



Total bacterial count and somatic cell count in bulk and individual goat milk around kidding: Two longitudinal observational studies

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ABSTRACT

Total bacterial count (TBC) and SCC are important quality parameters in goat milk. Exceeding the bulk milk TBC (BMTBC) thresholds leads to price penalties for Dutch dairy goat farmers. Controlling these milk quality parameters can be challenging, especially around kidding. First, we describe the variation and the peaks around kidding of TBC and SCC in census data on Dutch bulk milk over the last 22 yr. Second, to explore causes of these elevations, we studied the variation of TBC and SCC in individual goat milk from 3 wk before to 5 wk after kidding and their association with systemic response markers IFN- γ , calprotectin, BHB, BCS, and fecal consistency. We visited 4 Dutch dairy goat farms weekly for 10 to 16 wk around kidding. Some of the goats had been dried off; other goats were milked continuously throughout pregnancy. A total of 1,886 milk samples from 141 goats were collected for automated flow cytometric quantification of TBC and SCC measurement. IFN- γ , calprotectin, and BHB were determined twice in blood of the same goats; most samples were collected after kidding. The BCS and fecal consistency were scored visually before and after kidding. We found a strong correlation between TBC and SCC (Spearman's $\rho = 0.87$) around kidding. Furthermore, in the third week before kidding, the average TBC ($5.67 \log_{10}$ cfu/mL) and SCC ($6.70 \log_{10}$ cells/mL) were significantly higher compared with the fifth week after kidding, where the average TBC decreased to $4.20 \log_{10}$ cfu/mL, and the average SCC decreased to $5.92 \log_{10}$ cells/mL. In multivariable linear regression models, farm and stage of lactation were significantly associated with TBC and SCC, but none of the

systemic response markers correlated with TBC or SCC. In conclusion, TBC and SCC in dairy goats were high in late lactation and decreased shortly after parturition. For SCC, the dilution effect might have caused the decrease, but this was not plausible for TBC. Moreover, the excretion of bacteria and cells in goat milk was not associated with the selected systemic response markers that were chosen as a readout for general immunity status, intestinal health, and metabolic diseases. Therefore, we assume that the TBC increase before kidding and the decrease after parturition are caused by other systemic, possibly hormonal, processes. To reduce BMTBC and bulk milk SCC, it would be advisable to keep milk of goats with highest numbers of bacteria and cells in their milk out of the bulk milk during end lactation. Further studies are needed to investigate the effects of withholding this end-lactation milk from the bulk tank.

Key words: dairy goat, bulk milk, total bacterial count, somatic cell count

INTRODUCTION

Together with the SCC, the number of bacteria per milliliter of milk (i.e., the total bacterial count, **TBC**), is an important milk quality parameter. The TBC is often measured by flow cytometry (such as the Bacto-Scan FC+), which measures the number of individual bacterial count (**ibc**) per milliliter, and after conversion the TBC is expressed in the number of colony-forming units (cfu) per milliliter. Enzymes such as proteases and lipases excreted by bacteria can alter the flavor and disturb the cheese making process and decrease shelf life (Muehlherr et al., 2003; Chen et al., 2010; Leitner et al., 2016; Stocco et al., 2018; Podhorecká et al., 2021). Due to the potential effects this poses to the industry, milk price penalties are often imposed if the bacterial load of the bulk milk exceeds a threshold. European Regulation

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EC No. 853/2004 (European Union, 2004) dictates a maximum plate count of 1.5×10^6 cfu/mL, but in many countries lower thresholds are used. The bulk milk total bacterial count (**BMTBC**) is influenced by several factors such as milking hygiene, milk collection frequency, cooling temperature of the bulk milk and cleaning of the milking machine (Delgado-Pertíñez et al., 2003; Zweifel et al., 2005; Friker et al., 2020), and mastitis (Koop et al., 2009). In addition, recurrent yearly elevations of both bacteria and SCC in bulk milk (**BMSCC**) coinciding with the kidding season have been reported in Dutch dairy goats (Koop et al., 2009). In sheep (Gonzalo et al., 2006; Lianou et al., 2021) and in goats (Koop et al., 2009), BMSCC and BMTBC are correlated, but the causes of this correlation and why both parameters are elevated around kidding are unclear. It is unlikely that herd-level factors, such as milking technique, milk collection frequency, and cooling of the milk change dramatically during the kidding season. Hygiene may be under pressure during the kidding season, but the elevated TBC is often reported before the actual start of the kidding, making this a less plausible explanation. Hence, shedding of bacteria by individual goats may be the cause of the high BMTBC in this period. If goats do shed high numbers of bacteria in the milk around kidding, it is important to know whether this only happens before kidding, or whether it stays high around or after kidding and whether there are differences in levels of shedding between animals. Finally, to further understand this phenomenon, and possibly prevent high BMTBC during the kidding season, it is also important to identify factors associated with the level of bacterial shedding.

In analogy with what has been shown in cows, it seems plausible that the transition period around kidding leads to changes in the functioning of the immune system (Horst et al., 2021). Also, metabolic diseases occur more often in this periparturient period (Smith and Sherman, 2009). In addition, Koop et al. (2009) showed an association between BMTBC and fecal consistency in dairy goats, suggesting that gut health might be related to the shedding of bacteria in milk. Therefore, we investigated whether parameters that are measures of the immunological, metabolic, and intestinal health status of goats were associated with TBC and SCC.

