

## Changes in Milk Composition as Affected by Subclinical Mastitis in Sheep

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### ABSTRACT

The mechanism of the effects of glandular-level subclinical mastitis in dairy sheep on milk yield and on its composition as expressed in curd yield was studied. Thirty-six Israeli-Assaf dairy sheep with one udder half infected with identified coagulase-negative staphylococci and the contralateral gland free of bacteria were chosen. The milk yield of the infected halves was significantly lower than that of the uninfected ones (0.36 vs. 0.76 kg/milking). The somatic cell count and *N*-acetyl- $\beta$ -D-glucosaminidase activity were significantly higher in the infected halves than in the uninfected ones. The plasminogen activator and plasmin (PL) activities were significantly higher in the infected glands than in the uninfected ones, whereas plasminogen (PLG) activity and the ratio PLG:PL were significantly lower in the infected glands. Concentrations of  $\text{Ca}^{2+}$  did not differ, whereas  $\text{Ca}^{2+}$  activity was significantly lower and proteose peptone concentration was 2.4 times as high in the infected glands than in the uninfected ones. Curd yield was significantly lower in the infected glands than in the uninfected ones.

**(Key words:** milk composition, sheep, subclinical mastitis)

**Abbreviation key:**  $\text{a}_{\text{Ca}^{2+}}$  = calcium activity, **NAGase** = *N*-acetyl- $\beta$ -D-glucosaminidase, **p-p** = proteose peptones, **PA** = plasminogen activator, **PL** = plasmin, **PLG** = plasminogen, **Tc** = clotting time, **Yc** = curd yield.

### INTRODUCTION

Udder infection in dairy sheep has major negative effects on both yield and quality of milk, and leads to

greater economic losses than those reported for dairy cattle (Watson and Buswell, 1984). In an earlier study on 1000 sheep from 20 Israeli dairy flocks, we found that the milk yield of the uninfected udder halves was significantly higher than that of the infected halves, whereas fat and total protein contents were lower in the uninfected halves than in the infected ones (Leitner et al., 2003a). However, a direct comparison between infected and uninfected glands was not possible in that study because the measurements were done on the animal level and not on the single-gland level. Also, in that study, we measured only total protein content. That information was insufficient in the case of dairy sheep because almost all of the milk was processed into cheese; thus, any change in total casein concentration would have a disproportionate effect on the industrial value of the milk.

The present study focused on gaining better insight into how subclinical mastitis on the glandular level in dairy sheep affects milk yield and the milk composition as expressed in curd yield (**Yc**). In order to achieve this goal, we chose animals in which one udder half was infected with identified CNS species and the contralateral gland was free of bacteria. In each gland, we analyzed inflammation indices, total milk protein subdivided into CN and whey protein, plasmin (**PL**) activity, and measures of proteolysis. The PL system was analyzed because PL is the main proteolytic enzyme in milk and because PL was found to be associated with enhanced CN degradation in subclinically mastitic dairy cows. Plasmin is found in milk mostly as the inactive zymogen plasminogen (**PLG**), which is activated by plasminogen activators (**PA**) (Politis, 1996).

### MATERIALS AND METHODS

#### Animals

Thirty-six Israeli-Assaf dairy sheep with one udder half infected with a single species of CNS and the contra-

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lateral gland bacteria-free were selected from 2 flocks. Milk samples from each udder half were tested for bacterial infection, SCC and *N*-acetyl- $\beta$ -D-glucosaminidase (NAGase) activity, in 3 consecutive weekly examinations. The sheep were selected 40 to 120 d after lambing and their daily milk yield exceeded 1.5 L. In both farms, the sheep were milked twice daily at 0400 and 1400 h, with teat postdipping only. Animals were kept in an open shelter that provided 4 m<sup>2</sup> of shaded slatted floor and 4 m<sup>2</sup> of concrete-surfaced yard for each sheep. Food was offered in mangers located in the sheds.

