, \mathbf{I}\sigma^2_{pe\_across}\right)$ and $\sigma^2_{pe\_across}$ represent the across-lactation permanent environmental variance and **I** the identity matrix; and e_{ijklmn} was the residual term, where $e \sim N\left(0, \mathbf{I}\sigma_e^2\right)$ and σ_e^2 represent the residual variance and **I** the identity matrix. In a supplementary series of univariate analyses, when the dependent variable was yield of (group of) fatty acids, fat yield for that corresponding sample was included as a covariate in the model. The coefficient of genetic variation was calculated as the direct genetic SD for a given trait divided by its respective raw mean.

A series of bivariate animal linear mixed models were used to estimate the genetic and phenotypic covariances among all fatty acid concentration traits. A series of trivariate analyses were used to calculate the correlations between the yield of each (group of) fatty acid and both milk and fat yield, where the latter 2 traits were included in all analyses. The models used were as described for the univariate model.

RESULTS

Variance Components

Summary statistics for individual and groups of fatty acids, expressed either as a concentration in fat or milk or total yield per milking, are in Table 1; also included in Table 1 are the summary statistics for the yield of each fatty acid per milking adjusted phenotypically for the respective fat yield. Regardless of whether defined as a concentration in fat or milk or as total yield per milking, the coefficient of genetic variation for the individual fatty acids varied from 2% (C17:0 expressed as a concentration in fat) to 13% (C10:0 expressed as a concentration in fat). The coefficient of genetic variation for the individual fatty acids was always greater when expressed as yield per milking compared with when expressed as a concentration in fat; when expressed as a concentration in fat, it was, in turn, consistently higher compared with when expressed as a concentration in milk. On average, the coefficient of genetic variation of the yield of individual fatty acids halved once adjusted (phenotypically) for differences in fat yield. The mean coefficient of genetic variation for the groups of fatty acids was 4%, 8%, and 8% when expressed as a concentration in fat, concentration in milk, or total yield, respectively (Table 1).

Irrespective of how defined, the heritability of the individual fatty acids (Table 1) varied from 0.08 (yield of C18:1 *cis*-9) to 0.37 (C12:0, C14:0, and C14:1 expressed as a concentration in milk). The mean heritability of the individual fatty acids was 0.28, 0.29, and 0.12 when expressed as a concentration in fat, as a concentration in milk, or as total yield. Of the 3 explored metrics of individual fatty acids, the heritability was always lower when expressed as total yield per milking (i.e., concentration in milk times milk yield); furthermore, adjusting the yield of individual fatty acids for differences in the associated fat yield increased the heritability for all individual fatty acids relative to when no adjustment for fat yield was made. The range in heritability estimates for the groups of fatty acids was similar to that for the individual fatty acids, although, on average, the heritability of the groups of fatty acids was lower.

Correlations Between the 3 Different Definitions of Fatty Acids

The genetic correlations between the 3 alternative definitions of fatty acids explored in the present study are in Table 2; the associated phenotypic correlations are in Supplemental Table S1 (see Notes). The genetic correlations between the different definitions of the individual fatty acids were, on average, stronger than between the different definitions of the groups of fatty acids. With the exception of the genetic correlation of −0.03 between C18:2 expressed as a concentration in fat and milk, all within-trait genetic correlations between the 3 different definitions of the individual fatty acids were positive, varying from 0.04 to 0.82. The mean genetic correlation between the groups of fatty acids expressed as a concentration in fat versus a concentration in milk was 0.23 (varied from −0.18 to 0.71), whereas the mean (range)

Table 1. Mean (μ), genetic SD (σ_g), and h^2 estimates for the different individual and groups[1] of fatty acids when expressed as grams per 100 grams of fat, grams per deciliter of milk, or as total yield (g) of the fatty acid per milking[2]

