

Changes in Milk Composition as Affected by Subclinical Mastitis in Goats

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ABSTRACT

The mechanism of the effects of subclinical mastitis (SM) at the glandular level in dairy goats on milk yield and its composition as expressed in curd yield (Yc) was studied. Twenty-five Israeli goats of various cross-breeds were chosen; one udder half was naturally infected with identified coagulase-negative staphylococci, and the contralateral gland was free of bacteria.

The milk yield of the infected halves was significantly lower than that of the uninfected ones. Somatic cell count and *N*-acetyl- β -D-glucosaminidase activity were significantly higher in the infected halves. The lactose concentration in the infected glands was significantly lower than that in the uninfected ones, casein concentrations did not differ, and the whey protein and albumin concentrations were significantly higher in the infected glands.

Plasmin activity was significantly higher in the infected glands, whereas plasminogen activity was undetectable. Concentrations of Ca²⁺ did not differ, whereas Ca²⁺ activity was significantly lower in the infected glands. The proteose peptone concentration was 1.5 times as great in the infected glands as in the uninfected ones. The Yc was significantly lower in the infected halves, and clotting time was significantly longer.

The mechanisms of the effects of SM on milk yield and Yc in goats and sheep are discussed and compared. (**Key words:** subclinical mastitis, goat, milk composition)

Abbreviation key: a_{Ca}²⁺ = calcium activity, CN = casein, CNS = coagulase-negative staphylococci, p-p = proteose peptones, PA = plasminogen activator, PL =

plasmin, PLG = plasminogen, SM = subclinical mastitis, Tc = clotting time, Yc = curd yield.

INTRODUCTION

The proportion of udder halves with subclinical IMI in goats in different countries ranges from 35 to 70% (Menzies and Ramanoo, 2001; Leitner et al., 2004b). In Israeli dairy goats, the main pathogen group in infected udder halves comprises various species of coagulase-negative staphylococci (CNS), mainly *Staphylococcus caprae* and *Staphylococcus epidermidis* (Leitner et al., accepted). A survey of 20 Israeli-Assaf dairy sheep (Leitner et al., 2003) revealed similar results; the main influence on SCC was IMI, and milk yield was significantly higher in uninfected than in infected halves. However, a direct comparison between infected and uninfected glands was not possible in that study because the measurements were based on the whole udder level and not on a single gland level. Therefore, a glandular level model was developed in which each animal had one udder half infected with an identified CNS species, and the contralateral gland was free of bacteria, to focus on how subclinical mastitis (SM) affected milk yield and compositional changes in relation to curd yield (Yc) (Leitner et al., 2004b). Applying this experimental design in sheep showed that subclinical IMI was associated with increased plasminogen activator (PA) and plasmin (PL) activities as a result of accelerated conversion of plasminogen (PLG) to PL in the infected glands. These changes were associated with accelerated apparent casein (CN) degradation, reduced Yc, and increased milk-clotting time (Tc). These modifications indicate that the changes in milk composition negatively affect the yield and quality of cheese made from milk that originates from infected glands.

Plasmin is the main proteolytic enzyme in cow and sheep milk (Politis, 1996; Leitner et al., 2004b) in which it occurs mostly as the inactive zymogen PLG, which

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is activated by PA. However, only residual PLG activity was found in goat milk, which was consistent with the unusually high PA activity compared with values for ovine and bovine milk. Nevertheless, PA and PL activities in late-lactating goats were negatively correlated with the coagulating properties of milk, which suggests that this system is important in goats as well (Fantuz et al., 2001).

The present study applied the glandular level model to dairy goats to test the effect of IMI on milk yield and on milk quality as reflected in Yc and Tc. To achieve this goal, animals were chosen that had one udder half infected with an identified CNS species and the contralateral gland free of bacteria. In each gland, inflammation indices were analyzed along with total milk protein, CN, whey proteins, the PA-PL system activity, and measures of proteolysis.