The first aim of our study was to describe the variations in BMTBC and BMSCC of goat bulk milk samples in the Netherlands from 2001 through 2022. Second, we aimed to describe the variations in individual goat milk TBC and SCC around kidding, and to quantify the association between individual TBC and SCC. Third, we analyzed associations between TBC and SCC and the systemic response markers IFN- γ , calprotectin, BHB, BCS, and fecal consistency.

MATERIALS AND METHODS

The bulk milk study on TBC and SCC in the Netherlands from 2001 to 2022 is described in Part 1. The study on the individual variations of TBC and SCC and the correlations of TBC with systemic response markers around kidding is described in Part 2.

Part 1: Bulk Milk—BMTBC and BMSCC

Selection Criteria Bulk Milk Records. In the period from January 2001 until January 2023, 207,990 bulk milk records from 1,463 Dutch dairy goat farms were analyzed for TBC, and 187,750 bulk milk records from 1,455 Dutch dairy farms were analyzed for SCC. Importantly, not every bulk milk sample was analyzed for both SCC and TBC. A total of 186,978 bulk milk records from 1,454 Dutch dairy goat farms were analyzed for TBC, and 191,411 records were analyzed for SCC. Qlip Laboratories (Zutphen, the Netherlands) analyzed these samples on BMTBC and BMSCC. Data were provided by Qlip with the permission of the Dutch Association of Dairy Goat Farmers (NGZO) and included records of ~95% of all Dutch dairy goat farms over the reported period. On average, farms were sampled twice a month for BMSCC and for BMTBC. Only a small number of the farms had multiple bulk milk tanks; therefore the effect of the clustering of these bulk tanks within farm was expected to be minor and was ignored.

Flow Cytometry Analysis. Within 42 h after sampling, bulk milk samples were analyzed by flow cytometric methods for TBC and SCC. The BactoScan instrument (Foss Analytics, Hillerød, Denmark) measures the individual bacterial count (ibc/mL). To convert the ibc/mL values to cfu/mL, both the values from the standard plate count method and the ibc/mL values from bulk tank milk were used in establishing a conversion calibration curve. This was performed according to ISO 21187, IDF 196 standards. For ease of interpretation, we report cfu/mL in this paper. The BMTBC was subsequently determined using a BactoScan 8000 (2001–2004), a BactoScan FC (2004–2015), and, since 2015, with a BactoScan FC+ (FOSS), also according to ISO 21187 and ISO 4833-1. The BactoScan was calibrated with goat milk. Bulk milk SCC was determined with the Fossomatic 5000 from 2001 to 2013 and thereafter with the Fossomatic FC. The different Fossomatic types were calibrated with cow milk according to ISO 13366-II.

Data Processing and Analysis. Out of 207,990 bulk milk records analyzed for BMTBC, Qlip deemed 16,098 analysis results as unsuccessful, leading to their exclusion from further analysis. Among the remaining BMTBC records, 478 samples were reported with a value of

999,000 cfu/mL, reflecting the laboratory's practice of reporting BMTBC values $\geq 10^6$ cfu/mL as 999,000 cfu/mL to indicate the upper limit of accurate detection. Additionally, 3 milk samples reported a BMTBC of 0 cfu/mL. To allow for log transformation of the results, the values for these samples were adjusted to 1 cfu/mL. In the case of BMSCC, out of 187,750 records analyzed by Qlip, 771 were marked as unsuccessful and excluded from further analysis. Of the BMSCC records that were analyzed, 1 sample was reported with a value of 9,999,000 cells/mL, reflecting the laboratory's practice of reporting BMSCC values $\geq 10^7$ cells/mL as 9,999,000 cells/mL to indicate the upper limit of accurate detection. The final dataset comprised 191,892 valid BMTBC and 186,979 BMSCC results from 1,457 Dutch dairy goat farms. It is noteworthy that most bulk milk samples were analyzed either for BMTBC or BMSCC, but not for both on the same date.

To analyze whether the BMTBC and BMSCC increased in the period between the beginning of 2001 and the end of 2022, linear least-squares regression analyses were used. As the dependent variable, we computed the average \log_{10} -transformed BMTBC and BMSCC values per month. The independent variable, month number, was constructed by creating an array with increasing numbers from 1 (January 2001) to 264 (December 2022). Data analysis was performed using Python programming language version 3.11.4 and statsmodels version 0.14.0 (Van Rossum and Drake, 2009; McKinney, 2010).

Part 2: Individual Goats Around Kidding

Ethics. The Dutch Central Commission for Animal experiments approved this study (administration number AVD1080020199025).

Study Design. We performed a longitudinal observational study at 4 dairy goat farms located in 3 different provinces in the Netherlands (Overijssel, Gelderland, and Limburg) from January to May 2020. We collected milk samples from both udder halves of 35 to 38 goats weekly on each farm for 10 to 16 wk.

Farm Selection. Farm selection was based on a history of annual high BMTBC above 100,000 cfu/mL during the kidding period, which could not be explained by technical problems such as inadequate cooling or cleaning of the milking machine. Although some participating farms had multiple kidding periods, we studied the kidding period between January and May 2020 to avoid confounding by seasonal influences. The kidding period of the participating farms was defined as the days between the first and last kidding of the enrolled goats.

Animal Selection. Sample size was based on logistical limitations: the maximum number of farms that could be visited weekly was 4, and the total number of goats that could be sampled on one occasion was ~ 35 . Thus, we

arrived at a total sample size of 147 goats, which were randomly selected from all pregnant goats at the end of lactation on 4 farms: individual goat identification numbers were assigned a random number in Microsoft Excel (Microsoft Corp., Redmond, WA) and the animals were enrolled in the order of these random numbers starting from the lowest number until the required sample size was reached. In the study group, the number of animals enrolled varied between 35 and 38 animals per farm. The enrolled goats were confirmed pregnant by ultrasound. Some goats were dried off, whereas others were milked continuously throughout pregnancy.

