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Variation in udder health indicators at different stages of lactation in goats with no udder infection

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ABSTRACT

Mastitis is an important disease in dairy goat production. Subclinical mastitis is common in goats and is mainly caused by contagious bacteria. Several methods to diagnose mastitis in goats are available but have not all been investigated in healthy udders and at different stages of lactation. The purpose of the study was to investigate the variation in some udder health indicators at different stages of lactation in goats without intramammary infection (IMI). The udder health indicators were: somatic cell counts (SCC) measured by DeLaval Cell Counter (DCC) and estimated by California Mastitis Test (CMT), lactate dehydrogenase (LDH) activity, N-acetyl-β-D-glucoseaminidase (NAGase) activity and alkaline phosphatase (AP) activity.

Milk samples from twenty-four clinically healthy dairy goats were collected on two consecutive days in early, mid and late lactation. At milking, each goat's udder half was given a CMT score before udder half milk samples were collected. The milk samples were then analyzed for SCC, LDH, NAGase and AP, and investigated for bacterial growth. Variation in udder health indicators between udder half within goat, samples between sampling days and samples between stages of lactation were investigated using multivariable mixed-effect linear regression and multivariable ordinal logistic regression models.

Of the 24 goats, 18 were considered IMI negative at all samplings, 3 goats had inconclusive results for one udder half in late lactation and 3 (12.5%) had IMI positive udder halves in one or more lactation periods. Period of lactation was significantly associated with all udder health indicators with an increase in all indicators at late lactation compared to mid and early lactation. For NAGase and AP, period of lactation was significant as an interaction term with sampling day. NAGase was significantly higher on day 2 compared to day 1 in mid lactation and significantly lower on day 2 than day 1 in late lactation. AP was significantly higher on day 2 compared to day 1 in early lactation and significantly lower on day 2 than day 1 in late lactation. Moreover, for CMT there was a significant association with udder half with a higher general (over period and day) probability of higher CMT scores in the right udder half compared to the left.

This study shows that SCC, LDH, NAGase and AP were all affected by period of lactation but also to some extent by sampling day and udder half. This must be considered when interpreting udder health indicators sampled at different stages of lactation.

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1. Introduction

Subclinical mastitis in goats is common (Contreras et al., 2007) and considered an important disease since it can lead to decreased milk yield, impaired milk quality (Leitner et al., 2004a) and poor milk hygiene. Good milk hygiene is especially important when unpasteurized milk is used for cheese production (Oliver et al., 2005). Subclinical mastitis in goats is mainly caused by bacteria; coagulase-negative staphylococci (CNS) and *Staphylococcus aureus* (*S. aureus*) being the most common pathogens (Bergonier et al., 2003; Persson and Olofsson, 2011). It is important to detect goats with IMI at an early stage in order to prevent further spread of bacteria in the herd and to reduce the negative effect on milk production and milk composition. Presence of IMI may be diagnosed by bacterial culturing, but also indirectly by measuring inflammatory indicators in milk; e.g. somatic cell count (SCC), electrical conductivity, acute phase proteins and different enzyme activities. Bacterial culturing takes time and is costly. As many indirect measurements are faster and cheaper to perform, they have the potential to be effective diagnostic tools.

Somatic cell count is the most widely used indicator of udder health in cow, sheep and goat milk, but can be difficult to interpret in goats. Compared to sheep and cows, SCC in goat milk is relatively high also in the healthy udder and it increases throughout the lactation as well as with parity and during oestrus (Paape and Capuco, 1997; McDougall and Voermans, 2002; Christodoulopoulos et al., 2008). There is also a great variation in SCC among farms and among individuals (Schaeren and Maurer, 2006). Nevertheless, some authors claim that SCC is a good predictor of IMI in dairy goats (Poutrel et al., 1997; Persson and Olofsson, 2011). In two longitudinal field studies by Koop et al. (2013) investigating risk factors for subclinical IMI in goat, it was shown that stage of lactation, parity and milk yield influenced the sensitivity and specificity of using SCC at a cut-off of 2000×10^3 cell/mL for finding goats with an IMI caused by a major pathogen.

