

Interrelationships between the activities of the plasmin system in goats and sheep experiencing subclinical mastitis, casein degradation and milk yield

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Abstract

The present report focuses on how the differences in the plasmin (PL) system between sheep and goats are reflected in their response to subclinical intramammary infection (IMI), particularly in respect to casein (CN) degradation and milk yield (MY). Plasmin is the major proteolytic enzyme in milk, where it is found mostly in its inactive zymogen - plasminogen (PLG), which is activated by plasmin activator (PA). Both plasmin activator (PA) and PL activities in goats in late lactation and goats with subclinical IMI are negatively correlated with MY and the coagulating properties of milk, suggesting that this system is important in goats as well. The higher CN content along with higher PL activity in sheep compared with goats resulted in higher CN degradation products that include factors that down-regulate milk secretion, which explains the more acute response in MY. Sheep are more vulnerable than goats to sub-clinical infections in terms of MY.

Keywords: Subclinical mastitis, goat, sheep, plasmin, milk composition

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Introduction

Mastitis, mainly in its subclinical outward appearance, is the most distressing disease in dairy animals. The proportion of udder halves with subclinical intramammary infection (IMI) in goat and sheep flocks in different countries ranges from 35 to 70%, with various species of coagulase-negative staphylococci (CNS) forming the primary pathogen group in infected glands (Leitner *et al.*, 2003; 2004a; b; c). Milk yield (MY) and milk gross composition were significantly affected by subclinical IMI in goats (Leitner *et al.*, 2004a; c) and sheep (Leitner *et al.*, 2004b). In both species subclinical IMI was associated with increased plasmin (PL) activity in the infected glands (Leitner *et al.*, 2004b; c). These changes were associated with a reduction in MY from the infected gland and with increased measures of casein (CN) degradation, reduced curd yield and increased curd clotting time, indicating that the changes in milk composition negatively affect cheese yield and cheese quality.

Plasmin is the major proteolytic enzyme in milk, where it is found mostly in its inactive zymogen - plasminogen (PLG), which is activated by plasmin activator (PA). However, only residual PLG activity was found in goats along with unusually high PA activity in comparison to the values found in ovine and bovine (Fantuz *et al.*, 2001, Leitner *et al.*, 2004c). Nevertheless, PA and PL activities in goats in late lactation and goats with subclinical IMI were negatively correlated with MY and the coagulating properties of milk, suggesting that this system is important in goats as well.

The present report focuses on how the differences in the PL system between sheep and goats are reflected in their response to subclinical IMI, particularly in respect to CN degradation and MY.

Materials and Methods

Thirty-six Israeli-Assaf dairy sheep and twenty-five Israeli dairy goats (representing various breeds, Saanen, Shami, and crossbreds: Shami x Anglo Nubian, Saanen x Anglo Nubian) in which one udder half was infected with a single species of CNS and the contra-lateral gland was bacteria-free were selected from two flocks (Leitner *et al.*, 2004b; c). The selected goats and sheep were 40 to 120 d after parturition, milked twice daily at 04:00 and 14:00 with post teat dipping. Their daily milk yields exceeded 1.5 L in sheep and

2.5 L in goats. The goats and sheep were kept in an open shelter providing 4-m² shaded slatted floor and 4-m² concrete surfaced yard. A total mixed ration was offered in feeders located in the sheds.

Milk yield, sampling procedures, bacteriological tests, protein, proteose-peptone (p-p) and lactose analyses, activities of PA, PLG and PL, concentrations of free (ionized) calcium ($[Ca^{2+}]$) and calcium activity (a_{Ca}^{2+}) and statistical analysis of the data were determined as described by Leitner *et al.* (2004b).

Results and Discussion

The compared measurements are depicted in Table 1. In sheep, the reduction in MY from the infected glands was 53%, 1.9 times higher than the 30% reduction in MY in the infected glands of goats. In line with these differences, the reduction in lactose concentration in milk of infected glands of sheep was 25% in comparison to the uninfected glands, twice the reduction of 11% found in goats. One of the basic features of mammary secretion is that the total osmotic pressure of the secretion remains approximately constant and equal to that of the blood. As lactose is the main single osmotic component in milk besides mineral salts, the secreted milk volume follows the changes in the secretion of lactose very closely (Shamay *et al.*, 2000a). It may be concluded that the much higher reduction in lactose secretion in the infected glands of sheep (65%) in comparison to goats (37.5%) is the main reason for the more acute response in reduced milk volume in the infected glands of sheep than that of goats.

