



Colostrum somatic cell counts and their association with the bacterial microbiota at udder quarter level in Simmental cows

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ABSTRACT

Analysing the somatic cell count (SCC) together with the microbiota composition in colostrum can be useful for monitoring udder health immediately after parturition. The present study aimed to explore the bacterial community in colostrum of Simmental cows, and to assess whether the bacterial diversity and composition related to the SCC at the udder quarter level. The SCC were examined in colostrum and milk on days 14 and 28 postpartum, which were aseptically collected separately for each udder quarter from 16 cows. The SCC was determined immediately after milking. The DNA was extracted from defatted colostrum and used for 16S rRNA amplicon sequencing. Results showed higher SCC in colostrum than in milk on day 14 and 28 postpartum. Colostrum was predominated by environmental bacteria (e.g., *Delftia* and *Cutibacterium*). Season did not affect SCC but colostrum collected in summer months contained more *Delftia*, whereas *Methylobacterium-Methylorubrum* and *Acinetobacter* were more abundant in the fall. Quarters on the left body side tended to have higher SCC and increased species richness than the quarters on the right body side. Partial least squares-discriminant analysis indicated that colostrum samples with low SCC ($< 100 \times 10^3$ cells/mL) were characterized by high abundances of *Lactobacillus* and *Cutibacterium*, whereas *Methylobacterium-Methylorubrum* discriminated for colostrum with high SCC. Colostrum samples with high SCC ($> 1000 \times 10^3$ cells/mL) also tended to contain more *Streptococcus*. Pearson correlations supported a link between higher SCC on day 28 and abundance of *Streptococcus* and *Bacillus* in colostrum, which should be further exploited in the future.

1. Introduction

Bovine mastitis remains one of the most common inflammatory diseases of the mammary gland in dairy cattle worldwide, causing substantial economic losses in the dairy industry (Ruegg, 2017, 2022). Clinical mastitis presents with visible symptoms, such as inflammation and altered milk yield and quality (Ruegg, 2017). Subclinical mastitis, in turn, is often asymptomatic but can be identified through measurement of the somatic cell count (SCC; Fonseca et al., 2025). With a SCC of less than 100×10^3 cells/ml it is unlikely that an infection exists. However, when the SCC rises above 200×10^3 cells/ml the probability of an infection is high (Fonseca et al., 2025). In the presence of infection in the udder, the inflammatory reaction causes the blood-milk barrier to become more permeable, allowing somatic cells to enter the milk. As part of the innate immune response, the somatic cells comprise lymphocytes, macrophages and epithelial cells, and hence can be used as an indicator for infection in the respective udder quarter (Schukken et al., 2003). The risk of developing an intra-mammary infection is highest

immediately after calving, as the disease can start as a chronic sub-clinical infection present at dry-off or through the acquisition of a pathogen over the dry period (Bradley and Green, 2000a, 2000b; McMullen et al., 2021). When the animals are no longer milked, the microbes present in the udder are no longer regularly flushed out. This can lead to an overgrowth of bacteria in the udder, contributing to the development of mastitis with the onset of lactation. As the SCC is typically elevated in the respective udder quarter, measuring the SCC at dry-off is used to detect subclinical infections in udder quarters, allowing to initiate treatment if necessary to prevent mastitis (Persson Waller et al., 2022).

Another option is to measure the SCC and microbial composition in colostrum. The colostrum quality is often monitored in terms of its immunological properties for the newborn calf but hardly in relation to udder health with the beginning of lactation. This would allow detecting abnormalities in the udder quarters that have developed during the dry period and colostrum formation before clinical symptoms occur. While SCC determination in milk is routine, there is still a lack of information

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about SCC in colostrum and its relation to the development of mastitis during lactation. Recent research has indicated that higher SCC at calving, which linearly declined over the first 30 days of lactation (Somagond et al., 2025).

Determining the SCC is a simple undertaking, but it does not allow any conclusions to be drawn about the etiological agents causing the mastitis (Ruegg, 2017). Therefore, measuring SCC in colostrum should be done in combination with a microbial assessment. The pathogens involved belong to a variety of bacterial taxa, including staphylococci, streptococci, enterococci and *Escherichia coli* (Ruegg, 2017). Aside from testing for pathogens, relatively little is known about the diversity and composition of the microbiota in colostrum and whether certain taxonomic patterns relate to the SCC in colostrum and mature milk. Existing literature shows study-specific microbiota composition in colostrum (Lima et al., 2017; Vasquez et al., 2022; Scully et al., 2025). In one study, *Prevotella*, *Ruminococcaceae*, *Bacteroidales*, *Clostridiales* and *Pseudomonas* represented the main taxa of the colostrum microbiota (Lima et al., 2017), whereas others reported *Clostridiales*, *Lactobacillales*, and *Bacillales* as the dominating bacterial classes (Vasquez et al., 2022). Moreover, the colostrum microbiota of primiparous and multiparous cows seem to vary (Lima et al., 2017; Scully et al., 2025), supporting the need to understand more about the natural variation in microbial composition and diversity of colostrum. This is also vital knowledge to gain regarding the colonisation of the newborn's gut (Urrutia-Angulo et al., 2025).

The aim of the present study was to determine the bacterial taxonomic composition and diversity in the first colostrum of Simmental cows, and to assess whether the bacterial diversity and composition in colostrum related to the SCC at the udder quarter level after parturition and 14 and 28 days postpartum. The SCC were monitored in mature milk on day 14 and 28 postpartum to assess the changes in SCC with progressing lactation. We hypothesised that the composition of the microbiota in colostrum would be similar among udder quarters. However, we further assumed that colostrum of udder quarters with higher SCC would contain a less diverse microbiota. Moreover, we hypothesised that higher SCC in colostrum would be associated with characteristic bacterial patterns such as a higher relative abundance of potential mastitis pathogens.