Earlier investigations have revealed that enzyme activities in the udder epithelium change markedly (Bogin et al., 1976; Banga et al., 1989) due to mastitic inflammation. Similarly, enzyme activities in blood serum/plasma or fractions of blood cells have proven to be indicative of experimentally induced mastitis (Symons and Wright, 1974; Banga et al., 1989; Heyneman and Burvenich, 1992). More practical attention has been given to detection of enzyme activity in milk, and numerous enzymes have been proposed and listed as reliable markers of bovine mastitis (Kitchen, 1981; Korhonen and Kaartinen, 1995). Among milk enzymes, NAGase (N-acetyl-β-D-glucoseaminidase; EC 3.2.1.30) has obtained the greatest attention due to the relative simplicity of analysis and its high correlation with SCC of milk (Kitchen et al., 1978, 1980). NAGase has been known as an indicator for mastitis detection in the last 2 to 3 decades, but other enzymes have also been suggested, i.e. alkaline phosphatase (AP; EC 3.1.3.1) (Rasmussen et al., 2008; Larsen et al., 2010) and β-glucoronidase (EC 3.2.1.31) (Larsen and Aulrich, 2011). Recently lactate dehydrogenase (LDH; EC 1.1.1.27) proved comparable qualities to NAGase for use for mastitis detection (Chagunda et al., 2006). Other studies also imply that LDH activity is one of the most reliable enzymes evaluated for the detection of IMI (Katsoulos et al., 2010; Stuhr et al., 2013). Moreover, Leitner et al. (2004a,b) as well as Stuhr et al. (2013) found that NAGase activity was significantly higher in infected udder halves compared to non-infected. Furthermore, Katsoulos et al. (2010) showed that AP activity was higher in infected udder halves compared to non-infected udder halves. However, as Stuhr et al. (2013) could show that both SCC and LDH was significantly influenced by week of lactation and parity, stage of lactation and parity must be taken into consideration when interpreting these udder health markers. To our knowledge, no public study has investigated the variation of LDH, NAGase or AP in healthy goats over an entire lactation.

The aim of the study was to investigate the variation in some udder health indicators at different stages of lactation in clinically healthy goats without IMI.

2. Methods

2.1. Animals

Every other dairy goat ($n = 24$), of the Jämtlandic breed (a typical Scandinavian dairy goat), in one farm in central Sweden was sampled in 2010. The farm was chosen because of known very good udder health and also since it was one of few farms close to the National Veterinary Institute. Of the sampled goats 6 were primiparous and the median parity was 4th parity (range 1st–10th parity). From earlier studies in this herd, we knew that the only isolated species were Coagulase-negative staphylococci (CNS) To investigate the effect of stage of lactation, the goats were sampled at three occasions during one lactation; early, mid and late lactation. The mean days in milk (DIM) in the period called early lactation was 40.2 (SD: 5.9), in mid lactation the mean DIM was 117.2 (SD: 5.9) and in late lactation the mean DIM was 223.2 (SD: 5.9). Samples in early and mid lactation were collected during morning milking and samples in late lactation were collected during afternoon milking. In early and mid lactation, does where milk twice a day with 12 h milking interval and in late lactation once a day with a milking interval of 24 h. The same person collected all samples. Only clinically healthy animals without any changes in udder consistency or milk appearance were included in the study (IDF, 1999). The herd was free from Caprine Arthritis Encephalitis Virus (CAEV).