Table 1 Means \pm s.e. of plasmin (PL), plazminogen (PLG), plasminogen activator (PA), PLG/PL, free (ionized) calcium ($[Ca^{2+}]$), calcium activity (a_{Ca}^{2+}) and proteose peptones (p-p) for sheep (A) and goats (B)

Parameter	Bacteriological status		Effect	
	Uninfected	Infected	Infection	Sheep
PL (unit*/mL)	33.85 \pm 5.1	58.94 \pm 4.8	0.0007	NS
PLG (unit*/mL)	92.2 \pm 8.1	62.5 \pm 5.3	0.001	0.0004
PA (unit*/mL)	148 \pm 29	354 \pm 56	0.0002	0.0091
PLG/PL	3.54	1.04	0.002	NS
$[Ca^{2+}]$ (mmol)	3.52 \pm 0.58	4.14 \pm 0.4	NS	NS
A_{Ca}^{2+} (mmol)	1.01 \pm 0.06	0.70 \pm 0.05	0.002	NS
p-p (mg/mL)	0.98 \pm 0.04	2.42 \pm 0.05	0.0001	NS

Parameter	Bacteriological Status		Effect	
	Uninfected	Infected	Infection	Goat
PL (unit*/mL)	20.32 \pm 2.4	39.81 \pm 6.1	0.0003	0.005
PA (unit*/mL)	3376 \pm 404.1	4334 \pm 565.5	0.05	0.002
A_{Ca}^{2+} (mmol)	1.89 \pm 0.1	1.62 \pm 0.1	0.002	< 0.0001
$[Ca^{2+}]$ (mmol)	4.80 \pm 0.4	5.05 \pm 0.3	NS	NS
p-p (mg/mL)	0.35 \pm 0.05	0.53 \pm 0.05	0.0005	0.0002

* Activity unit: 1 unit is the amount of PL that produces a change in absorbance of 0.1 at 405 nm in 60 min

In goats and sheep the reduction in whey protein secretion was lower than the reduction in MY, with the outcome of increased concentration in the infected glands of the two species. The gross CN concentration in goat milk (28 g/L) resembles that of cow milk and is much lower than in sheep milk (40-46 g/L). Thus, the p-p (boiling resistant peptide formed by CN degradation) concentration in uninfected glands in goats is consistent with a lower CN concentration. Moreover, the increase of p-p in the infected glands as a response to doubled PL activity was still much lower than the corresponding figures in sheep.

Silanikove *et al.* (2003) found a negative linear relationship between CN concentration in the milk of humans, goats, cows, sheep and mice and a_{Ca}^{2+} , a finding which is consistent with the fact that CNs are powerful Ca chelators. In both species, a_{Ca}^{2+} was related to measures of proteolysis (p-p content), reflecting the release of phosphorilated p-p with Ca chelating properties from CN. Thus, the lower increase of p-p and

lower reduction in a_{Ca}^{2+} in the infected glands of goats than in the infected glands of sheep indicate lower degradation of CN in goats than in sheep.

In goats, PLG activity was close to zero and may be explained by unusual high PA activity, consistent with the report of Fantuz *et al.* (2001) and Baldi *et al.* (2002). Silanikove *et al.* (2000) proposed that regulation of PA activity serves as a bridge between systemic hormonal influences and the local regulatory system and that CN degradation products down-regulate milk secretion. Thus, the high basal level of PA in goats may make the system irresponsive to systemic effects. In line 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 MY (Shamay *et al.*, 2000a), as is the case with dairy cows (Shamay *et al.*, 2000b).

Conclusions

The higher CN concentration along with higher PL activity in sheep compared with goats resulted in higher CN degradation products that include factors that down-regulate milk secretion, which explains the more acute response in MY. Accordingly, sheep are more vulnerable than goats to sub-clinical infections in terms of MY, though definite conclusions should wait for accumulation of additional independent data.

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