### Milk Sampling and Analysis

Milk yield was measured and sampling procedures were carried out during the morning milking. Milking was by hand and the yield was determined by weighing the milk of each udder half of each sheep individually. For the bacteriological tests, NAGase activity measurement and SCC determination with a Coulter counter model Z1 (Coulter Electronics Ltd., Beds, UK), the udder halves were cleaned, disinfected, sampled, and analyzed as described by Leitner et al. (2003a). Three additional sets of samples were taken from each udder half and distributed for analysis as follows: one set was preserved with Broad Spectrum Microtabs II (D & F Control Systems, Inc., Dublin, CA) and was sent to the Israel Cattle Breeders Association Central Laboratory (Caesarea, Israel) for analysis of milk gross composition (protein, fat, and lactose) with the Milkoscan 6000, and of SCC with a Fossomatic 360 (Foss Electric, Hillerød, Denmark). The second set was defatted, and the skim milk was subjected to analysis of the concentrations of casein, total whey protein, albumin, and proteose peptones (**p-p**), as described by Shamay et al. (2000, 2003), and measurement of the activities of PA, PLG, and PL, according to Silanikove et al. (2000). In these samples, within 5 h after sampling, the concentrations of free (ionized) calcium ([Ca<sup>2+</sup>]) was determined by the repeated addition procedure and the calcium activity (**a**<sub>Ca<sup>2+</sup></sub>) by the uncorrected procedure, with a calcium-specific electrode (Silanikove et al., 2003). The third set of samples was used to determine the percentage of curd and Yc, calculated as the percentage of curd multiplied by milk yield. Clotting time (**Tc**) was performed according to Berridge (1952). The curd percentage was determined by a modification of the method described by Calvo and Balcones (1998), with Fromase 15 TL (Gist-Brocades nv, Delft, The Netherlands) used as the coagulating enzyme. The modifications included using 5 mL of the fresh milk, the addition of lactic acid to imitate the culture growth, and weighing the pellet after centrifugation at 1650  $\times$  g.

### Bacteriological Examinations

Bacteriological analysis was performed according to accepted standards (Hogan et al., 1999). From every milk sample, 0.01-mL subsamples were spread onto blood-agar plates (Bacto-Agar; Difco Laboratory, Detroit, MI) containing 5% of washed sheep red blood cells, and onto 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) (Anilab, 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. If the percentage fell below 90%, the strain was recorded as unidentified CNS.

### Statistical Analysis

Data were analyzed with the SAS Software Release 8.2 (SAS Inst., Inc., Cray, NC) with the Proc GLM and Proc CORR procedures. Dependent variables were SCC and Log SCC (Coulter counter), SCC and Log SCC (Fossomatic 360), NAGase, milk, fat, protein, lactose, CN, whey proteins, albumin, p-p, Ca, PA, PLG, PL, curd, Tc, and the calculated values of CN:protein, whey proteins:protein, and PLG:PL ratios and Yc. The independent variables—bacteriological status and sheep—were examined according to the model:

$$Y_{ij} = u + B_i + S_j + e_{ij},$$

where  $Y_{ij}$  = dependent variable,  $u$  = overall mean,  $B_i$  = bacteriological status,  $i = 1$  (infected) or 2 (uninfected),  $S_j$  = sheep,  $j = 1 \dots 36$ ,  $e_{ij}$  = error term.

No significant difference in the significance level was found between the results of the analyses based on SCC and those based on log SCC; therefore, the results presented are those based on the arithmetic SCC.

## RESULTS

The distribution of CNS species causing udder infections was as follows: *Staphylococcus chromogenes* (11), *Staphylococcus simulans* (10), *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and *Staphylococcus xylosus* (5 each). No clinical abnormalities were visible or palpable in more than 50% of the sheep tested. In the remainder, infected halves were smaller and exhibited some degeneration. However, there were no significant differences in any of the dependent variables between halves with visible and those with invisible clinical abnormalities. In our previous study (Leitner et al., 2003a) on a large data set, all of the CNS species induced

**Table 1.** Mean values and SE of bacteriological status vs. the various independent variables and its effects.

Parameter	Bacteriological status		Effect		
	Uninfected	Infected	Infection		Sheep
			P Estimate		
Milk, kg/milking	0.76 ± 0.04	0.36 ± 0.03	0.0001	-0.4	0.0009
SCC ± SE (× 1000)	270 ± 38	2358 ± 278	0.0001	2087	NS
Coulter counter					
SCC ± SE (× 1000) (Fossomatic)	311 ± 37	4999 ± 1219	0.0004	4688	NS
NAGase <sup>1</sup>	22.3 ± 1.91	95.5 ± 9.82	0.0001	73.2	NS
Fat, g/L	64.9 ± 0.26	61.7 ± 0.21	0.05	-3.2	0.0004
Protein, g/L	58.5 ± 0.07	53.5 ± 0.10	0.0009	-5.1	0.0001
Lactose, g/L	44.7 ± 0.08	33.5 ± 0.16	0.0001	-11.2	0.02
Whey, g/L	11.9 ± 0.38	12.8 ± 0.16	0.0731	0.85	0.03
Casein, mg/mL	45.9 ± 1.36	40.5 ± 1.59	0.0002	-5.5	0.0001
Albumin, µg/mL	517 ± 31	759 ± 59	0.0047	268.7	0.0568