The study goats differed in breed, although most were of the Saanen breed, age 2 to 7 yr, and in the second through fifth parity at time of enrollment. Primiparous does and nonlactating does were not included in the study because no milk samples before parturition could be collected from these goats.

Goats diagnosed with a clinical disease (e.g., diarrhea or fever) or clinical mastitis, as defined by abnormalities in milk or the udder, whether or not accompanied by general illness according to the criteria by Pinzón-Sánchez and Ruegg (2011), were excluded from the study at the time of enrollment. However, goats that became diseased or acquired clinical mastitis during the study period were included in statistical analyses.

The farms enrolled in the study varied in their dry-goat management. Goats in farm D were milked throughout the dry period, unless goats became dry themselves ($n = 4$). In the other 3 farms goats were dried off by discontinuing milking when the individual milk production dropped below 0.4 kg/d before kidding. The duration of the dry-off period varied between 1 and 3 wk. At 3 wk before parturition, $\sim 15\%$ of the animals had been dried off, which increased to 20% at 2 wk before parturition, and to 37% 1 wk before parturition. None of the farms employed dry-off treatments with antibiotics. Farmers provided information on the dry-off date and kidding date via an animal administration system. During the study, farmers noted disease occurrence and medical therapies. During the weekly visits the researchers registered clinical mastitis cases

Sampling Scheme. The milk sampling interval was weekly (6–9 d). However, farm A had 1 delayed sampling visit with an interval of 14 d between the visits. Because we sampled the goats according to a fixed weekly schedule and the goats had their own individual kidding date, the timing of the samplings relative to kidding varied between the goats. Thus, the period before kidding covers the period starting 3 wk (-3 wk) before the kidding period. The kidding period (wk 0) starts 3 d before kidding (-3 d) and ends 3 d after the parturition ($+3$ d). The post-kidding period starts from wk $+1$ to $+5$ after the parturition. The systemic response markers that could be

measured in blood (IFN- γ , calprotectin, and BHB) were determined twice. Some animals ($n = 23$) were measured before parturition, 54 goats were measured during the kidding week, and 190 goats were measured after parturition. The BCS was determined before and after kidding at 5 or 6 sampling moments per farm. Fecal consistency was scored for each goat during 3 samplings before and after kidding. Supplemental Table S1 (see Notes) shows per farm which variables were measured at which sampling occasions.

Farm C was the only farm with individual milk production data. Based on this milk production data, we estimated whether the decrease in TBC and SCC after kidding could be caused by the increased milk production after parturition (the dilution effect). We measured the average milk yield of the preceding 10 d, SCC, and TBC of 29 goats before (-3 wk to -3 d) and after ($+3$ to $+5$ wk) parturition on farm C. Thereafter we quantified the increase in milk production and the decrease in TBC and SCC after parturition.

Milk Sampling. Milk samples from each udder half were taken aseptically according to the National Mastitis Council (NMC, 2017) during the morning milking period. The first 3 squirts of milk were discarded; thereafter the teat-ends were thoroughly disinfected with cotton wool balls drenched in 70% alcohol. Per udder half, a 100-mL capped sterile vial (Aptar CSP Technologies, Auburn, AL) was filled with ~ 75 mL of milk for both TBC and SCC analysis. Immediately after sampling, the milk samples were stored on melting ice for a maximum of 6 h and then stored at 4°C until further investigation.

Flow Cytometry Analysis. Within 36 h after sampling, individual TBC and SCC were determined with Bacto-Scan FC+ and the Fossomatic FC (FOSS) at Qlip Laboratories.

Systemic Response Markers. Blood samples were collected from the jugular vein using the Vacutainer system with Precision Glide Multiple Sample Blood Collection Needles, 0.9×38 mm, and Vacutainer heparin LH 170 IU 10-mL tubes or Vacutainer SSTT II advanced serum 10-mL tubes (Beckton Dickson and Company, Franklin Lakes, NJ).

The IFN- γ was determined in heparinized blood samples. Within 8 h after the collection, whole blood stimulation assays were started with either 0.03 mg/mL pokeweed mitogen (Sigma L8777, Sigma-Aldrich, Burlington, MA) or with 30 μL of PBS (Gibco, Thermo Fisher Scientific) as an unstimulated control. After gentle mixing, the plates were incubated in a humidified incubator with 5% CO_2 , at 37°C for 24 h. After 24 h, the plates were spun down at $500 \times g$ for 3 min. A volume of 500 μL of plasma supernatant was carefully pipetted to avoid aspiration of any blood cells. The supernatants were stored in 250- μL Micronics tubes at -20°C until

further analysis. The IFN- γ levels were analyzed using the Bovigam TB kit (cat. no. 63320, Thermo Fisher Scientific, Prionics AG, Schlieren-Zurich, Switzerland).

Calprotectin concentration (ng/mL) was determined in blood serum. After sampling, the serum tubes were centrifuged, and serum was stored at -20°C until further analysis. We used the calprotectin assay (cat. no. MBS 2601308) for goats' serum (My Biosource). For determination of calprotectin, we followed the procedure described by Bortoluzzi et al. (2021), but we used the ELISA kit MBS 2601308 for goats' serum instead of the MBS1601938 kit.

The BHB concentrations (mmol/L) were determined in heparinized blood using the Abbott Freestyle Precision Neo test using the Freestyle Optium β -ketone test strips (Abbott GmbH & Co. KG, Wiesbaden, Germany) as described by Doré et al. (2013).

