

## Intramammary infections in dairy goats: recent knowledge and indicators for detection of subclinical mastitis

Tanja Stuhr\* and Karen Aulrich\*

### Abstract

Intramammary infections (IMI) in goats constitute an enormous animal health problem, alter milk composition, lower the hygienic value of milk, impair the processing properties of milk and cause high economical losses in dairy farming today. A great part of IMI in dairy goats can be assigned to the group of subclinical mastitis (SCM) with no outward clinical symptoms. Staphylococci are certainly the most common type of pathogens in goat milk, but the frequency of occurrence of this pathogenic group varies greatly. This review tries to summarise the most recent knowledge about IMI in dairy goats and possible parameters for detecting this disease with the main focus on SCM.

The gold standard for mastitis detection is the determination of bacteriological status, but analysis is time-consuming, costly and not relevant for practical use. Suggestions have been made to use parameters like somatic cell count (SCC), the California-Mastitis-Test (CMT), electrical conductivity (EC), milk composition (fat, protein, lactose), N-acetyl- $\beta$ -D-glucosaminidase (NAGase), lactoferrin (Lf),  $\beta$ -Glucuronidase and lactate dehydrogenase (LDH) alternatively. SCC, the most established parameter for the diagnosis of the udder health status in cows, seems to be no reliable parameter for the detection of SCM at the proposed static level of  $1 \cdot 10^6$  cells  $\text{ml}^{-1}$ , which has been proposed by many authors. SCC in goats shows great variations (e.g., during lactation) and often higher numbers of  $> 1 \cdot 10^6$  cells  $\text{ml}^{-1}$  without incidence of SCM were measured. SCC can increase solely due to physiological factors (like breed, parity, stage of lactation, estrus), hygienic standards and milking equipment. The impact of IMI on the parameters mentioned above, as well as the efficiency of diagnostic tools is discussed in this review.

*Keywords: goats, mastitis, detection, staphylococci*

### Zusammenfassung

#### Euterinfektionen bei Milchziegen: Stand des Wissens und Indikatoren zum Nachweis subklinischer Mastitis

Euterinfektionen, eins der vordringlichen Gesundheitsprobleme bei Milchziegen, führen zu Veränderungen der Milchezusammensetzung, zur Minderung der hygienischen Wertigkeit, zu Beeinträchtigungen der Käseerzeugung und verursachen hohe wirtschaftliche Verluste in der Milchviehhaltung. Ein großer Anteil intramammärer Infektionen (IMI) tritt in der subklinischen Form, also ohne Anzeichen klinischer Symptome, auf. Staphylokokken stellen bei Milchziegen die häufigste Erregergruppe innerhalb subklinischer Mastitiden dar, wobei die Häufigkeit des Auftretens stark variiert. Die vorliegende Studie versucht einen Überblick über Erkenntnisse zu Euterinfektionen bei Milchziegen zu geben und mögliche Parameter zur Diagnose subklinischer Mastitiden aufzuzeigen. Die bakteriologische Untersuchung stellt den Goldstandard für die Erkennung von Mastitiden dar, diese Form der Analyse ist jedoch zeitaufwändig, teuer und wenig praxisgeeignet. Die Eignung von Zellzahl (SZZ), California-Mastitis-Test, elektrischer Leitfähigkeit, Milchinhaltsstoffen (Fett, Protein, Laktose), N-Acetyl- $\beta$ -D-Glucosaminidase, Lactoferrin,  $\beta$ -Glucuronidase oder Lactat-Dehydrogenase zur Erkennung von Euterinfektionen bei Milchziegen werden in dieser Studie untersucht. SZZ, der am besten etablierte Parameter zur Einschätzung der Eutergesundheit bei Kühen, scheint wenig geeignet für den Nachweis subklinischer Mastitiden bei Milchziegen zu sein. Die SZZ zeigt bei Ziegen große Schwankungsbreiten (z. B. im Verlauf der Laktation) und oft werden Werte  $> 1 \cdot 10^6$  Zellen  $\text{ml}^{-1}$  ohne Anzeichen einer Euterinfektion gemessen. Die SZZ kann allein aufgrund physiologischer Faktoren (Rasse, Laktationsstadium, -nummer, Brunst), des Hygienestandards oder der Melktechnik ansteigen. Die Auswirkungen IMI auf die oben genannten Parameter, sowie die Eignung der untersuchten Parameter als diagnostische Methode soll in diesem Beitrag diskutiert werden.

*Schlüsselwörter: Milchziegen, Mastitis, Bestimmung, Staphylokokken*

\* Johann Heinrich von Thünen-Institut (vTI), Institute of Organic Farming, Trenthorst 32, D-23847 Westerau, Germany  
corresponding author: karen.aulrich@vti.bund.de

## 1 Introduction

Intramammary infections (IMI) cause changes in the milk composition, lowers the hygienic value of milk and impairs the suitability for cheese dairy. Obviously subclinical mastitis (SCM) is one of the most important infectious diseases in small ruminants. Furthermore, SCM represents a constant risk of infection for the whole stock. As there is a need for higher milk yields and more stringent requirements on milk quality in dairy goat herds, udder infections must be prevented or detected at an early stage not only to protect the farmer but rather the consumer. Indirect tests suitable for setting limit values indicating SCM are yet to be found.

Udder infections in goats play a similarly important role as in dairy cows. Common parameters used in practice for the diagnosis of mastitis in dairy cows are the electrical conductivity (EC) and the somatic cell count (SCC). The assessment of udder health in goats, however, seems to be more difficult. Most studies have examined the suitability of SCC and California-Mastitis-Test (CMT) for detecting SCM in goat's milk. Suggested threshold values for SCC range between  $750 \cdot 10^3$  and  $1 \cdot 10^6$  cells  $\text{ml}^{-1}$ , but lack practicability as SCC shows greater ranges depending on parity, breed, lactation stage and variety of pathogens (Hamann, 1999; Bergonier et al., 2003). Despite the unique characteristics of goat milk, limit values for the SCC already exist in some countries (Pirisi et al., 2007).

Leading authors investigating the function of lysosomal enzymes as anti-inflammatory agents proposed  $\beta$ -Glucuronidase and N-acetyl- $\beta$ -D-glucosaminidase (NA-Gase) as possible early indicators for SCM. The enzyme lactate dehydrogenase (LDH) is already in use for quality assurance of cow milk and was also investigated as a possible parameter for infection diseases in goats indicating differences in status of infection. As even light infections of the udder lead to a change in milk composition, ingredients like lactose or lactoferrin (Lf) are also mentioned in studies regarding SCM in goats. Lf has bacteriostatic properties which could indicate an udder infection at an early stage. The paper reviews the recent knowledge about IMI in goats and tried to reflect the published investigations on possible indicators of SCM in goats.