<sup>1</sup>NAGase = *N*-Acetyl- $\beta$ -D-glucosaminidase.

an increase of SCC to approximately  $10^6$  cells/mL, which is consistent with the present results. Thus, the question of visibility or invisibility of abnormalities was disregarded, as statistical analysis was applied to clinical abnormalities and CNS species.

Milk yield of the infected halves (0.36 kg/milking) was significantly lower ( $P < 0.001$ ) than that of the uninfected halves (0.76 kg/milking) (Table 1). The indicators of infection response (SCC and NAGase activity) were significantly higher ( $P < 0.0001$ ) in the infected halves than in the uninfected ones. The correlation coefficient ( $r$ ) between the measurement of SCC with the Coulter counter and with the Fossomatic was 0.81, the values being higher with the Fossomatic than with the Coulter counter, mainly in the infected halves. Sheep effects on these parameters were not significant.

The concentrations of milk components in the infected halves were significantly lower than those in the uninfected halves, as follows: fat ( $61.7 \pm 0.21$  vs.  $64.9 \pm 0.26$  g/L), protein ( $53.5 \pm 0.11$  vs.  $58.5 \pm 0.07$  g/L), lactose ( $33.5 \pm 0.16$  vs.  $44.7 \pm 0.08$  g/L), and casein ( $40.5 \pm 1.59$  vs.  $45.9 \pm 1.36$  g/L). In contrast, the concentrations of total whey proteins and albumin were significantly higher in the infected than in the uninfected halves:  $12.8 \pm 0.16$  vs.  $11.9 \pm 0.38$  g/L and  $759 \pm 59$  vs.  $517 \pm 31$  µg/L, respectively (Table 1). The sheep effects on these variables were significant (Table 1). In comparison with the uninfected halves, the CN:total protein ratio was significantly lower ( $P < 0.01$ ) in the infected halves, whereas the whey proteins:total proteins ratio was significantly higher ( $P < 0.004$ ) (Figure 1).

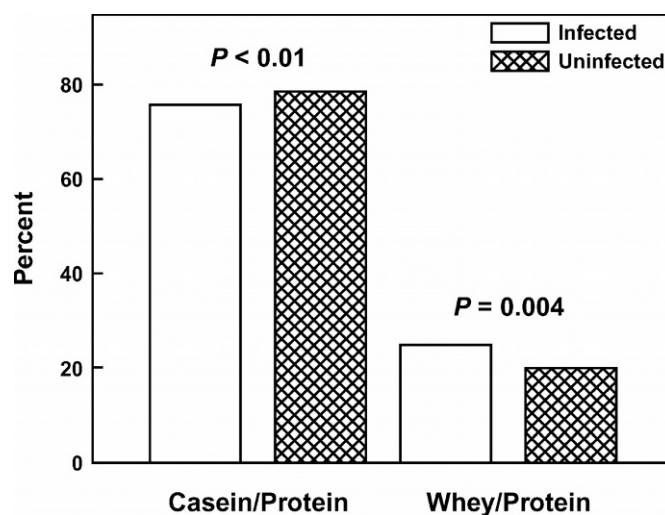
The PA and PL activities were significantly higher in the infected glands than in the uninfected ones, whereas PL + PLG was similar, and PLG activity and the PLG:PL ratio were significantly lower in the infected glands (Table 2). The data clearly indicate that the increase in PL activity in the infected glands was due to conversion of

PLG to PL by PA without an apparent increase in total PL + PLG activities.

Concentrations of  $[Ca^{2+}]$  did not differ, whereas  $a_{Ca^{2+}}$  was significantly lower ( $P < 0.002$ ) and the p-p concentration was 2.4 times as high ( $P < 0.0001$ ) in the infected glands than in the uninfected ones (Table 2).