2.2. Milk sampling and measurement of SCC

At each occasion two consecutive samples were collected 24 h apart in order to get a more reliable status for IMI (Sears et al., 1990), and to investigate if there is a day-to-day variation in the analyzed indicators. All milk samples were collected just before machine milking. The first milk was discharged. Milk samples were tested using CMT and graded from 1 to 5. The scores are ranked according to an increase in viscosity, where the highest viscosity (CMT 5) is more or less correlated to the highest SCC (modified from Schalm et al., 1971). After cleaning the teat ends with alcohol (70%), an aseptic milk sample was collected (IDF, 1999) from each udder half in sterile test tubes and sent to the National Veterinary Institute, Uppsala, Sweden, for bacteriological analysis. Milk from each udder half was also collected in additional test tubes, with bronopol as a preservative, for cell counting and measurement of enzymes. Milk aliquots from each bronopol test tube were analyzed individually at the laboratory the same day with the DeLaval Cell Counter (DCC) (DeLaval International AB, Tumba, Sweden (Berry and Broughan, 2007)).

2.3. Enzymes

Test tubes with bronopol preserved milk were deep frozen at −20 °C and sent to the faculty of Agricultural Science, Aarhus University, Foulum, Denmark for analysis of LDH, NAGase and AP. Enzyme activities were determined by kinetic fluorometric measurements. Lactate dehydrogenase activity was analyzed according to Larsen (Larsen, 2005);

N-acetyl-β-D-glucosaminidase activity and AP were analyzed according to Larsen et al. (2010).

2.4. Bacteriological examinations

Bacteriological analysis was performed according to accredited routines (National Mastitis Council, 1999) at the National Veterinary Institute, Uppsala, Sweden. Briefly, milk samples (10 μl) were cultured on blood (5%) agar plates with esculine, which were incubated at 37 °C for 16–24 h and re-evaluated at 48 h. A milk sample was classified as negative if less than one colony-forming unit (CFU) of *Staphylococcus aureus* or *Streptococcus agalactiae* was isolated. For other bacteria, the presence of less than three CFUs was needed for negative classification. Samples were classified as contaminated if three or more bacterial types were isolated from one milk sample and growth of a major udder pathogen was not identified. Coagulase-negative staphylococci were identified by typical colony morphology and negative coagulase reaction, but were not further characterized for this paper. In addition, all isolates of staphylococci were examined for betalactamase production by the "clover-leaf" method as described by Bryan and Godfrey (1991).

2.5. Statistics

Descriptive statistics were used to summarize SCC, LDH, NAGase and AP over lactation period, sampling day and udder halves. An udder half was considered IMI negative if no udder pathogen had been isolated at two consecutive samplings in respective lactation period and IMI positive if udder pathogens had been isolated on two consecutive samplings in respective lactation period. If an udder pathogen had been isolated at one of the samplings (within lactation period) the results were considered inconclusive. Inconclusive results were not included in the analyses. To investigate the associations between, and variation within, the dependent variables (udder half SCC, LDH, NAGase and AP) and udder halves, consecutive sampling days and period of lactation, four multivariable mixed-effect linear regression models were constructed, taking into account the repeated sampling within goat. The following model was used:

$$Y_{ijklm} = \mu + S_i + D_j + U_k + (g)_{lm} + e$$

where Y_{ijklm} was the observation (SCC, LDH, NAGase or AP activity in an udder half), μ was the general mean, S_i was the fixed effect of the ith stage of lactation (early/mid/late lactation), D_j was the fixed effect of the jth sampling day (day1/day2), U_k was the fixed effect of the kth udder halves (left/right), $(g)_{lm}$ was the random effect of the mth udder half within the lth goat (1–24) and e was an error term representing the residual error specified by an identity covariance structure. A manual stepwise backward variable selection procedure was used including all main effects. All plausible interactions between the main effects were tested. Variables with a significant association ($P < 0.05$) were kept in the model. To obtain normally distributed residuals all the dependent variables were transformed using the natural logarithm or Box-Cox power transformation. The normality of the residuals was then tested (using quantile plots) after the analyses to confirm the correctness of the transformation.

To investigate associations between the dependent variable "udder half CMT" (ordered categories 1–5) and udder halves, consecutive sampling day and period of lactation a multivariable ordinal logistic regression analysis, allowing the standard errors for intra-group correlation (within goat), was used.

All statistical analyses were performed using Stata Software (StataCorp., 2010; Stata Statistical Software: Release 11.0; College Station, TX, USA: StataCorp LP.).

