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Original Scientific Article

SOMATIC CELL COUNTS AND TOTAL BACTERIAL COUNT IN BULK TANK MILK OF SMALL RUMINANTS

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Summary: Total bacterial count (TBC) is a good tool for monitoring the hygienic environment of small ruminants. TBC is significantly correlated with the number of somatic cells in bulk milk (BMSCC). Programs to improve the hygienic quality of bulk milk are more effective if you take into account both variables (TBC and BMSCC). A significant impact on BTSCC in ewe bulk milk was found for the following factors: herd/flock, sampling month, dry therapy practice, breed of sheep, and type of milking. Factors associated with the mean standard plate count (SPC) included the month of bulk milk sampling and the number of milkings contained in bulk tank milk, as well as the adopted milking technique. In fresh sheep chesses the casein fractions (αs1-1-CN and β-CNs) were significantly lower, when BTSCC was high. These cheeses have lower fat contents and as a result an increased pH and fat acidity. Yoghurts made from milk with a high BTSCC were characterized by a higher protein content and the highest level of syneresis. To improve the udder health status of small ruminants it is necessary to ensure hygienic conditions of animal maintenance and optimization of milking machine standards and parlor systems.

Key words: small ruminants; somatic cell count; total bacterial count; bulk tank milk

Introduction

Total bacterial count (TBC) can serve as a tool in monitoring hygiene of dairy sheep flocks and can also provide a basis for payment systems for milk (4, 8, 15). A statistically significant correlations was found between TBC and the number of somatic cells in bulk milk (BMSCC). Programs to improve the hygienic quality of bulk milk are more effective if you take into account both variables (8). More recent results indicate that predictors of quality milk for cheese production include effectiveness of lactose, casein % and somatic cell count (SCC) of bulk milk (15). Milk microbiological standard stipulated by the European Council Directive 92/46/EEC may be used as a basis to develop specific quality parameters in the standard system for assessing milk of small ruminants, as it has been the case in Switzerland (27). In recent years, the Single Strand Conformation Polymorphism (SSCP) method proved to be a powerful tool for distinguishing milks and classifying them on the basis of the balance between different bacterial populations (26). In goats apocrine secretion mechanisms cause in milk the presence of cytoplasmic particles (CP), which are mostly anuclear and part of physiological secretions (22). Application of flow cytometry facilitates a rapid differentiation of somatic cells; however, it is necessary

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to use DNA-specific fluorescent dyes to avoid overlapping of SCC and CP (2). This new method allows a differentiation between viable and non-viable polymorphonuclear neutrophils (PMN), macrophages, and lymphocytes.

The aim of the study is to review the scientific literature in the field of hygienic and microbiological quality of bulk milk and the quality of products (cheese and yogurt) made from the milk of small ruminants.