## 2 Intramammary infections in dairy goats: recent knowledge

Intramammary infections constitute an enormous animal health problem and causes high economical losses in dairy farming today. Mastitis is defined as an infectious disease of the mammary gland and leads in its clinical form to pathological changes of the udder accompanied by changes in milk composition. SCM reveals no outward

symptoms of inflammation, with the exceptions of an increased number of cells and the detection of pathogens in the milk. Investigations from Boscós et al. (1996) demonstrated that the prevalence of bacteria in examined udder halves differed between herds with percentages from 19.0 to 35.7 %. In addition, Contreras et al. (1995) found ranges from 7 % to 34 % in infected glands. Bergonier et al. (2003) reviewed the literature data until 2002 and described the prevalence of SCM in goats ranging from 20 to 50 % related to means of SCC in bulk milk. More recent studies found prevalence in the upper and lower range of these data. Moroni et al. (2005) found an average prevalence in individual samples of two goat herds of 49.8 %. Contreras et al. (2007) summarized the results from various research groups and noticed a prevalence of SCM in goats of 5 to 30 %. Other studies from Manser (1986) and Kalogridou-Vassiliadou et al. (1992) found significantly higher percentages of bacteriologically positive milk samples (up to 79.0 % and 81.4 %, respectively). Only recently, Vasiu et al. (2008) diagnosed a prevalence of 70.21 % of SCM in individual goat milk samples taken from flocks with somatic cell counts  $> 1 \cdot 10^6$  cells  $\text{ml}^{-1}$ .

IMI can be caused by several different groups of pathogens. Depending on the risk of infection udder infections are classified into contagious or environmental associated mastitis (Hogan & Smith, 1987). Besides this discrimination it can be distinguished between minor and major pathogenic bacteria (Schalm et al., 1971). While minor pathogens (coagulase negative staphylococci, *Corynebacterium* spp.) usually lead to mild forms of disease in the glandular tissue, the patterns of infections with major pathogens (*Escherichia coli*, *Staphylococcus (S) aureus*, *Streptococcus (Sc) dysgalactiae*, *Streptococcus uberis*) are much more obvious and lead to increased cell numbers (Lerondelle & Poutrel, 1984; Harmon, 1994).

Coagulase negative staphylococci (CNS) are capable of persisting in the udder and lead to subclinical cases but could also cause clinical mastitis (Deinhofer & Pernthaler, 1995; Paape et al., 2001). Thus, CNS are viewed as the most prevalent organisms indicating SCM (Contreras et al., 2007; Leitner et al., 2007) and were detected in more than 50 % of SCM cases (Vihan, 1989; Contreras et al., 2003). Bergonier et al. (2003) found in their review of several studies a prevalence of CNS-induced SCM ranging from 25 to 93 % in different flocks. The more recent bacteriological studies of goat milk show a significant increase in CNS infections. The percentage of CNS out of mastitis pathogens isolated in the years 1998 to 2003 in goats rose from 50 % to 68.8 % (Winter, 2009). Moroni et al. (2005) found a prevalence of 80.7 % of CNS in cases of SCM. The level of CNS might also change during lactation. SCM often lasts over 3 to 4 months caused by only one pathogen and is frequently followed by other infections

and normally not eliminated during lactation (Bergonier et al., 2003). Studies from Aulrich & Barth (2008), investigating one dairy goat herd, showed a decreasing level of CNS during the lactation period. Prevalence sank over time of lactation starting from 33 % in the first days and finally to 15 % after 263 days.

Important pathogens of the group of CNS are *S.caprae*, *S.chromogenes*, *S.epidermidis*, *S.haemolyticus*, *S.warneri* and *S.xylosum* (Valle et al., 1991). Staphylococcal species isolated from goat milk samples by Deinhofer & Pernthaner (1993) were *S.epidermidis*, *S.caprae*, *S.lentus*, *S.simulans*, *S.capitis*, *S.lugdunensis*, *S.xylosum*, *S.chromogenes*, *S.hominis*, *S.arlettae*, *S.warneri*, *S.sciuri* and *S.saprophyticus*. Some CNS in goat might even be attributed to the group of major pathogens. Especially *S.simulans* and *S.hycus* can cause persistent and stable infections in cows (Aarestrup et al., 1999) and are correlated with the occurrence of clinical symptoms of mastitis (Myllys, 1995). CNS can be divided into more than 30 different species and subspecies (Aarestrup et al., 1999), but it is still unclear what role CNS, either considered as non-pathogenic or of low pathogenicity, play in clinical or sub-clinical infections of the mammary gland of dairy goats.

Bergonier et al. (2003) described *S.caprae* as the most important pathogen in goats and furthermore *S.epidermidis*, *S.xylosum*, *S.chromogenes* as well as *S.simulans*. Several studies (Moroni et al., 2004; Moroni et al., 2005) have shown that *S.epidermidis* is also one of the most prevalent species. Moroni et al. (2005) found *S.epidermidis* in 48.1 % of all bacteria isolates in one herd with total absence of *S.caprae*, while *S.caprae* was the dominant isolate with 43.5 % in the other herd with total absence of *S.epidermidis*. This study shows that varying prevalence of different CNS pathogens can be found in different goat herds. An overview supporting this statement can be found in studies summarized by Raynal-Ljutovac et al. (2007).

Coagulase-positive staphylococci (CPS) are a staphylococcal species with high pathogenic potency. An important species of CPS is *S.aureus*, which is one of the most common pathogens in dairy cows and is predominantly found in the affected mammary gland. *S.aureus*, mainly transmitted via the milking equipment, is characterized by pathogen specific virulence factors that can damage the immune defense of the udder. Therefore, *S.aureus* is a highly contagious germ with poor treatment success. As *S.aureus* has the ability to produce toxins it can cause food poisoning after consumption of contaminated dairy products. It is expected that the prevalence of udder infections particularly caused by other staphylococci and *S.aureus* is 20 % to 50 % higher in goats than in cows (Maurer et al., 2004).

Although the role of *S.aureus* in clinical mastitis in the goat has clearly been identified (Maisi & Riipinen, 1991) a statement on *S.aureus* influencing SCM is difficult.

Incidence of clinical mastitis in small ruminants tends to be lower than 5 % (Bergonier et al., 2003). In the study of Moroni et al. (2005), *S.aureus* was found in 3.6 % of infected samples. Vasiiu et al. (2008) found *S.aureus* in 7.4 % of 94 individual milk samples taken at the end of lactation, which is equivalent to 10.6 % *S.aureus* findings with regard to all infected samples. One of the most recent studies analysing milk samples from seven goat herds showed a prevalence of *S.aureus* in 6.2 % of bacteriologically positive samples (Mork et al., 2010). But as with CNS, the prevalence of *S.aureus* might clearly be higher. Boscós et al. (1996) found a prevalence of *S.aureus* in 18.5 % of bacteriologically positive milk samples. According to studies of Winter (2009) for the years 1998 to 2003, *S.aureus* can be detected in between 20.8 % and 46.6 % as a pathogen of SCM. The study of Ravnal-Ljutovac et al. (2007) found *S.aureus* in from 0 % and up to 36.9 % of infection cases.

Streptococci are among the classic animal associated pathogens, the different species of streptococci differ significantly in their occurrence and pathogenicity. *Sc.agalactiae* is one of the highly contagious pathogens, leading to massive losses in dairy farming (Wolter et al., 2004). In cases of IMI, *Sc.dysgalactiae* occurs sporadically and leads only to mild forms of disease and is generally easy to cure. *Sc.uberis* and *E.coli* are pathogens associated with the environment and as such widespread. They often occur in the context of other factors that promote mastitis. Streptococcal intramammary infections can occur in both subclinical and clinical cases. Corynebacteria, in particular *Corynebacterium bovis*, are common colonizers of the teat canal flora. These animal associated agents may be mainly responsible for a large part of subclinical forms of mastitis in the goat. *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Mannheimia haemolytica*, Corynebacteria and fungi occur less frequently in cases of IMI in goats (Contreras et al., 2007).