MATERIALS AND METHODS

Animals

Twenty-five Israeli goats of various crossbreeds, mainly Shami × Anglo Nubian and Saanen × Anglo Nubian, in which one udder half was naturally infected with an identified single species of CNS and the contralateral gland was bacteria free, were selected from 2 flocks. Prior to animal selection, milk samples from each udder half were subjected to 3 consecutive weekly examinations to test for bacterial infection, SCC, and NAGase activity. The selected goats were 40 to 120 d post-kidding, and their daily milk yield exceeded 2.5 L. In both farms, the goats were machine milked twice daily at 0500 and 1500 h. Post teat dipping was practiced, and they were kept in an open shelter that provided 4 m² of shaded slatted floor and 4 m² of concrete-surfaced yard for each goat. Feed was offered in mangers located in the sheds.

Milk Sampling and Analysis

The milk sampling and yield measurements were carried out during the morning milking. Yield was determined by weighing the milk of each udder half of each goat after hand milking. For the bacteriological tests and NAGase activity measurements, the teats were cleaned and disinfected, and the milk was sampled and analyzed as described by Leitner et al. (2004b). Three additional sets of samples were taken from each udder half and distributed for analysis as follows. One set was preserved by means of Broad Spectrum Microtabs II (D & F Control Systems, Inc., CA) and sent to a central laboratory (Cattle Breeders Association Laboratory, Caesarea, Israel) for analysis of the milk gross composition—protein, fat, and lactose contents—with the Mil-

koscan 6000 and analysis of the SCC with a Fossomatic 360 (Foss Electric, Hilleröd, Denmark); both were calibrated with goat milk. The second set of samples was used to determine Yc and Tc; Tc was measured according to Berridge (1952). A third set was defatted, and the skim milk was used for analysis of CN concentration, whey proteins, albumin, and proteose peptones (**p-p**) (Shamay et al., 2000b, 2003) and for PA, PLG, and PL activities (Silanikove et al., 2000). The repeated addition procedure was used to measure the concentrations of free (ionized) calcium ([Ca²⁺]) and the uncorrected procedure was used to determine calcium activity (**a_{Ca}²⁺**) in these samples within 5 h of sampling by means of a specific calcium electrode (Silanikove et al., 2003).

Bacteriological Examinations

Bacteriological analysis was performed according to accepted standards (Hogan et al., 1999). From every milk sample, 0.01 mL was spread onto blood agar plates (Bacto-Agar; Difco Laboratory) containing 5% of washed sheep red blood cells and on MacConkey plates. All plates were incubated at 37°C and examined for growth after 18 and 42 h. Colonies suspected to be staphylococci were tested for coagulase (tube test) (Ani-lab, Rehovot, Israel). Strain identification was carried out with the API STAPH-IDENT, 32 Staph kit (bioMérieux S.A., Marcy-l'Etoile, France). When the percentage of micrococci-like bacteria that matched the test strain exceeded 90%, the strain was regarded as specific.

Yc Determination

Curd yield was determined according to Leitner et al. (2004a); Fromase 15 TL (Gist-Brocades nv, Delft, The Netherlands) was used as the coagulating enzyme. To determine the accuracy of the Yc measurement, Yc was measured for a sample in both dry and wet states. The dry curd was tested according to Melilli et al. (2002), whose procedure was modified by weighing the wet curd after centrifugation and drying it overnight in an oven at 100°C. The curd sample was not transferred to another container for drying, as suggested by Melilli et al. (2002), but was left in the centrifugation tube to prevent any possible loss of curd during transfer of the centrifuged curd pellet. The correlation between Yc based on wet curd vs. dry curd is presented in Figure 1; the regression coefficient was 0.96.