	g/100 g of fat			g/dL			Yield[3] (g)				
Trait	μ	σ_g	h^2	μ	σ_g	h^2	μ	σ_g	σ_g adj	h^2	h^2 adj
C4:0	2.87	0.116	0.35	1.23	0.096	0.28	15.78	1.279	0.584	0.11	0.25
C6:0	1.82	0.070	0.30	0.78	0.081	0.33	10.06	0.915	0.353	0.13	0.22
C8:0	1.20	0.054	0.28	0.51	0.058	0.35	6.62	0.630	0.272	0.14	0.20
C10:0	2.72	0.175	0.29	1.17	0.155	0.36	15.03	1.604	0.872	0.15	0.19
C12:0	3.48	0.209	0.26	1.49	0.190	0.37	19.11	1.955	1.064	0.15	0.18
C14:0	12.00	0.364	0.19	5.13	0.513	0.37	65.66	5.596	1.958	0.13	0.14
C14:1	1.06	0.047	0.21	0.45	0.045	0.37	5.71	0.488	0.246	0.14	0.15
C16:0	29.91	1.033	0.29	12.89	1.402	0.35	163.8	15.068	5.441	0.14	0.21
C16:1 *cis*	1.61	0.078	0.25	0.70	0.071	0.25	8.75	0.754	0.398	0.10	0.18
C17:0	0.66	0.014	0.30	0.28	0.025	0.29	3.6	0.271	0.072	0.10	0.23
C18:0	11.42	0.359	0.21	4.94	0.420	0.23	62.76	5.042	1.823	0.10	0.14
C18:1 *cis*-9	21.04	1.195	0.29	9.08	0.629	0.15	115.0	8.513	5.934	0.08	0.18
C18:2	2.49	0.101	0.36	1.07	0.081	0.25	13.67	0.989	0.516	0.09	0.27
C18:2 *cis*-9,*cis*-12	1.44	0.077	0.33	0.62	0.049	0.23	7.97	0.602	0.397	0.09	0.25
C18:2 *cis*-9,*trans*-11	0.8	0.092	0.29	0.34	0.040	0.28	4.27	0.542	0.476	0.17	0.22
C18:3 *cis*-9,*cis*-12,*cis*-15	0.55	0.026	0.25	0.24	0.023	0.26	3.01	0.265	0.139	0.11	0.20
SCFA	8.93	0.326	0.27	3.84	0.397	0.34	49.29	4.417	1.656	0.13	0.20
MCFA	51.61	1.325	0.22	22.19	2.292	0.36	282.4	24.535	7.042	0.13	0.15
LCFA	43.45	1.640	0.25	18.75	1.346	0.18	237.9	17.137	8.362	0.08	0.16
Saturated	70.2	1.401	0.27	30.23	3.016	0.34	385.1	32.602	7.106	0.12	0.18
Unsaturated	33.49	1.503	0.30	14.44	1.032	0.18	182.8	13.199	7.558	0.08	0.20
MUFA	29.51	1.371	0.29	12.73	0.905	0.18	161.1	11.640	6.859	0.08	0.19
PUFA	4.03	0.187	0.29	1.73	0.145	0.26	22.0	1.738	0.980	0.10	0.22
OCFA	3.87	0.104	0.29	1.66	0.146	0.33	21.13	1.641	0.534	0.12	0.22
n-3	0.67	0.029	0.23	0.29	0.027	0.25	3.64	0.313	0.155	0.09	0.18
n-6	2.59	0.102	0.34	1.11	0.091	0.26	14.24	1.055	0.526	0.09	0.26
Total C18:1	25.85	1.351	0.29	11.15	0.774	0.16	141.2	10.331	6.775	0.08	0.19
Total C18:1 *cis*	22.67	1.277	0.29	9.78	0.671	0.15	123.9	9.130	6.333	0.08	0.19
Total C18:1 *trans*	3.73	0.226	0.21	1.60	0.135	0.22	20.33	1.782	1.184	0.11	0.16

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

[2]SE of the heritability estimates were all ≤0.01.

[3]σ_g adj and h^2 adj = genetic SD and heritability, respectively, of fatty acid yield phenotypically adjusted for differences in fat yield.

genetic correlation between the yield of groups of fatty acids per milking with those defined as a concentration in fat or a concentration in milk was 0.34 (0.11 to 0.57) and 0.46 (0.33 to 0.63), respectively. Almost all genetic correlations between the same (group of) fatty acid expressed as yield or as concentration in milk were weaker than the respective correlation when defined as a concentration in fat versus as a concentration in milk.

Correlations Among the Individual Fatty Acids

The genetic correlations among the individual fatty acids when expressed using the same definition are in Tables 3 and 4. The pairwise genetic correlations between the yields of individual fatty acids were all positive (Table 3), as were the genetic correlations between individual fatty acids when defined as a concentration in milk (Table 4); with the exception of the fatty acids C18:1 *cis*-9, C18:2 *cis*-9 *cis*-12, and C18:2 *cis*-9 *trans*-11, all genetic correlations were >0.50. When defined as a concentration in fat, the pairwise correlations between the individual fatty acids were highly variable, ranging from −0.81 (between C14:0 and C18:1 *cis*-9) to 0.98 (between C10:0 and C12:0). Irrespective of definition, there was a tendency for the individual fatty acids with similar carbon numbers to be more strongly genetically correlated with each other, at least those with less than 14 carbons.

Correlations Between Fatty Acids and Both Milk and Fat Yield

The genetic correlations between both fat yield and milk yield with each of the individual fatty acids and groups of fatty acids when expressed using the different definitions are in Table 5. The genetic correlation between fat yield and milk yield was 0.46 (SE = 0.04). Expressed as a concentration in fat, the genetic correlations between the individual and groups of fatty acids with fat yield were all relatively weak, around zero, varying from −0.36 to 0.36; all the individual fatty acids with 6 to 16 carbons, when expressed as a concentration in fat, were negatively genetically correlated with milk yield. The yield of all individual fatty acids or groups of fatty acids was positively genetically correlated with both fat

Table 2. Genetic correlations (SE) between the 3 different definitions of individual or groups[1] of fatty acids either expressed as grams per 100 grams of fat, total grams, or grams per deciliter of milk

