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Age at First Calving and Udder Health as Factors Associated with Milk Fatty Acid Composition in Dairy Cows

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Abstract

This exploratory single-farm study investigated age at first calving (AFC), somatic cell count (SCC), and Fourier-transform mid-infrared (MIR)-predicted milk fatty acid concentrations and fat-normalized proportions in Black-and-White dairy cows. After removal of one record with a repeated ear-tag identifier, 190 unique milk records were analyzed; individual AFC data were available for 180 cows and paired udder temperatures for 185. AFC was not significantly associated with SCC (Spearman $\rho = +0.103$, $p = 0.167$; $n = 180$). Across 190 samples, SCC was modestly associated with higher saturated fatty acid (SFA) proportion ($\rho = +0.270$, $p = 0.00017$, false-discovery-rate $q = 0.00184$) and lower polyunsaturated fatty acid (PUFA) proportion ($\rho = -0.245$, $p = 0.00065$, $q = 0.00358$). In 185 paired thermal records, mean post-minus-pre-milking udder temperature changes were +0.146 °C ($n = 137$) and +1.494 °C ($n = 48$) in lower- and elevated-SCC groups, respectively (Welch $p = 0.0083$). The fat-normalized fatty acid patterns and thermal findings are observational; no independent effects of AFC or diagnostic performance can be inferred without parity, days-in-milk, milk-yield, and standardized imaging information.

Keywords: milk fatty acid fractions; mid-infrared spectroscopy; somatic cell count; dairy cow; milk composition; infrared thermography; ultrasonography; age at first calving

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1. Introduction

Milk and dairy products are important components of human diets and provide proteins, lipids, minerals, and other nutrients [1]. Biomolecular evidence recovered from archeological material also indicates a long history of milk consumption from several animal species [2–4].

Bovine milk accounts for approximately 80.0% of global milk production [5,6]. According to the OECD-FAO Agricultural Outlook 2025–2034, global milk production is projected to grow by an average of 1.8% annually and to reach approximately 1.146 billion tonnes by 2034 [7]. Maintaining milk quality while supporting animal health therefore remains relevant to sustainable dairy production.

Milk is a complex biological fluid containing lipids, proteins, carbohydrates, minerals, and a broad range of minor metabolites [8]. Its nutritional and technological properties vary among animal species and according to milk composition [9].

Within dairy cattle, milk yield and composition are influenced by multiple factors, including diet [10], housing and milking systems [11], environmental conditions and stress [12], physiological status and udder health [13–15], and the milk microbiome [16]. Consequently, interpretation of milk-quality traits requires consideration of both animal-related and management-related sources of variation.

Milk protein and fat, fatty acid composition, somatic cell count (SCC), casein, and β-hydroxybutyrate are among the traits used to characterize milk composition, metabolic status, or udder health [17–19]. Genetic variation also contributes to fatty acid composition, with specific genes and single-nucleotide polymorphisms associated with concentrations of individual milk fatty acids [20].

Breed-related differences in milk fatty acid profiles have been reported [21]. The milk fat-to-protein ratio requires interpretation in the context of parity, lactation stage, and energy balance rather than as a stand-alone health indicator [22]. Somatic cell count (SCC) is a useful indicator of udder health, but its interpretation is influenced by physiological and management factors and does not establish the etiology of mastitis by itself [23,24]. AFC is a historical reproductive trait, whereas SCC is assessed at the time of sampling. Their associations with MIR-predicted fatty acid concentrations and fat-normalized proportions must therefore be interpreted cautiously, particularly when parity, days in milk, and individual milk yield are unavailable.

This observational study evaluated the associations of SCC and AFC with MIR-predicted concentrations and fat-normalized proportions of milk fatty acid groups. Infrared thermography and descriptive ultrasonography provided secondary information about the mammary gland. We treated AFC as a continuous variable and did not seek to determine an optimal calving age, demonstrate causal effects, or validate a disease-diagnostic test.

2. Materials and Methods

2.1. Ethics Approval

The study protocol was approved by the Ethics Committee of the Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences (Protocol No. 2, 4 June 2025).