The body condition of goats was visually determined by 1 of 2 observers, who received the same training. They scored the lumbar region with scores ranging from 0 to 5 with 0.25-point divisions (Smith and Sherman, 2009), where a score of 0 is very thin and 5 is very fat. These BCS scores were then recoded into 3 classes: normal BCS (≥ 2.75 to ≤ 3.50), low BCS (> 0 to < 2.75), or high BCS (> 3.50 to ≤ 5.00); and in the analysis 2 classes were used: normal or non-normal BCS.

Feces were collected per rectum and then scored by 1 of 3 observers, who received the same training. We used a fecal consistency scoring protocol used in cows (Ireland-Perry and Stallings, 1993) with some adjustments, because no stool form scale has been described for goats yet. The fecal consistency was scored 1 when feces were watery to very soft. A score of 2 was assigned if feces were smooth but no pelleted feces had been formed. A score of 3 was given when feces were dry and pelleted.

Statistical Analysis

Some results of the TBC, SCC, calprotectin, and BHB had values of 0, which would have resulted in an undefined value after taking the logarithm. To address this, all values were increased by 1. For SCC, values $\geq 10^7$ cells/mL were reported by the laboratory as 9,999,000 cells/mL as a maximum threshold value. The correlation between TBC and SCC was determined using Spearman's rank-based correlation test. Because the systemic response markers were often not measured at the same moment, each independent variable, IFN- γ , calprotectin, BHB, BCS, and fecal consistency, was tested in a separate model for its association with the dependent variables \log_{10} TBC and \log_{10} SCC in a separate mixed regression model, with udder half as the unit of observation. Weeks relative to kidding and farm were fixed effects, and udder half nested within goat was modeled

as random effect. To check the fit of the linear mixed effects (LME) models and whether model assumptions were met, we visually inspected quantile-quantile plots of standardized residuals, plots of standardized residuals against standardized predicted values, and plots of standardized residuals against predictor variables. The plots showed insufficiently normally distributed residuals. The abnormal distribution of the residuals was likely caused by extremely high TBC values and the fact that a cut-off value of SCC (9,999,000 cells/mL) was used. Because linear quantile mixed models (LQMM) do not require normal distribution of the residuals, we also assessed our data in a LQMM with package for Laplace quantile regression (Geraci, 2014). Because the results of both models were very similar, we decided to present the LME because of easier interpretability. Statistical analyses were performed in R version 4.3.1, using the package ggResid panel (R Core Team, 2023).

RESULTS

Part 1: Bulk Milk TBC and SCC

The linear regression analyses, using monthly average \log_{10} -transformed BMTBC and BMSCC as dependent variables and month number as the independent variable, revealed an increase in both BMTBC and BMSCC over the 22-yr period (Figure 1). For BMTBC, the regression coefficient was 0.0015 (95% CI: 0.0013 to 0.0016) \log_{10} cfu/mL per month, and for BMSCC, the regression coefficient was 0.0013 (95% CI: 0.0011 to 0.0014) \log_{10} cells/mL per month. Supplemental Figure S1 illustrates the yearly seasonal fluctuations in both BMTBC and BMSCC, although this was more pronounced for BMSCC than for BMTBC. The peak of these fluctuations was typically seen at the beginning of January, aligning with the start of the main kidding period in the Netherlands. Visual inspection of Figure 1 and Supplemental Figure S1 suggests that the amplitude of these fluctuations has decreased over time, although this trend was more pronounced for BMSCC than for BMTBC.

Part 2: Individual Goat Milk Around Kidding

Descriptive Statistics. Table 1 summarizes the basic characteristics of the farms (A–D). Milking was performed twice a day on all farms, except farm C, which changed the milking frequency from 2 to 3 times a day when the goats were on average 3 wk into lactation. All farms fed a transitional feed with high-fiber pellets combined with silage during the study period. Until 3 wk after kidding, no major feed changes took place during the study. The selected goats were housed in their own lactation groups to minimize changes in environment and hierarchical order during the study.

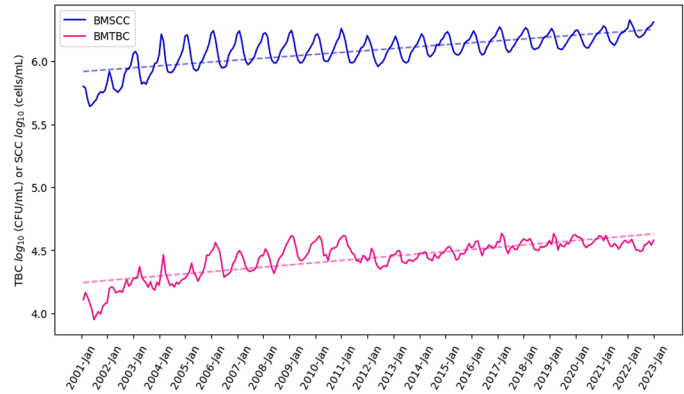


Figure 1. Each monthly average \log_{10} BMSCC and each average \log_{10} BMTBC of Dutch dairy goat farms between 2001 and 2022, based on an average of 2 measurements per farm. The dashed lines are regression lines through the monthly averages of BMTBC and BMSCC, respectively. Estimates were based on 390 farms per month on average (minimum = 316, maximum = 559).