Trait	g/100 g fat vs. total grams	g/100 g fat vs. g/dL	Total grams vs. g/dL
C4:0	0.35 (0.04)	0.04 (0.03)	0.48 (0.03)
C6:0	0.58 (0.03)	0.58 (0.02)	0.63 (0.02)
C8:0	0.64 (0.03)	0.69 (0.02)	0.67 (0.02)
C10:0	0.72 (0.02)	0.82 (0.01)	0.75 (0.02)
C12:0	0.68 (0.02)	0.78 (0.01)	0.73 (0.02)
C14:0	0.46 (0.04)	0.53 (0.03)	0.60 (0.03)
C14:1	0.43 (0.04)	0.47 (0.03)	0.58 (0.03)
C16:0	0.63 (0.03)	0.74 (0.02)	0.66 (0.02)
C16:1 *cis*	0.46 (0.03)	0.52 (0.02)	0.59 (0.03)
C17:0	0.07 (0.02)	0.09 (0.01)	0.38 (0.01)
C18:0	0.29 (0.04)	0.10 (0.03)	0.49 (0.03)
C18:1 *cis*-9	0.31 (0.04)	0.05 (0.04)	0.35 (0.04)
C18:2	0.19 (0.04)	−0.03 (0.03)	0.40 (0.04)
C18:2 *cis*-9,*cis*-12	0.37 (0.04)	0.20 (0.03)	0.48 (0.03)
C18:2 *cis*-9,*trans*-11	0.74 (0.02)	0.72 (0.01)	0.72 (0.02)
C18:3 *cis*-9,*cis*-12,*cis*-15	0.47 (0.03)	0.41 (0.03)	0.55 (0.03)
SCFA	0.56 (0.03)	0.59 (0.02)	0.63 (0.02)
MCFA	0.57 (0.03)	0.71 (0.02)	0.62 (0.02)
LCFA	0.11 (0.05)	−0.18 (0.04)	0.34 (0.04)
Saturated	0.55 (0.03)	0.70 (0.02)	0.60 (0.03)
Unsaturated	0.18 (0.04)	−0.07 (0.03)	0.34 (0.04)
MUFA	0.19 (0.04)	−0.06 (0.03)	0.34 (0.04)
PUFA	0.32 (0.04)	0.21 (0.03)	0.47 (0.03)
OCFA	0.21 (0.04)	0.21 (0.03)	0.51 (0.03)
n-3	0.43 (0.04)	0.36 (0.03)	0.53 (0.03)
n-6	0.22 (0.04)	0.11 (0.03)	0.45 (0.04)
Total C18:1	0.26 (0.04)	−0.01 (0.04)	0.33 (0.04)
Total C18:1 *cis*	0.30 (0.04)	0.03 (0.04)	0.35 (0.04)
Total C18:1 *trans*	0.47 (0.03)	0.33 (0.03)	0.51 (0.03)

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

yield (0.48 to 0.98) and milk yield (0.18 to 0.65). When expressed as a concentration in milk, the genetic correlations with fat yield were all positive (0.15 to 0.52), whereas the genetic correlations with milk yield were all negative (−0.52 to −0.26).

DISCUSSION

Dairy cow breeding objectives have evolved over the past 2 decades (Miglior et al., 2005; Cole and VanRaden, 2018) primarily by considering new traits that either influence profit or address societal concerns. Three suites of traits underrepresented in many dairy cow breeding objectives are animal health and disease, animal efficiency, and product quality. Improving product quality is particularly important as consumer focus on the nutritional value of food intensifies (Willett et al., 2019; McGaugh and Barthel, 2022). The role of fatty acids in human diets is well established (Calder, 2015), as is the effect of milk fatty acid profile on the processing characteristics and the properties of the resulting product portfolio (Bobe et al., 2003). While cow dietary strategies to enrich dairy products with healthy fatty acids have been proposed (for review, see Kholif and Olafadehan, 2022), genetic variability in the concentration of individual fatty acids of dairy cow milk has also been documented (for review, see Hossein-Zadeh, 2021). One of the underlying objectives in the present study was to estimate genetic parameters for alternative definitions of fatty acids, thereby helping inform more strategic breeding decisions for improved product quality. Whereas the exploration strategy used in the present study focused specifically on fatty acids, the methodology is equally applicable to other milk quality attributes such as the concentration of protein or different protein fractions.

Continuous Versus Ratio Traits

The perils of genetic selection for ratio traits have received attention in both animal and plant breeding (Turner, 1959). If both the numerator and denominator are normally distributed, then their ratio is not normally distributed, thus violating one of the assumptions of standard parametric analyses; where the numerator and denominator are uncorrelated and normally distributed, then their ratio follows a Cauchy distribution. Ratio traits such as fatty acid concentration therefore violate statistical assumptions, which can affect inferences on the linear relationship between traits as defined by correlations; correlations between traits are used in selection index

Table 3. Genetic correlations[1] between the individual fatty acids when defined as a concentration in fat (above the diagonal) or as yield per milking (below the diagonal)