2. Material and methods

2.1. Animals and experimental design

All procedures involving the handling and treatment of animals were approved by the institutional ethics committee of the University of Veterinary Medicine Vienna and the National authority under the Law for Animal Experiments in Austria (GZ: 2023-0.300.442). In total, 16 Simmental cows of the dairy cattle herd (Vetfarm) of the University of Veterinary Medicine Vienna were used in this study. Cows included in the study were in parity 1 to 8 (Supplementary Table 1) and calved consecutively between June and December 2023. The housing, feeding and management of the dry cows close to parturition and the lactating cows corresponded to the standard protocol at the dairy cattle farm. All cows were kept in free stall barns. Close to parturition, each single cow of the study was kept in a separate pen (4 m × 4 m) on deep straw bedding. Afterwards (2 to 4 days postpartum), she returned to the main herd to be milked in a herring-bone milking parlour twice daily. Data on parity and current incidences of clinical mastitis were taken from the veterinary register of the farm. Clinical mastitis examination was performed by experienced farm employees at the milking parlour twice daily.

2.2. Sampling

Quarter-level colostrum samples were collected within three hours of birth. In contrast, quarter-level milk samples on days 14 and 28

postpartum were collected in the milking parlour during morning milkings between 05:00 and 07:00 am. As samples were used for 16S rRNA amplicon sequencing, extra measures were taken to ensure that colostrum and milk samples were not contaminated. Colostrum and milk samples were always collected in the same order from the four quarters after aseptic cleaning of the udder and teats using the conventional hand milking technique (Lima et al., 2017; Dahlberg et al., 2020; Vasquez et al., 2022). The udder was first cleaned of coarse dirt, then with a wet paper towel, and finally with a dry paper towel. Fresh paper towels were used for each udder quarter. Afterwards, the teats and the teat canal were disinfected with gauze soaked in 70%-ethanol to avert microbial contamination of the colostrum and milk samples. This procedure was repeated until there was no visible dirt left on the teat end and the gauze. Each quarter was then stripped three times, and the milk was discarded before taking the milk sample. Then, the desired volume of colostrum or milk of 10 mL was retrieved into a sterile container. Udder quarters were always milked in the same order: starting with the left cranial quarter of the udder, then the sequence was continued clockwise (left cranial quarter = CRL, right cranial quarter = CRR, right caudal quarter = CAR, left caudal quarter = CAL). Approximately 10 mL of colostrum or milk were taken from each quarter. Subsamples for microbiota analysis were aliquoted and kept on ice before storing at -80°C . Colostrum and milk samples for SCC determination were stored on ice for less than 30 min before being processed.

2.3. Determination of the somatic cell counts

An automatic cell counter (DeLaval CellCounter, DeLaval, Sweden) was used to determine the SCC in milk samples according to the protocol of the manufacturer. To ensure even distribution of cells, colostrum and milk samples were mixed 15 to 25 times by slowly inverting the sample tubes before measurement. Based on the SCC, the colostrum samples were categorized to evaluate the risk of developing mastitis or the presence of subclinical mastitis (SCM) using the following thresholds: < 200 (low likelihood of intramammary infection), $\text{SCC2} \geq 200 < 1000$ (moderate likelihood of intramammary infection), and $\text{SCC3} \geq 1000 \times 10^3$ cells/mL (high likelihood of intramammary infection).

2.4. DNA extraction

First, the colostrum samples were thawed and centrifuged for four minutes at $4000 \times g$ to separate the fat layer. The defatted colostrum was used for DNA extraction. Total DNA was extracted from 350 mg defatted colostrum using the DNeasy PowerSoil Pro kit (Qiagen, Hilden, Germany) with some modifications to the manufacturer's protocol. The modifications included an additional heating step for ten minutes at 70°C and homogenisation of the samples using the SpeedMill Plus homogeniser (Analytik Jena GmbH, Jena, Germany). The SpeedMill protocol included three cycles of one minute each with a one-minute break between cycles. The DNA concentration in each eluate was quantified using the Qubit DNA HS Assay Kit on the Qubit 4 Fluorometer (Thermo Fisher Scientific Inc., Waltham, USA).

2.5. 16S rRNA amplicon sequencing and bioinformatics

Targeted 16S rRNA (V3-V4 hypervariable region) sequencing was performed in an external laboratory (Novogene, Cambridge, UK). For this purpose, aliquots of the DNA extracts were sent for library preparation (NEBNext Ultra II DNA Library Prep Kit, Illumina, San Diego, CA, USA). The 16S rRNA amplicon was amplified using primers 341F-ill (5'-CCTACGGGNGGCWGCAG-3') and 806R-ill (5'-GACTACHVGGGTATCTAATCC-3'). Equimolar pools of samples were sequenced to generate 250 bp pair-end raw data on the Novaseq 6000 platform (Illumina). Demultiplexing and trimming of raw sequences was performed by Novogene. Bioinformatic analysis was performed according to the protocol described in Yosi et al. (2024). Raw sequence data (Fastq files)