Statistical Analysis

The dependent variables were SCC, NAGase, milk yield, fat, protein, lactose, CN, whey proteins, albumin,

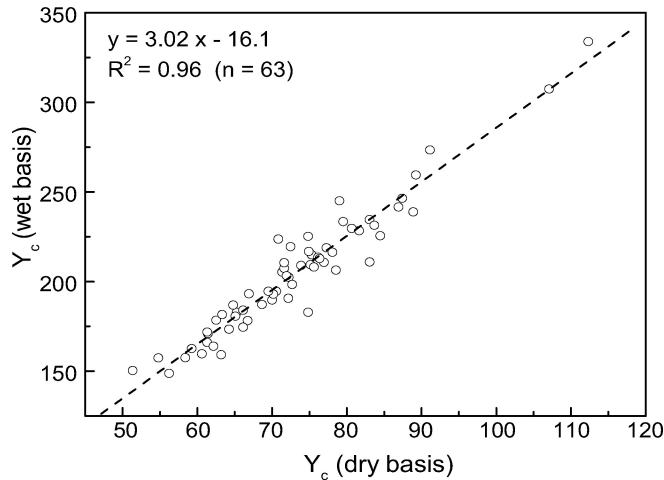


Figure 1. Comparison of curd yield (Y_c) on dry basis vs. wet basis (g/L).

p-p, $[Ca^{2+}]$, $a_{Ca^{2+}}$, PL, PA, Y_c , and Tc. Data were analyzed with the JMP statistical software (SAS, 2002) under a randomized block design in which goats served as blocks. Within each block, there were 2 levels of bacteriological status, as follows:

$$Y_{ij} = \mu + G_i + B_j + e_{ij}$$

where Y_{ij} = dependent variable, μ = overall mean, G_i = goat_{*i*} where *i* = 1 to 25, B_j = bacteriological status where *i* = 1 (infected) or 2 (uninfected), and e_{ij} = error term (experimental variation between udder halves within a goat).

No significant difference in SCC was found between the different CNS species, which was consistent with previous results (Leitner et al., 2004a,b); therefore, the analysis was completed over species.

RESULTS

The pathogens causing the udder infections were various species of CNS: *S. epidermidis* (*n* = 11), *Staphylococcus simulans* (*n* = 6), *S. caprae* (*n* = 2), *Staphylococcus chromogenes* (*n* = 3), and *Staphylococcus xylosus* (*n* = 3). The bacterial species isolated from any given gland was identical at each sampling. No clinical abnormalities were visible or palpable in >80% of the goats tested.

Milk yield of the infected halves (0.69 kg/milking) was significantly lower ($P < 0.0001$) than that of the uninfected halves (0.98 kg/milking) (Table 1). The measured indications of infection response—SCC and NA-Gase activity—were significantly higher in the infected halves than in the uninfected ones.

Table 1. Effects of mammary gland infection on milk production and composition using half-udder design with 25 Israeli dairy goats tested 2 or 3 times at 10- to 20-d intervals.

Parameter	Bacteriological status		Effect	
	Uninfected	Infected	Infection	Goat
Milk, kg/milking	0.98 ± 0.04	0.69 ± 0.04	<0.0001	<0.0001
SCC ± SE ($\times 10^3$)	417 ± 72	1750 ± 197	<0.0001	0.07
NA-Gase ¹	13.6 ± 2.7	37.9 ± 4.5	0.05	NS ²
Fat, g/L	38.9 ± 1.1	38.8 ± 1.2	NS	0.0002
Protein, g/L	34.2 ± 0.5	35.0 ± 0.5	0.07	<0.0001
Lactose, g/L	47.0 ± 1.0	41.7 ± 1.3	0.004	0.004
Whey, g/L	6.1 ± 0.3	6.8 ± 0.4	<0.0001	<0.0001
Casein, mg/mL	28.1 ± 0.7	28.2 ± 0.8	NS	<0.0001
Albumin, μ g/mL	279.9 ± 22.2	471.8 ± 49.8	0.003	0.04

¹A value of 100 corresponds to a product release of about 5 μ mol/L per min at 25°C.

²NS: $P > 0.1$.