Trait	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C14:1	C16:0	C16:1	C17:0	C18:0	C18:1 *cis*-9	C18:2	C18:2 *cis*-9,*cis*-12	C18:2 *cis*-9,*trans*-11	C18:3 *cis*-9,*cis*-12,*cis*-15
C4:0		0.55	0.25	−0.22	−0.32	−0.14	−0.39	−0.21	−0.37	−0.44	0.35	0.15	−0.06	−0.19	0.23	0.09
C6:0	0.93		0.88	0.60	0.48	0.51	−0.16	0.30	−0.56	−0.6	−0.07	−0.58	−0.43	−0.46	−0.09	0.12
C8:0	0.87	0.98		0.86	0.83	0.80	0.05	0.35	−0.63	−0.47	−0.22	−0.77	−0.31	−0.30	−0.14	0.19
C10:0	0.72	0.90	0.96		0.98	0.86	0.24	0.39	−0.44	−0.24	−0.39	−0.79	−0.24	−0.18	−0.21	0.10
C12:0	0.69	0.88	0.94	0.99		0.89	0.39	0.35	−0.36	−0.1	−0.49	−0.75	−0.15	−0.13	−0.14	0.10
C14:0	0.83	0.94	0.97	0.95	0.96		0.42	0.41	−0.54	−0.3	−0.5	−0.81	−0.11	−0.07	−0.17	0.03
C14:1	0.69	0.79	0.81	0.81	0.85	0.9		−0.07	0.32	0.37	−0.78	−0.11	0.15	−0.19	0.43	0.18
C16:0	0.81	0.90	0.89	0.85	0.85	0.93	0.81		−0.25	−0.39	−0.17	−0.75	−0.77	−0.34	−0.71	−0.54
C16:1	0.67	0.67	0.63	0.59	0.62	0.71	0.82	0.76		0.63	−0.28	0.55	0.14	−0.03	0.29	0.05
C17:0	0.80	0.83	0.82	0.78	0.80	0.89	0.88	0.87	0.91		−0.24	0.37	0.55	0.29	0.48	0.38
C18:0	0.88	0.85	0.81	0.72	0.70	0.82	0.67	0.86	0.74	0.88		0.34	−0.05	0.10	−0.12	0.05
C18:1 *cis*-9	0.70	0.57	0.49	0.39	0.40	0.55	0.62	0.57	0.85	0.80	0.79		0.48	0.31	0.42	0.12
C18:2	0.74	0.72	0.73	0.69	0.71	0.80	0.79	0.68	0.77	0.91	0.80	0.80		0.70	0.37	0.38
C18:2 *cis*-9,*cis*-12	0.62	0.61	0.82	0.62	0.63	0.72	0.60	0.68	0.66	0.80	0.76	0.70	0.89		−0.21	−0.07
C18:2 *cis*-9,*trans*-11	0.51	0.44	0.41	0.33	0.37	0.58	0.61	0.24	0.53	0.57	0.40	0.58	0.62	0.23		0.64
C18:3 *cis*-9,*cis*-12,*cis*-15	0.78	0.81	0.82	0.75	0.76	0.82	0.80	0.69	0.73	0.88	0.81	0.70	0.86	0.65	0.72	

[1]SE of the genetic correlations were all ≤0.0382.

Table 4. Genetic correlations[1] between the individual fatty acids when defined as a concentration in milk

Trait	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C14:1	C16:0	C16:1	C17:0	C18:0	C18:1 *cis*-9	C18:2	C18:2 *cis*-9,*cis*-12	C18:2 *cis*-9,*trans*-11
C6:0	0.95														
C8:0	0.91	0.98													
C10:0	0.81	0.94	0.98												
C12:0	0.78	0.91	0.96	1.00											
C14:0	0.85	0.95	0.98	0.98	0.96										
C14:1	0.71	0.82	0.84	0.87	0.89	0.91									
C16:0	0.91	0.92	0.92	0.90	0.89	0.95	0.85								
C16:1	0.69	0.72	0.69	0.69	0.71	0.76	0.85	0.80							
C17:0	0.80	0.79	0.86	0.86	0.87	0.91	0.82	0.91	0.93						
C18:0	0.87	0.87	0.84	0.80	0.78	0.84	0.53	0.89	0.79	0.89					
C18:1 *cis*-9	0.61	0.56	0.43	0.49	0.58	0.55	0.83	0.45	0.90	0.80	0.77				
C18:2	0.71	0.76	0.79	0.81	0.82	0.84	0.64	0.73	0.82	0.93	0.8	0.76			
C18:2 *cis*-9,*cis*-12	0.60	0.67	0.36	0.74	0.75	0.77	0.66	0.75	0.71	0.82	0.76	0.64	0.90		
C18:2 *cis*-9,*trans*-11	0.40	0.37	0.36	0.34	0.36	0.36	0.55	0.22	0.50	0.50	0.32	0.51	0.53	0.13	
C18:3 *cis*-9,*cis*-12,*cis*-15	0.79	0.84	0.85	0.84	0.82	0.84	0.83	0.76	0.79	0.9	0.83	0.71	0.88	0.68	0.65

[1]SE of the genetic correlations were all ≤0.0319.

Table 5. Genetic correlations (SE in parentheses) between the 3 different definitions of individual and groups[1] of fatty acids with fat yield and milk yield