were processed, aligned and categorized using the Divisive Amplicon Denoising Algorithm 2 (DADA2; version 1.26.0) (Callahan et al., 2016), which was performed in RStudio (version 1.4.1106). Firstly, the quality profiles of the forward and reverse sequences were evaluated. Then, the total length of the sequences was trimmed to 220 (forward sequences) and 210 nucleotides (reverse sequences) with a maximum error rate of 5 for both, forward and reverse sequences, to account for the degradation of the quality score of the remaining nucleotides, using the filter-AndTrim function. Ambiguous sequences and sequences exceeding the estimated error of five nucleotides were removed from the amplicon set. After estimation of the error rates, amplicon sequence variants (ASV) were derived (Callahan et al., 2016). Subsequently, the deduced forward and reverse sequences were merged, paired sequences that did not match perfectly were removed to control for remaining errors, and the sequence table was generated. Chimeras were eliminated using the removeBimeraDenovo command. Subsequently, the taxonomy was assigned using the SILVA 138.2 ribosomal RNA (rRNA) database for bacteria (Quast et al., 2013) with a 3% dissimilarity threshold. Alpha-diversity (Shannon, Simpson, Observed features) was determined using R phyloseq (version 1.42.0). At genus level, the sequences of the different bacterial taxa were totalled and presented as a proportion of the total sequences per sample. For the bacteria, the genera with a relative abundance of >0.1% of all sequences were statistically analysed using ANOVA. Beta-diversity was assessed using permutational multivariate analysis of variance (PERMANOVA) on the Bray-Curtis distance matrices to assess the dissimilarities among the bacterial communities using the R package 'vegan' (version 2.6.4; Oksanen et al., 2022). The statistical significance was determined after 999 random permutations.

2.6. Statistical analysis

For ANOVA, the SCC, bacterial taxonomy and alpha-diversity were first tested for normal distribution using the Shapiro-Wilk test and the UNIVARIATE procedure in SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA). For genera and the SCC on day 1, 14 and 28 postpartum with non-normally distributed residuals, data were root square-transformed before subjecting the data to ANOVA using the MIXED procedure in SAS. The SCC were analysed using repeated measures over time. To characterize the impact of the season, months of parturition were categorized into summer (June, July and August) and fall months (October, November and December). Fixed effects were day postpartum, season (summer and fall), udder quarter, body side and SCC group. Significant two-way interactions of season with udder quarter, body side or SCC group did not exist. Therefore, two-way interactions were removed from the final model. To identify bacterial patterns in colostrum, the colostrum microbiota data were analysed using three random models based on the udder quarter, body side and SCC group. The random effect was parity. The experimental unit was quarter nested within cow. Degrees of freedom were approximated by the Kenward-Rogers method (ddfm = kr). Data were reported as the least-squares means $\pm$ standard error of the mean (SEM). Multiple pairwise comparisons among least-square means were performed using the pdiff statement. The Bonferroni correction was applied to adjust the raw *P*-values (Lin and Peddada, 2020). Significance was defined at $P \leq 0.05$. Trends were defined at $P < 0.10$. Descriptive statistics were generated using the PROC MEANS procedure in SAS.

Data integration analysis was performed on log₁₀-transformed data for SCC, alpha-diversity and relative abundances at genus level (> 0.1% of all sequences) using partial least-squares regression in the R package 'mixOmics' (version 6.30.0; Rohart et al., 2017). Results for season, at udder quarter level, body side level and SCC groups were visualized in biplots displaying the five most important variables as vectors. Moreover, pattern search was performed using feature correlation analysis (Pearson) to associate SCC on day 1, 14 and 28 postpartum (feature) with colostrum alpha-diversity and bacterial genera profiles. The FDR-adjusted *P*-values were used to indicate significance ($P < 0.05$).

3. Results

3.1. Somatic cell counts

Season did not influence the SCC in colostrum and mature milk (Fig. 1A). The SCC were higher in colostrum compared to day 14 and 28 postpartum ($P < 0.05$; Fig. 1A). While some colostrum and milk samples from day 14 postpartum contained more than 1000×10^3 cells/mL, SCC on day 28 postpartum remained below 1000×10^3 cells/mL. Descriptive statistics indicated considerable variation in SCC in the four quarters, ranging from 3 to 3114×10^3 cells/mL in colostrum, from 1 to 1869×10^3 cells/mL on day 14, and from 1 to 775×10^3 cells/mL on day 28 postpartum (Table 1). There was a general trend that the two quarters of cow's left body side had a higher SCC than the two quarters of the right body side when analysed across all three sampling timepoints (334 versus 212 ± 65.01 (SEM) $\times 10^3$ cells/mL in quarters on left and right body side, respectively; $P = 0.077$). In particular, the SCC were 81.1%-higher ($P = 0.021$) in quarters on the left body side compared to the quarters on the right body side on day 14 postpartum (Fig. 1B). When comparing the SCC in the individual quarters, SCC were higher in the left cranial quarter compared to the right cranial and caudal quarters on day 14 postpartum ($P < 0.05$; Fig. 1C). The average SCC in colostrum and milk samples for the three SCC groups are displayed in Fig. 1D. From the six quarters that had SCC above 200 or 1000×10^3 cells/mL in colostrum, four remained above 200×10^3 cells/mL and two above 1000×10^3 cells/mL on day 14 postpartum, whereas on day 28 postpartum only two quarters had SCC above 200×10^3 cells/mL. Mastitis-associated changes in milk texture were only detected for four udder quarters in the milking parlour (i.e., cow 2 and cow 10: CRL and CAL) with SCC above 200×10^3 cells/mL in colostrum (Supplementary Table 1).