Lactose concentration in the infected glands was significantly lower ($P < 0.004$) than that in uninfected glands. The concentration of fat did not differ between the uninfected and infected halves. Protein concentration tended to be higher in the infected glands ($P < 0.07$). Casein concentration did not differ, whereas whey protein and albumin concentrations were significantly higher in the infected glands than in the uninfected glands. The goat effect on these variables was significant (Table 1).

Plasminogen activator and PL activities were significantly higher in the infected glands than in the uninfected ones (Table 2). Plasminogen activity was very low to undetectable; therefore, these data are not presented. Free (ionized) calcium did not differ, whereas $a_{Ca^{2+}}$ was significantly lower ($P < 0.002$) in the infected glands vs. the uninfected glands. Concentration of p-p was 1.5 times higher ($P < 0.0005$) in the infected glands than in the uninfected ones (Table 2). The goat effect was significant for $a_{Ca^{2+}}$ and p-p and insignificant for $[Ca^{2+}]$.

Table 2. Effects of mammary gland infection on plasmin (PL), plasminogen activator (PA), calcium activity ($a_{Ca^{2+}}$), free (ionized) calcium ($[Ca^{2+}]$), proteose peptones (p-p), curd yield (Y_c), and clotting time (Tc). A half-udder experimental design was utilized with 25 Israeli dairy goats.

Parameter	Bacteriological status		Effect	
	Uninfected	Infected	Infection	Goat
PL, unit ¹ /mL	20.32 ± 2.4	39.81 ± 6.1	0.0003	0.005
PA, unit ¹ /mL	3376 ± 404.1	4334 ± 565.5	0.05	0.002
$a_{Ca^{2+}}$, mmol	1.89 ± 0.1	1.62 ± 0.1	0.002	<0.0001
$[Ca^{2+}]$, mmol	4.80 ± 0.4	5.05 ± 0.3	NS	NS
p-p, mg/mL	0.35 ± 0.05	0.53 ± 0.05	0.0005	0.0002
Y_c , g/L	231.6 ± 2.9	207.8 ± 2.7	<0.001	<0.0001
Tc, s	167 ± 18.6	295 ± 43.4	<0.02	<0.08

¹Unit = activity unit; 1 unit is the amount of PL that produces a change in absorbance of 0.1 at 405 nm in 60 min.

²NS: $P > 0.1$.

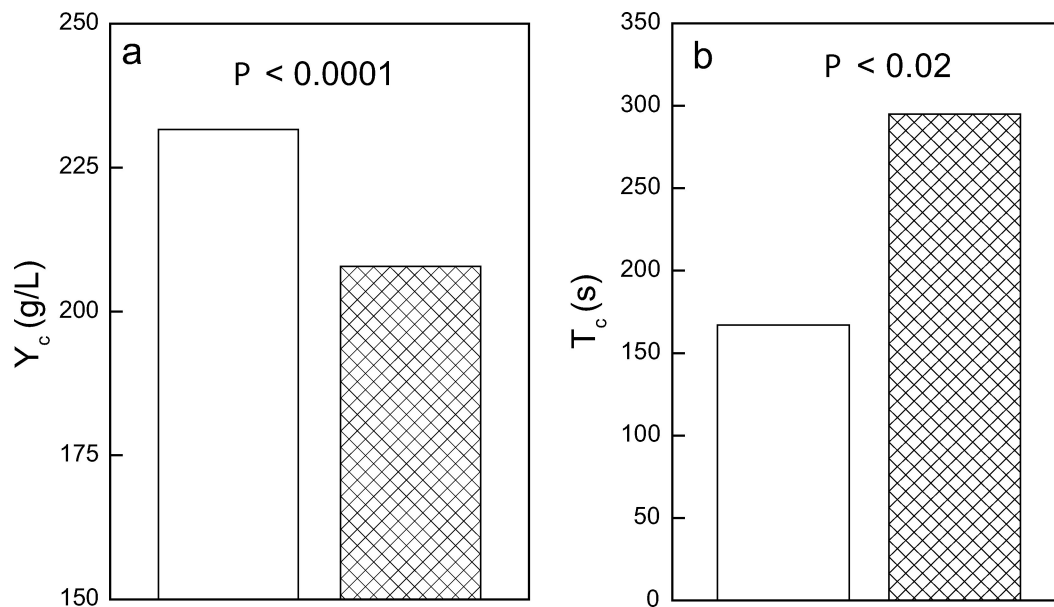


Figure 2. Effect of subclinical mastitis on a) curd yield (Y_c) and b) clotting time (T_c) in milk of infected vs. uninfected udder halves. Open bars = uninfected glands; hatched bars = infected glands.