3. Results

3.1. Bacteriology

Of the 24 goats, 18 were considered IMI negative at all samplings (i.e. 2 negative udder halves in each lactation period = 216 IMI negative milk samples), 3 goats had inconclusive results for one udder half in late lactation (=30 IMI negative milk samples), one goat was IMI positive in one udder half in early and mid lactation (=4 IMI positive milk samples and 8 IMI negative milk samples), one goat was IMI positive in one udder half in early and mid lactation, and had inconclusive results for one udder half in late lactation (=4 IMI positive milk samples and 6 IMI negative milk samples) and one goat was IMI positive in both udder halves in early and mid lactation and IMI positive in one udder half in late lactation (=10 IMI positive milk samples and 2 IMI negative milk samples). This resulted in a total of 262 IMI negative milk samples that were used in the statistical analyze of the variation in the udder health indicators. In total, 3 of the 24 goats (12.5%) had IMI positive udder halves in one or more lactation periods and 18 of the 288 (6.3%) milk samples were IMI positive. The only isolated bacterial species were CNS. Of the CNS, 14% were positive for betalactamase production. No mixed infections were detected and only two of all milk samples were considered as contaminated (no specific pathogen were identified).

3.2. Dynamics of the udder health indicators

The median (50% central range (CR)) SCC, LDH, NAGase and AP in early, mid and late lactation were 137,000 cells/ml (77,000–274,000), 3.2 U/l (2.3–4.5), 1.9 U/l (1.4–2.3), 43.2 U/l (21.7–70.0), 190,500 cells/ml (82,500–402,500), 3.3 U/l (2.6–4.3), 1.3 U/l (0.9–1.9), 53.2 U/l (28.5–65.8), 449,000 cells/ml (291,000–976,000), 7.7 U/l (6.2–10.4), 4.5 U/l (2.8–7.0), 72.5 U/l (48.7–119.3), respectively (Fig. 1).

Period of lactation was significantly associated with all udder indicators with an increase in all indicators in late lactation compared to mid and early lactation (Figs. 1 and 2 and Tables 1 and 2).

For NAGase and AP, period of lactation was significant as an interaction term with sampling day (Table 1). NAGase was significantly higher on day 2 compared to day 1 in mid lactation and significantly lower on day 2 than day 1 in late lactation. AP was significantly higher on day 2 compared to day 1 in early lactation and significantly lower on day 2 than day 1 in late lactation.

The median CMT (50% CR) in early, mid and late lactation were 2 (2–2), 2 (2–3) and 3 (2–3), respectively. In addition to lactation period, CMT was significantly associated with udder half with a higher general (over period and day) probability of higher CMT scores in the right udder half compared to the left (Fig. 2 and Table 2).

4. Discussion

This study shows some of the variations of SCC, LDH, NAGase and AP in clinically healthy goats. However, as only 24 goats from one herd were included in this study, the results are not representative for goats in general, but give an indication of how these mastitis indicators vary.

The results show that all measured udder health indicators increase significantly in late lactation compared to early and mid lactation. This is in accordance with Wilson et al. (1995) who showed that SCC in goats increases significantly with increasing days in milk both in IMI negative and positive goats. In a review (Haenlein, 2002) of the relationship of SCC in goat milk with mastitis and productivity,