The percentage of curd in the infected glands (34.81) tended ( $P < 0.1$ ) to be lower than that in the uninfected glands (36.40), but the curd yield in the infected glands (13.9 g/milking) was significantly lower ( $P < 0.0001$ ) than that in the uninfected ones (30.1 g/milking) (Figure 2). The sheep effect on this variable was significant ( $P < 0.002$ ). The curd clotting time in the infected halves (909 s) was significantly longer ( $P < 0.0001$ ) than that in the uninfected ones (413 s) (Figure 2).

A matrix of correlations among milk components is presented in Table 3. Milk yield was negatively related



**Figure 1.** Casein/protein and whey proteins/protein ratios in milk of infected vs. uninfected udder halves.



**Table 2.** Means  $\pm$  SE of plasmin (PL), plasminogen (PLG), PLG/PL, plasminogen activator (PA), free (ionized) calcium ( $[Ca^{2+}]$ ), calcium activity ( $a_{Ca^{2+}}$ ), and proteose peptones (p-p) and their effects.

Parameter	Bacteriological status		Effect		
	Uninfected	Infected	Infection	Sheep	
			P Estimate		
PL, U/mL	33.9 $\pm$ 5.1	58.9 $\pm$ 4.8	0.0007	25.1	NS
PLG, U/mL	92.2 $\pm$ 8.1	62.5 $\pm$ 5.3	0.001	-29.7	0.0004
PA, U/mL	148 $\pm$ 29	354 $\pm$ 56	0.0002	206	0.0091
PL+PLG, U/mL	126.05	121.44	NS	-4.59	NS
PLG/PL	3.54	1.04	0.002	-2.5	NS
$[Ca^{2+}]$ , mmol	3.52 $\pm$ 0.58	4.14 $\pm$ 0.41	NS	0.62	NS
$a_{Ca^{2+}}$ , mmol	1.01 $\pm$ 0.06	0.70 $\pm$ 0.05	0.002	-0.32	NS
p-p, mg/mL	0.98 $\pm$ 0.01	2.42 $\pm$ 0.12	0.0001	1.4	NS

to PA and PL, and positively, but not significantly so, to PLG and the PLG:PL ratio; it was also negatively related to the indices of proteolysis (p-p and p-p:CN). The SCC was positively related to PA and PL, and negatively to the PLG:PL ratio; it was also negatively related to  $a_{Ca^{2+}}$  and positively to p-p and the p-p:CN ratio. Lactose was negatively related to PA, p-p, and the p-p:CN ratio.

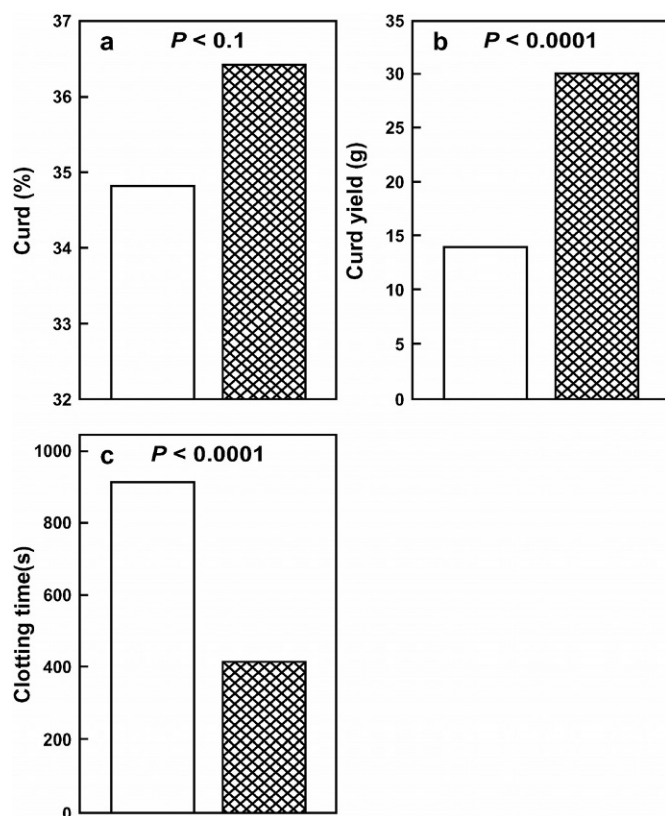
The whey proteins content was positively related to PA, PL, PLG, p-p, and the p-p:CN ratio, whereas the casein content was negatively related to PA, PL, p-p, and the p-p:CN ratio, but positively related to the PLG:PL ratio.