2.2. Study Site and Animals

The observational study was conducted on a commercial dairy farm in the Nizhny Novgorod region, Russian Federation (54.788559, 44.844092), where Black-and-White dairy cows were housed in tie-stalls. The original farm registry contained 192 animal records (Supplementary Table S1), whereas the laboratory dataset comprised 191 complete milk-sample records. No corresponding laboratory record was available for the animal identified by ear tag 33,227. According to the farm breeding records, the source population comprised 177 purebred Black-and-White cattle with no Holstein ancestry (0%) and 15 Holstein-crossbred Black-and-White cattle with documented Holstein ancestry ranging from 69% to 97%. Individual breed classifications and ancestry proportions are provided in Supplementary Table S1. Ear-tag identifier 73,950 appeared twice in the laboratory dataset, corresponding to records 69 and 70. As the identity of the animal associated with the second occurrence could not be independently verified, record 70 was excluded, and the first occurrence was retained. This procedure resulted in a final analytical dataset comprising 190 records with unique ear-tag identifiers. Individual age-at-first-calving (AFC) data were extracted from the farm records and linked to the laboratory measurements using ear-tag

identifiers and sample sequence information. Valid AFC measurements were successfully matched for 180 cows. Although calving-number information was available in the farm registry, its temporal correspondence to the milk-sampling date was not independently verified. Therefore, it was not included as a parity covariate in the regression models. Paired pre- and post-milking temperature values were available for 185 cows. Parity, days in milk, current age, and actual cow-level milk yield were not verified in the analysis-ready dataset. Farm personnel supplied a reference ration formulation and calculated nutritional specifications (Tables 1 and 2). Its correspondence to the exact sampling period could not be independently established, and individual feed intakes were unavailable; therefore, these values describe an unverified farm reference rather than observed cow-level exposure and were not used as model covariates. No controlled dietary intervention was imposed.

Table 1. Investigator-supplied farm reference ration: ingredients and dry matter per cow per day. This formulation was not independently verified as the diet fed to the sampled animals.

Feed Ingredient	As-Fed kg/Cow/Day	Dry Matter kg/Cow/Day
Corn silage	20.00	6.46
Alfalfa haylage	17.00	5.36
Wheat straw	2.20	1.89
Fresh brewers' grains	10.00	2.75
Rolled corn	3.00	2.05
Dairy concentrate	7.50	6.65
Total	59.70	25.16 (rounded)

Table 2. Investigator-supplied calculated nutritional characteristics of the reference ration; these are not verified individual intake measurements.

Nutritional Characteristic	Reported Amount	Units
NEL	152.76	MJ/cow/day
NEL per dry matter	6.07	MJ/kg DM
Crude protein	3578.49	g/cow/day
Crude protein in DM	14.23	% DM
NDF	38.61	% DM
ADF	24.97	% DM
ADL	3.48	% DM
NFC	35.26	% DM
Starch	5469.99	g/cow/day
Starch in DM	21.75	% DM
Rumen nitrogen balance	−6.33	g/cow/day

2.3. Milk Sampling and Analysis

One representative milk sample per cow was obtained during morning milking by collecting portions at the beginning, middle, and end of milk ejection; morning and evening samples were not pooled. Samples were transported under refrigeration (reported 2–4 °C). Milk components and grouped fatty acid concentrations were predicted by Fourier-transform mid-infrared spectroscopy using a MilkoScan 7 RM analyser within the CombiFoss 7 system, and SCC was measured using a Fossomatic 7 DC unit (all manufactured by FOSS Analytical A/S, Hillerød, Denmark). No fatty-acid extraction, methylation, GC, or GC–MS was performed on the study samples. Fatty acids are recorded in g/100 g milk and as percentages of milk fat (100 × fatty acid concentration [g/100 g milk]/milk fat [%]). The latter values represent calculated MIR-based proportions rather than independent reference measurements. The version and external accuracy of the specific fatty acid calibration applied to these samples were not available and require verification. The

MilkoScan 7 RM method complies with ISO 9622/IDF 141:2013 [25] and AOAC Official Method 972.16 [26]; the instrument is CE marked. The Fossomatic 7 DC uses somatic cell counting technology conforming to ISO 13366-2/IDF 148-2:2006 [27], is CE marked, and has EURL/MicroVal (Europe) and FDA/NCIMS (USA) approvals.

2.4. Thermography and Ultrasonography

Ultrasonography was conducted on the caudal udder quarters of 40 cows using a KX5600G ultrasound scanner and a 3.5L160E2 linear probe (Xuzhou Kaixin Electronic Instrument Co., Ltd., Xuzhou, China; 2.0–5.0 MHz, operated at 3.5 MHz). The displayed ultrasound images are qualitative examples, not a systematically scored or statistically analyzed series; anatomical restriction to the caudal quarters limits generalization to the whole udder. Thermographic imaging before and after milking was performed using a Guide H2 thermal imaging camera (Wuhan Guide Sensmart Tech Co., Ltd., Wuhan, China) with a detector resolution of 256 × 192 pixels and a spectral range of 7.5–14 µm. A representative image record documented an emissivity of 0.95, reflected temperature of 23 °C, relative humidity of 70%, and acquisition distance of up to 3 m; these settings have not been verified across all examinations. The 185 matched pairs of numerical udder temperatures were independently analyzed, defining ΔT as post-milking minus pre-milking temperature. Acquisition intervals and reproducible image-region definitions were not documented. These imaging measurements should not be interpreted as independent diagnoses of mastitis.