	Fat yield			Milk yield		
Trait	g/100 g fat	g/dL	Yield	g/100 g fat	g/dL	Yield
C4:0	−0.12 (0.04)	0.49 (0.03)	0.89 (0.01)	0.29 (0.03)	−0.41 (0.03)	0.57 (0.03)
C6:0	0.23 (0.04)	0.51 (0.03)	0.92 (0.01)	−0.04 (0.04)	−0.44 (0.03)	0.38 (0.04)
C8:0	0.26 (0.04)	0.49 (0.03)	0.90 (0.01)	−0.13 (0.04)	−0.46 (0.03)	0.32 (0.04)
C10:0	0.25 (0.04)	0.45 (0.03)	0.84 (0.01)	−0.26 (0.04)	−0.47 (0.03)	0.18 (0.04)
C12:0	0.20 (0.04)	0.44 (0.03)	0.84 (0.01)	−0.25 (0.04)	−0.48 (0.03)	0.20 (0.04)
C14:0	0.14 (0.05)	0.49 (0.03)	0.94 (0.01)	−0.11 (0.04)	−0.49 (0.03)	0.38 (0.04)
C14:1	−0.07 (0.04)	0.4 (0.03)	0.87 (0.01)	−0.09 (0.04)	−0.50 (0.03)	0.37 (0.04)
C16:0	0.33 (0.04)	0.51 (0.03)	0.94 (0.01)	−0.22 (0.04)	−0.48 (0.03)	0.30 (0.04)
C16:1 *cis*	−0.07 (0.04)	0.37 (0.04)	0.85 (0.01)	−0.11 (0.04)	−0.48 (0.03)	0.36 (0.04)
C17:0	0.17 (0.06)	0.45 (0.03)	0.96 (0.004)	0.02 (0.06)	−0.52 (0.03)	0.47 (0.04)
C18:0	−0.05 (0.05)	0.48 (0.03)	0.93 (0.01)	0.18 (0.04)	−0.44 (0.03)	0.51 (0.03)
C18:1 *cis*-9	−0.35 (0.04)	0.30 (0.04)	0.77 (0.02)	0.26 (0.04)	−0.41 (0.04)	0.65 (0.03)
C18:2	−0.32 (0.04)	0.39 (0.04)	0.87 (0.01)	0.21 (0.03)	−0.47 (0.03)	0.58 (0.03)
C18:2 *cis*-9,*cis*-12	−0.27 (0.04)	0.35 (0.04)	0.78 (0.02)	0.19 (0.03)	−0.42 (0.04)	0.56 (0.03)
C18:2 *cis*-9,*trans*-11	−0.21 (0.04)	0.15 (0.04)	0.48 (0.03)	0.11 (0.04)	−0.26 (0.04)	0.41 (0.03)
C18:3 *cis*-9,*cis*-12,*cis*-15	−0.04 (0.04)	0.42 (0.04)	0.85 (0.01)	0.06 (0.04)	−0.44 (0.03)	0.46 (0.04)
SCFA	0.22 (0.04)	0.50 (0.03)	0.93 (0.01)	−0.07 (0.04)	−0.46 (0.03)	0.37 (0.04)
MCFA	0.33 (0.04)	0.51 (0.03)	0.96 (0.004)	−0.23 (0.04)	−0.49 (0.03)	0.33 (0.04)
LCFA	−0.33 (0.04)	0.39 (0.04)	0.89 (0.01)	0.26 (0.04)	−0.47 (0.05)	0.61 (0.03)
Saturated	0.36 (0.04)	0.52 (0.03)	0.98 (0.003)	−0.22 (0.04)	−0.49 (0.03)	0.53 (0.03)
Unsaturated	−0.35 (0.04)	0.35 (0.04)	0.85 (0.01)	0.24 (0.04)	−0.46 (0.04)	0.62 (0.03)
MUFA	−0.35 (0.04)	0.51 (0.03)	0.84 (0.01)	0.24 (0.04)	−0.49 (0.03)	0.62 (0.03)
PUFA	−0.25 (0.04)	0.34 (0.04)	0.83 (0.01)	0.10 (0.04)	−0.46 (0.04)	0.52 (0.03)
OCFA	−0.12 (0.04)	0.46 (0.03)	0.95 (0.004)	−0.04 (0.04)	−0.52 (0.03)	0.44 (0.04)
n-3	−0.05 (0.04)	0.43 (0.04)	0.90 (0.004)	0.08 (0.04)	−0.43 (0.03)	0.61 (0.01)
n-6	−0.27 (0.04)	0.39 (0.04)	0.88 (0.01)	0.12 (0.03)	−0.48 (0.03)	0.53 (0.03)
Total C18:1	−0.36 (0.04)	0.32 (0.04)	0.8 (0.02)	0.26 (0.04)	−0.43 (0.04)	0.64 (0.03)
Total C18:1 *cis*	−0.35 (0.04)	0.30 (0.04)	0.77 (0.02)	0.27 (0.04)	−0.41 (0.04)	0.65 (0.03)
Total C18:1 *trans*	−0.20 (0.04)	0.35 (0.04)	0.76 (0.02)	0.17 (0.04)	−0.38 (0.04)	0.53 (0.03)

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

theory to, for example, calculate the expected response to selection (Cameron, 1997).

Another concern related to selection for a ratio trait is that it can inadvertently cause undesirable changes in the individual traits that constitute the ratio. For example, selection for fatty acid concentration in milk fat could result in animals that have either a greater yield of the fatty acid or unfavorably low concentrations of fat. Exploring the genetic correlation between the numerator and denominator with their ratio, Turner (1959) proved that positive genetic correlations likely exist between the ratio and the numerator across a wide range of different parameters for the numerator and denominator. However, Turner (1959) also stated that the genetic correlation between a ratio and its denominator is nearly always negative and, in fact, can often be strongly negative. This could imply that selection for greater fatty acid concentration in fat could negatively affect the rate of genetic response in fat yield.

Because ratios combine 2 traits, each with their own genetic basis, it can be challenging to predict the outcomes of selection on these ratios. Similarly, by focusing on a ratio, breeders may overlook individuals that excel in one trait but not the other, thereby missing opportunities to improve both traits concurrently. This may occur because the ratio hides information about the absolute values of the contributing traits, leading to potential imbalances. The issues could also be exacerbated in situations where the individual fatty acids are grouped, thereby further hiding the intricacies of selection for the individual fatty acids. When selection is applied to an individual trait (such as increasing C18:1, a desirable MUFA), breeders can have clearer control over the direction of genetic change. In all, many (Gunsett, 1984; Mather et al., 1988) have concluded that direct selection for ratio traits (i.e., fatty acid concentration) is less effective than selection using a linear index of the numerator and denominator for improving the value of the ratio itself.