Bulk milk somatic cell counts related to bulk milk total bacterial counts

The base level of SCC in bacteria free udders of goats (~ 300,000 cells/ml) and sheep (~ 200,000 cells/ml) is generally higher than in dairy cows (~ 70,000 cells/ml); hence, the level of SCC in animals with udder infection is usually much higher in small ruminants than in dairy cows (24). Intramammary infection (IMI) is a major cause of an increased SCC in milk of dairy ruminants (7, 8, 9, 13, 14, 21, 22). The innate immune system plays a major role, especially polymorphonuclear neutrophils (PMN), that appear in the mammary gland in response to invading pathogens (21). However, non-infectious factors are the cause of the variability of SCC in goat milk and some species of bacteria cause different levels of somatic cells as well as varying degrees of *mastitis* (22). Similarly in sheep, the increase in SCC is related to the stage of lactation and parity, but mainly results from intramammary infections (21). Many authors emphasize that the use of SCC is a good indicator in the assessment of sanitary conditions in herds/flocks, especially in herds with maintained control of IMI (8, 9, 22). In herds of Murciano-Granadina goats several animals infected with mycoplasma do not cause an increase in BTSCC, provided that basic rules of hygiene are kept in the herd, and infected animals are rapidly removed from the herd (6). From the 1068 collected goat milk bulk-tank samples, 84 (7.9%) were positive for the presence of *Mycoplasma* species (4). Most of the isolated species were *Mycoplasma agalactiae*, which were found in 69 samples of milk (82%), and in 14 samples (17%) was detected *Mycoplasma mycoides* subsp. *Mycoides* large colony type (*MmmLC*) in the herd. Higher BTSCC levels (1,176,000 cells/ml) were recorded in bulk-tank samples containing mycoplasmas in comparison to the count recorded for samples testing negative (875,000 cells/ml), and these differences were statistically significant. Bulk goat milk generally has a high SCC level; however, less than 50% of milk supplied by producers comply with the standards, i.e. 1 x 10^6 cells/ml (7). In a high percentage of milk samples neutrophils and variable bacterial counts, and a lack of correlation with SCC indicate problems with milk contamination. These problems may be caused by residual lactation and low milk production, but further research needs to be carried out on the high percentage of neutrophils reported in goat milk. In a study by the year 2010 in 53 herds of dairy goats 3 bulk milk samples were taken at 2-week intervals to assess how different bacterial groups in bulk milk are related to BTSCC, TBC and bulk milk standard plate counts (SPC) (13). It was found that the staphylococcal count was correlated to SCC (r = 0.40), TBC (r = 0.51) and SPC (r = 0.53). Coliform count was correlated only to TBC (r = 0.33); however, *Staphylococcus aureus* was not correlated to SCC. The share of the staphylococcal count in the SPC was 31%, while coliform count was only 1% of the SPC. Somatic cells in goat bulk milk showed a moderate correlation to the bacterial counts, and Koop et al. (14) hypothesized that IMI is an important factor affecting SCC in bulk milk. However, many other factors associated with the management of the herd, as well as nutritional factors and diseases, of which an important role is played by *mastitis*, affect SCC in bulk milk. In the literature there is little information showing an association between bulk tank total bacterial count (TBC) and bulk tank SCC (BTSCC) in dairy sheep (8, 10, 18). Dry therapy practice and milking the ewes in parlor systems were associated with low TBCs (8). A study on milk quality in Assaf ewes indicated that antibiotic residue (AR), SCC and TBC in bulk tank milk can be used as variables to monitor mammary health, milk hygiene, and safety in dairy ewes (10). High SCCs were associated with an increased AR occurrence. It was confirmed that the prevalence of AR in the milk of sheep is inversely proportional to milk yield and the highest degree of AR incidence was in the autumn. The milk from small ruminants, similarly as milk of other species in the EU countries cannot contain antibiotic residues (5). In raw goat and ewe bulk tank milk in Switzerland a wide distribution of subclinical infection with *Mycobacterium avium ssp. Paratuberculosis* (MAP) was found, amounting to 23% and 24%, respectively (18).

