



Influence of udder infection status on milk enzyme activities and somatic cell count throughout early lactation in goats

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ABSTRACT

At present the analysis of somatic cell count (SCC) used for the detection of intramammary infections (IMI) in bovine milk is also recommended for goat milk, but due to the various factors influencing SCC it allows only limited conclusions on the udder health of goats. The research on enzyme activity in milk appears to show promise in finding an approach with more suitable indicators of the early detection of IMI in goats. Therefore, the present study aimed to investigate the influence of goat udder infection status on different milk enzyme activities and SCC throughout early lactation. A total of 60 dairy goats were sampled at weekly intervals over a period of 6 weeks after kidding and the bacteriological status, milk SCC and the activity of N-acetyl- β -D-glucosaminidase (NAGase), β -glucuronidase and lactate dehydrogenase (LDH) of udder halves were analysed. Infections with minor or major pathogens were identified in 47% of all animals over the sampling period. Coagulase-negative staphylococci (CNS) represented the main group of pathogens in bacteria isolates (16.4%). *Corynebacteria* and major pathogens were detected in 7.2% and 5.7% of udder half samples. Excluding the first week after parturition, the study revealed a highly significant influence of lactation week on \log_{10} SCC ($F_{4,255} = 11.63$, $p < 0.001$) and \log_{10} LDH ($F_{4,285} = 5.02$, $p < 0.01$) and must be acknowledged as the most dominating predictor on NAGase activity ($F_{4,250} = 29.62$, $p < 0.001$) in early lactation. Levels in β -glucuronidase activity were not influenced by the stage of lactation. The infection status of udder halves had a highly significant effect on \log_{10} SCC ($F_{3,528} = 18.88$, $p < 0.001$), \log_{10} β -glucuronidase ($F_{3,407} = 11.02$, $p < 0.001$) and \log_{10} LDH ($F_{3,534} = 12.39$, $p < 0.001$). We revealed no effect of different pathogenic groups on NAGase activity, thus this parameter proved not to be suitable as an indicator for udder infections in early lactation. The proposed milk enzymes β -glucuronidase and LDH might be indicative of inflammatory processes, but the influence of parity and lactation stage on the overall results should be considered in the assessment of udder health in dairy goats.

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1. Introduction

As a multifactorial disease mastitis is influenced in its expression by the type of pathogen, the animal's

constitution and by environmental conditions, and can take a broad spectrum of variable courses. Most cases of intramammary infection (IMI) can be assigned to the subclinical form, which shows no outward symptoms of inflammation, such as udder swelling, increased body temperature, changes in secretion or flocculation in the milk, and is often diagnosed at a late stage representing a constant risk of infection for the whole flock. Mastitis has a negative impact in terms of changing milk composition,

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reducing milk quality and causing high economic losses. Thus, udder infections must be detected at an early stage, while the reliable diagnosis must be based on objective parameters that have not yet been established for goat milk. The current state of knowledge of the subject investigated was recently summarised in an overview study (Stuhr and Aulrich, 2010). Until now a reliable detection of IMI has only been possible with a bacteriological examination and the accompanied identification of pathogens, which incurs high costs and time delays for mastitis treatment in practice.

At present the somatic cell count (SCC) in milk has been used widely as an indicator for the detection of mastitis in cows and it is also propagated for goat milk. Recent studies discuss the practicability of indirect and direct methods in cell count measurement for the determination of IMI in dairy goats (Persson and Olofsson, 2011), but the major part of investigations found no significant relationship between a rise in SCC and the occurrence of IMI (Kyozaire et al., 2005; Moroni et al., 2005; Vihan, 1989). In dairy cows a SCC < 100,000 cells/ml classifies healthy udders (DVG, 2002), but SCC in goat milk is knowingly higher than in milk from cows (Escobar, 1999) and shows much larger fluctuations with values above 1 million cells/ml during the lactation period even without signs of an udder infection (De Crémoux et al., 1995; Haenlein, 2002; Paape et al., 2001). Despite the additional influence of physiological factors like breed (Jendretzke, 2009), stage of lactation (Paape et al., 2001), parity (Sanchez et al., 1999) and estrus (Christodouloupoulos et al., 2008; McDougall et al., 2002; Moroni et al., 2007) as well as hygiene standards (Delgado-Pertinez et al., 2003) or milking equipment (Souza et al., 2009) on SCC, efforts are made to establish SCC limit values for grading milk quality of raw goat milk (Silanikove et al., 2010). The Pasteurized Milk Ordinance (PMO) implemented cell count limits as a legal basis for the assessment of goat milk quality in the USA with 1 million cells/ml (PMO, 2003) as the threshold, whereas the EU Directives comprise no legal requirements for goat milk SCC at this time.