Curd yield was significantly lower ($P < 0.0001$) in the infected halves, and there was a significant goat effect ($P < 0.0001$). Clotting time was significantly longer ($P < 0.02$) in the infected halves than in the uninfected ones, with a relatively small goat effect ($P < 0.08$) (Figure 2).

DISCUSSION

Model and Individual Animal Effect

Use of the half udder as the experimental unit has been found to enable quantification of the negative effect of SM on milk yield with high statistical reliability, even for a relatively small data set of 20 to 40 animals. This finding is consistent with that of a recent study on sheep (Leitner et al., 2004a). The half-udder model is an effective tool for isolating the experimental effect from masking effects. An alternative approach based on conventional whole-udder sampling would require a data set of the order of 100 animals to account for the large and significant individual variations in the sheep or goat effect on most of the indices measured in these studies. These significant sheep or goat effects most likely reflect the diversity of genetic (breed) sources in the Israeli flocks, and they are further complicated by the effects of factors such as farm management, environmental conditions, age, and stage of lactation. Obviously, variations caused by all of these factors are neutralized when the unit of comparison is 2 udders of the same animal.

Infection and Milk Yield

Various CNS bacteria are the most abundantly occurring in isolates associated with SM in goat herds in a number of countries (Kalogridou-Vassiliadou, 1991; Contreras et al., 1997; 1999; Haenlein, 2002). The CNS are not considered as major pathogenic bacteria, and their occurrence is usually ignored by farmers and veterinarians (Leitner et al., 2004a). However, in the present study, CNS infection induced the inflammatory response, reflected in the high SCC, which is consistent with previous findings in goats and sheep (Lerondelle et al., 1992; Contreras et al., 1999; Haenlein, 2002; Leitner et al., 2003). The inflammatory response was associated with a marked reduction in milk yield in the infected gland compared with that of the uninfected one, which is consistent with earlier results in sheep (Leitner et al., 2003, 2004). In sheep with both glands infected, the reduction in milk yield was significant, whereas when only one gland was infected, the contralateral gland compensated for about 80% of the reduction. Thus, although the point has not yet been tested, we cannot rule out the possibility that in goats too, compensation in the uninfected gland mitigates the effect on milk yield as measured on a whole-animal basis when only one gland is infected. Nevertheless, the extensive survey of milk records of goat farms in France by Baudry et al. (1997) leaves no doubt that increased SCC associated with IMI reduced milk yield in comparison with farms with low SCC.

$a_{Ca^{2+}}$ as a Measure of CN Degradation

Silanikove et al. (2003) demonstrated a negative linear relationship between CN concentrations in milk of humans, goats, cows, sheep, and mice, on one hand, and $a_{Ca^{2+}}$ on the other, a finding that is consistent with the fact that CN are powerful Ca chelators. In the present study, $a_{Ca^{2+}}$ was negatively related to measures of proteolysis (p-p), which was consistent with similar findings in sheep (Leitner et al., 2004a). The association between CN degradation and the reduction in $a_{Ca^{2+}}$ may be related to the exposure of phosphoserine groups that are hidden within the casein micelles because these molecules are responsible for the Ca-chelating properties of CN. Casein degradation occurs in the gland during the intervals between milkings (Le Roux et al., 1995; Urech et al., 1999). Thus, the differences in $a_{Ca^{2+}}$ between the infected and uninfected glands may represent the additional CN degradation in the infected glands. The present results further support the conclusion that measurement of $a_{Ca^{2+}}$, which is rapid and cheap, appears to be a valuable tool for monitoring the extent of CN degradation under various conditions.