### 3 Detection of SCM in dairy goats

The determination of bacteriological status (BS) of milk samples is regarded as a "Gold Standard" for the determination of the udder health status. The BS gives the number of aerobic microorganisms developing at a temperature of 30°C and is expressed as a number of colony forming units (CFU) per ml. According to the International Dairy Federation (Kästli, 1967) the DVG (1994) defined cell count threshold values for categorisation of udder health in cows. Values above 100 10<sup>3</sup> cfu ml<sup>-1</sup> when associated with evidence of udder pathogens are referred to as mas-

titis and result in a reduction of processing properties and lead to large losses in milk production (Hamann, 2002). In Regulation (EC) N° 853/ 2004 thresholds as geometric mean for maximum CFU are set within the EU for raw cow milk at this level of  $100 \cdot 10^3$  cfu ml<sup>-1</sup>, for raw goat milk used for the manufacture of products with heat treatment at  $1.5 \cdot 10^6$  cfu ml<sup>-1</sup> and for products without any heat treatment at  $500 \cdot 10^3$  cfu ml<sup>-1</sup>. Zangerl & Kupfner (2009) suggested a limit value for total bacterial count of  $< 100 \cdot 10^3$  cfu ml<sup>-1</sup> for a goat milk payment system in Austria. The reason for having BS as one criteria within a payment system is explained by the need for bacteriological screening since SCC was not highly correlated to the infection status (Min et al., 2007) and the other indirect methods used in evaluating the quality of cows milk are not applicable for evaluating the bacteriological quality of goat milk. Unfortunately, the analyses needed for the determination of CFU are too costly and time-consuming. Thus, methods for the indirect detection of udder health status and bacterial infection are urgently being searched for.

### 3.1 Somatic Cell Count (SCC)

The use of the somatic cell count is one of the most established methods for the diagnosis of udder health in cows (Brade, 2001; Paape et al., 2007). Unfortunately, SCC could yet not be established as a proven marker for SCM in goats. Factors like parity, stage of lactation, estrus and breed contribute to significant changes of SCC in milk of dairy goats. SCC is also affected by the nature of infection with minor or major pathogens. Furthermore, mycoplasmal infections can lead to highly increased SCC in goat milk (Corrales et al., 2004). In contrast, lentiviral infections by Caprine Arthritis Encephalitis Virus (CAE) may also lead to higher SCC, but seem to be a minor contributor to SCC in cases of persistent SCM (Bergonier et al., 2003). Thus, so far no reliable thresholds values could yet be defined for SCC in goat milk. Some authors even state that SCC can be viewed as not suitable for the monitoring of caprine mastitis (Vihan, 1989).

Depending on the individual study, goat milk has a significantly higher cell count than milk from cows (Maurer et al., 2004; Winter, 2009) and higher variability in SCC (Kloppert et al., 2000). While the health of udder quarters of cows are confirmed by SCC up to  $100 \cdot 10^3$  cells ml<sup>-1</sup> (DVG, 2002), the maximum SCC for goats range from of  $200 \cdot 10^3$  cells ml<sup>-1</sup> up to a few million cells ml<sup>-1</sup> (Raynal-Ljutovac et al., 2007).

In a summarising study conducted by Bergonier et al. (2003), SCC from annual bulk milk samples from Spain, France and Italy for the years 1995 to 2000 geometric means lay at 1.2, 1.2 and  $1.6 \cdot 10^6$  cells ml<sup>-1</sup>, respectively. In this study, arithmetic means between  $520 \cdot 10^3$  and  $1.1 \cdot 10^6$

cells ml<sup>-1</sup> and geometric means between  $223 \cdot 10^3$  and  $396 \cdot 10^3$  cells ml<sup>-1</sup> for SCC of healthy udders were mentioned.

Souza et al. (2009) examined bulk milk samples of 1,400 dairy goats resulting in an average SCC of  $779 \cdot 10^3$  cells ml<sup>-1</sup>. Jendretzke (2009) found an average of  $990 \cdot 10^3$  cells ml<sup>-1</sup> in goat milk samples (n = 863) whereat 25 % of all samples had a SCC of over  $2 \cdot 10^6$  cells ml<sup>-1</sup>. According to Wilson et al. (1995), SCC can even sometimes exceed numbers of 10 million cells ml<sup>-1</sup>.

In the EU the SCC threshold for raw cow milk was set at  $400 \cdot 10^3$  cells ml<sup>-1</sup> (EC, 2004), but so far no limit values for goat milk exist (Paape et al., 2007). Nevertheless, some national thresholds do exist for SCC in bulk milk ranging from  $750 \cdot 10^3$  to 1 million cells ml<sup>-1</sup> (Pirisi et al., 2007). In the United States SCC is not allowed to exceed 1 million cells ml<sup>-1</sup> (US/Public Health Service, 2003). This limit value was already proposed by many authors (Poutrel & Leron-delle, 1983; Kalogridou-Vassiliadou et al., 1992; Perrin et al., 1997). Contreras et al. (1996) suggested a SCC threshold of  $500 \cdot 10^3$  cells ml<sup>-1</sup> for the differentiation between subclinical mastitis and healthy udders, but the proposed value classified only 62.3 % of samples correctly linked to a possibility of 28 % to deliver positive results and to a possibility of 90 % to deliver negative results.

Vasiu et al. (2008) also recommended using a maximum level of SCC of  $5 \cdot 10^3$  cells ml<sup>-1</sup> (based on geometric mean) to differentiate between udders free of infection and udders with SCM. Below this threshold they expect to classify 75 % of healthy udders correctly. Of the remaining udders, 6 to 11 % might be infected with minor, 2 % with major and the rest with undetermined status. Nevertheless, these limit values can be exceeded without seeing signs or bacteriological proof of infection (White & Hinckley, 1999).

In a study of 155 French goat herds, the geometric means of SCC thresholds of  $750 \cdot 10^3$  cells ml<sup>-1</sup>,  $1.0 \cdot 10^6$  cells ml<sup>-1</sup> and  $1.5 \cdot 10^6$  cells ml<sup>-1</sup> corresponded to a theoretical infection rate of 30 % ( $\pm 12$  %), 39 % ( $\pm 8$  %) and 51 % ( $\pm 8$  %), respectively (de Cremoux, 2000; 2001). De Cremoux (2003) estimates that using a geometric annual mean between 1.0 to  $1.2 \cdot 10^6$  cells ml<sup>-1</sup> 52 % ( $\pm 6.3$  %) of goats should be non-infected, 34.5 % ( $\pm 6.6$  %) are possibly infected with minor and 8.1 % ( $\pm 1.9$  %) possibly with major pathogens.