For the entire study period we selected 147 study goats, of which 126 goats were sampled at each sampling moment during the study period. There were 21 goats with incomplete sampling records, of which 10 goats died during the study due to acetonemia, 2 goats became otherwise diseased, and 9 goats were missed during one or more sampling moments. All goats were included in the analysis. Our period of interest was between -3 and $+5$ wk relative to kidding, so for all statistical analyses we used the samples derived from this period, resulting in 141 study goats. To determine the variation and the correlation of TBC and SCC over time, we randomly used 1 udder half per goat ($n = 961$ udder halves of 141 goats) for the analyses. For correlation of TBC and SCC between the left and the right udder halves, we used both udder halves of the goats. For the mixed models, a total of 1,886 observations of 141 goats were used.

Variation of Individual TBC and SCC Around Kidding. The median TBC in individual milk samples of goats on the 4 farms was elevated 3 wk before kidding (5.94 \log_{10} bacteria/mL) and significantly decreased to 4.00 \log_{10} bacteria/mL in wk 5 after kidding. The SCC showed a similar pattern, with a median of 6.93 \log_{10} cells/mL 3 wk before kidding, decreasing to 5.78 \log_{10} cells/mL in wk 5 after kidding. Thus, the median TBC decreased by ~ 2 orders of magnitude, whereas SCC showed a smaller decrease of ~ 1 order of magnitude between pre- and post-kidding (Figure 2). In farm C, the average milk yield increased after parturition by a factor of 11 (range 0.3 to 80). In the goats on this farm, the TBC decreased with an average factor of 310 (range 0.3 to 2,901), and the SCC decreased with an average factor of 18 (range 0.1 to 141) after kidding.

Correlation Between TBC and SCC. Left and the right udder halves were strongly correlated for both

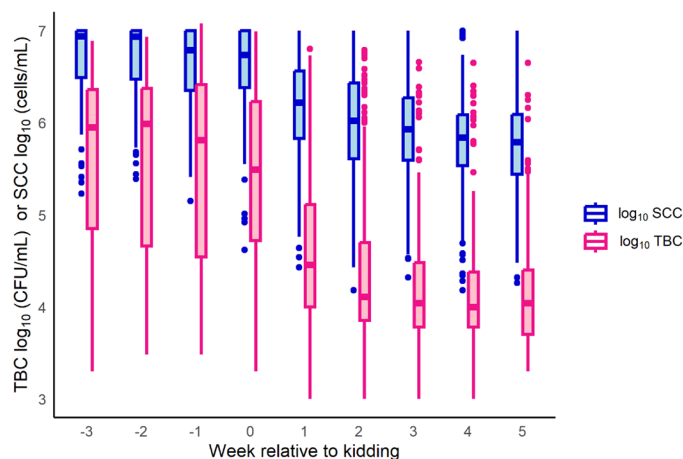


Figure 2. Boxplots of the median individual \log_{10} TBC and \log_{10} SCC. A total of 141 goats on 4 Dutch dairy goat farms were measured during wk -3 to +5 relative to kidding, with wk 0 as the kidding week. Per goat, 1 randomly selected udder half was measured, resulting in $n = 961$ TBC and $n = 969$ SCC measures. The bottom and top edges of the boxes indicate the first and third quartiles, respectively. The middle line shows the median, the whiskers extend up to 1.5 times the interquartile range. The dots denote outliers for BMTBC and BMSCC.

TBC and SCC (Spearman's $\rho = 0.83$). We also found a strong Spearman's correlation ($\rho = 0.87$) between the \log_{10} TBC and \log_{10} SCC in individual goat milk

samples. When we stratified the data over time relative to kidding (Figure 3), similar correlation coefficients were observed in the period before kidding ($\rho = 0.86$), in the kidding week ($\rho = 0.86$), and after kidding ($\rho = 0.76$).

Associations of Systemic Response Markers With Individual \log_{10} TBC and \log_{10} SCC.

In both the multivariable LME (Table 2) and the LQMM (Supplemental Table S2), the mean TBC and SCC were higher in the third week before kidding than any of the weeks around and after kidding. In all 5 wk after kidding the mean TBC and SCC were significantly lower than in the kidding week (Table 2). In both models the intercepts for SCC were higher than the TBC values. Farm A had a lower mean TBC and SCC than farms B, C, and D. Both the multivariable LME and the LQMM showed similar outcomes between the systemic response markers and the dependent variables TBC or SCC. Because the systemic response markers were not measured during all sampling points, each of these was modeled separately and based on a subset of the data, and hence the number of observations varied per variable (Table 2). No significant associations between the systemic response markers and TBC and SCC were demonstrated.

Table 1. Farm characteristics of 4 Dutch dairy goat farms participating in a longitudinal study in 2020 about individual TBC and SCC in goat milk around kidding

Item	Farm			
	A	B	C	D
Herd size (no. of female goats)	877	922	3,000	1,681
Milk yield (kg/goat per yr)	1,170	1,180	1,400	1,025
BMTBC $\times 10^3$ (cfu/mL)	40	49	57	78
Median	18	20	26	27
Minimum	193	536	156	174
Maximum	1,556	2,264	1,508	1,844
BMSCC $\times 10^3$ (cells/mL)	1,089	1,704	1,313	1,490
Median	1,971	3,703	1,883	2,555
Milking parlor	Side by side	Side by side	Rotary	Rotary
Mainly Saanen (S) breed; also crossings with Nubian (N), Toggenburger (T), and Alpine (A) breeds	S, N, T, A	S, N, T, A	S, A	S, N, T
CAEV ¹ certified-free status	Yes	Yes	Yes	No
CL ² certified-free status	Yes	Yes	Yes	Yes
Pre-milking teat end cleaning or disinfection	No	No	No	No
Teat end disinfection after milking	No	No	No	No
Staphylococcal vaccination	No	Yes	No	No
Kidding period (in 2020)	March 23–April 5	January 26–February 26	February 4–February 21	January 25–March 6
Enrolled goats (n)	38	37	37	35
Goats with dry period ≥ 1 wk (%)	77	38	43	11

¹CAEV = caprine arthritis encephalitis virus.

