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Pathogen-specific changes in composition and quality traits of milk from goats affected by subclinical intramammary infections

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Research Article

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Abstract

We investigated the effects of pathogens associated with subclinical intramammary infections on yield, composition and quality indicators of goat milk. By means of a longitudinal study, individual half udder milk samples ($n = 132$) were collected at different lactation periods and assessed for milk yield and physicochemical composition, somatic cell count (SCC), total bacteria count (TBC) and microbiological culture. Staphylococci species accounted for the great majority of the isolates (96.1%). Intramammary infections significantly reduced fat and total solids in goat milk and increased both SCC and TBC. However, these indicators were significantly higher in udder halves affected by *S. aureus* compared with other staphylococci species.

Intramammary infections (IMI) causing inflammation of the udder (mastitis) can lead to serious economic losses to the dairy industry because of the reduced milk production (Leitner *et al.*, 2011; Gelasakis *et al.*, 2016), treatment costs and animal welfare issues (Rainard *et al.*, 2018), as well as physicochemical changes that negatively impact the quality of milk and dairy products (Forsbäck *et al.*, 2010). Changes in goat milk composition caused by subclinical IMI have been reported, such as the reduction of lactose (Leitner *et al.*, 2011; Silanikove *et al.*, 2014), and increased serum protein levels (Leitner *et al.*, 2004b), as well as alterations in somatic cell count (SCC) and total bacteria count (TBC) (Koop *et al.*, 2010).

Staphylococcus other than *S. aureus* species, also referred as non-*aureus* staphylococci (NAS) have been reported as leading subclinical IMI agents in the caprine species (Gosselin *et al.*, 2018), and have been associated with negative changes in goat milk yield, quality, and nutritional value (Silanikove *et al.*, 2014). However, such impact is controversial, since in bovine species, NAS have been reported to cause only moderate and transitory inflammation leading to limited changes in milk (Tomazi *et al.*, 2015).

The accurate identification of staphylococci by means of conventional biochemical methods has been a bottleneck to evaluate the real impact of those NAS species on milk quality traits in goats. In the last decade, matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has been increasingly used for a rapid and accurate species-level diagnostic tool both in human and veterinary medicine, including the identification of mastitis-causing agents (Gosselin *et al.*, 2018). By means of MALDI-TOF, the aim of this study was to investigate the effects of pathogens associated with subclinical intramammary infections on yield, composition and quality indicators (total bacteria and somatic cells counts) of goat milk.

Material and methods

Study design and samplings

In this longitudinal study, half udder milk samples were collected at early (50 d), intermediate (100 d), and late stages of lactation (150 d). Based on sampling guidelines (NMC, 2004), a total of 132 milk samples were collected from ¾ Alpine ($n = 12$) and Anglo Nubian ($n = 10$) multiparous dairy goats in a commercial herd in Paraiba state, Brazil. Goats were milked twice a day manually and milk yield determined weekly. Milk production per animal was calculated considering both morning and afternoon yields.

Somatic cell count (SCC) and physicochemical composition

Milk samples (50 ml) from each udder half were collected in sterilized plastic bottles containing bronopol, and immediately shipped to a national reference lab (PROGENE, UFRPE, Brazil) for milk compositional analysis and somatic cell count by means of Fourier Transform Infrared Spectroscopy (FTIR, Bentley Instruments, USA) and flow cytometry (Somacount 300, Bentley), respectively. Milk samples were also evaluated by titratable acidity and cryoscopy (MK 540 FLEX, Brazil).

Total bacterial count (TBC), microbial isolation and identification

TBC was determined by means of aerobic mesophilic bacteria count using *pour plate* technique. Microbial isolation was performed by means of conventional microbiological procedures. All isolates were further identified in triplicates by means of Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany).

Data analyses

Based on a longitudinal design, the response variables were considered independent among the animals since the repeated measurements were nested within the individual animal. We considered mixed infections whenever two or more different bacterial species were detected in a same udder half. Estimated reductions of daily milk yield were determined for each of the following categories: no infection, *S. aureus* infection, coagulase negative *Staphylococcus* infection, and mixed infection.

The putative effects of the intramammary infections on milk production, physicochemical composition, SCC, and TBC were evaluated using two linear mixed models using the PROC MIXED from Statistical Analysis System (SAS) version 9.2 package. Model 1 was applied to determine the effects of the subclinical intramammary infection on milk production (kg/day), SCC, TBC, and physicochemical composition. The values of the dependent variables SCC and TBC were logarithmically transformed (Log). The animal was used as random effect and intramammary infection and lactation period were used as fixed effects, according to the following mathematical model:

$$Y_{ijl} = IM_i + PL_j + A_{l+} e_{ijl}$$

where: Y_{ijl} is the dependent variable; μ is a general constant inherent to each observation; IMi is the fixed effect of the intramammary infection; PL_j is the fixed effect of lactation period; A_l is the random effect of the animal; e_{ijl} is the error associated to each observation.