A similar challenge has been encountered when considering selection for feed efficiency, which is also commonly expressed as a ratio trait; feed conversion efficiency is defined as the ratio of feed or energy intake relative to (energy) output (Beever and Doyle, 2007). To circumvent the challenges of selecting on a ratio trait, Byerly (1941) proposed an alternative approach that later became known as residual feed intake (Koch et al., 1963), wherein feed intake is corrected for differences in energy sinks. The present study attempted to mimic this

strategy where the yield in individual and groups of fatty acids was adjusted phenotypically for the corresponding differences in fat yield. Of particular interest was the extent of genetic variability in this adjusted yield trait, reflecting therefore the capacity to alter fatty acid yield without affecting fat yield. The coefficient of genetic variability in (groups of) fatty acid yield when adjusted phenotypically for fat yield was, on average, half that of the fatty acid yield trait itself. Adjusting the yield of a given fatty acid for genetic differences in fat yield is also possible using the covariance components estimated in the present study; the genetic standard for the yield of a given fatty acid conditional on genetic differences in fat yield (i.e., $\sigma_{FA|FAT}$) is simply

$$\sigma_{FA|FAT} = \sigma_{FA}\sqrt{1 - r_g^2},$$

with σ_{FA} being the genetic SD of the fatty acid yield trait and r_g being the genetic correlation between that fatty acid yield and fat yield. When adjusted genetically for differences in fat yield, the coefficient of genetic variation for the (groups of) fatty acids was similar to that following phenotypic adjustment, varying from 0.02 to 0.11.

The genetic correlation between the ratio of the yield of fatty acid relative to fat yield and the residual from regressing fatty acid yield on fat yield was, on average, moderate (0.46), varying from 0.07 to 0.74 (Table 2). An explanation (with an accompanying equation to calculate the expected correlation) as to why the correlation between these 2 measures is not perfect despite comprising the same 2 component traits was provided by Tavernier et al. (2025), who compared the ratio of nitrogen intake to nitrogen output versus the difference in the 2 traits in dairy cows.

Also of note was that moderate to strong positive genetic correlations existed between fatty acids expressed as a concentration in fat versus milk, while weak or even negative genetic correlations exist for other traits. A clear example is the strong positive genetic correlation of 0.70 between SFA concentration in fat and SFA concentration in milk, while the genetic correlation was −0.07 for UFA. Because total fat composition must sum to 100%, should the SFA concentration in fat increase, the UFA concentration must decrease; the UFA concentration in milk may, however, still increase due to more total fat. This explanation is aided by the positive genetic correlation between the SFA concentration in fat and fat yield (correlation of 0.36; Table 5) with a corresponding negative correlation for UFA (correlation of −0.35; Table 5).

The heritability estimates of the concentration of fatty acids in fat in the present study (0.19 to 0.36) are similar to those reported elsewhere in the study of different fatty acid concentrations in the milk fat of dairy cows in Belgium (0.09 to 0.28; Soyeurt et al., 2007) and New Zealand (0.07 to 0.62; Tiplady et al., 2022). Similarly, the heritability estimates of the concentration of fatty acid groups in fat in the present study (0.21 to 0.30) are similar to those reported elsewhere in the study of different fatty acid groups in the milk fat of dairy cows in Belgium (0.05 to 0.38; Soyeurt et al., 2007) and Canada (0.26 to 0.51; Fleming et al., 2018). Moreover, the heritability of the ratio traits (i.e., the fatty acid concentration) in the present study was, on average, over 2.5 times the heritability of either the yield of the respective fatty acid or fat yield itself; the heritability of fat yield was 0.10. This could be due to several reasons. One reason could be the cancelling out of shared environmental effects between the numerator and denominator caused by management influences having a common effect on both traits or indeed both fatty acid concentration and fat concentration using the same MIR spectrum in their prediction. With the exception of C18:2 *cis*-9 *trans*-11, the residual correlation between each individual fatty acid and fat yield was >0.85, with the mean residual correlation being 0.93.

Extent of Genetic Variability

The accuracy of selection as a contributing factor to genetic gain is a function of the heritability of the trait as well as the information available, be it phenotypic information, genomic information, or a combination of both. The usefulness of MIR spectra for predicting fatty acids in milk has been explored in several dairy cow populations (Soyeurt et al., 2011; Lopez-Villalobos et al., 2014; Fleming et al., 2017), with the accuracy of prediction varying by fatty acid. Because milk MIR data are being routinely captured in many large dairy cow populations, arguably the extent of genetic variability is more important than heritability because the vast quantities of available data will compensate for any low heritability in the pursuit of high accuracy of selection. Even more important, though, is the extent of genetic variability in the fatty acid trait independent of other traits that are being selected on—one such trait is fat yield. The coefficient of genetic variation in the present study for fat yield and milk yield was 0.075 and 0.077, respectively; clear evidence of genetic gain for both traits has been demonstrated in dairy cow populations (Guinan et al., 2023) owing, in part, to the routine access to vast quantities of phenotypic data for these traits. The average coefficient of genetic variability of 0.089 for the yield of individual fatty acids in the present study is therefore larger than that for both fat and milk yield; the coefficient of genetic variation for the individual fatty acids, once adjusted for differences in fat yield, was still, on average, 0.044, suggesting that, once individual cow phenotypic data exists for fatty acids (i.e., from milk MIR predictions) and with

sufficient selection pressure within an overall breeding objective, then changing the fatty acid composition of fat is indeed possible. For example, when expressed as a concentration in fat, cows ranked in the lowest 10% genetically for SFA concentration are expected to have a mean SFA concentration of 67.7%, whereas those in the highest 10% are expected to average 72.7%.