2.5. Statistical Analysis

Data analyses included 190 uniquely identified cow-level milk records. AFC ($n = 180$) and paired udder temperatures ($n = 185$) were linked from the farm worksheet by ear-tag identifier, with a sequence check for repeated tags. Descriptive results are mean ± SD, median (Q1–Q3), or n, as appropriate. Distributions were assessed with the Shapiro–Wilk test. Two-sided Spearman rank correlations were used for exploratory relationships involving AFC, SCC, fatty acid concentrations (g/100 g milk), and fatty acid proportions (% of total fat). For the family of 11 SCC–fatty-acid proportion comparisons, Benjamini–Hochberg q values were computed. AFC associations with the five main fractions were separately adjusted using Benjamini–Hochberg correction. An exploratory standardized rank-based OLS regression with HC3 robust standard errors tested AFC, fat percentage, and true protein as simultaneous predictors of ranked SCC ($n = 180$); rank models for PUFA and SFA proportions additionally included SCC. Coefficient estimates were reported with robust confidence intervals. Sensitivity analyses were conducted using only positive SCC values. These models cannot control for unavailable parity, days in milk, or cow-level milk yield. Of the 190 records, 27 showed SCC = 0; primary rank correlations retained those reported values and a sensitivity analysis restricted SCC to positive records. The thermographic ΔT difference for the two SCC groups (≤200 vs. >200 × 10^3 cells/mL) was tested using Welch's unequal-variance t-test with a 95% confidence interval. Statistical analyses were independently reproduced from the supplied source files using Python (v3.14.6), SciPy (v1.18.0), and statsmodels (v0.14.6).

3. Results

Of 192 initially recorded animal/sample entries, 191 complete laboratory records were available; one record with a duplicate ear tag was removed, leaving 190 independent identifiers. Individual AFC values were available for 180 cows and matched paired thermal values for 185 cows (Table 3). Milk fat averaged 4.19 ± 1.90% (mean ± SD); true protein averaged 3.22 ± 0.64%; lactose 4.39 ± 0.66%. SCC had a skewed distribution (median

45.05 × 10^3 cells/mL; IQR 11.43–216.35); 27 records showed SCC = 0, and 49/190 (25.8%) exceeded 200 × 10^3 cells/mL. The AFC mean was 29.16 ± 6.28 months (n = 180).

Table 3. Sample inclusion and descriptive characteristics of the analyzed records, including available age at first calving and thermographic measurements.

Parameter	Unit	*n*	Mean ± SD	Median (Q1–Q3)
Initial registry	entries	192	—	—
Complete laboratory records	samples	191	—	—
Unique retained ear tags	cows	190	—	—
Age at first calving	months	180	29.16 ± 6.28	28.00 (24.00–33.00)
Milk fat	%	190	4.19 ± 1.90	4.05 (2.77–5.34)
True protein	%	190	3.22 ± 0.64	3.16 (2.87–3.50)
Lactose	%	190	4.39 ± 0.66	4.57 (4.29–4.78)
SCC	×10^3 cells/mL	190	467.72 ± 1275.12	45.05 (11.43–216.35)
SFA, fat-normalized	% of fat	190	59.39 ± 6.53	58.52 (55.58–61.65)
PUFA, fat-normalized	% of fat	190	3.80 ± 0.77	3.70 (3.35–4.10)
Udder temperature change	°C	185	0.50 ± 3.19	1.60 (−2.60–3.20)

The Shapiro–Wilk statistics were W = 0.921, $p < 0.0001$ for AFC (n = 180); W = 0.979, $p = 0.0064$ for milk fat (n = 190); and W = 0.399, $p < 0.0001$ for SCC (n = 190). Additional variable-wise tests are reported in Supplementary Table S2.

After matching individual AFC values from the farm records, no statistically significant association was observed between AFC and SCC (Spearman ρ = +0.103, $p = 0.167$; n = 180). AFC–PUFA proportion showed an exploratory negative unadjusted association (ρ = −0.218, $p = 0.0033$, $q = 0.0164$ across five principal fatty acid groups). AFC showed no statistically supported relationship with SCC after adjustment for milk fat and true protein (standardized-rank β = +0.104, 95% CI −0.056 to +0.265, $p = 0.202$). These models do not adjust for parity, days in milk, or milk yield and remain exploratory.

In the same n = 180 AFC-matched subset, the Spearman association between AFC and milk fat was ρ = +0.140 ($p = 0.060$); the SCC–milk fat correlation was ρ = +0.073 ($p = 0.330$). The three-variable rank correlations are displayed in Figure 1.