Yc and Milk Tc

Curd yield was lower from the infected halves than from the uninfected ones, although the CN contents were almost equal in the 2 glands. Negative effects of mastitis on CN content and cheese yield were also reported for cow milk (Auldism and Hubble, 1998). Thus, our data suggest that knowledge of the gross CN content in the milk is insufficient for predicting Yc, probably because of modifications in the CN micelles or in the various CN micelle components that are more detrimental to curd formation than they are to the CN concentration itself. The primary enzymatic coagulation is based on the action of rennin on κ -CN, which, thereafter, exposes hydrophobic sites on the CN micelle, thus making it available for the secondary aggregation reaction (Ernststrom and Wong, 1974). However, the effect of rennet and, therefore, the coagulation process may be impeded by only partial hydrolysis of κ -CN and more pronounced hydrolysis of the other caseins by enzymes such as PL and cathepsin (Srinivasan and Lucey, 2002; Moussaoui et al., 2003). Thus, the conversion of CN to whey components (p-p) by PL may partially explain the reduction in Yc. In support of this hypothesis, a significant positive correlation was observed between the PL and PA activities, on one hand, and the rennet Tc, on the other hand, which is consistent with similar interactions found in late-lactating goats (Fantuz et al., 2001) and in subclinically infected sheep (Leitner et al., 2004a). The changes in p-p concentration and $a_{Ca^{2+}}$ in the infected glands suggest that CN was modified by the

release of certain peptides or because of a change in the CN micelle compaction that was caused by changes in $a_{Ca^{2+}}$ (Leitner et al., 2004a) and that this hampered the coagulum formation. The combination of higher PL activity in the infected gland and the long interval of about 12 h between the evening and morning milkings resulted in a correspondingly and considerably higher proteolysis of CN by PL, because of the extended exposure of CN to its action, as was found elsewhere for added PL in vitro (Srinivasan and Lucey, 2002).

Interrelationships Between Milk Yield and Composition in Subclinically Mastitic Goats

The present finding of an upregulation in the activity of the PL system in glands infected with SM is consistent with previous findings in dairy cows (Schaar and Funke, 1986; Auldism et al., 1996; Urech et al., 1999) and dairy sheep (Leitner et al., 2004a). Higher protein and fat concentrations were found in infected glands than in uninfected ones (Leitner et al., 2003, 2004a); at the same time, milk volume decreased, suggesting that this response is related to a mild increase in PL activity (30 to 50%) over the basal level. Under such conditions, plasmin-induced hydrolysis of CN liberates a peptide from β -CN (β -CN 1-28), which in turn down-regulates milk secretion in cows and goats; its activity was correlated with its ability to block potassium channels in the apical membranes of mammary epithelia (Silanikove et al., 2000). However, in some individual cases, the protein and fat concentrations were found to be lower in infected glands than in uninfected ones. Such a response was observed when the increase in PL activity was large (2-fold or more), as is the case during milk stasis (Shamay et al., 2002, 2003). It has been shown that CN hydrolysis under high PL activity induces rapid drying-off of mammary secretions in goats (Shamay et al., 2002) and cows (Shamay et al., 2003).

In the present study, despite the doubled PL activity, the reduction in milk yield more or less matched the reductions in protein and fat secretion, so that overall there was no net change in protein and fat concentrations. However, as discussed previously, there is compelling evidence that the CN was degraded and modified in the infected gland. The marked reduction in lactose concentration resembled the response in sheep and cows under high PL activity. Thus, the degree of PL activation determines not only the reduction in milk volume, but also the changes in the secretion of organic components and, consequently, milk composition and Yc, probably because certain structural modifications in the molecule affect its ability to aggregate, owing to the proteolytic action of enzymes on the CN micelle.