## DISCUSSION

### Infection and Milk Yield

The CNS bacteria, mainly *S. epidermidis* and *S. chromogenes*, are the most abundant bacterial isolates that are associated with subclinical mastitis in sheep flocks in various countries (Fthenakis, 1994; Gonzalez-Rodriguez et al., 1995; Las Heras et al., 1999; Leitner et al., 2001, 2003a). In most countries, CNS in small ruminants are not considered by veterinarians to be cases requiring treatment, for 2 main reasons: 1) in most cases, CNS infections do not develop sufficiently to elicit clinical symptoms; therefore, it is assumed that the negative effects on milk yield are minor; and 2) payment schemes and milk grading based on SCC, designed to ensure the proper hygiene of milk in the dairy sheep and goats industry, are still under consideration (unlike the regulations—long-established in most developed countries—for cow's milk). However, the high SCC and high *NAGase* activity found in the infected glands in the present study are consistent with previous findings in sheep (Leitner et al., 2001, 2003a) and goats (Leitner et al., 2003b), indicating that the response to IMI with respect to SCC and *NAGase* activity is more acute in these species than in cows.

Intramammary infection, even if restricted to subclinical levels, has been reported to affect the milk yield of sheep negatively (McCarthy et al., 1988; Fthenakis et al., 1991; Leitner et al., 2003a). However, quantifying this effect is difficult because 1) in most cases, only one gland is infected; therefore, the effect is diluted when measurements are made on a whole-animal basis; and 2) individual animal variability, breed (in the case of mixed breeds in the same flock), age, and stage of lacta-



**Figure 2.** Effect of subclinical mastitis on percentage of curd (panel a), calculated curd yield  $Y_c$  (panel b), and clotting time  $T_c$  (panel c), in milk of infected (□) vs. uninfected (■) udder halves.  $Y_c = \text{milk, kg} \times \% \text{curd/milking/udder half}$ .

**Table 3.** Matrix of correlations among milk yield, milk composition (lactose, whey, casein), and indices of inflammation (SCC, PL system) and indices of proteolysis (calcium activity [ $a_{Ca^{2+}}$ ] proteose peptones [p-pl]).

Item	PA	PL	PLG	PLG/PL	$a_{Ca^{2+}}$	p-p	p-p:CN
Milk	-0.239	-0.301				-0.362	-0.338
<i>P</i>	0.05	0.01	NS	NS	NS	0.002	0.005
SCC	0.492	0.392		-0.332	-0.499	0.501	0.440
<i>P</i>	<0.0001	0.0009	NS	0.048	0.001	<0.0001	0.0002
Lactose	-0.657					-0.577	-0.631
<i>P</i>	0.0002	NS	NS	NS	NS	0.002	0.0004
Whey	0.477	0.279	0.509			0.291	0.297
<i>P</i>	<0.0001	0.02	0.001	NS	NS	0.01	0.01
Casein	-0.263	-0.423		0.428		-0.344	-0.557
<i>P</i>	0.03	0.0004	NS	0.009	NS	<0.004	<0.0001
PA		0.509		-0.408	-0.464	0.266	0.314
<i>P</i>		<0.0001	NS	0.01	0.003	0.03	0.009
PL	0.509			-0.454	-0.404	0.390	0.508
<i>P</i>	<0.0001		NS	0.005	0.01	0.001	<0.0001
PLG				0.665			
<i>P</i>	NS	NS		<0.0001	NS	NS	NS
PLG/PL	-0.408	-0.454	0.665		0.331	-0.337	-0.321
<i>P</i>	0.01	0.005	<0.0001		0.05	0.04	<0.05
$a_{Ca^{2+}}$	-0.464	-0.404		0.331		-0.426	-0.415
<i>P</i>	0.003	0.01	NS	0.05		0.008	0.009

tion introduce considerable variability. In addition, the practice of milk recording in sheep still lags behind that applied to cattle.