3.2. Bacterial diversity and taxonomy

After quality filtering of the reads from the 16S rRNA amplicon sequencing, a total of 3,485,828 reads remained, with an average of 55,331 reads per sample. Taxonomic classification identified 705 genera. The majority of them were very low abundant taxa and/or only occurred in few samples. Taxa that were abundant >0.1% were analysed (Fig. 2). Of these, *Delftia* was by far the dominating genus (75.4% of all reads). Further genera with relative abundance >0.5% were *Stenotrophomonas*, *Methylobacterium-Methylorubrum*, *Staphylococcus*, *Streptococcus* and *Acinetobacter*.

While the species richness remained unaffected, characterisation of the impact of season on the bacterial communities in colostrum showed a trend for a 45.1 and 34.8%-higher Shannon and Simpson diversity, respectively in the summer compared to the fall months (adjusted $P < 0.10$; Table 2). Similarly, season influenced the community composition with higher relative abundance of *Delftia* (26.7%) in the summer months compared to the fall, whereas *Methylobacterium-Methylorubrum*, *Acinetobacter*, *Comamonas* and *Christensenellaceae* R-7 group were largely more abundant in the fall compared to the summer months (adjusted $P < 0.05$, Fig. 2A). The impact of season on the bacterial community structure and composition was similar for the individual udder quarters, quarters at the left and right body side and SCC groups.

Quarters on the left body side had a higher species richness (observed species, adjusted $P = 0.024$; Table 2) but similar Shannon and Simpson diversity as the quarters on the right body side (Table 2). However, comparison at udder quarter level and the three SCC groups showed high within-quarter and within-SCC group variation, which may have masked differences between individual quarters and SCC groups for alpha-diversity. Beta-diversity analysis, based on Bray-Curtis dissimilarity (PERMANOVA), indicated an effect of season on the bacterial community ($P = 0.011$), whereas the individual udder quarter, quarters per body side and SCC groups only explained a small proportion of community structure variation (PERMANOVA, $P > 0.10$). Due to the

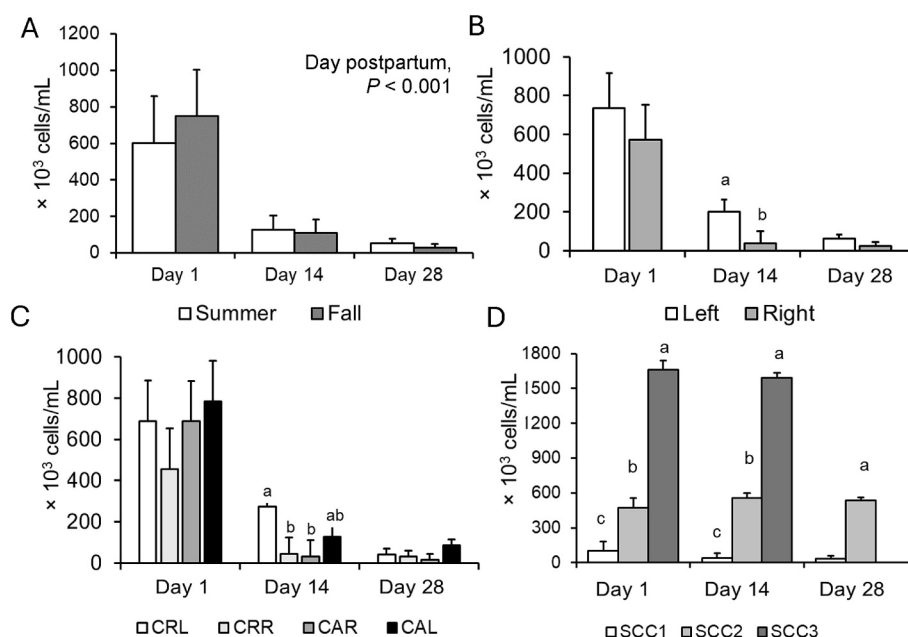


Fig. 1. Somatic cell counts (SCC) in colostrum (day 1) and milk on day 14 and 28 postpartum. Comparison of SCC for A) season and day postpartum; B) body side; C) individual quarters; and D) SCC group (SCC1, $< 200 \times 10^3$ cells/mL; SCC2, $< 1000 \times 10^3$ cells/mL; SCC3, $> 1000 \times 10^3$ cells/mL). Season: time of parturition (number of cows): Summer months: June (2), July (3) and August (5); fall months: October (3), November (1), December (2). a,b differences in SCC on separate sampling days ($P < 0.05$).

Table 1

Description of somatic cell count (10^3 cells/mL) in individual udder quarters used in the statistical analysis.

Quarter	Day postpartum	Mean	SD	Minimum	Maximum
CRL	1	657	679	40	2294
	14	273	557	3	1869
	28	46	80	3	287
CRR	1	423	580	3	2078
	14	44	54	3	175
	28	36	55	1	185
CAR	1	654	762	29	3114
	14	31	44	3	141
	28	21	30	2	95
CAL	1	774	639	10	2474
	14	131	200	1	693
	28	90	197	1	775

CRL = left cranial quarter; CRR = right cranial quarter; CAR = right caudal quarter; CAL = left caudal quarter; SD = standard deviation.

high within-quarter variation among cows, no differences in relative bacterial abundances at genus level among udder quarters and body sides could be detected (Fig. 2B and C). When the bacterial data were analysed based on SCC groups, bacterial diversity (Table 2) and relative abundances of bacterial genera in colostrum were similar across SCC groups (Fig. 2D), except for the genus *Streptococcus*. The genus *Streptococcus* was mainly abundant in colostrum samples grouped as SCC3 compared to the other two groups (adjusted $P = 0.084$).