Comparisons Between Goats and Sheep

Application of the half-gland model to the same set of measurements performed with similar methodologies in sheep and goats provides us with a unique opportunity to compare the responses of the 2 species to subclinical IMI with CNS.

The SCC levels in uninfected glands of goats and sheep (around 200,000 cells/mL) were higher than those reported for uninfected cows (Maisi et al., 1987; Fthenakis et al., 1991; Baro et al., 1994; Gonzalo et al., 1994; Gonzalez-Rodriguez et al., 1995; Paape and Capuco, 1997). In both goats (Leitner et al., 2004b) and sheep (Leitner et al., 2004a), CNS IMI increased SCC to $>10^6$ cells/mL, suggesting that diapedesis in response to the latter in goats and sheep is more acute than in cows. Whether this more severe influx of leucocytes to the mammary gland is associated with improved antibacterial defense capacity is a question that remains to be answered.

In sheep, the reduction of milk yield in the infected glands was 53% (Gonzalo et al., 1994), which is considerably higher than the 30% reduction in milk yield in the infected glands of goats, which was found in the present study. It is consistent with these differences that the reduction of lactose concentration in the infected glands was 25% compared with that in the uninfected glands, i.e., twice the reduction of 11% found in goats in the present study. It may be concluded that the much higher reduction in lactose secretion in the infected glands of sheep (65%) than in those of goats (37.5%) is the main reason for the finding that the reduction in milk volume in the infected glands was greater in sheep than in goats.

In both goats and sheep, the reduction in whey protein secretion was less than the reduction in milk yield, because of the increased protein concentration in the infected glands of both species. The gross CN concentration in goat milk (28 g/L) resembles that of cow milk and is much lower than that in sheep milk (40 to 46 g/L). Thus, the p-p concentration in uninfected glands in goats is consistent with the lower CN concentration. Moreover, the increase of p-p in the infected gland in response to doubled PL activity was still much lower than the corresponding figure in sheep, and the reduction in a_{Ca}^{2+} in the infected glands was lower in goats than in sheep.

It is noteworthy that the PL activity found in the present study in uninfected glands (Figure 3a) was essentially similar to that found in goats by Baldi et al. (2002), who used the same methodology. In both studies, PLG activity was close to zero (Figure 3b), which may be explained by the unusually high PA activity (Figure 3c). The PA activity found in the present study