Deployment

How fatty acids are defined serves a different scientific or practical purpose depending on the context of interpretation—nutrition, breeding, milk processing, or animal physiology. The motivation for exploring the alternative definitions in the present study, especially that of the yield of fatty acids independent of fat yield, was to meet the desire of breeders to try and avoid the use of ratio traits in breeding objectives; nonetheless, most dairy cow breeding objectives include SCC, which is, in itself, a ratio, with some dairy cow breeding objectives also including milk fat or protein concentration. Therefore, which definition of fatty acid to use will be a function of the motivation and eventual end use.

Consumers may be interested in the total yield of individual fatty acids consumed—this could be of use in, for example, marketing campaigns, stating what quantity of individual fatty acids are within a serving. The concentration of fatty acids in milk may also be relevant to consumers, as food labels typically report the levels of various constituents per unit of product. From the perspective of milk processing and product quality, it is usually the concentration of selected fatty acids in fat that is of importance (Bobe et al., 2003), although the yield of fatty acid in a milk pool provides information on the total quantity of potentially recoverable fatty acids. Milk processors in many countries currently pay on the yield of fat (and protein) and not actually on the concentration of fat (and protein). Fatty acid concentration in fat is also deemed to be important in understanding and monitoring animal physiology and metabolism (Giannuzzi et al., 2022).

Nonetheless, given the information provided in this study, it is possible to derive the (co)variance components (and therefore also heritability estimates) for a whole plethora of different formulations as well as for new groups of fatty acids. For example, the genetic and phenotypic variance for the sum of individual fatty acids (i.e., a group of fatty acids) can be calculated from the reported covariances among them (Appendix 1). Using this approach, the genetic SD for the group of short-chain fatty acids is 0.304 g/100 g fat, whereas a genetic SD of 0.325 g/100 g fat was estimated in the present study for MIR-predicted short-chain fatty acids. The difference is simply due to the fact that predicted short-chain fatty acids used in the present study were predicted directly as a trait in itself. If, however, the phenotypic values for the individual C4, C6, C8, and C10 fatty acids were summed to generate an overall predicted short-chain fatty acid record, then the genetic SD for this reconstituted predicted short-chain fatty acid trait estimated using REML was indeed 0.304 g/100 g fat. The provided variance components can also be used to estimate the genetic correlation between any individual fatty acid (or group of fatty acids) with this new group of fatty acids (Appendix 1). Furthermore, the variance components for a ratio trait (i.e., concentration) can be estimated from the (co)variance components and means of the individual traits in the numerator and denominator (Appendix 2). The calculated genetic SD for C4 concentration in fat based on the covariance components for C4 and fat yield was 0.107 g/100 g fat, whereas that estimated using REML directly for C4 concentration in fat was 0.116 g/100 g fat. From this also, the expected correlation between the ratio traits and either the denominator or numerator can be calculated (Appendix 2). The expected genetic correlation between C4 concentration in fat and fat yield was calculated to be −0.111, whereas the genetic correlation estimated using REML was −0.118. Similarly, the approach can be used to calculate genetic parameters for other ratios, such as the ratio of n-6 to n-3 with its known health benefits (Simopoulos, 2008; Appendix 2).

Whereas a precedent exists to publish estimates of genetic merit for individual stand-alone traits without explicitly considering these traits in breeding objectives, genetic gain is arguably best achieved when the trait, for instance a fatty acid, is explicitly included in a breeding objective. This then begs the question as to what relative emphasis should be placed on the fatty acid trait within an overall breeding objective and how that emphasis is justified. The emphasis on individual traits in many dairy cow breeding objectives is economic-based, where the weight on a given trait reflects the expected change in profit per unit change in that trait, holding all other traits in the breeding objective constant. An alternative strategy to determine the weighting factor on an individual trait, especially those with no obvious (current) monetary value, is a desired gains index (Cameron, 1997), where the weight on the trait is such to expect a desired rate of genetic gain. Henchion et al. (2016) proposed using a Delphi survey of stakeholders to gauge the relative emphasis that could be placed on a trait in a dairy cow breeding objective and, in their case study of dairy cow breeding in Ireland, concluded that a relative emphasis of 4% to 10% should be placed on milk quality. A similar approach is a preference-based approach (Byrne et al., 2012), which also leverages stakeholder preferences. Should fatty acid yield be considered a goal trait in a breeding objective, then the relative emphasis on the fatty acid trait relative to fat yield (and other traits in the

breeding objective) is important, as it will dictate the rate of genetic change in fatty acid concentration. Applying an economic weight to a ratio (i.e., fatty acid concentration) could, nonetheless, prove difficult because the change in the ratio can be affected by either a change in the numerator or the denominator (Pym, 1985).

CONCLUSIONS

Several alternative definitions of milk fatty acids exist, each with their own statistical properties and uses. Each definition, however, reflects variability not captured in the other definitions. This was demonstrated by only moderate genetic correlations between the different formulations. Large genetic variability in the yield of individual and groups of fatty acids exists, albeit half of this genetic variability can be attributable to genetic differences in fat yield. Nonetheless, an opportunity does exist to alter either the yield or concentration of fatty acids in bovine milk. Having routine access to low-cost predictions of individual fatty acids from milk MIR data means that delivering accurate genetic evaluations is possible—the strategy for applying selection pressure on fatty acids, however, warrants further investigation.