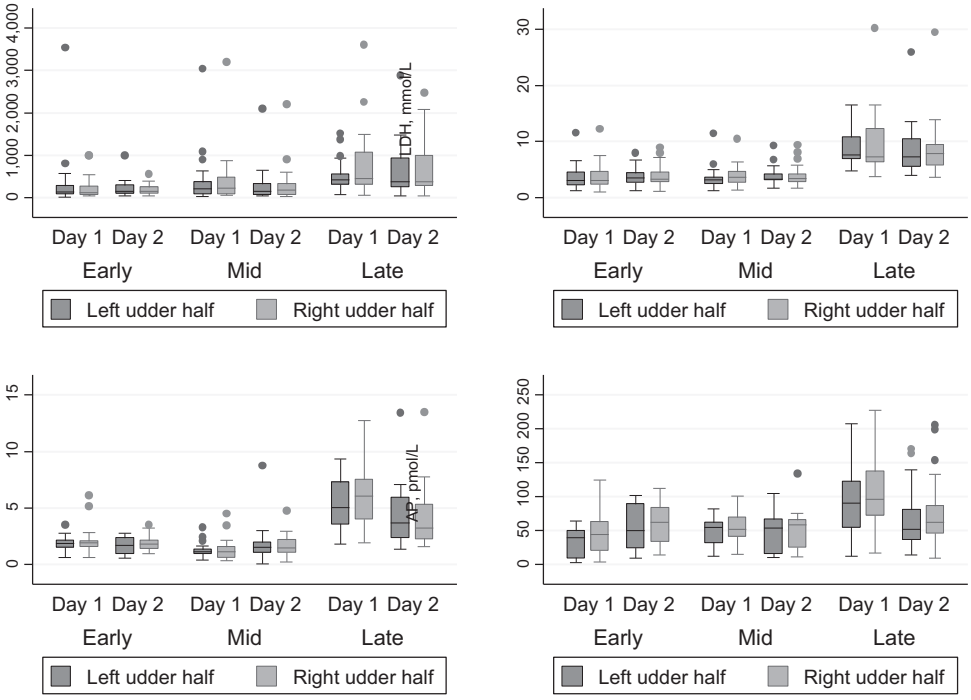

Fig. 1. Box plots of original data for somatic cell count (SCC), lactate dehydrogenase (LDH), N-acetyl-β-D-glucosaminidase (NAGase) and alkaline phosphatase (AP) of milk samples from dairy goats without intra-mammary infection as a function of udder halves within sampling day and period of lactation (n = 262 milk samples from 24 goats).

Table 1
Estimated marginal means (±SEM) for somatic cell count (SCC, 1000 cells/ml), lactate dehydrogenase (LDH, mmol/L), N-acetyl-β-D-glucosiminidase (NAGase, mmol/L) and alkaline phosphatase (AP, pmol/L) of milk samples from dairy goats without intramammary infection related to lactation period and sampling day (n = 262 milk samples from 24 goats) from mixed linear regression models.

	lnSCC	lnLDH	lnNAGase[a]	bcAP[b]
Lactation period				
Early	4.936 ± 0.156a	1.070 ± 0.069a		
Day 1			0.647 ± 0.095a	8.033 ± 0.569a
Day 2			0.585 ± 0.095a, b	10.11 ± 0.569b, c
Mid	5.480 ± 0.146b	1.505 ± 0.061a		
Day 1			0.209 ± 0.095c	9.770 ± 0.569b
Day 2			0.470 ± 0.095b, d	9.464 ± 0.569b
Late	6.024 ± 0.155c	1.940 ± 0.069b		
Day 1			1.691 ± 0.095e	13.52 ± 0.571 c, d
Day 2			1.300 ± 0.095f	11.11 ± 0.571c, e

Means within the same column with different letters (a, b, c, d, e, f) differ significantly (P < 0.05).
[a] ln(NAGase + 0.096764).
[b] Box-cox transformed: $((AP^{0.4199091}) - 1)/0.4199091$.

Table 2
Estimated predicted probabilities (margins (±SEM)) for California mastitis scores (CMT) of milk samples from dairy goats without intramammary infection related to lactation period and udder half (n = 262 milk samples from 24 goats) from an ordered logistic regression model.