The use of the half-udder as the experimental unit enabled us to quantify the negative effect of subclinical mastitis on milk yield with high statistical reliability, with a data set of 36 animals, compared with approximately 100, which would most likely be required with a conventional approach. Our previous data (Leitner et al., 2003a) clearly demonstrated the association between CNS infection and milk yield. However, a comparison between a large data set based on sheep in which one gland was infected and the contralateral one was free of bacteria, with a set based on sheep in which both glands were free of bacteria, revealed only a small difference in milk yield, whereas a significantly lower yield was found in sheep in which both glands were infected. These findings suggest that when one gland is infected, the contralateral gland compensates for the reduced milk yield from the infected gland. On the other hand, when both glands are infected, milk yield losses are severe (Leitner et al., 2003a). Thus, the assumption that subclinical CNS infection affects milk yield only moderately is justified if only one gland is infected and not if both are.

The current situation, in which relatively little effort is invested in preventing bacterial infection in dairy sheep flocks in most countries, will change if their milk comes to be graded according to SCC and farmers are made to pay penalties for low-quality milk. Moreover, in the case of dairy goats and sheep, most if not all of the milk is processed into cheese; therefore, any change in the DM—mainly CN—concentration, will have an amplified in-

dustrial influence, as will be shown in the following sections relating to milk composition and curd yield.

#### Milk from Halves Infected with Subclinical Mastitis

The increase in the index of inflammation (SCC) is correlated with a greater potential for proteolysis (activation of the PA-PLG-PL system) and, consequently, with increased proteolysis indexes (p-p, p-p:CN, and  $a_{Ca^{2+}}$ ). The present finding of an increase in the activity of the PL system in glands infected with subclinical mastitis is consistent with previous findings in dairy cows (Schaar and Funke, 1986; Auldust et al., 1996; Urech et al., 1999). However, the increase in PL activity and the reduction in the PLG:PL ratio were much more acute in sheep than in cows; in the latter, similar responses were recorded only in quarters with severe subclinical mastitis.

The correlations between PL activity and measures of proteolysis were significantly positive, in agreement with previous findings in dairy cows (Le Roux et al., 1995; Urech et al., 1999). Thus, PL activity appears to be the major proteolytic enzyme in the milk of sheep. The more acute activation of the PL system in sheep in comparison with cows is most likely associated with more pronounced changes in curd yield and coagulation time than those in cows.

In dairy cows, both PL activity in the mammary gland and the content of PLG increased as SCC increased (Politis et al., 1990). On the other hand, at late lactation, the source of increased PL activity is accelerated conversion of PLG to PL without an increase of PLG content in the gland (Politis, 1996). Thus, the mechanism for

increased PL activity in sheep is consistent with the mechanism found in cows at late lactation and differs from that found in mastitic cows. One possible explanation for these species differences is the difference in the response to clinical (Politis et al., 1990) and subclinical mastitis (present study). It is possible that the tight junction opening, and consequently leaking of PLG from systemic fluids, in subclinical cases is lower than in clinical cases. Another possible explanation is that species differences in extent of tight junction opening during intramammary infection and/or differences related to the acuteness of SCC influx to the gland. Heegaard et al. (1994) and Zachos et al. (1992) found a large increase in PA activity in mastitic cows in close association to bovine neutrophils. Thus, the more acute the influx of SCC to the mammary gland in sheep in subclinical mastitis in comparison with cows may be associated with increased leukocyte-related PA activity, and consequently with increased conversion of PLG to PL. Notwithstanding, the leukocyte-related PA activity (mostly urokinase-PA) was not detected in the present experiment because most of the leukocytes were discarded during the preparation of the samples and because urokinase-PA is closely associated to the leukocytes through urokinase-PA receptor (Politis et al., 2002a, 2002b).

### Calcium Activity ( $a_{Ca^{2+}}$ ) as a Measure of Casein Degradation

The  $a_{Ca^{2+}}$  was negatively related to measures of proteolysis (p-p, p-p:CN). The activity of ions in solution is affected by the presence of a chelator in that solution. Silanikove et al. (2003) demonstrated a negative linear relationship between CN concentrations in the milk of humans, goats, cows, sheep, and mice on the one hand, and  $a_{Ca^{2+}}$ , a finding that is consistent with the fact that CN are powerful Ca chelators. The association between CN degradation and reduction in  $a_{Ca^{2+}}$  may be accounted for by 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 represent the additional CN degradation in the infected glands. Because the measurement of  $a_{Ca^{2+}}$  is rapid and cheap, it appears to be promising as a valuable tool to monitor the extent of CN degradation under various conditions.