Vasiu et al. (2008) found an average SCC of  $1.345 \cdot 10^6$  cells ml<sup>-1</sup> (geometric mean) and  $1.403 \cdot 10^6$  cells ml<sup>-1</sup> (arithmetic mean) from 642 bulk goat milk samples. Within this study 5.6 % were identified with  $> 2.0 \cdot 10^6$  cells ml<sup>-1</sup>, 17 % between 1.5 and  $2.0 \cdot 10^6$  cells ml<sup>-1</sup>, 32.4 % between 1.0 and  $1.5 \cdot 10^6$  cells ml<sup>-1</sup>, 28.6 % between 0.5 and  $1.0 \cdot 10^6$  cells ml<sup>-1</sup> and the remaining 16.4 % with SCC  $< 500 \cdot 10^3$  cells ml<sup>-1</sup>. Later bacteriological examinations were carried out from samples of late lactation with SCC  $> 1.0 \cdot 10^6$

cells ml<sup>-1</sup> (n = 94) from which only 29.76 % were classified free of germs after bacteriological examination. From the samples with > 2.0 10<sup>6</sup> cells ml<sup>-1</sup>, they expect to classify more than 50 % correctly with IMI connected to minor and 15 % connected to major pathogens.

Thus, a universal definition of a cell number threshold to distinguish between healthy and infected udder halves does not exist yet. Both physiological and pathological factors influence SCC in goat milk. However, as for other parameters, the comparison of the two udder halves leads to more reliable results (Poutrel & Lerondelle, 1983).

Thus, SCC of udder halves in the same animal has to be compared with reference to parity and stage of lactation in order to be able to define standards for SCC in goat milk (Haenlein, 2002). Using a linear regression model recognizing lactation stage the study proposed threshold values between 556 10<sup>3</sup> cells ml<sup>-1</sup> and 1.2 10<sup>6</sup> cells ml<sup>-1</sup>, according to 90 and 305 days in milk (DIM), to distinguish between infected and non-infected milk.

In a study from Aulrich & Barth (2008) no statistical correlation could be found between parity and the level of SCC. These results contradict the findings of Paape et al. (2007), but correlate with the results of Zeng & Escobar (1995) which also stated that parity does not affect the SCC. Jendretzke (2009) found the highest average SCC in goat milk samples from animals during first lactation, in tendency SCC decreased until the age of six and then stayed more or less constant, also depending on the stage of lactation (early vs. late).

SCC is generally influenced by the stage of lactation. It is well known that SCC tends to increase during and towards the end of lactation period, without necessarily seeing an increased incidence of IMI during this period (Wilson et al., 1995; Zeng & Escobar, 1995; Luengo et al., 2004; Min et al., 2005; Moroni et al., 2005; Paape et al., 2007; Aulrich & Barth, 2008). At the end of lactation SCC can increase to such an extent that it can not be distinguished between infected and non-infected udders. e.g., Moroni et al. (2005) could not find significant differences in SCC between healthy and infected animals at the end of the lactation period.

Increasing levels of SCC may be linked to a decreasing amount of milk produced by goats during late lactation, leading to a higher concentration of pathogens in the milk (Zeng & Escobar, 1995).

The SCC increases every year in autumn by an estrus synchronization (McDougall & Voermans, 2002). Significantly higher values of SCC during the first days of estrus and lower values of SCC during the following two days were found compared to the control group. Aulrich & Barth (2008) also investigated the relationship between estrus and daily variations of SCC in goats. They observed the highest variation of SCC in October during the breed-

ing period. SCC increased significantly during estrus starting from 800 10<sup>3</sup> to over 1 Million cells ml<sup>-1</sup> (Christodoulou et al., 2008).

According to literature, the status of infection represents the most important factor influencing milk SCC (DVG, 2002; Luengo et al., 2004). In contrast Kyozaire et al. (2005) investigated 270 udder samples of goat milk from 3 different dairy goat farms regarding SCC and infection status and no significant correlation was found.

Recently published data (Aulrich & Barth, 2008) shows also, that infected udder halves had significantly higher SCC than non-infected udder halves. SCC levels in CNS induced IMI increased to > 10<sup>6</sup> cells ml<sup>-1</sup> (Leitner et al., 2004a). CNS infections with *S.epidermidis* seem to show the highest values of the SCC (Deinhofer & Pernthaner, 1995; Contreras et al., 1996). In contrast to these findings, Moroni et al. (2005) observed that SCC of infected udder halves was greater with *S.caprae* than with *S.epidermidis* and other CNS. In the study from Aulrich & Barth (2008) infections with *S.epidermidis* and *S.xylopus* showed a tendency to increase the SCC in infected udder halves when compared to non-infected halves. Nevertheless, it was also found that there was no independent reaction of udder halves of one animal. If one udder half was infected with CNS, the other udder half tended to show higher SCC. Other authors showed that the differences between infected and non-infected udder halves are not significant as non-infected udder halves can also have high cell counts (Schaeren & Maurer, 2006). SCC in goat milk samples also differs significantly before, during and after milking and hand milking might cause higher SCC than machine milking (Haenlein, 2002). Souza et al. (2009) found an average of 848 10<sup>3</sup> cells ml<sup>-1</sup> in machine and 1.1 10<sup>6</sup> cells ml<sup>-1</sup> in hand milked samples of goat bulk milk. These findings, but also all the other factors mentioned above that might influence SCC in goat milk, must be taken into consideration when it comes to setting SCC criteria for assessing the quality of goat milk. Leitner et al. (2008) made a proposal to differentiate between high-, medium- and low quality milk from goat herds with reference to bulk milk samples considering SCC and infection rate: high-quality milk SCC < 800 10<sup>3</sup> cells ml<sup>-1</sup>, associated with infection of about 25 %, medium quality milk < 1.5 10<sup>6</sup> cells ml<sup>-1</sup>, associated with infection rate between 25 and 50 %, and low-quality milk > 1.5 10<sup>6</sup> cells ml<sup>-1</sup>, associated with infection rate above 50 %, respectively. Goat milk with SCC values of > 3.5 10<sup>6</sup> cells ml<sup>-1</sup> should not be accepted for human consumption. The usefulness of this proposal should be verified under the different production conditions existing in the several goat milk producing countries.

### 3.2 California Mastitis Test (CMT)

The California Mastitis Test (CMT) is based on a reagent destroying the membranes of the somatic cells in milk and binding to the cellular DNA. This process results in an increase of the milk viscosity depending on the amount of cells. Thus, CMT allows to roughly estimate the number of cells of the immune system and epithelial cells in a given milk sample (Schaeren & Maurer, 2006). CMT is influenced by factors causing variations in SCC as well. Therefore CMT levels correlate well with SCC levels found in caprine milk (Pettersen, 1981; Poutrel & Lerondelle, 1983; Maurer & Schaeren, 2007), but the comparison between studies is hindered by the different scores used by the various authors. Furthermore, classification in CMT tests is solely based on individual assessment of visual changes of the sample. CMT can be classified from 1 to 5 according to Klastrup and Schmidt-Madsen (1974). Recently the scores range on a scale of 0 (negative), 1, 2 or 3 (Höhn, 2006), but usually authors use a scale of 0 (negative), trace, 1, 2 or 3 (Upadhyaya & Rao, 1993; Boscos et al., 1996; Escobar, 1999; Schaeren & Maurer, 2006; Maurer & Schaeren, 2007; McDougall et al., 2010). Some authors decided to combine 0 and trace to one CMT score for a more informative value (Poutrel & Lerondelle, 1983; Contreras et al., 1996).

