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Pathogens in milk of goats and their relationship with somatic cell count

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Abstract

We evaluated the presence of bacterial pathogens in the milk of goats and their relationship with somatic cell count (SCC) and milk composition. The study was performed on a dairy farm in northern Slovakia. Half udder milk samples were collected from goats in June and July. The samples were divided on the basis of SCC into 4 bands (SCC1 lowest to SCC4 highest). Bacterial pathogens were only detected in 13% of samples. SCC3 and SCC4 had 15 and 25% positive samples respectively compared with SCC1 (2%) and SCC2 (14%). Coagulase-negative staphylococci (CNS) were the most common isolates (73%), of which Staphylococcus caprae was the most frequently isolated (65%). In samples with \geq 1000 × 10³ cells ml⁻¹ (SCC3, SCC4) there was higher somatic cell score (SCS) in the presence of a pathogen (7.48 ± 0.11) than without a pathogen (7.16 ± 0.05, P < 0.01). Statistically significant but weak negative correlations were observed between SCS and lactose, dry matter and non-fat dry matter. In conclusion, a higher percentage of bacteriologically positive milk samples was observed in both SCC3 and SCC4 groups but this does not explain the aetiology of high SCC in the milk of goats that are apparently free of bacteria. As a diagnostic tool, SCC is probably less useful in goats than in cows.

The breeding of goats has a rich tradition and history in Slovakia. The majority are dairy goats, predominantly the White Shorthaired, then the Brown Shorthaired breeds and finally the dual-purpose Anglo-Nubian breed (Oravcová, 2013). Recently, the demand for goat milk and its products has increased, so attention is paid to the best nutritional, techno-functional and sanitary qualities of dairy goat products, all of them depending on the udder health (Kováčová et al., 2021). Mastitis, an intramammary inflammation mostly resulting of bacterial infection, is the most important disease of the udder in dairy animals. The health of the udder is critical for dairy farms and is correlated with milk yield, quality of milk and food safety (Spuria et al., 2017; Zigo et al., 2022). Mastitis in goats is responsible for a drop in milk production and protein, lactose and fat contents (Novac and Andrei, 2020) as also observed in dairy ewes (Tvarožková et al., 2019). Intramammary infection is also the main cause of somatic cell count (SCC) increase in milk (Raynal-Ljutovac et al., 2007) which is used for mastitis detection in goats as in other ruminants. However, other factors that also affect SCC in goat milk include parity, stage of lactation, oestrus cycle and breed (Paape et al., 2007; Persson et al., 2014). Udder and teat morphologies, milking frequency, grazing management, milking machine equipment and settings (Marnet et al., 2018) and viral co-infection with CAEV (Sanchez et al., 2001) can all influence SCC, making high SCC difficult to interpret in goats, compared with cows and ewes (Persson and Olofsson, 2011). Further, subclinical mastitis is a problem in goats where prevalence rates are important (reported as 35% to 70%: Leitner et al. 2004a; Hall and Rycroft, 2007). The major types of pathogens causing subclinical mastitis in dairy goats are coagulase-negative staphylococci (CNS) (Bergonier et al., 2003; Dore et al., 2016), in particular Staphylococcus caprae and Staphylococcus epidermidis (Leitner et al., 2004b). However, the main pathogens affecting goats in Slovakia and their effects on udder inflammation are still unknown.

The hypothesis of this work was that the high level of somatic cells in the milk of goats is caused by mastitis pathogens and that the increased SCC changes milk composition. Therefore, the aim of this study was to describe the frequency of distribution of SCC from half udder milk samples, identify causative bacteria of mastitis and evaluate effects on milk composition in dairy goats.

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174 Kristína Tvarožková et al.

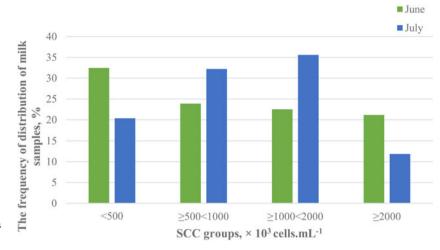


Figure 1. Frequency of distribution of half udder milk samples for four SCC groups ($\times 10^3$ cells ml⁻¹) in June and July.