²CL = caseous lymphadenitis.

Table 2. Coefficients (β) and 95% CI of the LME models for \log_{10} TBC and \log_{10} SCC, and the week relative to kidding and farm were used as fixed effects, and the random effect was udder half nested within goat¹

Variable and level	Goat (n)	Observation (n)	LME					
			Log ₁₀ TBC			Log ₁₀ SCC		
			β	95% CI (β)	P-value	β	95% CI (β)	P-value
Intercept			5.48	5.29 to 5.68		6.70	6.57 to 6.83	
Week relative to kidding								
-3	65	129	0.19	0.04 to 0.35	0.01	0.09	0.02 to 0.17	0.02
-2	75	149	0.10	-0.05 to 0.24	0.18	0.06	-0.02 to 0.13	0.12
-1	77	147	0.03	-0.12 to 0.17	0.71	-0.01	-0.09 to 0.06	0.73
0	91	173	Ref ²			Ref		
+1	133	263	-0.76	-0.89 to -0.64	0.00	-0.36	-0.42 to -0.29	0.00
+2	136	285	-1.06	-1.18 to -0.93	0.00	-0.53	-0.60 to -0.47	0.00
+3	130	259	-1.23	-1.36 to -1.10	0.00	-0.65	-0.71 to -0.58	0.00
+4	124	245	-1.30	-1.43 to -1.17	0.00	-0.73	-0.80 to -0.67	0.00
+5	120	236	-1.28	-1.41 to -1.15	0.00	-0.78	-0.84 to -0.71	0.00
Farm								
A	36	394	-0.28	-0.52 to -0.03	0.03	-0.42	-0.59 to -0.25	0.00
B	36	537	Ref			Ref		
C	36	509	-0.02	-0.26 to 0.23	0.89	-0.07	-0.24 to 0.10	0.41
D	33	446	0.16	-0.09 to 0.41	0.22	-0.11	-0.28 to 0.07	0.23
INF- γ , ng/mL	135	269	-0.13	-0.29 to 0.04	0.13	-0.11	-0.22 to 0.01	0.08
Calprotectin, log ₁₀ ng/mL	136	271	-0.14	-0.49 to 0.22	0.45	-0.20	-0.44 to 0.04	0.10
BHB, log ₁₀ mmol/L	137	273	-0.03	-0.04 to 0.33	0.86	-0.06	-0.31 to 0.19	0.63
BCS								
≥ 2.75 to ≤ 3.50	117	472	Ref			Ref		
< 2.75 to > 3.50	94	340	-0.02	-0.12 to 0.09	0.078	0.01	-0.05 to 0.07	0.73
Fecal consistency								
Dry, pelleted	68	176	Ref			Ref		
Smooth	105	294	-0.11	-0.26 to 0.03	0.013	-0.04	-0.13 to 0.05	0.34
Watery, very soft	33	69	-0.17	-0.36 to 0.02	0.009	-0.05	-0.16 to 0.07	0.46

¹The study period during wk -3 to +5 relative to kidding includes a total of 1,886 observations of 141 goats on 4 Dutch dairy goat farms. The separate models of the independent variables IFN- γ , calprotectin, BHB, BCS, and fecal consistency were corrected for week and farm, and the models are based on a subset of the complete dataset. For each variable the number of observations is noted.

²Ref = reference category.

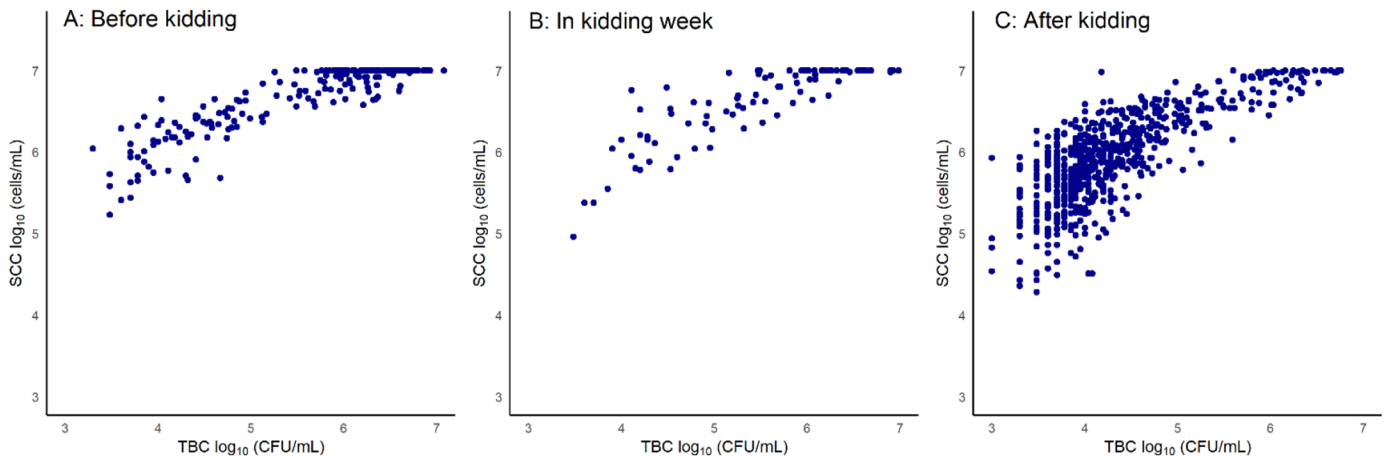


Figure 3. Scatter plots of individual \log_{10} TBC versus \log_{10} SCC of 4 Dutch dairy goat farms. For each goat, a randomly selected milk sample of the left or right udder half was used in the analysis. Before kidding in wk -3, -2, and -1, a total of 103 goats with 218 udder halves (A) were sampled; in the kidding wk 0, a total of 91 goats and 91 udder halves were sampled (B); and after kidding in wk +1, +2, +3, +4, and +5, 138 goats with 652 udder halves (C) were sampled.