To examine potential relationships among udder quarters, relative genera abundances, alpha-diversity and SCC, multivariate analyses were performed. The PLS-DA indicated that the bacterial communities in colostrum clustered closer together for parturitions during the summer months compared to the fall months (Fig. 3A). *Delftia* and *Lactobacillus* were the most discriminant genera for the summer months, whereas *Methylobacterium-Methylorubrum*, *Ruminococcaceae* UCG-005 and *Acinetobacter* were the five most discriminative taxa for the fall months. However, there was no clear clustering of bacterial communities in colostrum samples among body sides and at individual quarter level (Fig. 2B and C). When assessing relationships for quarters per body side,

the PLS-DA indicated that *Methylobacterium-Methylorubrum*, *Ruminococcaceae* UCG-005 were more discriminative for quarters on the left body side and *Staphylococcus* and *Sphingomonas* for quarters on the right body side (Fig. 3B). The PLS-DA further identified *Saccharopolyspora*, *Romboutsia*, *Streptococcus*, *Methylobacterium-Methylorubrum* and *Delftia* as the five most discriminative variables for the individual udder quarters (Fig. 3C). The relative abundances of *Saccharopolyspora*, *Romboutsia*, *Streptococcus* were positively correlated among each other, and negatively with *Delftia* and *Methylobacterium-Methylorubrum* but were not distinctive for udder quarter. Despite overlapping clusters, PLS-DA based on SCC groups demonstrated that *Cutibacterium* and *Lactobacillus* discriminated for SCC group 1, *Corynebacterium* for SCC group 2, and *Methylobacterium-Methylorubrum* for SCC group 3, whereas *Delftia* was not specifically discriminative for one SCC group (Fig. 3D). Feature correlation analysis showed positive association between SCC in mature milk on day 14 and 28 ($r = 0.51$, FDR-adjusted $P = 0.001$). Moreover, weak positive correlations existed between SCC on day 28 and relative abundance of *Bacillus* ($r = 0.43$, FDR-adjusted $P = 0.004$) and *Streptococcus* ($r = 0.38$, FDR-adjusted $P = 0.017$).

4. Discussion

Intra-mammary infection can develop during the dry period (Bradley and Green, 2000a, 2000b). Therefore, determination of the SCC together with the bacterial microbiota in colostrum may improve our understanding about the predictive power of the SCC and the potential role of the bacterial profile in colostrum on early mastitis development (Lima et al., 2017). The results of the present study align with previous research (Puppel et al., 2020; Somagond et al., 2025), demonstrating considerably higher SCC in colostrum compared to milk on days 14 and 28 postpartum. Although we cannot exclude the possible presence of subclinical intramammary infection, as only two cows were diagnosed with clinical mastitis in two udder quarters in the present study, it is more probable that the high SCC were not due to disease but to physiological processes during colostrum production (McGrath et al., 2016) and possibly related to the passage of immune cells through permeable tight junctions between the udder epithelial cells (Nguyen and Neville,