The relationship between SCC, CMT and infection status was reviewed within many studies (Kalogridou-Vassiliadou et al., 1991; Contreras et al., 1996; Schaeren & Maurer, 2006; Jendretzke, 2009). It was proposed that low levels of CMT indicate an absence of mammary gland infection (Maisi, 1990b). Within this study, using scores from 1 to 5, CMT scores of udder half samples lay at 1 or 2 throughout the lactation with the exception of the colostral period. Infected halves gave higher scores ranging from 3 to 5. Maisi (1990b) suggested the use of a threshold score of 4 for CMT as an indication of infection, but scores of 3 may already give a hint of an infection. In a summarising study, Haenlein (2002) concluded that CMT might be able to identify infected udder halves. By Contreras et al. (1996) specificity lay at 79 % and sensitivity at moderate 47 %. Thus, a CMT score of 0 + 1 together as threshold value detects non-infected glands the best (specificity of 73 %). This correlates with results from studies in France, where CMT scores of 0 + 1 and 2 + 3 were combined (Perrin et al., 1997). McDougall et al. (2010) concluded from the distribution of CMT scores in 211 goat milk samples that a cut point greater than trace would result in a specificity and sensitivity of 74 % each.

The comparison between udder halves of one animal showed one point higher scores of CMT for subclinical infected halves when compared with the adjoining uninfected half. Using this method Maisi (1990b) showed that

the CMT predicted only 18 % of the present IMI wrongly as not infected.

Kalogridou-Vassiliadou et al. (1992) found a significant correlation between CMT and SCC in three goat herds. In this case the groups differed in level of infection with minor or major pathogens. In 81 % of infected cases with major pathogens, a CMT of 2 or 3 was found, in contrast to 65 % in udders infected with minor pathogens.

Boscos et al. (1996) found *S.aureus* in 89 % of all samples with CMT scores higher than 2, therefore CMT is a suitable parameter indicating the presence of major pathogens. Maurer et al. (2004) found that over 20 % of the infected udder halves with CNS were assessed as negative by CMT. Earlier Upadhyaya & Rao (1993) already concluded that CMT can not be used as direct proof of CNS in SCM without accompanying analytical tests on leucocytes or lactose content. Their samples were classified in those of non-mastitic and mastitic goats, the latter were classified according to a CMT score of  $\geq 1$  and at least two violations of the three threshold values: leukocyte count ( $> 0.63$  millions/ml), lactose ( $\leq 4.33$  %) and chloride contents ( $> 0.109$  %). Of the mastitis samples 78.8 % were positive for bacteria comprising *S.aureus* (26.5 %), *E.coli* (14.6 %), *Streptococcus* sp. (9.8 %) and occasionally other pathogens.

A study conducted by Boscos et al. (1996) showed fluctuations in CMT scores connected to breed, parity and stage of lactation. CMT scores increased, for example, with DIM, which was also observed for SCC. Due to the mentioned physiological factors, CMT scores can be correlated to a higher presence of epithelial particles (Schaeren & Maurer, 2006), than to infection. Thus, absolute values of CMT of goat milk sample do not seem to be a sufficient method for predicting CNS and hence SCM in goats. Overall, the presented data confirm that the presence of bacteria in milk increases CMT scores while the level of increase depends on the individual type of microorganism isolated. Oppositely, high levels of CMT do not necessarily indicate an infection (Avila et al., 1982). Nevertheless, CMT can be used for classification of samples in order to avoid unnecessary bacteriological examinations and might be suitable for the detection of major pathogens in cases of IMI.

### 3.3 Electrical Conductivity (EC)

Due to a change of the permeability of the udder tissue, the concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  in milk increase during an intramammary infection not only in cows (Krömker, 2007) but also in goats. This change is detectable by measurement of the electrical conductivity (EC). Although EC measurement is well established to monitor the udder health in dairy cows, it is not very common to use this indicator in small ruminants (Barth, 2009). Besides the lack-

ing knowledge of the factors influencing the EC in dairy goats, the wide range of EC readings might be another reason that this parameter is usually not used. Schüppel & Schwöpe (1999) found a mean EC of  $6.6 \pm 0.5 \text{ mS cm}^{-1}$ , which was confirmed by Boulaaba (2009), who measured  $6.33 \pm 0.76 \text{ mS cm}^{-1}$ . However, a reliable absolute threshold to differentiate between infected and non-infected udder glands was not found, yet (Barth, 2009; Tangorra et al., 2010). Significant correlation between EC and SCC as known as in dairy cows seems not to exist in dairy goats (Park, 1991). However, Tangorra et al. (2010) measured EC during milking of 8 Saanen goats over a whole lactation, and they revealed a relationship between the 20 highest EC readings during milking in early and mid lactation and the infection status of the udder halves. Nevertheless, the potential of EC to monitor mastitis in goats seems to be weak.

### 3.4 Milk composition (fat, protein, lactose)

The milk as the mammary gland secretion contains organic and inorganic ingredients. Milk fat, milk protein and lactose are the quantitatively predominant organic ingredients. Furthermore, various trace elements, vitamins and low molecular weight organic compounds can be found in the milk. In general, milk composition of goat's milk is similar to cow's milk, although the milk of goats showed lower levels of fat, protein and lactose, when compared to cow's milk (Maurer et al., 2004). The composition of the milk is determined by the regulation of the blood-udder barrier between the blood and the parenchyma. The integrity of the blood-udder barrier largely influences the health status of the udder. Mastitis results in a damage of the blood-udder barrier by the action of microbial toxins and metabolic products (Wagner et al., 2009). The milk fat concentration and its composition will be determined by the type and quantity of feed, breed, the stage of lactation, the lactation number, seasonal changes and other factors. In a study conducted by Souza et al. (2009), all milk components in 913 bulk goat milk samples from seven herds were significantly affected by season and herd. The mean content of fat within this study was 3.44 % with minimum values of 3.15 % and maximum values of 3.87 %. Leitner et al. (2004a) analysed the fat content in 25 crossbreed goats and found no differences between infected halves ( $3.88 \% \pm 0.12$ ) and uninfected halves ( $3.89 \% \pm 0.11$ ). In samples from 10 different herds (50 goats each) Leitner et al. (2004b) again found no significant influence regarding fat content in infected (3.75 %) and uninfected halves (4.2 %). These results were confirmed by investigations with 35 mixed-age Alpine goats (Min et al., 2007). No significant interaction between infection status and milk fat was found. Experi-

ments from Ying et al. (2002) showed significant, positive correlations of infection status, expressed as logarithm of standard plate count (SPC), with fat content in early lactation, but not in late lactation milk samples.

The milk protein is primarily composed of caseins and milk serum proteins. In addition there are various enzymes and proteins, such as lactoferrin. Protein represents a relatively autonomous parameter that is essentially affected mainly by the feeding. For goats the physiological range of reference for the total milk protein is between 2.9 and 5.0 % (Haenlein, 2006). Souza et al. (2009) found an average of 2.95 % with ranges from 2.85 % to 3.00 %. Leitner et al. (2004a) found higher protein concentrations in infected than in uninfected glands ( $3.50 \pm 0.05 \%$  vs.  $3.42 \pm 0.05 \%$ ), but without significance. Another investigation from Leitner et al. (2004b) shows significant higher protein concentrations in infected than in uninfected udders (3.99 vs. 3.91 %). Min et al. (2007) could not measure a significant effect of IMI on protein concentration. Investigations from Ying et al. (2002) showed different results regarding the influence of bacteriological status on protein content: in early lactation they found a significant correlation of standard plate count with protein content, in late lactation this correlation was not found.