Material and methods

Sampling

The study was carried out on a goat dairy farm in northern Slovakia on dairy goats of the White Shorthaired breed. A total of 458 half udder milk samples from 129 goats (44 goats in their first lactation, 61 in second and 24 in third and higher lactations) were collected during evening milking in June (222) and July (236 samples). The goats had kidded from mid-February to mid-March, so the samples were from mid- to late lactation. Only 22 animals were not sampled in both months. Only clinically healthy goats without any visual abnormalities in udder or milk were included. The first squirts of milk from teats were discarded and subsequently the teat end was cleaned with 70% alcohol. Then the milk samples were collected for bacteriological cultivation using sterile tubes (5 ml) and followed by sampling for determination of SCC and milk composition (50 ml). Samples were frozen at -20°C until thawing and cultivation (Sánchez *et al.*, 2003).

Microbiological analysis

Milk samples (10 ul) were incubated aerobically on blood agar plates (MkB Test a.s., Rosina, SR) for 24 h at 37°C. Bacterial colonies were identified by haemolysis, a catalase test, aesculin hydrolysis, Gram staining and cell morphology. Presumptive Staphylococcus aureus were identified with the clumping factor test (DiaMondiaL Staph Plus Kit, Germany). Aesculin-positive streptococci were subcultured to identify Streptococcus uberis or Enterococcus sp. on modified Rambach agar (Watts et al., 1993). Aesculin negative streptococci were characterised by Lancefield serotyping (DiaMondiaL Strept Kit, Germany). Gram and catalase positive small colonies were identified as coryneform bacteria. Large colonies, Gram and catalase positive, capable of forming endospores were identified as Bacillus sp. All Gram positive and Gram negative colonies were classified using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) (Tvarožková et al., 2021). Presence of contagious pathogens (Staphylococcus aureus, Streptococcus agalactiae) was reported as positive if one or more colonies were found. Presence of other pathogens was reported as positive if at least five colonies were found. Samples were considered contaminated and removed from data analysis if more than two different colony types were isolated on blood agar.

Somatic cell analysis

SCC were determined using a Somacount 150 (Bentley Czech, USA). Milk composition was determined using MilkoScan FT 120 (Foss Electric, Hillerød, Denmark).

Statistical analysis

Milk samples were divided into four SCC groups on the basis of half udder SCC. Group SCC1 comprised samples of less than 500×10^3 cells ml⁻¹. SCC2 ranged from 500 to 1000, SCC3 from 1000 to 2000 and SCC4 comprised samples above 2000, all \times 10^3 cells ml⁻¹. For statistical evaluation SCC were recalculated to SCS: LOG₂ (SCC/100 000) + 3.

Relationships among traits were analysed using Pearson's correlation coefficients. Statistical analysis was performed using the GLM procedure in SAS9.2 (2009). The resulting models, based on preliminary analysis of possible sources of variability of investigated traits, are specified in the online Supplementary File. Results are presented as LSmeans \pm standard error. The effects in the models were tested using the F-test. Differences between LSmeans were tested using multiple ranging Sheffes tests. The differences were considered statistically significant at $P \le 0.05$.

Results

The overall mean SCC was $1250 \pm 1265 \times 10^3$ cells mL⁻¹ (SCS 6.10 \pm 1.30). Classification by SCC groups is shown in Figure 1. More than 50% of individual samples were below 10^6 cells mL⁻¹ and 32.43 and 20.34% of samples were classified in SCC1 group ($<500\times10^3$ cells ml⁻¹) in the months June and July, respectively (Fig. 1). Bacteria presence was detected from 12.88% of milk samples, none of which were contaminated. The most common bacteria found were CNS (72.88%). The most common CNS was Staphylococcus caprae (65.12%) (Table 1). Staphylococcus aureus was isolated from 6.90% and 6.66% of samples taken in June and July, respectively (Table 1). Seven goats had the same pathogen in both halves of udder and four goats had different species of pathogens in the two udder halves. Bacterial positive samples were found only in 1.67%, 13.95%, 14.93% and 25.33% in SCC1, SCC2, SCC3 and SCC4, respectively.