Risk factors associated with a deterioration of bulk milk quality

A basic condition for good quality milk from small ruminants, especially in case of small farms, is the observance of hygiene on the farm, mainly to maintain hygiene equipment and utensils for milking in good condition (17, 20). The IMI that cause subclinical and clinical *mastitis* has been estimated at 5 - 30% and 5%, respectively (5, 16). In problematic flocks mastitis incidence is much higher than 30 - 50% and may reach up to 70%. Many pathogens can cause *mastitis*; however, *Staphylococcus* spp. are the most commonly diagnosed microorganism of IMI in goats and sheep (5). Manual milking was associated with a 62% risk of bacterial infection of milk compared to mechanical milking, while mechanical milking with portable devices was associated with a 40% higher risk of bacterial infection compared to mechanical milking with fixed plants (16). In Switzerland for goat milk median SPC was 4.68 log cfu/ml and mean SPC 6.92 log cfu/ml, whereas for ewe milk median SPC was 4.79 log cfu/ml and mean SPC was 6.05 log cfu/ml, respectively (27). Factors associated with SPC included the month of bulk milk sampling, while among the four evaluated months (April, May, June and July) the highest median SPC (5.24 log cfu/ml) and mean SPC (7.13 log cfu/ml) were found in June. Differences in SPC results between the months of sample collection were significant. The number of milkings contained in bulk tank affected SPC (log cfu/ml). The highest median value of the SPC (5.70 log cfu/ml) and mean SPC (7.24 log cfu/ml) were found in bulk tank milk containing four milkings. According to Zweifel et al. (27) also milking technique affected SPC value, the highest median SPC (5.06 log cfu/ml) and mean SPC (6.90 log cfu/ml) were found for bucket milking without a parlor; however, the hand milking technique showed the lowest SPC results (median SPC 4.48 log cfu/ml, and mean SPC 6.87 log cfu/ml). Flock size also affected SPC value, as the highest median SPC (4.85 log cfu/ml) and mean SPC (6.86 log cfu/ml) were found in larger flocks (> 25 animals). In case of BTSCC in ewe bulk milk a significant impact was found for the following factors: flock/herd, sampling month, dry therapy practice, breed of sheep (Spanish Assaf, Awassi, Churra, Castel), type of milking (hand vs. machine, and type of installations used for machine milking (milking buckets and parlors: looped type milkline, dead-milkline ended, midlevel and low-level systems) (9).

Effects of somatic cell counts and contamination on quality products from goat milk and sheep milk

Bulk milk from ewes of three breeds, i.e. Churra, Castelana, and Assaf, with different levels of somatic cells was used to produce hard cheese (23). Chesses were produced from bulk milk of three classes: low (< 500,000 ml^{-1}), medium (1,000,000 – 1,500,000 ml^{-1}) and high (>2,500,000ml^{-1}) SCCs. A significant increase has been shown in proteolysis with an increase in SCC levels. In fresh cheeses the casein fractions (α_{s1}-1-CN and β-CNs) were significantly lower as SCC values increased. In cheeses made from milk with a high SCC a reduced fat content was recorded, and it followed an increase of pH and fat acidity. Yoghurts were made from milk of the Baluchi breed with two SCCs, i.e. <200,000 ml^{-1} and >750,000 ml^{-1} (19). Different levels of SCC are associated with changes in milk composition and properties of yoghurt; the SCC had no effect on acidity, while for pH of the yoghurt after 24 h this effect was significant after 168 h of storage. Somatic cell count had no effect on total solids and fat content of the yoghurt. A high SCC was associated with a higher protein content and the highest level of syneresis. In Norway the presence of potential-poisoning bacteria was evaluated during the process of small scale cheese production using caprine and bovine raw milk (12). The prevalence of *Staphylococcus aureus* was varied during the manufacturing process of cheese from the milk of both species. The prevalence of *Staphylococcus aureus* in caprine cheese ranged from 91.8% in milk at 0 h and 95.9% at 24 h, and reduced to 42.9% after 30 days. The prevalence of *Staphylococcus aureus* in bovine cheese was 47.2% at 0 h, peaked to 80.8% after 5 - 6 h, and amounted to 24.7% after 30 days. The highest contamination levels of *Staphylococcus aureus* were observed in both caprine and bovine cheese after 5-6 h. Somatic cell count in bulk goat milk below the admissible limit according to the current Pasteurized Milk Ordinance regulation (PMO) amounting to 1,000,000 cells/ml in grade. A goat milk, did not affect milk composition and semisoft cheese yield (3). However, the high SCC in goat milk resulted in a lower texture score as well as a lower total sensory score. Cheeses made with high SCC milk exhibited higher lipolysis during ripening compared with cheeses made from low SCC milk. The high levels of SCC in goat and ewe milk are associated with milk yield losses, changes in milk composition end affect the quality and stability of cheese (22).