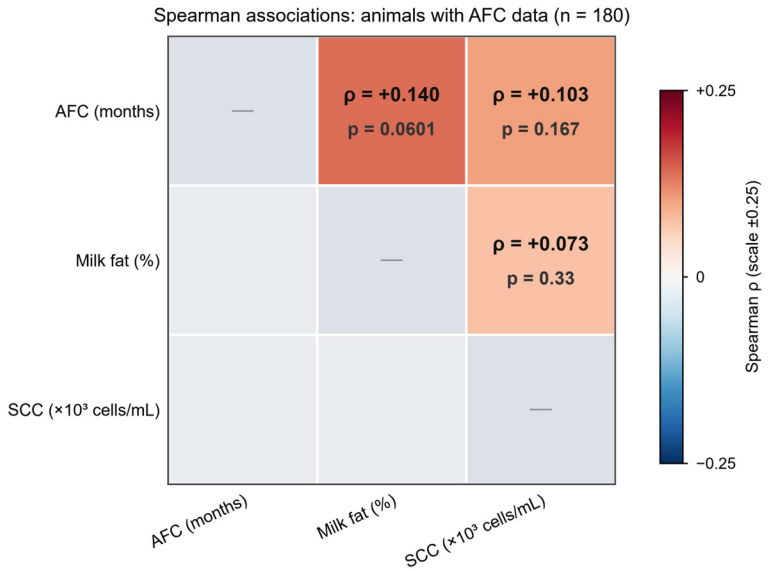

Figure 1. Spearman rank correlations between AFC, milk fat, and SCC, calculated in the same 180 cows with recorded AFC. All coefficients and two-sided p values were recalculated from linked individual observations; AFC, age at first calving; SCC, somatic cell count.

Matched pre- and post-milking temperature values were obtained for 185 uniquely identified cows. The SCC ≤ 200 × 10^3 cells/mL group included 137 cows; the >200 × 10^3 cells/mL group included 48 cows. Mean ΔT and variability are reported in Table 4.

Table 4. Udder surface temperature change (ΔT = temperature after milking minus temperature before milking) according to SCC group (n = 185).

SCC Group (×10^3 Cells/mL)	n	Mean ΔT (°C) ± SD
≤200	137	+0.146 ± 3.228
>200	48	+1.494 ± 2.886

Mean ΔT was +0.146 ± 3.228 °C (mean ± SD) in the lower-SCC group and +1.494 ± 2.886 °C in the elevated-SCC group. The mean between-group difference was 1.348 °C (95% CI 0.355–2.340 °C; Welch's t-test p = 0.0083). This observational comparison does not demonstrate thermographic diagnostic accuracy.

All 11 MIR-predicted fatty acid fractions were analyzed using the 190 retained records (Table 5). Absolute predicted fatty acid concentrations depended strongly on the amount of milk fat; fat-normalized estimates were therefore examined separately. SCC was positively correlated with SFA proportion (ρ = +0.270, p = 0.00017, q = 0.00184) and negatively correlated with PUFA proportion (ρ = −0.245, p = 0.00065, q = 0.00358). Associations with MUFA (ρ = −0.116, p = 0.111) and SCFA (ρ = −0.128, p = 0.078) were not significant in the full dataset. Spearman correlations between SCC and selected MIR-predicted fatty acid proportions are shown in Figure 2. Total fat-normalized proportions are calculated from the same MIR prediction family and must not be interpreted as independently measured chromatographic fractions.

Table 5. MIR-predicted fatty acid concentrations and fat-normalized proportions, with recalculated two-sided Spearman correlations and false-discovery-rate q values (n = 190).

Fatty Acid Group	Conc. g/100 g Milk Mean ± SD	% Total Fat Mean ± SD	ρ SCC: Conc. (p)	ρ SCC: % Fat (p)	q (% Fat)	ρ with Fat
C14:0	0.378 ± 0.141	10.13 ± 4.18	+0.123 (0.091)	−0.085 (0.242)	0.29549	+0.872
C16:0	0.961 ± 0.405	24.77 ± 8.19	+0.322 (<0.0001)	+0.233 (0.0012)	0.00446	+0.863
C18:0	0.380 ± 0.192	8.85 ± 2.42	+0.048 (0.509)	+0.036 (0.625)	0.62529	+0.931
C18:1	1.401 ± 0.638	33.69 ± 4.85	+0.026 (0.718)	−0.169 (0.020)	0.04307	+0.965
SCFA	0.436 ± 0.251	9.73 ± 2.52	+0.005 (0.948)	−0.128 (0.078)	0.12269	+0.957
MCFA	1.382 ± 0.547	36.10 ± 12.27	+0.272 (0.0001)	+0.141 (0.053)	0.09707	+0.870
LCFA	1.707 ± 0.879	39.38 ± 9.43	−0.020 (0.780)	−0.181 (0.013)	0.03439	+0.957
SFA	2.481 ± 1.153	59.39 ± 6.53	+0.162 (0.026)	+0.270 (0.00017)	0.00184	+0.973
MUFA	1.374 ± 0.617	33.24 ± 4.49	+0.050 (0.492)	−0.116 (0.111)	0.15201	+0.968
PUFA	0.152 ± 0.063	3.80 ± 0.77	+0.023 (0.755)	−0.245 (0.00065)	0.00358	+0.938
TFA	0.139 ± 0.079	3.21 ± 1.18	+0.061 (0.402)	+0.059 (0.419)	0.46133	+0.859