DISCUSSION

Bulk Milk TBC and SCC

The mean BMTBC and BMSCC values trended upwards over the last 22 years. Simultaneous with this increase of the BMTBC and BMSCC, also the average herd size of the Dutch dairy goat farms increased (Statline, 2023). In cows it has been shown that a larger herd size was associated with higher BMSCC (Archer et al., 2013), but Wenz et al. (2007) showed an inverse effect. As herd size increases, management often changes, which can lead to improved udder health (e.g., through stricter implementation of udder health protocols) or to worse udder health (e.g., because a bigger herd makes a herdsman spend less time on udder health). In general, Dutch dairy goat farmers do not perceive udder health and high SCC as major problems, and therefore farmers probably direct their attention more to other aspects of goat dairying. Using a part of the same dataset (2005–2007) that we used in this study, Koop et al. (2009) already showed that BMTBC and BMSCC showed annual peaks that occurred simultaneously with the kidding period.

The BMSCC showed a clearer and more stable pattern than BMTBC. Somatic cells in the tank only come from cells secreted by lactating goats into their milk, whereas bacteria can also come from environmental contamination (e.g., during the milking process, in the milking machine, in the bulk milk tank). Hence, bacteria can come from different routes, and, moreover, during these different contamination processes bacteria can multiply, which is not the case with cells. This may explain the more erratic pattern of BMTBC compared with BMSCC. Yet we still see peaks followed by decreases in the bacterial count around kidding.

The amplitude (range between peak and bottom values) of both BMTBC and BMSCC within a year decreased over the years, which may be related to the fact that more farmers use extended lactations, and a higher proportion of goats in a herd with extended lactations has been shown to be associated with less fluctuation in BMSCC (Koop et al., 2009). We have shown that individual goats have high TBC and SCC at the end of lactation. Assuming that this is related to pregnancy and not to DIM, a larger proportion of animals with extended lactations should lead to less pronounced peaks in BMSCC and BMTBC. In addition, it is becoming increasingly common on Dutch goat farms to have multiple kidding periods instead of just one kidding period per year in which all pregnant goats have their parturition. Spreading the number of goats over several periods could also contribute to less pronounced fluctuations in bulk milk. Recurring seasonal BMSCC patterns are described in other countries (Zeng et al., 1999; Foschino et al., 2002; Haelein, 2002).

Whether recurring BMTBC patterns are also present in other countries could not be determined because, to our knowledge, no seasonal effects on BMTBC have been published.

Although the BMTBC were determined with different BactoScan types, all measurements were according to ISO 21187 and ISO 4833-1. Therefore, we assume that the variation in bulk milk over time is not caused by the different BactoScan types or calibration methods. Moreover, since 2015 to 2022, all bulk milk measures were performed with BactoScan FC+, and in this period the increasing trends are also clearly visible.

Individual TBC and SCC Around Kidding

Both TBC and SCC decreased significantly after kidding (Figure 2). The strong increase before parturition and the steep decrease afterward are intriguing, and there may be several explanations for this finding.

First, IMI in late lactation could have caused the high TBC and SCC before kidding, and these infections are cured after kidding. It seems unlikely that these IMI occurred massively during end lactation because it is known that IMI also frequently occurs at the start of lactation (McDougall et al., 2014) or during mid-lactation (Albenzio et al., 2016). Moreover, IMI with *Staphylococcus* spp. are common in goats (Bergonier et al., 2003; Contreras et al., 2007), and these udder infections are generally chronic (Koop et al., 2012). Thus, it is unlikely that all udder infections cured after kidding. In addition, it seems unlikely to find bacterial counts at the level we observed during late lactation as a result of IMI, because NAS in cows were shown to be shed in milk in low numbers (Hamel et al., 2020).

A second explanation could be that because milk yield decreases at the end of lactation and increases in early lactation, cells and bacteria are diluted in larger volumes of milk after kidding. Although we only measured the milk yield at one farm before and after kidding, it seems that the decrease of SCC was similar to the milk yield increase. So, the SCC decrease after kidding could possibly be explained with a dilution effect (or, conversely, the increase before kidding could be caused by concentration). However, the extent to which TBC decreased after parturition was not proportional to the increase in milk production after parturition. Milk production increased on average by a factor of 11, whereas TBC decreased by a factor of 310. The reduction in TBC seems therefore insufficiently explained by a dilution effect after kidding.

A third explanation would be that milk samples with a high cell count, above 4 million cells/mL, might increase the viscosity of the milk, which could lead to an overestimation of the TBC by the BactoScan (Ramsahoi et al., 2011). Similar to other researchers (Zeng and Es-

cobar, 1995; Paape et al., 2001; Contreras et al., 2007; Smistad et al., 2021) we also found very high individual SCC (>10 million cells/mL) in milk of goats during end lactation. Both BMTBC and BMSCC peak around kidding; however, our BMSCC data seldomly exceeded 2 million cells/mL. Hence, this finding in bulk milk makes it implausible that SCC drives TBC through a measurement error.