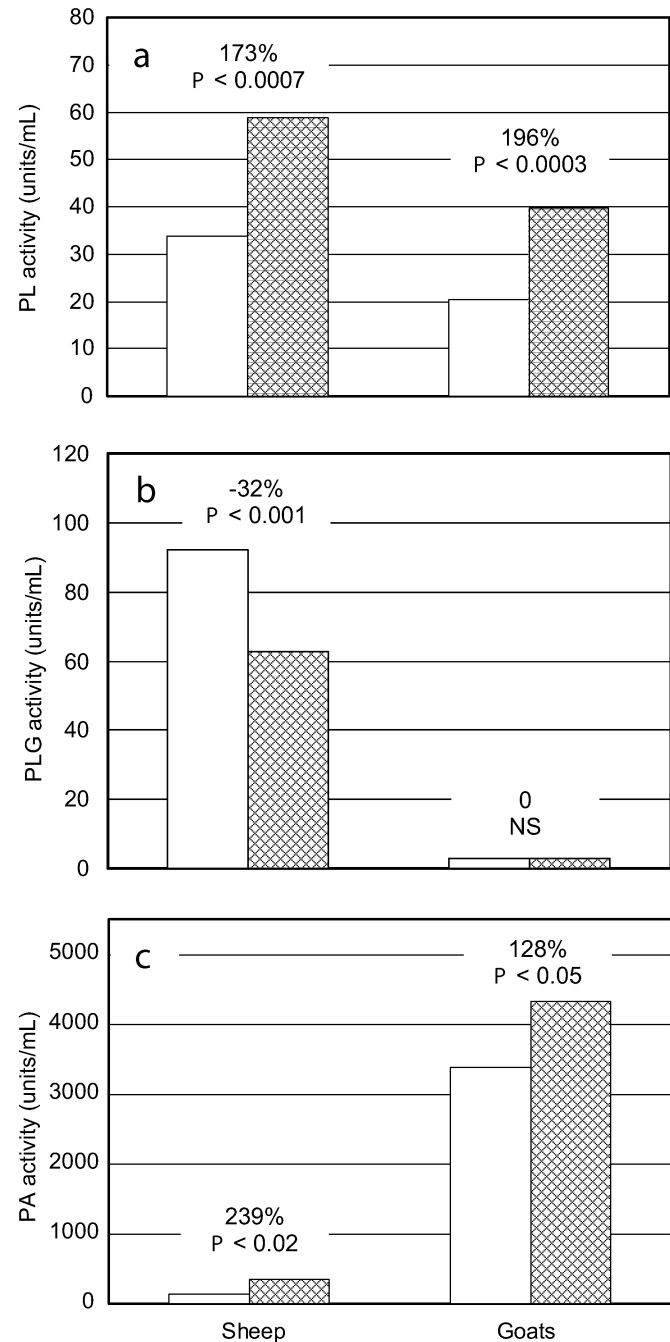


Figure 3. a) Plasmin (PL), b) plasminogen (PLG), and c) plasminogen activator (PA) activities in milk from uninfected vs. infected udder halves in sheep and goats. Open bars = uninfected glands; hatched bars = infected glands.

was even higher than that reported by Baldi et al., possibly because the PA was determined in whole skim milk, whereas Baldi et al. (2002) determined it in redissolved CN. When the PLG activity is added to that of PL to obtain the total PA-derived activity it becomes

clear that the latter is considerably higher in sheep than in goats. The initial level of the PLG pool in the goat gland ought to be much lower than that in the sheep gland, which may be an additional reason for the apparently rapid disappearance of PLG from the goat mammary gland.

It has been suggested that regulation of PA activity serves as a bridge between systemic hormonal influences and the local regulatory system (Silanikove et al., 2000). In the organ culture of the mouse mammary gland, hormones that fostered involution induced PA synthesis, whereas hormones that promoted lactation repressed PA secretion (Ossowski et al., 1979). The treatments of cows (Politis, 1996) and sheep (Baldi et al., 1997) with bovine somatotrophin increased milk yield and reduced the conversion of PLG to PL. On the other hand, stress, stress hormones, and estrogen, which induce down-regulation of milk yield, increased PL activity in milk (Athie et al., 1997; Silanikove et al., 2000). The high basal level of PA in goats may make the system irresponsive to systemic effects. Consistent with this suggestion, treating goats with somatotrophin did not affect the PA system (Baldi et al., 2002), whereas treating goats with adrenocorticotrophin and dexamethasone did not reduce their milk yield (Shamay et al., 2000a), as it did in the case of dairy cows (Shamay et al., 2000b).

CONCLUSIONS

The findings of the present study highlight the economic loss that can be attributed to IMI caused by CNS; this was clearly demonstrated by using the half-udder model. In goats, as in sheep, infection decreased Yc and increased curd Tc, and these changes were reflected in increased CN degradation, increased CN degradation products (p-p), and decreased $a_{Ca^{2+}}$. However, the higher CN content and higher PL activity in sheep than in goats results in a higher output of CN degradation products, including factors that down-regulate milk secretion. This accounts for the stronger effect on milk yield in sheep than in goats. This scenario suggests that in terms of milk yield, sheep are more vulnerable than goats to subclinical infections, though definite conclusions should await the accumulation of additional independent data. The trends in the responses of PA and PL activities to SM are similar in goats and sheep. The unusually high basal PA activity in goats results in a lack of PLG in their milk. Consequently, our results suggest that the PL system functions at a lower rate in goats than in sheep and that its response to external factors, such as infection, that augment its activity in other species is more attenuated in goats.

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