NOTES

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Nonstandard abbreviations used: LCFA = long-chain fatty acids; MCFA = medium-chain fatty acids; MIR = mid-infrared; OCFA = odd-chain fatty acids; SCFA = short-chain fatty acids.

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APPENDIX 1

The variance of the sum of individual fatty acids (FA) can be calculated as

$$var\left(\sum_{i=1}^{n} FA_i\right) = \mathbf{1}^T \sum \mathbf{1} = \sum_{i=1}^{n} Var\left(FA_i\right) + 2\sum_{i<j} Cov\left(FA_i, FA_j\right), \quad [1]$$

where $\mathbf{1}$ is an $n \times 1$ vector of ones and $\mathbf{\Sigma}$ is an $n \times n$ (co) variance matrix between the n FA, noted as FA_i and FA_j. The covariance between any one of the individual FA and that group of FA can be computed as

$$Cov\left(FA_i, \sum_{i=1}^{n} FA_i\right) = \sum_{i=1}^{n} Cov\left(FA_i, FA_j\right), \quad [2]$$

which is simply the sum of the ith row of the (co)variance matrix $\mathbf{\Sigma}$. This can then be used along with the variance of the ith fatty acid and the variance of the sum of the FA (Equation 1) to calculate the correlation between the ith fatty acid and the group of FA as

$$Corr\left(FA_i, \sum_{i=1}^{n} FA_i\right) = \frac{Cov\left(FA_i, \sum_{i=1}^{n} FA_i\right)}{\sqrt{var\left(\sum_{i=1}^{n} FA_i\right)}\sqrt{FA_i}}. \quad [3]$$

From this framework, it is also possible to calculate the covariance and, by extension, the correlation between groups of FA.

The group of short-chain fatty acids (**SCFA**) was used to illustrate the presented equations with the genetic (co) variance matrix between C4, C6, C8, and C10 described as (in that order)

$$\begin{bmatrix} 0.013358 & 0.004452 & 0.001545 & -0.004486 \\ 0.004452 & 0.004954 & 0.003332 & 0.007411 \\ 0.001545 & 0.003332 & 0.002911 & 0.008161 \\ -0.004486 & 0.007411 & 0.008161 & 0.030735 \end{bmatrix}.$$

The genetic SD of the SCFA group calculated using the covariance matrix was 0.304 g/100 g in fat. The genetic

SD of the direct milk mid-infrared prediction of SCFA in the dataset was 0.325 g/100 g in fat; however, once the SCFA was recalculated phenotypically as the sum of the individually predicted FA and the variance components re-estimated, then the genetic SD of the recalculated SCFA was 0.304 g/100 g in fat.

The correlation between C4 and SCFA calculated using the presented equation was 0.42; this was the same as the correlation estimated using REML from the dataset irrespective of whether the actual direct milk mid-infrared prediction of SCFA was used or the recalculated SCFA from the sum of the individually predicted FA.

Finally, the correlation between the sum of C4 and C6 with the sum of C8 and C10 was calculated using the (co)variance matrix. The calculated correlation was 0.21, which is the same as was estimated using REML, where 2 new phenotypic variables were calculated as C4 + C6 and separately as C8 + C10.

APPENDIX 2

It is possible to estimate the variance of the ratio of 2 traits using a Taylor series approximation:

$$var\left(\frac{X}{Y}\right) \approx \left(\frac{\sigma_X}{\mu_Y}\right)^2 + \left(\frac{\mu_X \sigma_Y}{\mu_Y^2}\right)^2 - 2 \cdot \frac{\mu_X \sigma_{XY}}{\mu_Y^3},$$

where μ_* is the mean of trait $*$, σ_* is the SD of trait $*$, and σ_{XY} is the covariance between X and Y. The mean and genetic variance for C4 yield is 15.78 g and 1.625 g^2, respectively, with the respective values for fat yield being 5.479 (100 g) and 0.1736 $(100\ g)^2$. The covariance between C4 and fat yield is 0.4713. The expected genetic variance for the ratio is 0.01153, whereas that estimated using REML for the concentration trait itself was 0.01336.

The expected correlation between the concentration trait and fat yield (i.e., Y) is

$$r_{\left(\frac{X}{Y},Y\right)} \approx \frac{\frac{\sigma_{XY}}{\mu_Y} - \mu_X \cdot \frac{\sigma_Y^2}{\mu_Y^2}}{\sqrt{\text{var}\left(\frac{X}{Y}\right)} \cdot \sigma_Y}.$$

Using the parameters already defined for C4 and fat yield (as well as the ratio just calculated), the expected genetic correlation between C4 concentration in fat and fat yield is −0.113, whereas that estimated directly using REML was −0.1181.

The ratio of n-6 to n-3 has been proposed as being important for chronic diseases, including cardiovascular disease (Simopoulos, 2008). The estimated genetic covariance matrix between n-3 (trait 1) and n-6 (trait 2) was

$$\begin{bmatrix} 0.000863 & 0.001197 \\ 0.001197 & 0.010500 \end{bmatrix},$$

and the mean of n-3 and n-6 was 0.6663 g/100 g in fat and 2.591 g/100 g in fat, respectively. From this, the estimated genetic SD of the ratio of n-6 to n-3 was 0.179 g/100 g in fat. The estimated genetic SD of the ratio trait using REML was 0.188 g/100 g in fat.