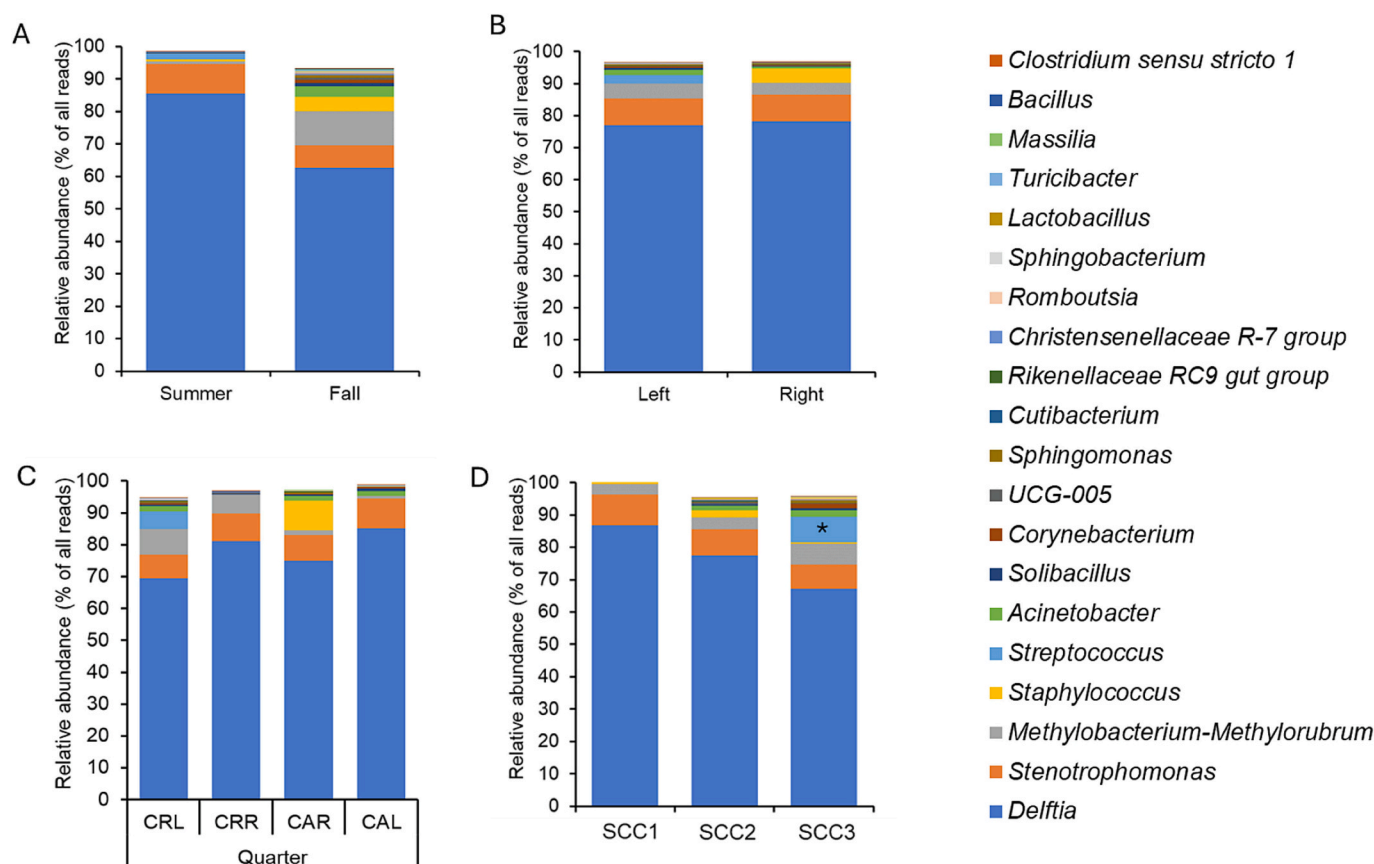


Fig. 2. Relative abundances of bacterial genera in colostrum (% of all reads). Comparison of relative abundances for A) season [time of parturition (number of cows): Summer months: June (2), July (3) and August (5); fall months: October (3), November (1), December (2)]; B) body side; C) individual quarter; and D) SCC group (SCC1, $< 200 \times 10^3$ cells/mL; SCC2, $< 1000 \times 10^3$ cells/mL; SCC3, $> 1000 \times 10^3$ cells/mL). *Trend for SCC group effect for *Streptococcus* ($P = 0.084$).

1998; Puppel et al., 2020). Therefore, different reference values for the likelihood of (sub-)clinical mastitis should be established for the assessment of SCC in colostrum compared to mature milk (Fonseca et al., 2025).

While there were no seasonal differences in SCC in colostrum and mature milk samples in the present study, results indicated great inter-animal variation of SCC in colostrum but also considerable differences between udder quarters, which may be related to the exposition of the udder parts and teats to the environment. Especially, the quarters on the left udder side had higher SCC and hence may have been more at risk to develop mastitis compared to those on the right udder side. One possible explanation for these findings could be that cows preferentially lied down on the left side as also reported in the literature due to the position of the rumen in the abdominal cavity (Forsberg et al., 2008). As a result, the left side of the udder would be in contact with the bedding and floor more often. This could lead to microbes from the environment entering the udder more easily via the left teats, explaining the higher species richness in these udder quarters, and potentially increasing the risk that mastitis-pathogens enter the teat. Accordingly, the udder quarters of cows 2 and 10 that showed changes in milk texture were on the left body side. As we did not determine the preferential lying position of the cows used in this study, the association between the preferential lying side of the cows and species richness should be verified in a future study. The samples taken on day 14 showed the greatest difference in SCC between left and right body sides. Therefore, regular monitoring of SCC in the first 14 days postpartum could be helpful in the early detection of mastitis.

While season did not influence the bacterial alpha-diversity, PERMANOVA, PLS-DA and ANOVA indicated seasonal differences in the bacterial community structure and composition. This seemed to be

caused by bacteria originating from the environment, including soil and skin bacteria such as *Delftia*, *Methylobacterium* and *Acinetobacter* (Ran et al., 2024; Ehinmitan et al., 2025). These differences may have been related to the charges of bedding material used in summer and fall months as well as higher environmental temperatures in the summer. Irrespective of the season, the PERMANOVA indicated similar bacterial communities in colostrum among quarters and quarters per body side, suggesting that the additional taxa in the quarters on the left body side were low abundant genera ($< 0.1\%$ relative abundance). In contrast to previous work (Lima et al., 2017; Taponen et al., 2019; Vasquez et al., 2022; Scully et al., 2025; Urrutia-Angulo et al., 2025), the colostrum microbiota was predominated by few taxa. Genera, such as *Stenotrophomonas*, *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Acinetobacter*, are frequently detected bacterial taxa in milk samples obtained from clinically healthy quarters (Catozzi et al., 2017; Derakhshani et al., 2018; Porcellato et al., 2020; Scully et al., 2025). *Delftia* and other *Comamodaceae* have been detected in colostrum and milk samples before but in lower proportional abundances (Derakhshani et al., 2018; Andriyanov et al., 2024). Therefore, we did not anticipate such a high predominance of *Delftia* in the present colostrum. *Delftia* is an aerobic genus, ubiquitous in the environment (Zheng et al., 2007; Guo et al., 2016; Chen et al., 2022; Ran et al., 2024) and thus may have originated from the barn environment (e.g., bedding material and floor) during the dry period. It is not yet known whether the presence of *Delftia* affects udder health. Currently, there is no evidence that the contained *Delftia* ASV, i.e., *Delftia tsuruhatensis*, *D. acidovorans*, *D. lacustris*, and *D. rhizosphaerae*, cause clinical or subclinical mastitis in cows but been related to multi-resistance to antibiotics (Andriyanov et al., 2024) and human diseases (Lee et al., 2024). The current PLS-DA and feature correlation analysis would support low influence of *Delftia* on SCC and