Note: ρ, Spearman's rank correlation coefficient; p, unadjusted two-sided p value; q, Benjamini–Hochberg-corrected p value for 11 SCCs versus fat-normalized fraction comparisons. All reported associations were recalculated after removing one record with an ambiguous duplicate ear tag. SCC = 0 records (n = 27) are retained in primary rank analyses. The listed fatty acid categories partly overlap (individual acids, chain-length classes, and saturation classes) and therefore must not be summed across all 11 rows.

Exploratory standardized-rank regression results are presented in Table 6 (n = 180). With ranked SCC as the outcome, AFC was not associated after adjustment for milk fat and true protein (β = +0.104, 95% CI −0.056 to +0.265, p = 0.2018). In models for fat-normalized outcomes, the AFC coefficient was −0.146 for PUFA proportion (95% CI −0.279 to −0.013, p = 0.0315) and −0.013 for SFA proportion (95% CI −0.156 to +0.130, p = 0.8568). SCC coefficients were −0.188 (95% CI −0.322 to −0.054, p = 0.0059) for PUFA and +0.253 (95% CI +0.109 to +0.398, p = 0.0006) for SFA. Coefficients were not adjusted for unrecorded

parity, DIM, or milk yield; all p values for these exploratory models are unadjusted for the number of models and coefficients tested.

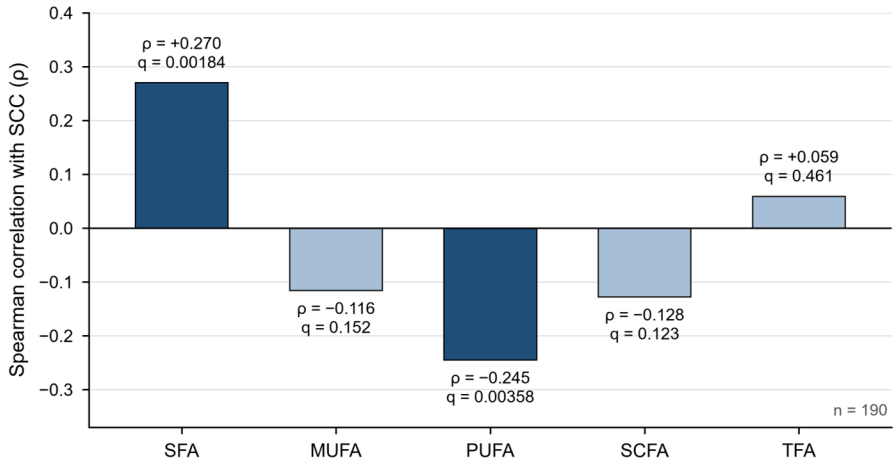

Figure 2. Spearman correlations of SCC with MIR-predicted fatty acid proportions in total milk fat after removal of the repeated identifier ($n = 190$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCFA, short-chain fatty acids; TFA, trans fatty acid isomers. The indicated q values are corrected over all 11 predicted fractions.

Table 6. Exploratory multiple regression on standardized within-sample ranks using HC3 robust standard errors ($n = 180$). β denotes standardized-rank coefficient; CI, confidence interval. Fat, milk fat concentration; protein, true milk protein. Models do not adjust for parity, days in milk, current age, or individual milk yield.

Outcome	*n*	Covariate	β	95% CI	*p*
SCC	180	AFC	+0.104	−0.056 to +0.265	0.2018
SCC	180	Milk fat	+0.082	−0.080 to +0.244	0.3186
SCC	180	True protein	+0.100	−0.054 to +0.255	0.2024
PUFA (% of milk fat)	180	AFC	−0.146	−0.279 to −0.013	0.0315
PUFA (% of milk fat)	180	SCC	−0.188	−0.322 to −0.054	0.0059
PUFA (% of milk fat)	180	Milk fat	−0.414	−0.552 to −0.275	<0.0001
PUFA (% of milk fat)	180	True protein	−0.045	−0.183 to +0.092	0.5174
SFA (% of milk fat)	180	AFC	−0.013	−0.156 to +0.130	0.8568
SFA (% of milk fat)	180	SCC	+0.253	+0.109 to +0.398	0.0006
SFA (% of milk fat)	180	Milk fat	+0.015	−0.144 to +0.174	0.8559
SFA (% of milk fat)	180	True protein	+0.158	+0.008 to +0.309	0.0386

The reported SCC = 0 entries ($n = 27$) were retained in the primary analysis. Excluding these values reduced the sample for SCC comparisons from 190 to 163 (Table 7). The SFA and PUFA associations remained directionally consistent; for PUFA the coefficient changed from $\rho = -0.245$ ($p = 0.00065$) to $\rho = -0.195$ ($p = 0.01279$). MUFA and SCFA findings were more sensitive to exclusion. Because the laboratory interpretation of reported zero SCC values is not documented, these comparisons should be regarded as sensitivity analyses rather than evidence that either zero-handling rule is definitive. Representative pre- and post-milking infrared thermograms and ultrasonographic images of the mammary gland are presented in Figure 3.

Table 7. Sensitivity analysis of Spearman correlations between SCC and MIR-predicted fatty acid proportions, including versus excluding reported zero-SCC observations. Two-sided *p* values shown without multiplicity correction for this sensitivity analysis.

Fraction	All *n*	ρ All	*p* All	SCC > 0 *n*	ρ SCC > 0	*p* SCC > 0
SFA	190	+0.270	0.00017	163	+0.315	0.00004
MUFA	190	−0.116	0.11056	163	−0.137	0.08227
PUFA	190	−0.245	0.00065	163	−0.195	0.01279
SCFA	190	−0.128	0.07807	163	−0.174	0.02658
TFA	190	+0.059	0.41939	163	+0.033	0.67266

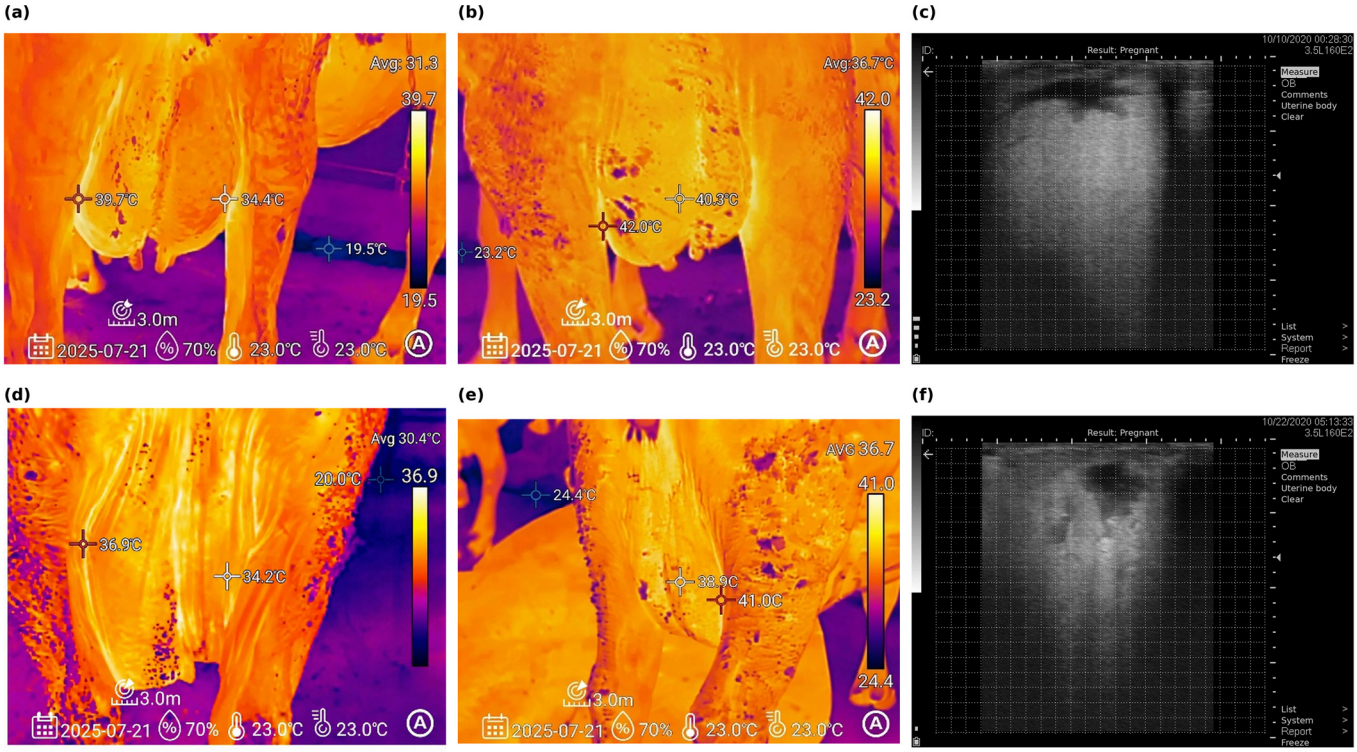

Figure 3. Illustrative pre- and post-milking infrared thermograms and rear-quarter ultrasonographic views of the bovine mammary gland. Forty cows underwent the ultrasound examination; two selected examples are shown without systematic echostructural scoring. Panels (**a**,**b**,**d**,**e**) present representative thermal images; panels (**c**,**f**) illustrate differences in parenchymal echostructure. These images were not used to estimate diagnostic sensitivity, specificity, or ultrasound lesion prevalence.

4. Discussion

The reanalysis differentiates MIR-predicted fatty acid concentrations per unit of milk from proportions relative to total fat. Because the absolute concentrations depend substantially on total milk fat, correlations involving concentration must not automatically be interpreted as compositional changes. In the 190 unique milk records, the corrected fat-normalized analyses found modest SCC associations with SFA and PUFA; correlations for MUFA and SCFA did not reach statistical significance.

Published studies indicate that dietary fat can affect milk composition [28], while farm, breed, lactation stage, parity, and genetic variation contribute to differences in milk fatty acid profiles [29]. The milk fat-to-protein ratio has also been evaluated in relation to ruminal pH, but it cannot substitute for direct assessment of nutritional or metabolic status [30]. More generally, milk fatty acid composition depends on physiology and nutrition [31], shows variation throughout lactation in MIR-predicted traits [32], and is affected by the regulation of mammary lipid synthesis and dietary manipulation of milk fat [33,34]. These

determinants were not all available as individual-animal covariates in the present study and may contribute to residual confounding.

AFC was available for 180 uniquely identified cows and was treated as a continuous variable. The newly verified AFC–SCC association was weak and nonsignificant ($\rho = +0.103$, $p = 0.167$), and an exploratory model incorporating milk fat and protein did not support an adjusted AFC association ($\beta = +0.104$, $p = 0.202$). A negative unadjusted AFC–PUFA proportion association ($\rho = -0.218$, $q = 0.0164$) should be treated as hypothesis-generating because parity, days in milk, and milk yield were not available as validated covariates. The former four k-means groups and their unsupported claims of AFC thresholds were excluded from inferential analysis.

The recovered individual milk data permit exploratory rank-based multivariable analysis with milk fat and true protein as covariates, and AFC values were matched for 180 cows (Table 6). AFC was not associated with ranked SCC in the adjusted exploratory model ($\beta = +0.104$, 95% CI -0.056 to $+0.265$, $p = 0.2018$). For PUFA proportion, AFC ($\beta = -0.146$, $p = 0.0315$) and SCC ($\beta = -0.188$, $p = 0.0059$) had negative rank-model coefficients; for SFA proportion, SCC had a positive coefficient ($\beta = +0.253$, $p = 0.0006$). These exploratory coefficients are not independent causal effects, and the p values were not corrected for multiple models. Physiological confounders, including parity, days in milk and yield, remained unmeasured. Removing the 27 zero-SCC records changed the magnitude and significance of some correlations (Table 7), further limiting strong clinical interpretation.

The continuous AFC data do not support an empirically determined optimal age at first calving or biological thresholds. The former four age clusters were generated for descriptive convenience without prespecification; after multiple-comparison correction their reported pairwise differences were absent. These categories and the associated one-way ANOVA/Kruskal–Wallis tests are therefore not used in the revised inferential framework.

Feeding strategies and Holstein genotype have been evaluated in relation to milk fatty acid profiles [35]. Solarczyk et al. examined reproductive characteristics, metabolic profiles, and fatty acid composition in dairy cattle [36]. Breed comparisons using MIR-predicted milk fatty acids [37] and studies contrasting early- and late-lactation lipid composition [38] further emphasize the potential importance of physiological context. These publications provide biological context but do not establish an independent AFC effect in the present cohort.

Stage-specific differences in mammary transcripts involved in milk component synthesis have been studied in ruminants [39], and experimental investigations have shown regulation of lipogenic gene networks in bovine mammary epithelial cells [40]. A multisite lipidomic study also identified parity and ageing as relevant sources of variation in dairy cow milk lipids [41]. Neither mammary gene expression nor parity-related variation could be directly evaluated in the present data.

Reviews of milk fat synthesis and nutritional manipulation describe multiple processes that can influence milk fatty acid distributions [42,43]. Associations of environmental conditions and DGAT1 polymorphism with milk production traits and fatty acid profiles have been reported in Holsteinized Black-and-White cows [44]. Mechanistic work has also investigated the determinants of fatty acid chain length during dairy fat synthesis [45]. These pathways are plausible biological context only; the current observational dataset does not test their activity or establish causal mechanisms.