Control and prevention strategies

In a study by Berthelot et al. (1) was demonstrated that in ewe milk individual SCC (iSCC) represent a useful tool for the detection of subclinical mastitis in dairy ewes. The authors propose a classification as a healthy udder (specificity = 75%), if throughout lactation iSCC are lower than 0.500 x 10^6 cells/ml, and infected (sensitivity = 82%) if at least two iSCC are higher than 1 or 1.2 million cells/ml. However, the decision should be adapted to different control strategies for SCC in bulk milk. Antibiotic dry-off therapy was found to significantly reduce IMI incidence in small ruminants (5, 9, 11, 25). This treatment significantly decreased BTSCC from log BTSCC 6.24 and the geometric mean of 1,725 x 10^3 cells/ml in the pretreatment lactation to log BTSCC of 6.15 and 1.422 x 10^3 cells/ml in the subsequent lactation (11). Some authors are of a different opinion that the dry treatment did not result in reduced SCC in the subsequent lactation (25). Ewes with a history of high SCC during the previous lactation (i.e. more than 3 monthly tests ≥ 400,000 cells/ml) were more likely to have IMI in the post parturition period. Treatment with antibiotics requires veterinary supervision to ensure proper hygiene, because some *mastitis* outbreaks originated from contamination of the syringe (5). Dry therapy is generally associated with improved milking hygiene in the subsequent lactation, thus reducing IMI and improving milk quality. This practice and milking in parlor systems were associated with low TBC, and a significant reduction of BTSCC (8, 9, 10). More pulsations (180 cycles/min) and low vacuum levels (34 to 36 kPa) are optimal for udder health during milking of ewes (9).

Conclusion

BTSCC of small ruminants depends mainly on TBC, that cause infection of mammary glands. Factors that may reduce the risk of IMI include the ability to keep milking hygiene and adherence to correct milking parameters. Cheeses made from milk with a high BTSCC had a reduced fat content and increased pH and fat acidity. In yoghurt high BTSCC was associated with a higher protein content and the highest level of syneresis. Further research is needed to determine the exact thresholds of BTSCC in the milk of sheep and goats related to dairy products.

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ŠTEVILO SOMATSKIH CELIC IN SKUPNO ŠTEVILO BAKTERIJ V ZBIRALNICAH MLEKA DROBNICE

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Povzetek: Skupno število bakterij (TBC, angl. total bacterial count) v mleku je dober pokazatelj higienskih razmer pri reji drobnice. TBC je povezan s številom somatskih celic v zbiralnicah mleka (BMSCC, angl. number of somatic cells in bulk milk), če pa upoštevamo obe spremenljivki, TBC in BMSCC, dobimo še zanesljivše rezultate. Pomemben vpliv na BMSCC v zbiralnicah ovčjega mleka imajo naslednji dejavniki: čreda, mesec vzorčenja, uporaba suhe terapije, pasma ovc in način molže. Na povprečno vrednost celic na plošči (MSPC, angl. mean standard plate count) pa vplivajo: mesec vzorčenja mleka in število molž v zbiralnici kakor tudi način molže. V svežem ovčjem siru so bile kazeinske frakcije (αs1-1-CN in beta-CNS) precej nižje, medtem ko je bila vrednost BMSCC visoka. Ti siri vsebujejo manj maščob in imajo zato višji pH in maščobno kislost. Jogurti, narejeni iz mleka z visoko vrednostjo BMSCC, imajo značilno višjo vsebnost beljakovin in visoko stopnjo sinereze. Potrebno je zagotoviti ustrezne higienske pogoje pri ravnanju z živalmi in določiti higienske standarde za molzne stroje in molzišča, da bi izboljšali zdravje vimen malih prežvekovalcev.

Ključne besede: drobnica; število somatskih celic; skupno število bakterij; zbiralnice mleka