Table 2

	Season ¹			Quarter			Body side			SCC group ²			SEM	P-value					
	Summer	Fall	SEM	P-value	CPL	CRR	CAR	CAL	SEM	P-value	Left	Right			SEM	P-value	SCC1	SCC2	SCC3
Observed	198	113	40.95	0.137	79	52	55	118	1.34	0.098	96	54	1.27	0.024	87	74	54	1.32	0.530
Shannon	1.40	0.77	0.246	0.095	1.18	1.11	1.17	1.26	0.335	0.862	1.22	1.14	0.249	0.798	1.24	1.30	0.83	0.307	0.546
Simpson	0.38	0.25	0.046	0.068	0.35	0.31	0.33	0.34	0.061	0.972	0.34	0.32	0.046	0.675	0.35	0.35	0.27	0.055	0.508

Values are least-squares means $\pm$ standard error of the mean (SEM). CRL = left cranial quarter; CRR = right cranial quarter; CAR = right caudal quarter.

¹ Time of parturition (number of cows): Summer months: June (2), July (3) and August (5); fall months: October (3), November (1), December (2).
$$^2 \text{ SCC1} < 200 \times 10^3 \text{ cells/ml}; \text{ SCC2} < 1000 \times 10^3 \text{ cells/ml}; \text{ SCC3} > 1000 \times 10^3 \text{ cells/ml}.$$

thus udder health. The presence of *Solibacillus* and *Methylobacterium*, which are genera commonly found in soil and plants, may support a certain contamination of the teat orifice and canal with environmental microbes (Rainard, 2017; Jo et al., 2024; Ehinmitan et al., 2025). Genera such as *Acinetobacter*, *Aerococcus*, and *Corynebacterium* may have also originated from the skin of the teat or gut (Braem et al., 2012; Luo et al., 2022) and may have entered from the teat orifice into the cistern. Species of *Cutibacterium* may have originated from the skin or rumen (Hackman et al., 2025). Gut-associated microbes are omnipresent in the dairy environment (Derakhshani et al., 2018) and are therefore a likely source for microbes entering the teat orifice and cistern, or via entero-mammary transmission (Luo et al., 2022) during the dry period.

Albeit belonging to the normal upper microbiota (Derakhshani et al., 2018), *Stenotrophomonas*, *Staphylococcus*, *Streptococcus*, *Corynebacterium* and *Acinetobacter* are genera which comprise mastitis agents (Derakhshani et al., 2018; Porcellato et al., 2020; He et al., 2023). Common mastitis pathogens within *Staphylococcus* and *Streptococcus* are *Staphylococcus aureus*, *Staphylococcus hyicus* and *Streptococcus dysgalactiae* (Porcellato et al., 2020; Winther et al., 2023; Kerro Dego and Vidlund, 2024), which were among the assignable ASV for these genera. Our results confirmed previous findings for mature milk that colostrum samples with higher SCC also comprised a higher abundance of *Streptococcus* (Oikonomou et al., 2014). Moreover, feature correlation analysis indicated that a higher relative abundance of *Streptococcus* and also of *Bacillus* may be predictive for higher SCC in mature milk on day 28 postpartum. Since the present study was only based on observations from 16 cows, these relationships should be verified in a larger number of animals. Nevertheless, *Streptococcus* appeared not to be the disease-causing agent in the udder quarters of cows 2 and 10, which were diagnosed with clinical mastitis in the current early lactation. Nevertheless, the presence of a *Strep. dysgalactiae* ASV may explain the persistently high SCC in the CRL quarter of cow 8 over the course of the three sampling time points. It should be kept in mind that targeting the V3–V4 region of the 16S rRNA gene allows taxonomic resolution primarily at the genus level, while the resolution at ASV level is incomplete because the discrimination between species or strains with different functional or pathogenic roles is limited (Yang et al., 2016). Due to this reason, the data at the ASV level were not statistically analysed in the present study. It is recommended to additionally analyse *Streptococcus* species and virulence factor patterns in colostrum using targeted quantitative PCR to overcome this bias.

Quantitative PCR of the milk samples revealed a predominance of environment-associated taxa, we cannot rule out that the chosen method of hand stripping and the timepoint of sample collection during the milking process may have influenced the results of the microbiota analysis. We followed the recommended method for the collection of colostrum and milk (Lima et al., 2017; Dahlberg et al., 2020; Vasquez et al., 2022). However, the bacterial load in healthy quarters is low because the gland cistern is a closed environment during the dry period (Derakhshani et al., 2018), resulting in low concentrations of bacterial DNA. Therefore, bacteria trapped within the keratin layer in the teat orifice, canal and cistern may have a more substantial influence on the overall bacterial community if not sufficiently flushed out. There is also a chance to detect DNA from bacteria that has been imported to the mammary gland via leukocytes (Metzger et al., 2018). Lastly, it cannot be ruled out that even the utmost care used to disinfect the teat apex may not have been sufficient to ensure that the colostrum sample was not contaminated by microbes residing on the teat apex, especially under field conditions (Rainard, 2017) like in the present study. In future studies on the colostrum microbiome, it should be therefore re-evaluated which portion of the first colostrum should be collected to remove microbial contaminations from the teat orifice and canal (Porcellato et al., 2020) and whether blunt cannulas are applicable under field conditions (Vangroenweghe et al., 2001).