The milk sugar, lactose, represents the most osmotically active component in milk (Rook, 1979). The healthy mammary gland is impermeable for lactose. Lactose is synthesized from glucose and galactose in the gland cells of the udder. During infections these functions are partly disabled leading to a lower percentage of lactose in the milk. The content of lactose in goat milk depends on many factors such as breed, feeding, lactation number and age, and can range on average from 3.8 to 4.6 % in goat milk (Jendretzke, 2009). The influence of breed could be seen from data published by Jenness (1980) with lactose concentration ranging from 4.01 % in Improved Fawn up to 6.30 % in African Dwarf goats. Souza et al. (2009) analysed 913 bulk milk samples from about 1400 European breed goats (Saanen, Toggenburg and Alpine) and found an average lactose content of 4.45 % with values ranging from 4.31 to 4.56 %. Upadhyaya & Rao (1993) analysed leukocyte counts, lactose and chloride contents in comparison to CMT of 204 milk samples collected 4 to 8 weeks postpartum (indigenous Ganjam breed in 2<sup>nd</sup> to 4<sup>th</sup> lactation). A total of 144 samples were analysed, the mean lactose values ranged from  $3.98 \pm 0.086 \%$  (CMT 3+) to  $4.73 \pm 0.35 \%$  (CMT 0) and showed a significant negative correlation with CMT scores ( $r = -0.72$ ). The threshold value for predicting mastitis was set at  $\leq 4.33 \%$  by Upadhyaya & Rao (1993), allowing the best prediction of negative samples among the analysed parameters.

Leitner et al. (2004a) found significantly higher levels of lactose in bacteriological negative ( $4.70 \pm 0.10 \%$ )

than in bacteriologically positive goat milk samples ( $4.17 \pm 0.13$  %). These results were found in weekly samples taken 40 to 120 days after parturition from 25 cross-breed goats over three weeks. The classification in negative and positive samples was linked to findings of CNS. In another study by Leitner et al. (2004b), again significantly higher lactose concentrations were found in uninfected than in infected udders (4.96 vs. 4.72 %).

Ying et al. (2002) observed negative correlations between logarithms of SPC and lactose content in early lactation, but not in late lactation milk samples. Experiments from Min et al. (2007) confirmed these findings: they found tendentially lower lactose concentrations in milk from infected halves in early and mid lactation. In late lactation the lactose concentration decreased independent of the infection status.

Bernacka (2006) investigated SCC and fat, protein and lactose among 30 goats (colour improved breed in 2<sup>nd</sup> and 3<sup>rd</sup> lactation) over a period of 288 days. The average monthly lactose concentration ranged from  $4.23 \pm 0.19$  % to  $4.7 \pm 0.23$  % with an overall average of  $4.43 \pm 0.30$  %. Again, higher levels of SCC were followed by proportionally lower content of lactose. SCC values of all samples were divided in four groups A to D (A:  $< 200 \cdot 10^3$  cells ml<sup>-1</sup>, B:  $202 \cdot 10^3$  to  $600 \cdot 10^3$  cells ml<sup>-1</sup>, C:  $601 \cdot 10^3$  to  $1 \cdot 10^6$  cells ml<sup>-1</sup>; D:  $> 1 \cdot 10^6$  cells ml<sup>-1</sup>) resulting in corresponding concentrations of lactose of 4.51 %, 4.40 %, 4.42 % and 4.36 %, respectively. Overall the levels of lactose from group A differed significantly from the levels of lactose from group D.

Jendretzke (2009) compared CMT scores with lactose concentration in goat milk from early ( $n = 485$ ) and late lactation ( $n = 186$ ) and found a significant average decrease of 0.82 % of lactose in milk from late lactation (average of 4.26 %) compared to early lactation milk (average of 5.08 %) with an overall average of 4.65 % among different breeds. Furthermore, a slight decrease in lactose was only observed in milk with CMT scores of 3+, which represented only a small fraction of samples ( $n = 3$ ). The lactose content with regard to breed and age was also investigated. The average lactose content varied among 6 different breeds in a maximum range of 0.4 % and varied from 5.02 % to 5.43 % in early lactation and from 4.20 to 4.46 % in late lactation among the different breeds. Only slight differences of a maximum of 0.2 % were found in 5 different groups referring to age. These results indicate that lactose is not a suitable parameter for detecting SCM.

Estrus seems to have no significant influence on goat milk's lactose content (Moroni et al., 2007, Christodoulou-poulos et al., 2008).

Overall, the presented data showed that none of the milk composition parameters could be a suitable indicator of IML in dairy goats.

### 3.5 *N-acetyl-Beta-D-Glucosaminidase*

Both in clinical and subclinical mastitis the increase of polymorphonuclear leukocytes (PMN) and macrophages indicates the body defense against the inflammatory process (Perdigon et al., 1986). Within this process hydrolytic enzymes that are either lysosomal such as N-acetyl- $\beta$ -D-glucosaminidase (NAGase) and  $\beta$ -glucuronidase or nonlysosomal (lactate dehydrogenase) are released in order to degrade damaged tissue.

Increased activities of NAGase in dairy cows are related to the immune defense during udder infections (Chagunda et al., 2006). NAGase activity would be the best way to diagnose bovine mastitis (Kitchen et al., 1981). Investigations from Maisi & Riipinen (1988) and Vihan (1989) view NAGase as a suitable parameter for the diagnosis of SCM in dairy goats although the level of NAGase activity in goat milk is nearly 4-fold lower as compared to bovine milk (Sharma et al., 2009).

Timms & Schultz (1985) determined NAGase activity with a marginal sampling (9 goats for 12 days over 3 weeks) however the study showed significant results regarding NAGase and SCC. Over 12 days of examination 3 of 18 udder halves were infected with CNS. NAGase activity increased significantly due to minor pathogen infections with CNS. The geometric means of NAGase activities for non-infected and infected halves were 1.51 and 2.58 nmol min<sup>-1</sup> ml<sup>-1</sup>, respectively.

Vihan (1989) examined two goat herds and found significant differences in NAGase activity in both flocks between infected and non-infected halves regardless of the type of infection. SCC and the NAGase activity were significantly higher with CNS and *Mycoplasma agalactiae* compared to uninfected goats. The author supposed a correlation of the higher NAGase levels within CNS infected halves with increased secretion of epithelial cytoplasmic particles. Vihan (1989) concluded from his own studies and the literature that NAGase activity seems to be a sensitive method for detecting SCM in goats.