We compared bacteria positive and negative milk samples within the SCC3 and SCC4 groups. In total we observed significantly higher SCS in milk samples with a pathogen (7.48 ± 0.11)

Journal of Dairy Research

Table 1. The incidence of pathogens in goat milk samples taken in June and July

	Month <i>n</i> , (%) positive samples	
Pathogens	June	July
Citrobacter braakii	1 (3.45)	
Enterobacter cloacae	2 (6.90)	
Enterobacter kobei	8 (27.59)	
Staphylococcus aureus	2 (6.90)	2 (6.66)
Staphylococcus caprae	10 (34.48)	18 (60)
Staphylococcus epidermidis	6 (20.69)	8 (26.67)
Staphylococcus xylosus		1 (3.33)
Steptococcus. pluranimalium		1 (3.33)
All positive samples, n (% of all samples)	29 (13.06)	30 (12.71)

compared with no pathogen $(7.16\pm0.05, P<0.001: \text{model }2)$. We found no effect of the month of sampling on SCC (online Supplementary Table S1), with more milk samples in SCC2 and SCC3 and fewer in SCC1 in July compared to June (Fig. 1). However, the mean SCS when only SCC3 and SCC4 were analysed (model 2) dropped significantly (P<0.05) from June (7.49 ± 0.09) to July (7.15 ± 0.08). Parity significantly influenced SCS, where SCS significantly increased from first (5.82 ± 0.10) to second (6.17 ± 0.08) and third and higher lactation (6.54 ± 0.16 : P<0.05, online Supplementary Table S2).

Significantly less protein, NFDM and lactose were found in July than in June, whereas fat content was the reverse (online Supplementary Table S1). Milk composition was not influenced by parity but SCS significantly increased with parity $(5.82 \pm 0.10, 6.17 \pm 0.08$ and 6.54 ± 0.16 for first, second and greater parities, online Supplementary Table S2). Milk composition in the four SCC groups is presented in Table 2. Statistically significant negative correlations were found between SCS and lactose content (-0.37), dry matter (-0.19) and non-fat dry matter (-0.30) (P < 0.001). The correlations between SCS and fat or protein were not significant.

Discussion

Our data confirm the presence of high SCC in goat milk samples, comparable to those of Moroni *et al.* (2005), Gosselin *et al.* (2020)

and Podhorecká *et al.* (2021) but almost twice that reported by Persson and Olofsson, (2011). We observed more than 50% samples with SCC less than 10^6 cells ml $^{-1}$ which we interpret as probably without infection. Albenzio *et al.* (2015) reported SCC of 700×10^3 cells mL $^{-1}$ as a threshold which represents changes in leucocyte distribution as a reflection of the immune status of the udder.

Persson and Olofsson (2011) and Bagnicka et al. (2011) reported the presence of pathogens in 18% and 35% of milk samples respectively, compared to our overall value of 15% and 25% in the highest SCC groups (SCC3 and SCC4, respectively). In our study CNS were the most common bacteria isolated. Our results also confirm the results of Leitner et al. (2004b) and Persson and Olofsson (2011) who reported that CNS were the most frequent pathogens in milk of goats. Among these pathogens Koop et al. (2012) and Gosselin et al. (2020) found Staphylococcus caprae as the second more frequent pathogen when we detected S. caprae as the most common pathogen. Staphylococcus aureus is considered the most important contagious pathogen in dairy goats, ranging from 4% to 40% of bacteriologically positive samples (Min et al., 2007; Marogna et al., 2010; Persson and Olofsson, 2011; Dore et al., 2016). Our data were at the bottom end of this range (7%). The number of same infection (7/129 goats) or dual pathogen infections (4/129 goats) in both half udder in our study is low, in agreement with Persson and Olofsson (2011) (9 and 3/111).