### Curd Yield and Milk Clotting Time

The conversion of casein to whey components (p-p) by PL accounts for the reduction in curd yield. In addition,

a positive significant correlation was observed between both PL and PA activities on the one hand, and rennet clotting time on the other hand, which is consistent with similar interactions found in late-lactating goats (Fantuz et al., 2001).

Curd yield was lower in the infected halves than in the uninfected ones. This phenomenon is directly related to the finding of lower casein content and higher p-p content in the milk, the latter being an indicator of casein breakdown. These findings reveal the losses that are not indicated by measurements of milk yield, and emphasize the severity of the effects of subclinical mastitis on cheese production through its effect on Yc. In addition to the direct loss of curd, in terms of volume, as presented above, Tc provides indirect evidence of poor milk quality since it is common to determine milk-clotting activity according to the rapidity with which the enzyme clots milk under a set of specified conditions. Moreover, the secondary, nonenzymatic phase of the aggregation of the casein micelles, which follows the first enzymatic phase, is particularly susceptible to variations in milk composition and to the presence of added salts (Ernstrom and Wong, 1974), and probably also to  $a_{Ca^{2+}}$ . The latter is probably influenced by the compositional changes caused by the subclinical infection, as manifested in  $a_{Ca^{2+}}$  and calcium concentration. The observed reduction in  $a_{Ca^{2+}}$  suggests that Ca ions were less available to induce their procoagulating effect on para- $\kappa$ -CN, most likely because of their close association with phosphorylated p-p. Thus, high PL activity in mastitic sheep negatively affects curd yield, both directly and indirectly, through its effect on the coagulation properties of the milk, most probably via the degradation of CN and the consequent modifications of  $a_{Ca^{2+}}$ .

### Interrelationships Between Milk Yield and Composition in Subclinically Mastitic Sheep

Enzymatic hydrolysis of CN liberates peptides that serve as local regulators of mammary gland function (Silanikove et al., 2000; Shamay et al., 2002, 2003). A peptide derived from the activity of PL on  $\beta$ -CN ( $\beta$ -CN 1-28) 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). This peptide reduces the output of lactose and other osmotic components from the alveoli into the gland lumen (Silanikove et al., 2000), which may result in increased concentrations of protein and fat, whose secretion was not affected when the activation of the PL system was relatively moderate (30 to 50% increase over the basal levels) (Shamay et al., 2000). This phenomenon was probably observed in previous



studies in sheep (Leitner et al., 2003b), goats (Leitner et al., 2003a), and cows (Urech et al., 1999), where the reduction in milk yield was modest and protein and fat concentrations were higher in infected animals than in uninfected ones. When the increase in PL activity is high (increase of 150% or more), as is the case during milk stasis or inflammation, CN hydrolysis in goats (Shamay et al., 2002) and cows (Shamay et al., 2003) induces rapid drying-off of mammary secretions, which is associated with lower secretion of fat and protein. Thus, the degree of PL activation dictates not only the reduction of milk volume, but also the changes in the secretion of organic components and, consequently, in the milk composition, as was found in the present study.

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### REFERENCES

- Auldust, M. J., S. Coats, J. B. Sutherland, J. J. Mayes, and H. G. McDowell. 1996. Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of cheddar cheese. *J. Dairy Res.* 63:269–280.
- Berridge, N. J. 1952. Some observations on the determination of the activity of rennet. *Analyst* 77:57–62.
- Calvo, M. M., and E. Balcones. 1998. Influence of heat treatment on rennet clotting properties of mixtures of cow's, ewe's, and goat's milk and on cheese yield. *J. Agric. Food Chem.* 46:2957–2962.
- Ernstrom, C. A., and N. P. Wong. 1974. Milk clotting enzymes and cheese chemistry. Pages 662–771 in *Fundamentals of Dairy Chemistry*. H. B. Webb, A. H. Johnson, and J. A. Alford, ed. Avi Publ. Co., Inc., Westport, CT.
- Fantuz, F., F. Plidori, F. Cheli, and A. Baldi. 2001. Plasminogen activation system in goat milk and its relation with composition and coagulation properties. *J. Dairy Sci.* 84:1786–1790.
- Fthenakis, G. C. 1994. Prevalence and aetiology of subclinical mastitis in ewes of Southern Greece. *Small Rumin. Res.* 13:293–300.
- