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Influence of lactation stage, parity, milk yield, and microbiological parameters on milk fatty acid profile

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Abstract

This study investigated the effects of lactation stage (LS), parity (PAR), milk yield (MY), total bacterial count (TBC), and somatic cell count (SCC) on the fatty acid (FA) profile of milk in 173 Holstein-Friesian cows, sampled at the morning milking. Milk fat was predominantly composed of saturated fatty acids (SFA), followed by monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), with palmitic acid (C16:0) as the most abundant individual FA. Among all examined factors, milk yield (MY) showed the most consistent effects, significantly influencing a wide range of short-, medium-, and long-chain FAs, as well as total SFA, MUFA, and PUFA. Higher milk production was associated with shifts in mammary lipid metabolism and FA synthesis pathways. LS also strongly affected FA composition, with early lactation characterized by increased long-chain FA content due to body fat mobilization, while later stages showed higher contents of *de novo* synthesized short- and medium-chain FAs. PAR had a moderate but significant influence, mainly affecting short-chain FAs and selected PUFAs, reflecting long-term physiological adaptations of the mammary gland. TBC showed selective effects on specific FAs, suggesting a potential link between microbial quality and lipid metabolism, whereas SCC had no significant effect. The R^2 values (0.04 to 0.25) suggest a moderate level of variance explained by the model. The results demonstrate that milk FA composition is primarily driven by physiological and production-related factors (MY and LS), while hygiene-related parameters have more limited or selective impacts. The findings confirm the highly dynamic and multifactorial nature of milk fat composition.

Keywords: milk quality; variability; fatty acids; physiological changes; Holstein-Friesian cows

Introduction

Cow's milk has been recognized as a high quality food of animal origin and has formed part of the human nutrition for millennia (Haug et al., 2007). It is a valuable source of energy and is especially suitable for consumption during growth and development. This energy is available through fat-soluble nutrients and bioactive lipids, including triacylglycerides, diacylglycerides, phospholipids, and fatty acids (FA) (Bilal et al., 2014). Bioactive FAs have attracted increasing attention from both producers and consumers because of their influence on the nutritional value, sensory properties, and technological quality of dairy products, as well as their association with beneficial health effects, including a reduced risk of cardiovascular diseases and diabetes (Wang et al., 2022; Rodríguez-Bermúdez et al., 2023). Therefore, it is important to optimize milk FA profiles in order to improve both the value and quality of dairy products (Hanuš et al., 2018).

There are around 400 different FAs present in milk fat, and their relative proportions vary considerably. The quantity of milk fat and its composition depend largely on metabolic processes (Hanuš et al., 2018). Milk FAs originate from *de novo* synthesis in the mammary gland, as well as from preformed FAs derived from the diet, ruminal biohydrogenation or bacterial degradation, or mobilization of body fat reserves (Stoop et al., 2009). Understanding the mechanisms of FA synthesis and transformation is therefore essential for interpreting variations in milk FA composition.

Given their diverse origins and metabolic pathways, FAs can be systematically classified according to their structural properties. Based on chain length, they are divided into short-chain FAs (C4:0-C6:0), medium-chain FAs (C8:0-C15:0), and long-chain FAs (≥C16:0). According to their degree of saturation, they are classified as saturated fatty acids (SFA) or unsaturated fatty acids (UFA), the latter including monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Additional groups include odd-chain fatty acids characterized by an odd number of carbon atoms, and branched-chain fatty acids which contain one or more methyl branches in their carbon backbone, typically in an iso- or anteiso- configuration (Vlaeminck et al., 2006). Changes in FA composition affect both the nutritional quality of milk and the functional properties of milk products, making it important to understand the factors driving these variations (Frizzarin et al., 2025).

The FA composition of milk is influenced by numerous factors, including genetics, breed, stage of lactation, parity, diet, and seasonal variation. In addition, metabolic disorders such as ketosis and subacute ruminal acidosis may alter both the fat content and FA composition (Sanjayaranj et al., 2022). Although diet is considered the main factor shaping the FA profile, individual animal differences, such as milk and fat yield, may also have an impact (Rodríguez-Bermúdez et al., 2023). Many of these factors have already been described in the literature, but they remain the subject of ongoing research because of their high variability and possible combined interactions (Hanuš et al., 2018). More recently, attention has also been directed to the potential effects of milk microbiota and somatic cell count on the FA profile (Turini et al., 2020; Coates et al., 2023; Pegolo et al., 2023).

Milk FA composition is partly genetically determined, with moderate heritability reported for individual FAs, indicating that selection may be used to improve the nutritional quality of milk fat (Arnould and Soyeurt, 2009). Breed significantly influences FA composition, as Jersey cows generally produce higher proportions of short- and medium-chain SFA, whereas Holstein cows exhibit relatively higher levels of long-chain SFA and MUFA (Kelsey et al., 2003; Sanjayaranj et al., 2022). Brown Swiss cows tend to produce milk with a more favorable FA profile than Holsteins, characterized by higher levels of conjugated linoleic acid (CLA) and UFA. These differences highlight the importance of breed selection in shaping milk fat quality and reflect variations in *de novo* synthesis and lipid metabolism among breeds.

Seasonal variation also strongly affects milk FA composition. Pasture-based feeding systems during spring and summer increase UFA concentrations, whereas winter feeding with conserved forages tends to elevate SFA concentrations (Heck et al., 2009). Additionally, heat stress during periods of high temperatures, as a seasonal factor, raises short and mediumchain FAs and reduces certain unsaturated longchain FAs, reflecting changes in lipid metabolism and rumen fermentation (Martínez Diez et al., 2025).

Lactation stage and energy balance contribute significantly to variation in milk fat composition by altering the activity of different FA pathways (Stoop et al., 2009). According to Hanuš et al. (2018), FA composition is significantly influenced by the energy balance of dairy cows. During early lactation, cows typically experience a negative energy balance (NEB) due to the high energy demands of milk production, which promotes the mobilization of body fat reserves and increases circulating non-esterified fatty acids. This metabolic state leads to a higher proportion of long-chain FAs, along with a concomitant reduction in short- and medium-chain FAs synthesized *de novo* in the mammary gland. As lactation progresses and the energy balance improves, reliance on body fat mobilization decreases, resulting in an increased proportion of SFAs and a reduction in MUFAs.

Parity is an important factor influencing milk FA composition. Studies have shown that primiparous cows tend to have a more favorable FA profile, characterized by higher proportions of UFAs and lower levels of SFAs compared to multiparous cows. This difference is partly attributed to lower metabolic activity and reduced *de novo* synthesis in the mammary gland of first-parity cows, resulting in lower concentrations of short- and medium-chain FAs. In contrast, cows in later parities are typically higher-yielding and are often fed diets richer in concentrates, which promote *de novo* synthesis and increase the proportion of these FAs in milk. Although some studies report no significant effect of parity, many have confirmed its influence on FA composition, particularly in relation to the degree of FA unsaturation, which is generally higher in primiparous cows (Bilal et al., 2014). Additionally, cows in their first and second lactations exhibit higher levels of milk fat odd- and branched-chain

FAs, a group of nutrients with emerging health benefits, compared to cows in later parities (Sun et al., 2022).

Milk yield is closely associated with changes in milk FA composition, as it reflects underlying energy balance and physiological adaptations during lactation. Since milk production varies with the lactation stage and parity, it is accompanied by shifts in metabolic processes, including body fat mobilization and *de novo* FA synthesis, which ultimately influence the FA profile of milk (Gross et al., 2011).

Elevated somatic cell count (SCC) in milk is associated with metabolic changes in the mammary gland. These changes reduce the synthesis of *de novo* short- and medium-chain FAs and increase the proportion of long-chain FAs derived from body fat. This indicates that SCC not only reflects udder health but also influences milk fat quality (Turini et al., 2020; Pegolo et al., 2023). Furthermore, recent research indicates significant associations between the milk microbiota and the FA profile of bovine milk, with specific bacterial genera showing consistent positive or negative relationships with various FA groups (Coates et al., 2023). These findings suggest that microbial composition may influence changes in milk lipid composition.

Therefore, the aim of the present study was to evaluate the effects of lactation stage (LS), parity (PAR), milk yield (MY), microbiological quality of milk expressed through the total bacterial count (TBC), and somatic cell count (SCC) on the FA profile of milk from Holstein-Friesian cows.

Materials and methods

Sample collection

This study was conducted on a commercial dairy farm. Cows were housed in a conventional system on a concrete floor, fed a total mixed ration (TMR) consisting primarily of corn silage (26 %), alfalfa haylage (12 %), alfalfa hay (5 %), concentrate mixtures (37 %), corn gluten feed (7 %), sugar beet pulp (3.35 %), molasses (5 %), fats and other supplements (4.65 %), to meet their nutritional requirements, and were milked three times daily.

In May 2025, a total of 173 lactating Holstein-Friesian cows were sampled during the morning milking. In addition to FAs composition, data on SCC and TBC were collected. Accordingly, two milk samples were obtained from each cow: one for milk quality analysis and the other for FA profile determination. Samples intended for FA analysis were frozen immediately after collection and stored at -20 °C until analysis.

Data on milk production, lactation stage, and parity were collected for all cows. It is important to note that milk yield was not expressed as daily production, but rather as yield per milking, because milking-level production records were consistently available due to technical specifications of the milking system during the study period. Although this approach may limit direct comparability with studies using daily milk yield, milking-level yield data still provide relevant information regarding production-related variation in milk FA composition. Although all cows originated from the same farm and were managed under relatively uniform feeding conditions, individual variation in feed intake, nutrient utilization, and rumen metabolism could not be taken into account in the present analysis.

Cows included in the study had an average milk yield of 14.94 kg per milking (≈45 kg per day), ranged from parity 2 to 6, and were in the first and second lactation stage (1-100 and 100-200 days in milk).

Milk quality analysis

The hygienic quality of milk: the somatic cell count and the total number of microorganisms were assessed according to the instructions described in the manual by Mikulec et al. (2023).

Determination of somatic cell count in milk

The somatic cell count in milk samples was determined using a fluoro-optoelectronic method with the BacSomatic instrument (Foss Electric, Denmark). The analysis was performed in accordance with the HRN EN ISO 13366-2:2007 standard. Results were expressed as the number of somatic cells per 1 mL of milk. Values obtained from the instrument (expressed per µL) were multiplied by 1,000 to convert them to mL units.

Determination of the number of microorganisms in milk

The total number of microorganisms in milk was determined using an instrumental method based on flow cytometry. Measurements were performed according to the manufacturer's instructions for the Bactoscan FC (type 73700, Foss Electric, Denmark). The conversion relationship between results obtained by the routine method and those obtained by the reference method was established in accordance with the standard HRN EN ISO 21187:2008. To eliminate interference from other milk components, such as fat globules, protein micelles, and somatic cells, samples were chemically treated prior to analysis. The number of viable bacteria capable of forming colonies (colony forming units, CFU) was calculated by converting Individual Bacterial Count (IBC) values using a linear regression model.

Fatty acid analysis

After thawing the milk samples, 1 mL of each sample was pipetted and prepared for further analysis.

Microwave method

In order to determine the FA profile, the milk samples were processed using a MARS 6 microwave device (CEM Corporation, Matthews, NC, USA) by applying microwave radiation at 1600 W in a two-stage process. In the first phase, a methanolic potassium hydroxide solution was added to the sample weighed into a reaction vessel. The vessels were

heated in the microwave system to 90 °C over eight minutes and held at that temperature for ten minutes. After cooling the samples, a methanolic sulfuric acid solution was added in the second phase. The samples were then heated to 120 °C over twelve minutes and maintained at that temperature for eight minutes. After the reaction, the samples were cooled again, followed by the addition of hexane and a saturated sodium chloride solution. After phase separation, an aliquot of the upper organic layer was collected, dried over anhydrous sodium sulphate, transferred into a vial, and stored in a freezer at -20 °C until gas chromatographic analysis.

Gas chromatography

Chromatographic analysis was performed using a SCION 436-GC gas chromatograph (SCION Instruments, Goes, Netherlands) equipped with a flame ionization detector (FID). A FAMEWAX (Restek Corporation, Bellefonte, PA, USA) capillary column (30 m×0.32 mm internal diameter × 0.25 µm film thickness) was used for the separation of FA methyl esters. The injection volume was 0.5 µL, with a split ratio of 1:10. Operating conditions were as follows: injector temperature of 230 °C, detector temperature of 230 °C, and carrier gas (hydrogen) flow rate of 2.5 mL/min. The oven temperature program was set as follows: from 60 to 160 °C at 20 °C/min, and from 160 to 230 °C at 10 °C/min, with a final hold at 230 °C for eight minutes. The total analysis time was 21 minutes. A standard mixture of 37 FAs (Food Industry FAME Mix, Restek Corporation, Bellefonte, PA, USA) was used to identify individual FAs in the chromatograms.

Results were expressed as the relative proportion (%) of each FA relative to the total identified FAs; in the analyzed samples, a total of 25 FAs were identified.

Data cleaning

Prior to statistical analysis, the dataset was subjected to standard data cleaning procedures. Records with missing or inconsistent values were removed, and extreme observations were examined. In the initial dataset, all variables were first inspected jointly to identify implausible or inconsistent records across the full data structure. Two milk samples were identified as having erroneous FA profiles, characterized by a total FA sum exceeding 100 %, indicating measurement or recording errors. These samples were excluded at the first stage of data cleaning. After this initial exclusion, the dataset consisted of 171 milk samples, which formed the basis for all subsequent analyses. In the next step, fatty acid-specific distributions were evaluated visually using diagnostic plots (distribution plots). Based on this assessment, additional outliers or missing values were removed on a variable-by-variable basis for individual fatty acids. Consequently, sample sizes varied slightly across FAs as reported in Table 2 by n (number of observations). Missing values were also present in explanatory variables. Parity data were missing for 10 cows, lactation stage for 13 cows, and milk yield for eight cows as reported in Table 1 by n. These missing observations were not imputed; instead, analyses were performed using available complete cases for each model.

The SCC and TBC were first log-transformed prior to analysis to improve normality and stabilize variance. Following transformation, the distributions of SCC and TBC were visually inspected using diagnostic plots to identify potential outliers. Based on this visual assessment, one outlier observation was removed for SCC and three outlier observations were removed for TBC due to clearly implausible values. These observations were excluded prior to statistical analysis to ensure data quality and robustness of the models.

Statistical analysis

Statistical analyses, including data transformation and model fitting, were performed using R version 4.5.2 (R Core Team, 2025). Separate general linear models were fitted for each individual fatty acid, as well as for total SFA, MUFA, and PUFA fractions, using the *lm* function in R. The FA concentrations were treated as dependent variables. TBC, SCC and MY were included as continuous covariates while LS and PAR, were treated as categorical fixed effects. The applied model can be expressed as:

$$y_{ijk} = \mu + \beta_1 \mathrm{TBC}_{ijk} + \beta_2 \mathrm{SCC}_{ijk} + \beta_3 \mathrm{MY}_{ijk} + \mathrm{LS}_j + \mathrm{PAR}_k + \varepsilon_{ijk}$$

where:
y_{ijk} = individual fatty acid concentration or total SFA, MUFA, or PUFA fraction for animal *i* within lactation stage *j* and parity class *k*,
μ = overall intercept of the model,
LS*j* = fixed effect of lactation stage
PAR*k* = fixed effect of parity
β_1-β_3 = regression coefficients describing the effect of each explanatory variable,
ε_{ijk} = residual error.

All models were fitted independently using an identical predictor structure to ensure comparability across fatty acid responses.

Interaction effects among explanatory variables (e.g., LS × MY and PAR × MY) were not included in the final models because the primary objective was to evaluate the independent contribution of each investigated factor to milk FA variability. Statistical significance was declared at $p<0.05$. Model coefficients, standard errors (SE), significance levels, and coefficients of determination (R^2) were extracted from the fitted models and used for interpretation of the results.

Model assumptions were assessed in R by visual inspection of residual plots, the Shapiro–Wilk test for residual normality, and the Breusch-Pagan test for homogeneity of variance. These procedures were applied systematically across all fitted models. Although some deviations from residual normality were detected, the affected results were interpreted with appropriate caution. Given the moderate sample size and the primary objective of evaluating the effects of lactation stage, parity, somatic cell count and bacterial count on milk fatty acid profiles, rather than developing predictive models for individual observations, minor deviations from residual normality were not considered to substantially affect the interpretation of the main findings, particularly where

diagnostic plots did not indicate severe departures from model assumptions.

Table 1 presents the average values of TBC, SCC, and MY, along with the number of animals in each lactation stage (1-100 and 100-200 days in milk) and parity group (2 - 6), thereby providing an overview of the analyzed dataset and supporting the interpretation of the subsequent statistical analyses.

Results and discussion

Distribution of fatty acids in milk according to saturation groups

Out of 25 identified FAs in the analyzed milk samples, 14 belonged to the group of SFAs, five to the group of MUFAs, and six to the group of PUFAs. In this study, milk fat was predominantly composed of SFAs (67.7 %), followed by MUFAs (28.23 %) and PUFAs (4.04 %).

Stoop et al. (2009) reported that approximately 71 % of milk fat consisted of SFAs, 26 % of UFAs, and about 3 % remained unidentified but were likely unsaturated. Similarly, Bilal et al. (2014) found average proportions of 68.1 % SFA, 23.1 % MUFA, and 3.1 % PUFA in milk fat. Mele et al. (2016) reported that SFAs accounted for 70 %, MUFAs for 25 %, and PUFAs for less than 5 % of total FAs. More recent results by Frizzarin et al. (2025) indicated that 69.76 % of FAs were SFAs, while 34.06 % were classified as UFAs. Overall, the results obtained in the present study fall within the range reported in the literature, confirming that milk fat is predominantly composed of SFAs, with a smaller proportion of UFAs.

A more detailed examination of the FA profile revealed that C16:0 (palmitic acid) was the most abundant SFA, as previously reported by Mele et al. (2016). Similarly, Sanjayaranj et al. (2022) confirmed that C16:0 was the dominant FA and identified C18:1n cis-9 as the dominant MUFA.

The results of the present study are consistent with these findings, with C16:0 being the most abundant FA (38.1 %), followed by C18:1n9 cis-trans (23.63 %) as the most abundant MUFA, and C18:2 (linoleic acid) as the most abundant PUFA. In addition, within the SFA group, C14:0 (myristic acid) and C18:0 (stearic acid) were also present in notable proportions, accounting for 11.01 % and 7.8 %, respectively. The FA composition observed in the present study is typical for the milk of Holstein-Friesian dairy cows (Rodríguez-Bermúdez et al., 2023).

The distribution of fatty acids, both by groups and individually, is presented in Figure 1.

Table 1. Overview of the dataset

VARIABLE									
TBC	SCC	MY	LS		PAR				
N=168	N=170	N=165	N= 82	N = 78	N=89	N= 40	N=19	N=9	N=6
μ=243503 CFU/mL	μ=167 SC/μL	μ= 14.94 kg	1-100 days	100-200 days	2	3	4	5	6

TBC - total bacterial count, SCC - somatic cells count, LS - lactation stage, PAR - parity, MY- milk yield, μ - average, N - number of observations

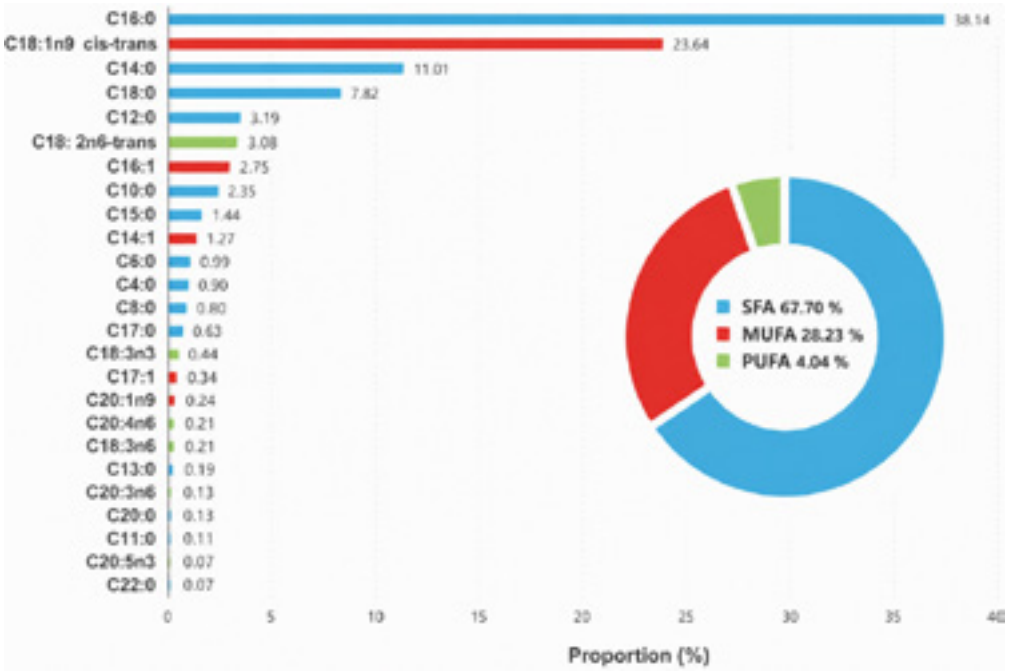

Figure 1. Distribution of fatty acids in milk by group and by individual fatty acid

Factors affecting the milk fatty acid profile

The effects of the investigated factors on milk FA composition were evaluated using statistical analysis, and the results including significance levels, coefficients of determination (R^2), standard errors (SE), and mean values are summarized in Table 2. In addition to the main findings, Table 2 also presents the number of observations for each analyzed FA, thereby providing important context for the interpretation of the statistical outcomes. It should be noted that two FAs (C22:0 and C20:5n3) were represented by a relatively low number of observations (n=42 and n=49, respectively). Although these variables were retained in the analysis to preserve the integrity and completeness of the milk FA profile, the limited sample size substantially reduces the robustness of the corresponding statistical estimates. Consequently, results related to these FAs, particularly significance levels and R^2 values, should be interpreted with considerable caution, as they may be more sensitive to random variation and less reliable than results obtained for FAs represented by larger sample sizes. Altogether, presented results provide a detailed understanding of the variability and explanatory power of the applied model.

MY significantly affected the majority of FAs, including short- and medium-chain FAs (C8:0-C14:0) and long-chain FAs (C16:0 and C17:0), selected MUFAs (C17:1 and C18:1n9 cis-trans), and PUFAs (C18:3n6 and C18:3n3), as well as total SFAs, MUFAs, and PUFAs content. The estimated coefficients indicated that increasing milk yield was generally associated with lower proportions of several SFAs, including C8:0 (ß=-0.020), C10:0 (ß=-0.070), C12:0 (ß=-0.090), C14:0 (ß=-0.101), C16:0 (ß=-0.358), and total SFAs (ß=-0.684), whereas positive associations were observed for C18:1n9 cis-trans (ß=0.611), total MUFAs (ß=0.628), and total PUFAs (ß=0.048). Notably, MY was the only factor that showed a significant effect on C18:2n6-trans (linoleic acid) which encompasses linoleic acid and its various isomers, including CLA. These findings suggest that milk yield is associated with variability in milk FA composition and may reflect metabolic adaptations related to milk fat synthesis. Previous studies have also identified MY as a contributor to FA variability in Holstein milk, particularly for C16:0 and other major FA groups (Rodríguez-Bermúdez et al., 2023; Frizzarin et al., 2025). Overall, the observed associations suggest a potential relationship between MY and milk lipid metabolism (Prado et al., 2019; Gallardo and Teixeira, 2023).

Table 2. Factors affecting milk fatty acid composition in Holstein-Friesian cows

Fatty acid	N	Mean	SE	R^2	significance
C4:0	169	0.90	0.01	0.19	LS*** PAR***
C6:0	171	0.99	0.01	0.17	LS*** PAR***
C8:0	171	0.80	0.01	0.16	LS*** PAR*** MY***
C10:0	171	2.35	0.04	0.11	LS* PAR* MY***
C11:0	171	0.11	0.00	0.11	MY**
C12:0	171	3.19	0.04	0.11	MY***
C13:0	171	0.19	0.00	0.05	⸸
C14:0	171	11.01	0.08	0.08	TBC* MY*
C15:0	171	1.44	0.03	0.07	PAR*
C16:0	171	38.14	0.21	0.09	MY**
C17:0	171	0.63	0.01	0.14	TBC* LS* MY**
C18:0	171	7.82	0.13	0.11	TBC* LS**
C20:0	150	0.13	0.00	0.08	TBC*
C22:0	42	0.07	0.00	0.25	TBC**
C14:1	171	1.27	0.03	0.14	LS***
C16:1	171	2.75	0.06	0.07	LS**
C17:1	171	0.34	0.01	0.08	MY**
C18:1n9 cis-trans	171	23.64	0.30	0.1	MY***
C20:1n9	161	0.24	0.00	0.04	LS*
C18: 2n6-trans	170	3.08	0.03	0.08	MY*
C18:3n6	169	0.21	0.01	0.13	TBC* MY***
C20:3n6	149	0.13	0.00	0.08	PAR*
C20:4n6	165	0.21	0.00	0.06	PAR*
C18:3n3	171	0.44	0.00	0.08	MY**
C20:5n3	49	0.07	0.00	0.18	⸸
Total SFA	171	67.70	0.37	0.08	MY***
Total MUFA	171	28.23	0.36	0.08	MY***
Total PUFA	171	4.04	0.04	0.08	MY*

*N - number of observations, SE - standard error, R^2 - coefficients of determination, TBC - total bacterial count , LS - lactation stage, PAR - parity, MY- milk yield, *** p≤0.001, ** p≤0.01, * p≤0.05, ⸸ - all explanatory variables were p>0.05 (non -significant)*

In addition to MY, another key physiological factor influencing milk FA composition is LS. LS had a pronounced effect on milk FA composition, influencing short-chain FAs (C4:0–C10:0), long-chain FAs (C17:0, C18:0), and certain MUFAs (C14:1, C16:1, C20:1n9). The estimated coefficients showed that early lactation was generally associated with lower proportions of short-chain FAs, including C4:0 (ß=-0.0800), C6:0 (ß=-0.1120), C8:0 (ß=-0.0896), and C10:0 (ß=-0.2088), whereas positive associations were observed for MUFAs such as C14:1 (ß=0.2729) and C16:1 (ß=0.3783). In the present study, cows were classified into early (1 - 100 days in milk) and mid-lactation (100-200 days in milk), thereby enabling the interpretation of physiological changes associated with the lactation stage. Previous studies have demonstrated that early lactation is associated with increased proportions of long-chain FAs due to the mobilization of body fat reserves, whereas de novo synthesized FAs increase during later lactation stages (Stoop et al., 2009; Gross et al., 2011). The present findings are consistent with these observations, as several FAs were significantly associated with LS. Our findings are consistent with more recent reports identifying LS as a major contributor to variability in milk FA composition across SFA, MUFA, and PUFA fractions (Rodríguez-Bermúdez et al., 2023; Nogalski et al., 2024; Frizzarin et al., 2025). Overall, the observed LS-related differences may reflect physiological and metabolic adaptations occurring throughout lactation. (Prado et al., 2019; Gallardo and Teixeira, 2023).

PAR showed significant effects on several FAs, including short-chain FAs (C4:0 - C10:0), C15:0, and selected PUFAs (C20:3n6 and C20:4n6). The model coefficients suggested positive associations between parity and short-chain FAs such as C4:0 (ß= .0562), C6:0 (ß=0.0603), C8:0 (ß=0.0470), and C10:0 (ß=0.0813), while negative associations were observed for C15:0 (ß=-0.0651). Although its effect was less pronounced compared to MY and LS, these associations may reflect physiological and metabolic differences between younger and older cows, particularly in terms of nutrient partitioning and mammary gland activity. Previous studies have also identified PAR as a contributor to variability in milk FA composition (Bilal et al., 2014; Mele et al., 2016), and more recent research have further confirmed this role (Sun et al., 2022; Rodríguez-Bermúdez et al., 2023; Frizzarin et al., 2025). Therefore, the observed parity-related associations may indicate long-term physiological differences related to lipid metabolism and mammary gland activity (Gallardo and Teixeira, 2023; Hanuš et al., 2018).

TBC showed selective but significant effects for specific FAs, including C14:0 and long-chain SFAs (C17:0, C18:0, C20:0, and C22:0), and the PUFA C18:3n6. Positive coefficients were observed for C17:0 ($ß=3.82\times10^{-8}$), C18:0 ($ß=7.95\times10^{-7}$), C20:0 ($ß=1.54\times10^{-8}$), and C22:0 ($ß=1.29\times10^{-8}$), whereas negative associations were identified for C14:0 ($ß=-5.73\times10^{-7}$) and C18:3n6 ($ß=-5.89\times10^{-8}$). Since TBC was expressed as CFU/mL and FAs as percentages of total fatty acids, the regression coefficients represent changes in FA percentage points per one-unit increase in bacterial count. Therefore, although statistically significant, the absolute magnitude of these effects should be interpreted in relation to the scale of TBC. The relatively high coefficient of determination observed for C22:0 (R^2=0.25) indicates a stronger model fit for this FA; however, this result should be interpreted with caution due to the limited sample size (n=42). Overall, the observed associations indicate a potential relationship between milk hygiene indicators and milk lipid composition. Previous studies have demonstrated that milk microbiota and intramammary infections can influence milk FA profiles through microbial metabolism, lipolytic activity, and oxidative processes affecting milk fat stability (Turini et al., 2020; Pegolo et al., 2023; Coates et al., 2023). Increased bacterial counts may be associated with enhanced lipolytic activity through microbial and endogenous lipases, potentially contributing to triglyceride hydrolysis and the release of free fatty acids. Such changes could increase susceptibility to oxidative processes, particularly in unsaturated fatty acids. In addition, bacterial metabolism may modify milk fat composition through enzymatic activity, biofilm formation, and interactions between microorganisms and milk substrates, potentially contributing to the degradation or transformation of specific FA fractions (Coates et al., 2023). Lipolytic and oxidative processes are particularly relevant in milk with elevated microbial contamination, where microbial enzymes may remain active even under cold storage conditions, which could contribute to alterations in milk fat quality and stability. In contrast, SCC showed no significant effect on overall milk FA composition in the present study, likely due to generally low SCC values and the absence of pronounced mastitis-related inflammation within the investigated herd, resulting in limited biological variability associated with udder health status.

Overall, the R^2 values observed across most FAs (0.04-0.25), ranging from low to moderate, indicate that the investigated factors explained only a limited proportion of the total variability in milk FA composition. Although several statistically significant associations were identified, the estimated coefficients were generally small, suggesting that the investigated factors exert modest individual effects on milk fatty acid composition. However, milk fatty acid composition is recognized as a highly complex and multifactorial trait influenced by numerous interacting factors, including diet, rumen metabolism, genetics, environmental conditions, physiological status, and management-related conditions (Mele et al., 2016; Hanuš et al., 2018; Rodríguez-Bermúdez et al., 2023). An important limitation of the present models is that some potentially influential variables, particularly detailed dietary composition and feeding management, were not included in the statistical analysis. Since diet and rumen biohydrogenation are known to be major determinants of milk FA composition, the omission of these variables likely contributed to the relatively low explanatory power of the models (Prado et al., 2019; Gallardo and Teixeira, 2023). Therefore, relatively low R^2 values are expected in milk datasets involving individual fatty acids and do not necessarily indicate poor model performance. Instead, they reflect both the inherent biological variability of milk lipid metabolism and the contribution of unmeasured or uncontrolled factors. The consistently low SE indicate high precision of the estimated means in this study, supporting the reliability of the observed average values across variables. Moreover,

despite the modest explanatory power of the models, the statistically significant associations observed for MY, LS, PAR, and TBC suggest that these factors may contribute to variability in specific FA fractions. Taken together, these findings indicate that milk FA composition is a highly dynamic trait, shaped by multiple biological, nutritional, environmental and management-related factors, and therefore only partially explained by models based on the investigated explanatory variables.

Conclusion

This study demonstrated that FA composition in Holstein-Friesian cows is predominantly shaped by physiological and production-related factors, particularly milk yield and lactation stage, which showed the most consistent effects across individual and grouped FAs. These effects reflect underlying metabolic adaptations, whereby early lactation is associated with increased proportions of long-chain FAs due to enhanced mobilization of body fat reserves under a negative energy balance, whereas *de novo* synthesized FAs (C4:0-C14:0) increase during later stages of lactation and with higher milk yield. Parity contributed to FA variability to a lesser extent, indicating additional but moderate long-term physiological effects. In addition, total bacterial count showed selective associations with specific FAs, suggesting a limited but measurable link between milk microbiological status and lipid metabolism, whereas somatic cell count did not significantly affect the FA profile under the conditions of this study. Overall, the low proportion of explained variance confirms the multifactorial and highly dynamic nature of milk FA composition, driven by complex interactions among genetic, nutritional, physiological, and environmental factors. Nevertheless, the identified significant associations highlight milk yield and lactation physiology as key determinants of milk fat quality. These findings may contribute to improved herd management strategies aimed at optimizing milk quality and its nutritional value.

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Utjecaj stadija laktacije, pariteta, prinosa mlijeka i mikrobioloških parametara na profil masnih kiselina u mlijeku

Sažetak

Ovim istraživanjem ispitani su učinci stadija laktacije, pariteta, proizvodnje mlijeka, ukupnog broja bakterija i broja somatskih stanica u mlijeku na profil masnih kiselina u mlijeku 173 krave holstein-frizijske pasmine, uzorkovane tijekom jutarnje mužnje. U mliječnoj masti pretežno su prevladavale zasićene masne kiseline, zatim mononezasićene i polinezasićene masne kiseline, pri čemu je palmitinska kiselina (C16:0) bila najzastupljenija pojedinačna masna kiselina. Od svih ispitivanih čimbenika, proizvodnja mlijeka pokazala je najizraženiji i najdosljedniji učinak, značajno utječući na širok raspon kratkolančanih, srednjelančanih i dugolančanih masnih kiselina te na ukupni udio zasićenih, mononezasićenih i polinezasićenih masnih kiselina. Veća proizvodnja mlijeka bila je povezana s promjenama u metabolizmu lipida mliječne žlijezde i putevima sinteze masnih kiselina. Stadij laktacije također je snažno utjecao na sastav masnih kiselina, pri čemu je rana laktacija bila obilježena povećanim udjelom dugolančanih masnih kiselina zbog mobilizacije tjelesnih masti, dok su kasnije faze pokazale veći udio *de novo* sintetiziranih kratkolančanih i srednjelančanih masnih kiselina. Paritet je imao umjeren, ali značajan utjecaj, uglavnom na kratkolančane masne kiseline i pojedine polinezasićene masne kiseline, što odražava dugoročne fiziološke prilagodbe mliječne žlijezde. Ukupan broj bakterija je pokazao selektivne učinke na pojedine masne kiseline, što upućuje na moguću povezanost mikrobiološke kvalitete i metabolizma lipida, dok broj somatskih stanica nije imao značajan učinak. Vrijednosti R^2 (0,04–0,25) ukazuju na umjerenu razinu varijabilnosti objašnjene modelom. Rezultati pokazuju da je sastav masnih kiselina mlijeka prvenstveno određen fiziološkim i proizvodnim čimbenicima, dok higijenski parametri imaju ograničeniji ili selektivan utjecaj. Dobiveni rezultati potvrđuju izrazito dinamičnu i multifaktorijalnu prirodu sastava mliječne masti.

Ključne riječi: kvaliteta mlijeka; varijabilnost; masne kiseline; fiziološke promjene; holstein-frizijske krave

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