The fatty acid groups were estimated using MIR and then expressed both per 100 g milk and as shares of milk fat. The share measurements are ratios involving total-fat measurements and remain subject to calibration error, denominator dependence, compositional constraints, and possible sample-level bias. Their associations with SCC cannot independently establish differences in mammary lipid synthesis.

Studies at the level of individual udder quarters have investigated relationships between SCC and milk fatty acid composition [46] and between subclinical intramammary infection and fatty acid profiles [47]. Relationships involving SCC, lipogenic gene expression, and antioxidant-related pathways have also been examined [48]. In our reanalysed dataset, SCC was modestly associated with a higher MIR-predicted SFA proportion and a lower PUFA proportion after correction for multiple testing. These observational findings do not establish that udder inflammation caused the observed differences in fatty acid estimates. Neither microbiological confirmation of intramammary infection nor its etiological agents were available in the source dataset.

Ultrasonography has been evaluated for mammary cistern assessment in cows with clinical or subclinical mastitis [49]. Non-contact udder assessment approaches [50] and infrared-thermography classification methods [51] have been investigated, while a review discusses thermography as a potential mastitis-assessment tool and its limitations [52]. Other studies have considered ultrasound for predicting outcomes in clinical mastitis [53], identifying mastitis-related findings and risk factors [54], and the wider role of on-farm diagnostic methods [55]. In the present study, paired thermographic values were available for 185 uniquely identified cows, and the elevated-SCC group showed a larger mean pre-to-post-milking temperature change (difference 1.348 °C, 95% CI 0.355–2.340, $p = 0.0083$). However, milking-related changes and incompletely controlled acquisition conditions may have contributed to the observed difference. The image acquisition intervals and region-of-interest definitions were not sufficiently documented. Ultrasonography was conducted in 40 cows, but the available images were not systematically scored. Accordingly, neither imaging approach can be regarded as a validated diagnostic test in this study.

The main limitations are the single-herd observational design, absence of verified days in milk, current age, parity and cow-level milk yield, and incomplete documentation of MIR calibration and imaging acquisition. The laboratory file contains 191 complete records, but exclusion of a repeated ear-tag occurrence reduced the primary analytic sample to 190 independently identified animals; 180 could be linked to AFC and 185 to paired temperatures. The farm worksheet and laboratory list contain one unresolved discrepancy in the first ear-tag identifier, and its AFC measurement was not imputed. SCC values of zero were retained as reported, with positive-SCC sensitivity analyses. The investigators supplied a reference ration and nutrient profile (Tables 1 and 2), but its temporal and animal-level representativeness was not independently verified. These constraints preclude causal inference or validated mastitis diagnosis.

5. Conclusions

After removal of one repeated identifier, 190 uniquely identified cow-level milk records showed a modest positive SCC association with the MIR-predicted SFA proportion ($\rho = +0.270$, $q = 0.00184$) and a modest negative association with the PUFA proportion ($\rho = -0.245$, $q = 0.00358$). No statistically significant association was observed between AFC and SCC ($n = 180$, $\rho = +0.103$, $p = 0.167$). In the matched thermographic subset ($n = 185$), cows with elevated SCC had a larger average pre-to-post-milking change in udder temperature (difference 1.348 °C, $p = 0.0083$). Given the observational design and incomplete physiological and measurement covariates, these findings remain exploratory and do not demonstrate a causal AFC effect or diagnostic performance of imaging.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/dairy7060101/s1, Table S1. Individual animal registry characteristics, including breed classification, Holstein ancestry, reproductive status, date of birth, age at first calving, and calving number; Table S2. Detailed results of Shapiro–Wilk normality tests for the analyzed variables.

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Informed Consent Statement: Written informed consent was obtained from the authorized representative of the commercial dairy farm for the participation of the animals in this study.

Data Availability Statement: The animal-level data underlying this study were obtained from a commercial dairy farm and are subject to contractual restrictions on disclosure and redistribution. Consequently, the complete dataset is not publicly available. Any request for access to restricted data requires prior authorization from the data owner. Requests to access the datasets should be directed to corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AFC	age at first calving
ANOVA	analysis of variance
CV	coefficient of variation
df	degrees of freedom
FM	milk fat content
GPAM	glycerol-3-phosphate acyltransferase, mitochondrial
MUFA	monounsaturated fatty acids
OECD-FAO	Organisation for Economic Co-operation and Development–Food and Agriculture Organization
PUFA	polyunsaturated fatty acids
r_s	Spearman rank correlation coefficient
SCC	somatic cell count
SCFA	short-chain fatty acids
SD	standard deviation
SE	standard error
SFA	saturated fatty acids
SNP	single-nucleotide polymorphism
TFA	trans fatty acid isomers

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