One of the main reasons for a high SCC in milk is the presence of mastitis pathogens, whether it be cows (Holko et al., 2019) or goats. Our findings confirm this result and seem to be different from a previous study done in dairy ewes in which we did not find effect of different pathogens on SCS over the range 6.68 ± 0.41 to 8.11 ± 0.63 (Tvarožková et al., 2020). Various observations have reported the effect of different pathogens on SCC in milk of goats (Moroni et al., 2005; Koop et al., 2012; Gosselin et al., 2020). Staphylococcus caprae was associated with higher SCC compared to other CNS (Moroni et al., 2005). Koop et al. (2012) recorded a higher SCC in milk samples with S. aureus compared to milk samples infected by CNS. The low number of S. aureus infections meant that we could neither confirm nor refute this observation. In another study S. caprae, S. epidermidis, S. simulans and S. xylosus were associated with higher SCC than other CNS species (Gosselin et al., 2020). We have considered the possibility that our high SCC values might indicate infection by microorganisms other than those we could detect using the methods employed (Mycoplasma, for example). Given the high number of such samples (high SCC in the absence of an identified pathogen) we consider this unlikely. Accordingly, it may be that the diagnostic value of SCC is lower

Table 2. Milk composition in the four SCC groups

		SCC group (×10 ³ cells ml ⁻¹)			
Component	<500	≥500 < 1000	≥1000 < 2000	≥2000	
Fat, %	4.15 ± 0.09	4.23 ± 0.09	4.13 ± 0.09	3.90 ± 0.12	
Protein, %	3.09 ± 0.03	2.99 ± 0.03	2.98 ± 0.03	3.10 ± 0.04	
Lactose, %	4.69 ± 0.04^{a}	4.61 ± 0.03^{ac}	4.52 ± 0.03 ^{bc}	4.26 ± 0.04^{d}	
DM, %	12.53 ± 0.11 ^a	12.42 ± 0.10 ^a	12.24 ± 0.10 ^{ac}	11.93 ± 0.14 ^{bc}	
NFDM, %	8.45 ± 0.05 ^a	8.25 ± 0.05 ^b	8.15 ± 0.05 ^{bd}	8.02 ± 0.06 ^{cd}	

Note: a,b,c,d LS Means and standard error within row with different letters are significantly different at P < 0.05, DM, dry matter; NFDM, non-fat dry matter.

176 Kristína Tvarožková *et al.*

in goats than in cows. Our data could also be interpreted as indicating that animals with a SCC $\geq 10^6$ cells ml⁻¹ have subclinical mastitis and those with a SCC $< 500 \times 10^3$ cells ml⁻¹ indicate absence of infection, as suggested by Persson and Olofsson (2011) who reported that the SCC of uninfected udder halves had a mean SCC of 478 \times 10^3 cells ml⁻¹. So far, the SCC threshold indicating mastitis in the udder of goats has not been agreed.

We did not observe an effect of month/stage of lactation on SCS contrary to other studies (Paape *et al.*, 2007; Persson *et al.*, 2017; Smistad *et al.*, 2021) but the relative proximity of our two samples, both in mid lactation when milk production was stabilized, could explain this observation. On the other hand, we detected a significant influence of parity on SCS, as did Smistad *et al.* (2021).

The milk composition of uninfected udder halves is similar to those described by Currò *et al.* (2019). Yakan *et al.* (2019) reported an increase in protein content in late lactation, which we did not observe at the earlier lactation stage we used. A statistically significant but weak negative correlation was observed between the content of lactose and SCS (-0.37, P < 0.001). Similar relationship between SCC and milk lactose content was reported by Ying *et al.* (2004) in goats and by Oravcová *et al.* (2018) in dairy ewes. Such a relationship is to be expected on the basis of tight junction integrity, 'leaky' tight junctions (as a consequence of infection and inflammation) allowing partial equilibration between plasma and milk such that somatic cells enter milk and lactose exits (Ben-Chedly *et al.*, 2009).

In conclusion, as in other goat studies, a high occurrence of milk samples with high somatic cell count at the half udder level was observed. Nevertheless, we also confirmed that only low percentage of samples with high somatic cell count were bacteriologically positive. Even if the bacteriologically positive samples had higher SCC in groups with high SCC (SCC3 and SCC4) we assume that SCC should not be regarded as a gold standard of infection in goats. More intensive study of the relationship between somatic cell count and caprine udder health status is needed.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029923000237

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Journal of Dairy Research

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