For the second model, the SCC values were categorized into low SCC (<2.5×10^5 cells/ml), mean SCC (>2.5×10^5 and < 1.3×10^6 cells/ml) and high SCC (>1.3×10^6 cells/ml) counts considering the 25th (2.5×10^5 cells/ml) and 75th (1.3×10^6 cells/ml) percentiles. Those categories were used as a fixed effect to evaluate the effect of SCC on the changes in physicochemical composition of milk, according to the following mathematical model:

$$Y_{ijkl} = SCC_i + PL_j + A_{l+} e_{ijl}$$

where: Y_{ijl} is the dependent variable; μ is a general constant inherent to each observation; SCCi is the effect of SCC; PL_k is the fixed effect of the lactation period; A_l is the random effect of the animal; e_{ijl} is the error associated to each observation.

Differences in the occurrence of IMI between different lactation stages and breed were analyzed using a binomial negative distribution. The Kruskal-Wallis test was also performed to evaluate differences in SCC among the three lactation stages for healthy mammary udder halves. Data were analyzed using a significance level of 5% with Statistical Analysis System (SAS) version 9.2 package.

Results

The reduction in milk yield in animals affected with subclinical mastitis caused by *S. aureus* (−0.2 Kg/animal/day), NAS (−0.1 kg/animal/day), or both *S. aureus* and NAS (−0.4 Kg/animal/day) were not statistically different from the milk yield of goats with no subclinical mastitis (1.8 kg/day), as observed in online Supplementary Table S1.

The pathogens cultured from the infected udder halves were identified as *S. aureus* (50%), non-*aureus* species (NAS) (46.1%), *Micrococcus luteus* (3.8%), and *Klebsiella pneumoniae* (1.9%). In the present study, only staphylococci were included in the statistical models because of the low frequency of infections attributed to other organisms. The most common NAS detected in the present study were *S. caprae* (9.6%), *S. epidermidis* (7.7%), and *S. simulans* (5.8%). The IMI effects (A) and the effects of etiological agent (B) on the physicochemical composition, SCC and TBC of milk are shown in Table 1.

In terms of quality indicators, SCC and TBC were higher ($P < 0.05$) in subclinical mastitis samples (A). Considering the effects of pathogen-specific agents on the quality traits of milk (B), SCC and TBC were higher in IMI associated with *S. aureus* compared to NAS ($P < 0.05$; Table 2). In terms of composition, IMI decreased both fat contents and total solids ($P < 0.05$). No other significant changes in physicochemical composition were attributed to IMI (A) or specific pathogens (B).

In our linear mixed model, it was observed that the increase of SCC in milk was associated with increased protein ($P < 0.001$), fat ($P < 0.05$) and total solids ($P < 0.05$), as well as with a decreased TBC ($P < 0.001$; Table 2). IMI occurrence was lower in the late lactation stage when compared to early and intermediate stages, whereas there was no difference in IMI between breeds (Supplementary Table S2). In non-infected udder halves, SCC counts were lower ($P < 0.05$) in the early lactation period as compared with the other stages. Mean values were 1.1×10^6, 2.2×10^6, and 2.7×10^6 cells/ml at 50, 100 and 150 d, respectively.

Discussion

Although CoNS have been reported as leading agents causing subclinical IMI in goats (Gosselin *et al.*, 2018), *S. aureus* was the most frequent pathogen found in our study, corroborating a previous report (Merz *et al.*, 2016). It must be noted that *S. aureus* is highly contagious and associated with persistent and hard-to-treat infections in both cows and small ruminants (Rainard *et al.*, 2018). Some strains can also produce thermostable enterotoxins capable of causing food poisoning in humans. Therefore, goat milk contamination by *S. aureus* is a matter of public health concern.

Our findings suggest no differences in terms of total milk yield between goats with and without IMI, corroborating previous studies that reported no effects of subclinical IMI on the total goat

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Table 1. Physicochemical composition, and logarithmically-transformed somatic cell counts (SCC) and total bacteria count (TBC) in milk produced by ¾ Alpine ($n = 12$) and Anglo Nubian ($n = 10$) goats according to the occurrence of subclinical intramammary infection (A) caused by *Staphylococcus aureus* (*S. aureus*) and non-aureus staphylococci (NAS) (B)[1]

	A				B			
Traits	No infection	Infection	SEM[2]	*P*	*S. aureus*	NAS	SEM	*P*
SCC (log SSC/ml)	5.9[b]	6.2[a]	0.13	0.03	6.5[a]	5.8[b]	0.23	0.005
TBC (log CFU/ml)	2.1[b]	3.5[a]	0.37	0.001	3.9[a]	2.8[b]	0.43	0.01
Fat (%)	3.6[b]	3.2[a]	0.16	0.02	2.9	3.2	0.39	0.27
Protein (%)	3.3	3.4	1.10	0.75	3.2	3.3	0.15	0.74
Lactose (%)	3.9	3.8	0.05	0.53	3.8	3.7	0.10	0.25
Total solids (%)	11.7[b]	11.2[a]	0.23	0.04	11.0	11.2	0.36	0.60
Acidity (%)	15.5	15.2	0.65	0.74	15.7	15.1	1.40	0.65
Freezing point (H°)	−0.571	−0.570	0.002	0.92	−0.570	−0.571	0.003	0.67

[1]Linear mixed model = Animal as random effect; period of lactation and intramammary infection as fixed effects.
[2]Standard error of the mean (SEM).
Means with different letters in the rows differ statistically by the Tukey-Kramer test ($P < 0.05$).