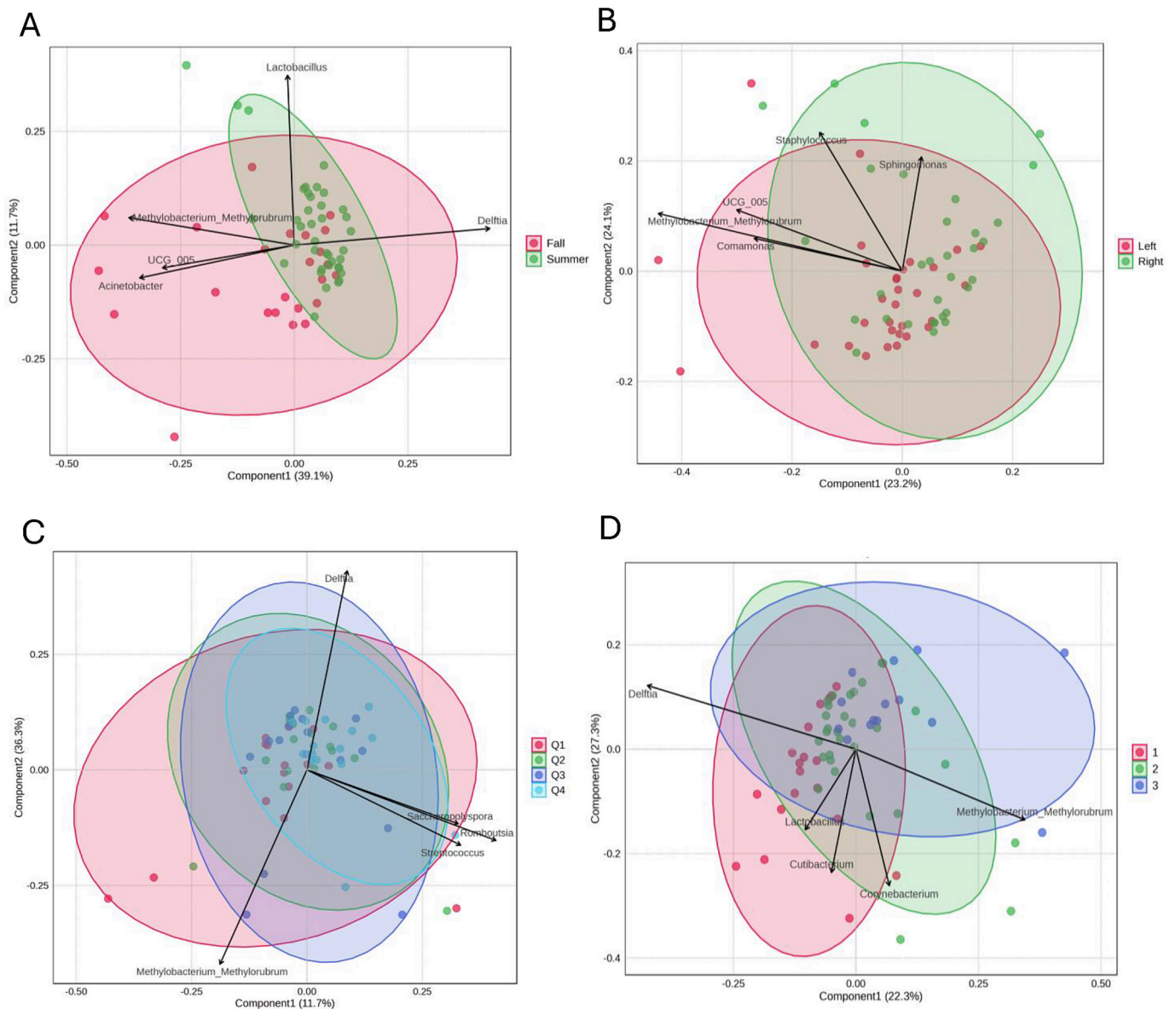


Fig. 3. Biplot of partial least squares-discriminant analysis on bacterial diversity and composition separated by A) season [time of parturition (number of cows): Summer months: June (2), July (3) and August (5); fall months: October (3), November (1), December (2)], B) body side, C) at quarter level, and D) somatic cell count (SCC) group (SCC1, $< 200 \times 10^3$ cells/mL; SCC2, $< 1000 \times 10^3$ cells/mL; SCC3, $> 1000 \times 10^3$ cells/mL). Arrows indicate most discriminative variables.

5. Conclusions

The present results added valid information about SCC ranges in colostrum, which were generally higher than in milk from day 14 and 28. The bacterial community structure and composition in colostrum was affected by season, whereas the bacterial composition was relatively similar across udder quarters and SCC groups. An interesting finding of the present study was that the SCC and bacterial species richness were higher on the left body side, which may have related to the preferred body side for lying down. The presented results supported a relationship between the relative abundance of the genus *Streptococcus*, which contains important mastitis-pathogens, and SCC in colostrum and mature milk. Due to the small number of cows included in the present study, this should be verified in a larger group of animals in the future. As the current bacterial community was dominated by environmental and skin bacteria, it is also recommended to re-evaluate the currently recommended sampling technique for colostrum and milk samples.

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CRediT authorship contribution statement

Carolina Kroboth: Writing – review & editing, Writing – original draft, Investigation, Data curation. **Felipe Penagos-Tabares:** Writing – review & editing, Investigation, Funding acquisition, Data curation, Conceptualization. **Daniela Klein-Jöbstl:** Writing – review & editing, Supervision, Project administration. **Barbara U. Metzler-Zebeli:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Formal analysis, Data curation.

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Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

The datasets generated for this study were deposited into the NCBI Bioproject databank under accession number PRJNA1364627.

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