The research on changes in enzyme activities in goat milk, linked to the occurrence of causative agents, might lead to more suitable parameters for the early detection of IMI. Studies focused on lysosomal enzymes delivered promising results, justifying further research: N-acetyl- β -D-glucosaminidase (NAGase, EC 3.2.1.52) is released by epithelial cells and polymorphonuclear leukocytes and catalyses cell reactions which help the immune defense against pathogens. This correlation is supported by in vitro studies of Hussain et al. (1992) that showed a bacterial reduction after the addition of NAGase. Thus, it can be assumed that this enzyme is an indicator for antimicrobial effects in the course of an inflammatory process. Both Maisi and Riipinen (1988) and Vihan (1989), expect NAGase to be able to diagnose mastitis in dairy goats. Leitner et al. (2004b) examined milk samples in 10 different herds and found NAGase activity significantly affected by the bacteriological status. In the same way Barth et al. (2010) found a significant effect of infection status on NAGase activity in milk samples taken from 58 goats in mid to late lactation.

Beta-glucuronidase (EC 3.2.1.31), which belongs to the group of hydrolases and split glucuronides in glucuronic

acid and its aglycone (Dohrmann, 1969), is also attributed to the immune response of the body. Due to the alleged lack of sensitivity and the time-consuming method, β -glucuronidase activity was rarely analysed in early studies (Kitchen, 1976). Later Perdigon et al. (1986) viewed the level of β -glucuronidase as a very sensitive and very effective parameter for the detection of bovine mastitis. Oliszewski et al. (2002) investigated β -glucuronidase levels in goat milk samples, supporting the usefulness of β -glucuronidase as a marker enzyme for the diagnosis of mastitis in conjunction with a threshold value for SCC, but due to the lack of bacteriological examinations no conclusive statement can be made on udder health status. Recently Larsen and Aulrich (2012) developed an optimised fluorometric method for a more precise determination of β -glucuronidase in ruminant milk, revealing its practicability in line with other indicators for mastitis.

The clarification of the role of the nonlysosomal enzyme lactate dehydrogenase (LDH, EC 1.1.1.27) within the inflammation process of udder infections might also lead to an objective marker. LDH can be released during immune response by leucocytes and the udder's parenchyma cells (Bogin et al., 1977). A release of enzymes into milk can be observed after an infusion of staphylococcal α -haemolysin into bovine mammary glands with LDH as the earliest indicator of rising mammary permeability (Symons and Wright, 1974). Chagunda et al. (2006a) described a dynamic deterministic model validated by measurements of LDH to detect clinical mastitis in cows. Friggens et al. (2007) successfully tested this mastitis risk model for the early identification of acute mastitis cases by using continuous analysis data of SCC in comparison to levels of LDH in cow milk. The verification of this model for goat milk still has to be carried out. Katsoulos et al. (2010) identified LDH activity as the most reliable indicator among three analysed enzymes, i.e., alkaline phosphatase, aspartate aminotransferase and LDH for the detection of IMI in dairy ewes and goats. LDH activity was found to be significantly higher in cases of subclinical IMI than in bacteriologically negative udder half samples. Compared to the investigations in dairy cows, there is still very little data on LDH as a reliable marker enzyme for IMI in dairy goats.

A major goal in goat farming is to bring healthy animals into lactation; therefore, information about the udder health status is needed early. Results from investigations in the first weeks also help to isolate infected animals from the herd as early as possible and to implement separate milking in order to prevent the infection of other animals. Criteria that are suitable for a safe monitoring of udder health in goats are still lacking under farm conditions. Therefore, the aim of this study is to investigate the effects of infection status on milk enzyme activities and SCC in early lactation in order to identify indicators to support the udder health management in dairy goat farms.

2. Materials and methods

2.1. Animals

From February to March 2010, milk samples of 60 lactating goats (German Improved Fawn) between their first ($n = 14$) and eighth lactation (2nd: 11, 3rd: 12, 4th: 9, 5th: 6, 6th and greater: 8) of the dairy goat

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