Maisi (1990a) analysed milk samples from 39 goats over a whole lactation and found that infected udder halves showed higher values for NAGase along with higher CMT values as compared to healthy udder halves during the whole period of lactation, with the exception of the colostrum period. In healthy udder halves average values for NAGase of  $0.9 \pm 1.5$  units were measured in contrast to infected halves with  $10.3 \pm 6.3$  units. Additionally the author (Maisi, 1990b) observed higher NAGase values not only in the infected udder halves but also in the adjoining non-infected halves. Investigations with 22 healthy goats showed a mean value for NAGase at  $0.93 \pm 20.0$  units for days 8 to 330 and confirm the results mentioned above. NAGase activity increased during the first week of

lactation and in late lactation after 270 days. Both studies (Maisi, 1990a; 1990b) showed the dependency of NAGase values from the stage of lactation and the lactation number. The analysis of the NAGase levels of the different age groups showed significant differences between the different age groups. The lowest levels of NAGase were found in goats having their first lactation. Nevertheless, NAGase tests gave a total of 31 % false negatives or false positives results regarding IMI (Maisi, 1990a).

Leitner et al. (2004b) examined NAGase in 10 different herds (50 goats each) and found a significant influence of the bacteriological status on the NAGase activity. Correlation between NAGase activity and SCC was low in this study, NAGase activity of uninfected udders with a mean of  $15.6 \pm 0.8$  units differed significantly from udders with infection that showed mean activities of  $59.2 \pm 5.3$  units. Within this study the flock also had significant influence on NAGase activity. Although correlation between NAGase and DIM as well as NAGase and parity was not significant, Leitner et al. (2004b) suggested using levels of NAGase (and SCC) not shortly after kidding and only up to 130 days after parturition in order to be able to detect SCM, which would avoid the effects from these factors.

A recent study by Barth et al. (2010) found a significant effect of infection status on NAGase activity in udder half milk samples taken from 58 goats in mid to late lactation (175 to 211 DIM) over three consecutive weeks. High correlation was found between SCC, Lf and NAGase. The study also stated a slight, but not significant difference of NAGase activity of complete healthy udders (both halves uninfected) and uninfected halves with adjoining infected halves. To evaluate this dependency, the authors suggest further investigations.

### 3.6 *Beta-Glucuronidase*

Beta-glucuronidase as a lysosomal enzyme released during the inflammatory process represents the most significant selectivity enzyme in these process (Schnyder & Baggiolini, 1978).

Despite these findings only few studies exist that prove the applicability of this parameter for the early detection of SCM in dairies. In early investigations with dairy cows beta-glucuronidase was not used because of the non sensitive and very time consuming method (Kitchen, 1976). Afterwards Perdigon et al. (1986) described the reaction of beta-glucuronidase as very sensitive as well as a very effective diagnostic tool for detection of bovine SCM. Within the study, 220 randomly taken samples of cow milk were examined regarding SCC and beta-glucuronidase along with microscopic evaluation on potentially pathogenic bacteria (detection of *S.agalactiae*, *E.coli*, *Klebsiella*, *P.aeruginosa*, *S.aureus*, *S.epidermidis* and *S.saprophyticus*). Positive cor-

relations between beta-glucuronidase, high SCC and the presence of pathogens were found. A total of 17 % of milk samples were considered normal milk with  $SCC < 500 \cdot 10^3$  cells  $ml^{-1}$  and showed all negative results on bacteriological cultures and in beta-glucuronidase testing. Out of the remaining 83 % of samples with  $> 500,000$  cells  $ml^{-1}$ , 76 % gave positive results regarding beta-glucuronidase and 74 % showed potentially pathogenic bacteria (with predominant pathogen *Sc. agalactiae* 43 %). The samples with positive enzyme reaction predominantly contained macrophages and PMN. In 6 % of samples with  $SCC > 500 \cdot 10^3$  cells  $ml^{-1}$  pathogen bacteria were detected, but no positive reaction to beta-glucuronidase was observed. Those samples contained mainly lymphocytes. The results permit the supposition that a significant release of beta-glucuronidase is only measurable if the process of phagocytosis is fully activated. This assumption was confirmed by studies from Nagahata et al. (1987) who monitored changes in beta-glucuronidase activities during the progress of clinical mastitis in cows. In contrast to normal milk secretion, elevated beta-glucuronidase activity has been found in mastitic milk.

It should be taken into account that the data cited above were from bovine studies, investigations in goats are rare.

Oliszewski et al. (2002) verified the beta-glucuronidase test in comparison with the SCC method and the CMT in goats but no bacteriological examination was included. Mixed samples (124) of udder halves were analysed. 79 of 124 samples showed a SCC lower than  $1.3 \cdot 10^3$  cells  $ml^{-1}$ , and 93 % of these samples showed low beta-glucuronidase activity ( $< 15$  U  $ml^{-1}$ ). Goat samples with higher SCC ( $> 1.3 \cdot 10^3$  cells  $ml^{-1}$ ) showed in 88 % low values in beta-glucuronidase activity ( $< 15$  U  $ml^{-1}$ ). CMT has shown similar results. The study confirms only the reliability of beta-glucuronidase as a tool to support SCC or CMT results. Conclusions to the udder health status could not be made from the published data because of the failure of pathogen detection. The applicability of beta-glucuronidase as indicator of SCM in dairy goats should be evaluated in future investigations.

### 3.7 *Lactate Dehydrogenase (LDH)*

LDH is released as an immune response and during changes in the cell membrane (Chagunda et al., 2006). After injury of the blood-milk barrier by a massive infusion of mastitis pathogens, a passage of LDH from the blood into the milk might take place, which can be measured and used for the diagnosis of SCM in dairy cows (Hiss et al., 2007). Injury of udder tissue from mastitis changes the enzyme activity in the mammary secretions (Grün et al., 1992).

Ying et al. (2002) examined individual animals and bulk milk samples over short periods and concluded that LDH is not a suitable parameter for the assessment of udder

health status in dairy goats. No research was carried out regarding infection status, lactation number and lactation stage during these studies. According to Batavani et al. (2003; 2007) LDH is probably also liberated from udder cells and from disintegrated leucocytes in sheep and cows, resulting in higher LDH activities in the milk of subclinical infected udders compared to milk from normal udders. Goat's milk was not included in his investigations.

Khodke et al. (2009) conducted a study on SCC, pH, LDH and alanine aminotransferase (ALT) in goat milk of 123 goats in dependence on different status of udder health (differentiation by udder examination and CMT reaction into normal, subclinical (grade 1<sup>+</sup>, 2<sup>+</sup>, 3<sup>+</sup>, 12 udder half samples in each group) and into clinical groups but irrespective of age, breed, stage and number of lactation. The mean LDH found in the different groups rose from  $263.97 \pm 13.74$  U ml<sup>-1</sup> in normal milk to  $1724.02 \pm 59.91$  U ml<sup>-1</sup> in the clinical milk and thus showed a significant increase. The subclinical samples gave LDH activities of  $389.86 \pm 15.24$  U ml<sup>-1</sup> (grade 1<sup>+</sup>),  $601.79 \pm 23.93$  U ml<sup>-1</sup> (grade 2<sup>+</sup>) and  $846.53 \pm 34.70$  U ml<sup>-1</sup> (grade 3<sup>+</sup>). The accompanying SCC values increased accordingly from normal over grade 1<sup>+</sup>, 2<sup>+</sup>, 3<sup>+</sup> to clinical milk:  $6.84 \pm 0.9$  10<sup>5</sup> cells ml<sup>-1</sup>,  $16.45 \pm 0.5$  10<sup>5</sup> cells ml<sup>-1</sup>,  $26.34 \pm 1.2$  10<sup>5</sup> cells ml<sup>-1</sup>,  $41.8 \pm 1.7$  10<sup>5</sup> cells ml<sup>-1</sup> and  $48.68 \pm 1.5$  10<sup>5</sup> cells ml<sup>-1</sup>.