Table 2. Physicochemical composition, and logarithmically-transformed total bacteria count (TBC) in milk produced by ¾ Alpine ($n = 12$) and Anglo Nubian ($n = 10$) goats according to somatic cell count levels (low, medium, and high)[1]

	Somatic Cell Count (SCC/ml)				
Variable	Low[2]	Medium	High	*P*	SEM[3]
TBC (log CFU/ml)	1.8[a]	2.5[b]	3.0[c]	<0.001	0.43
Fat (%)	3.2[b]	3.2[b]	3.6[a]	< 0.05	0.25
Protein (%)	3.2[b]	3.1[b]	3.6[a]	<0.001	0.13
Lactose (%)	4.0[a]	3.9[a]	3.7[b]	<0.001	0.05
Total solids (%)	11.3[b]	11.1[b]	11.9[a]	<0.01	0.35
Acidity (%)	16.5	15.34	14.6	> 0.05	0.81
Freezing point (H°)	−0.572	−0.567	−0.571	> 0.05	0.003

[1]Linear mixed model = Animal as random effect; lactation period and SCC categories as fixed effects.
[2]Low: $< 2.5 \times 10^5$; Medium: $>2.5 \times 10^5$ and $<1.3 \times 10^6$; High: $> 1.3 \times 10^6$ cells/ml.
[3]Standard error of the mean (SEM).
Means with different letters in the rows differ statistically by the Tukey–Kramer test ($P < 0.05$).

milk yield (Koop *et al.*, 2010). On the other hand, some other studies reported significant lower milk yield in infected goats (Leitner *et al.*, 2011; Koop *et al.*, 2012).

The increased SCC values observed in milk samples from udder halves affected by subclinical IMI corroborated previous studies (Koop *et al.*, 2010). The inflammatory process triggers leukocyte migration from the blood to the mammary gland (Leitner *et al.*, 2011) that causes increased SCC. In dairy cows, there is an inverse correlation between somatic cell counts and milk quality, which makes SCC a valuable and widely used indicator of subclinical mastitis. On the other hand, the relevance of SCC in dairy goats is very controversial (Merz *et al.*, 2016), as the correlation between increased SCC and subclinical IMI is not as clear as it is in the bovine species. In this study, we were able to correlate increased SCC levels with subclinical IMI using a SCC threshold of 1.3×10^6 cells/ml, which is similar the threshold used in some countries for goat milk regulatory purposes.

Higher SCC and TBC were expected in the samples positive for *S. aureus* compared to NAS, since *S. aureus* is known as a major mastitis pathogen, frequently reported to harbor several pathogenicity factors and greater potential to damage the mammary glandular tissue leading, therefore, to increased SCC. Despite predominance of NAS in IMI in goats, the influence of those microorganisms on SCC is not as evident as compared to *S. aureus* (Koop *et al.*, 2012). Although there were no differences in protein and lactose contents between udder halves with and without IMI, the linear mixed model indicated that the increase in SCC was associated with the increase of proteins, fat and total solids contents, as well as with a decrease in lactose contents (Table 2). Changes in milk composition are expected during mastitis as there might be increased permeability of the blood-milk barrier conferred by leakiness of the tight junctions of the alveolar epithelial cells (Stelwagen and Singh, 2014). Higher protein contents (Åkerstedt *et al.*, 2012), and reduced lactose in milk (Silanikove *et al.*, 2014) have been previously associated with increased SCC. In our study, we observed an increase in protein and a decrease in lactose along the lactation stages in non-infected animals, corroborating previous reports (Leitner *et al.*, 2004b, 2011).

The increase in SCC along the lactation was expected and might be attributed to the desquamation of alveolar epithelium that is more marked at the later stages of lactation, as reported by Leitner *et al.* (2012) in Saanen, Alpine and crossbred goats without IMI. The reduction of fat and total solids observed in milk from animals with IMI could be attributed to the inflammation process. Our results corroborate Sánchez *et al.* (2002), who reported a lower fat concentration in milk of Murcian goats with *S. caprae* intramammary infection. On the contrary, previous studies found no effect of subclinical IMI on fat contents in goat milk (Leitner *et al.*, 2004a). Such differences are probably associated with the different capacity of pathogens to trigger inflammatory reactions in the caprine udder and to the severity of the inflammation at a given lactation point, which reinforces the importance of knowing the effects of specific pathogens in longitudinal study designs.

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In conclusion, whilst staphylococcal subclinical intramammary infections are associated with a significant reductions in fat and total solids in goat milk, *Staphylococcus aureus* species lead to higher somatic cell and total bacteria counts compared to other species. These indicators cannot be overlooked in goat milk quality assessment considering the high importance of *S. aureus* species as a mastitis-causing pathogen and its role in public health.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029921000418

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