A fourth explanation would be that TBC is affected by another process in the goat around kidding. This is in line with the fact that TBC of the left and right udder halves were strongly correlated within goats, suggesting a determinant at goat level and not a local process at udder-half level, such as udder infection, which is often one-sided. As discussed below, we studied several systemic response markers, but none of them correlated with TBC. Perhaps this process could be caused by hormonal changes, which have been shown to affect SCC in goats (Paape et al., 2001; McDougall and Voermans, 2002; Contreras et al., 2007; Manica et al., 2022), but the mechanism through which this occurs is unknown.

The fact that the Fossomatic machines were calibrated with cow milk could have led to a small overestimation of the SCC in goat milk (Zeng, 1996). Because this overestimation occurred in the SCC of all milk samples that were taken before and after kidding, we expect no effect on the variation of SCC over time. The BactoScan methodology counts all bacterial cells, including dead, nonculturable, and culturable bacteria (Suhren and Reichmuth, 2000). Apart from bacteria, goats can also secrete somatic cells and cytoplasmic particles with the milk through the apocrine nature of milk secretion (Farkaš, 2015). Bacteria and cytoplasmic particles are distinguished by the BactoScan method based on fluorescence intensity. The signals resulting from cytoplasmic particles in the milk are compared with the signals of bacterial control samples according to the calibration standards of ISO/17025:2017. Hence, we assume that misclassification of cytoplasmic particles as bacteria in the BactoScan count is limited.

Unlike our study goats, where ~63% were still lactating before kidding, it is unclear whether other species such as cows and sheep experience an increased bacterial count at the end of lactation, because they are usually dried off before parturition, and in nonlactating animals it is not possible to examine milk for bacterial counts. Nevertheless, we hypothesize that the increase in the bacterial count in goats around parturition can possibly be explained by a difference in hormones between the animal species. For instance, the hormone ruminant placental lactogen has both lactogenic and somatogenic biological functions, and it is found in 100- to 1,000-fold higher concentrations in small ruminants than in cows around parturition (Byatt et al., 1992). Therefore, the

relation between ruminant placental lactogen and TBC should be investigated.

Based on our findings, we propose that drying off goats during at least the last 3 wk before kidding, as is standard practice in many countries, might reduce the contribution of bacteria and cells in the bulk milk. To determine whether a dry-off period or the length of the dry period is a feasible management measure for dairy goat farmers to control their BMTBC and BMSCC around kidding should be investigated. Moreover, the finding that SCC and TBC in goats are strongly correlated around kidding suggests that SCC can be used as a proxy for TBC during end and start lactation. Farmers can easily collect individual SCC data via routine milk production registration, which could help to identify goats with high probability of high TBC. As individual TBC elevations may contribute substantially to the BMTBC, it needs to be investigated whether farmers can control BMTBC by identifying and excluding individual goats based on individual SCC around kidding.

Associations of Systemic Response Markers With Individual Log₁₀ TBC and Log₁₀ SCC

We broadly assessed whether selected systemic response markers were correlated with individual TBC and SCC. Systemic response markers used as proxies for the immune system were IFN- γ for intestinal health, calprotectin, and fecal consistency, and BCS and BHB for metabolic disease parameters. We found no statistically significant correlations between the systemic response markers after correcting for stage of lactation and farm (Table 2).

The BCS and the fecal consistency were scored by 2 to 3 different people; because the scorers were trained well and the numbers were limited, we assume that these scores were reliable. The dataset contained more samples taken before than after kidding, and our sample size might have lacked power to significantly show associations between the systemic response markers and the individual TBC and SCC. We feel that if a strong relationship had existed between the systemic response markers and TBC and SCC, we would also have found signals in our sample set, but we cannot rule out the possibility that biologically meaningful differences have been missed. Thus, it is more likely that an underlying mechanism which occurs around parturition plays a role in the pattern of change of TBC and SCC around kidding. The residuals of the models of TBC, SCC, and the systemic response markers did not show a sufficiently normal distribution. Nevertheless, the linear regression models and the quantile regression models yielded the same variables as statistically significant, suggesting that this has limited effect on our conclusions.

CONCLUSIONS

During the kidding season, BMTBC and BMSCC levels are high. In individual goat milk, bacteria and somatic cells increase before kidding and decrease quickly after, suggesting that late-lactation goats cause high BMTBC and BMSCC. The pattern of change of SCC could be explained by the changed milk production around kidding, but this dilution effect seems not to fully explain the pattern shown by TBC. This, together with the strong correlation of TBC between both udder halves, suggests that the high bacterial counts around kidding likely result from systemic processes rather than IMI, immunity, intestinal health, or metabolic diseases. Future studies on the bacterial microbiome could provide more insights into these processes. To control the BMTBC and BMSCC, farmers could exclude milk from animals that excrete high levels of bacteria and cells from the bulk milk, for example, by drying off these goats. Because individual TBC and SCC are strongly correlated, we propose that using individual SCC derived from the milk production registration could be used as a proxy for TBC for the identification of high-TBC goats.

NOTES

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Nonstandard abbreviations used: BMSCC = bulk milk SCC; BMTBC = bulk milk total bacterial count; CAEV = caprine arthritis encephalitis virus; CL = caseous lymphadenitis; ibc = individual bacterial count; LME = linear mixed effects; LQMM = linear quantile mixed models; Ref = reference category; TBC = total bacterial count.

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