Katsoulos et al. (2010) investigated 162 samples of goat milk from equal udder halves (only one half of each udder) divided in a no-infection (total of 108 samples) and a subclinical infection group (differentiation by bacteriological and udder examination resulting in 97 samples) regarding the enzymes LDH, alkaline phosphatase (ALP) and aspartate aminotransferase (AST). LDH activity was found to be significantly higher in the subclinical infection group ( $354.07 \pm 13.33$  U/l) than in the no-infection group ( $103.79 \pm 3.75$  U/l). According to Katsoulos et al. (2010) LDH activity was identified as the most reliable indicator for SCM among the analysed enzymes LDH, ALP and AST leading to diagnostic sensitivities and specificities of over 92 %.

Several studies have been carried out regarding LDH and the infection status of cow udders (Chagunda et al., 2006). Compared to the investigations in dairy cows there is as yet very little data on LDH as a marker enzyme in IMI for dairy goats. At this stage no conclusive statement about LDH as reliable marker enzyme of SCM can be made.

### 3.8 Lactoferrin (Lf)

Lactoferrin is synthesized in the mammary glands as part of the non-specific immune response and has antibacterial properties (Nagasako et al., 1993; Conneely, 2001). Oram & Reiter (1968) as well as Masson & Heremans (1971) already examined Lf in milk from different species such as goat's milk and showed its antibacterial traits in inhibiting

the growth of bacteria. Lactoferrin acts both as an antibacterial and anti-inflammatory agent in the mammary glands and prevents binding of lipopolysaccharide endotoxin to inflammatory cells, but also inhibits inflammatory cytokine production through interaction with epithelial cells at local sites of inflammation (Conneely, 2001). Lf can also regulate the iron absorption and promote the growth of lymphocytes, which might lead to an alteration in milk Lf levels postpartum and during physiological changes at the end of lactation (Hiss et al., 2008).

Recent studies from Chen et al. (2004) investigated the relationship between the variation of Lf in bulk milk from 70 goats and IMI. The quality of milk was measured by the reaction time in the methylene blue reduction test (MBRT), a common test to set the milk price in Taiwan and indirect expression of the bacteriological quality of milk, and then categorized. The mean values of Lf corresponding to the reaction time of MBRT of more than 8 hours (high quality milk), 5 to 8 hours (normal quality milk) or below 5 hours (not acceptable for sale) were 167, 218 or 304 mg ml<sup>-1</sup>, respectively. Moreover, the mean Lf concentration found in mastitic milk originating from 10 infected goats (SCM persisted more than 20 days) was at 587 µg ml<sup>-1</sup> (Chen et al., 2004). In addition, Chen et al. (2004) inoculated one udder half of three goats with *S.aureus* causing a significant increase of the Lf content in the milk from the inoculated halves. Thus, the Lf in clinical and subclinical mastitic milk was observed to be significantly higher than that in normal milk, suggesting that Lf may be a good indicator for SCM in goats.

Hiss et al. (2008) analysed Lf in milk from 19 goats over an entire lactation period in order to evaluate the correlation between Lf and lactation stage, lactation number and SCC, unfortunately the bacteriological status was not included in the study. They found significantly higher levels of Lf in goats with SCC medians higher than 430 10<sup>3</sup> cells ml<sup>-1</sup>. In addition, sampling week and parity significantly affected the Lf concentration. Lf content only increased during the colostrum phase and at the end of lactation. Colostrum milk samples showed Lf values of  $387 \pm 69$  µg ml<sup>-1</sup> (SCC  $1.1$  10<sup>6</sup> cells ml<sup>-1</sup>  $\pm$   $643$  10<sup>3</sup> cells ml<sup>-1</sup>), in the 1st week  $62 \pm 25$  µg ml<sup>-1</sup> and then between 10 to 28 µg ml<sup>-1</sup> until week 32 (5th week: SCC  $487$  10<sup>3</sup> cells ml<sup>-1</sup>  $\pm$   $111$  10<sup>3</sup> cells ml<sup>-1</sup>). Whereas the SCC already increased during week 31 ( $1.0$  10<sup>6</sup> cells ml<sup>-1</sup>  $\pm$   $187$  10<sup>3</sup> cells ml<sup>-1</sup>), the Lf began to increase close to the end of lactation during week 33 to a maximum value of  $107 \pm 19$  µg ml<sup>-1</sup> in week 44 (SCC  $4.3$  10<sup>6</sup> cells ml<sup>-1</sup>  $\pm$   $954$  10<sup>3</sup> cells ml<sup>-1</sup> in week 43). Sampling week and lactation number significantly influenced the Lf concentration. Because of the failure of bacteriological examinations no statements on the usefulness of the parameter Lf for diagnosis of SCM could be made from that study.

Barth et al. (2010) analysed the influence of an intramammary infection on the SCC, the content of Lf and the activity of NAGase by examining foremilk samples of 58 dairy goats (German Improved Fawn). Parameters were determined weekly over a period of three weeks including the accompanying detection of infection status. Clear correlation between SCC, NAGase and Lf was found. Lf values of non-infected udder halves were significantly lower than those of infected halves. Lf showed the best differentiation between infected and non-infected halves even of the same udder when compared to SCC or NAGase. The investigations show that primiparous goats had significantly lower Lf contents in milk than multiparous goats and furthermore Lf was influenced by the day of sampling (Barth et al., 2010).

Undecided whether SCC or Lf is the better indicator for IMI, both indicators increase during late lactation (Hiss et al., 2008). As a consequence it is necessary to involve influencing factors like sampling day, stage of lactation and lactation number if Lf should be used as indicator for SCM.

#### 4 Conclusion

From the previously published data for dairy goats, no definite conclusions can be drawn for the applicability of one single parameter or a combination of several parameters for the evaluation of udder health status in goats. No comprehensive study including all of the mentioned possible indicators, collecting results over a longer period of time and comprising a sufficient set of data has been carried out so far. The data base is also not sufficient to make recommendations for the use in practice. The main reason for the dissatisfying situation in evaluation of the applicability of a parameter or a combination of parameters for detecting SCM is the lack of consistent bacteriological investigations in various studies published so far. The bacteriological analysis as gold standard is still the only method to make a clear statement on the infection status in dairy goats. The most common indirect methods used for detecting udder infections in dairy cows, namely SCC and CMT, are of limited value for goats because of the multitude of physiological influencing factors independent from the infection status. One promising approach for the early detection of SCM might be the combination of the parameters SCC, Lf and NAGase considering factors like stage of lactation and parity. Since IMI, caused by CNS mainly in its subclinical form represents the most common disease complex in dairy goats, resulting also in reduced milk production and affecting the processing properties of milk, it seems very necessary for maintaining animal health, for the preventive consumer protection and for securing the income of goat farmers to establish criteria that are appropriate to monitor udder health of goats easily

and safely. In order to find needed thresholds for practice, further studies comprising all possible parameters are yet to be carried out.

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