CMT score	1	2	3	4	5
Lactation period					
Early	0.197 ± 0.053	0.633 ± 0.042	0.135 ± 0.043	0.031 ± 0.016	0.004 ± 0.003
Mid	0.082 ± 0.020	0.558 ± 0.061	0.270 ± 0.048	0.080 ± 0.031	0.010 ± 0.008
Late	0.031 ± 0.010	0.361 ± 0.065c	0.394 ± 0.052	0.187 ± 0.054	0.027 ± 0.022
Udder half					
Left	0.133 ± 0.032	0.620 ± 0.051	0.192 ± 0.045	0.048 ± 0.021	0.006 ± 0.005
Right	0.053 ± 0.014	0.472 ± 0.065	0.338 ± 0.048	0.121 ± 0.042	0.016 ± 0.013

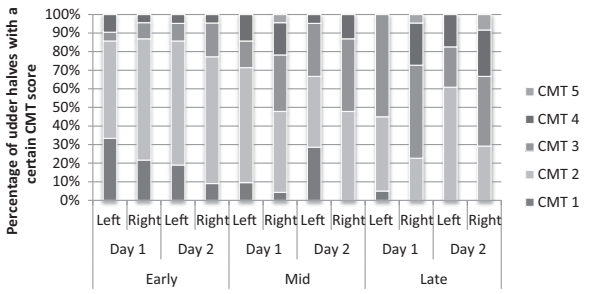

Fig. 2. Distribution of original data for California mastitis test scores of milk samples from dairy goats without intra-mammary infection as a function of udder halves within sampling day and period of lactation (n = 262 milk samples from 24 goats).

it was concluded that stage of lactation always influences SCC in goats. Moreover, Stuhr et al. (2013) also found a significant association between lactation week and SCC, LDH and NAGase, though they only recorded data for the first six weeks of lactation. The estimated marginal mean SCC measured by DCC in early lactation in the present study (139,000 cells/ml), is very similar as reported from Stuhr et al. (2013) (138,000 cells/ml) and findings in a study by Aulrich and Barth (2008) (158,000 cells/ml), but lower than e.g. Wilson et al. (1995) (303,000 cells/ml). Moreover, the early lactation estimated marginal mean LDH of 2.9 mmol/L is also very similar to that of Stuhr et al. (2013) (2.97 mmol/L), while the studies differ somewhat in early lactation estimated marginal mean NAGase (1.73 mmol/L vs. 1.03 mmol/L). The latter might be caused by differences in analyzing NAGase. The late lactation estimated marginal mean SCC of 426,000 cells/ml is similar to findings in another Swedish study (Persson and Olofsson, 2011) (481,000 cells/ml), but somewhat lower than reported by Wilson et al. (1995) (650,000 cells/ml). The many similarities in levels/activity in the investigated udder health indicators in the present study with that of others indicates that our results can be useful for others.

Other studies have reported a prevalence of CNS positive udder halves ranging from 6 to16% (Aulrich and Barth, 2008; Barth et al., 2010; McDougall and Prosser, 2010; Stuhr et al., 2013). Our result (6.3%) is within that range.

In the present study there was no difference between number of goats with IMI and different sampling occasions. Min et al. (2007) reported higher rates of IMI during the early stage of lactation and in the late stage of lactation. The reason for this is somewhat unclear, but in Min' study many different pathogens were studied, compared to our, where CNS was the only pathogen found. Also, the very limited data in our study might explain some of the differences.

The significant differences in CMT scores between right and left udder halves is difficult to explain, especially as no significant differences were seen between udder halves in the other measured udder health indicators. It could be due to a repeated error in scoring CMT, but it is unlikely since the sampler is an experienced person. It could also be caused by the milking machine, but one would expect to see the same differences in other parameters, especially SCC measured by DCC.

The difference in NAGase and AP levels between sampling days is also hard to explain, especially as they did not show consistently the same pattern, though, similar in late lactation. The results show that there can be a significant day to day variation so repeated sampling should be recommended to be able to distinguish between a true increase due to IMI (that would then probably last more than one day) and variation due to e.g. management.

5. Conclusions

This study shows that SCC, LDH, NAGase and AP all were affected by period of lactation but also to some extent by sampling day and udder half. This must be considered when interpreting udder health indicators sampled at different stages of lactation.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YP conceived of the study and was responsible for its coordination, participated in its design and drafted the manuscript. TL carried out the analysis of the enzymes and helped to draft the manuscript. AKN carried out the statistics of the study, participated in its design and helped to draft the manuscript. All authors read and approved the final manuscript.

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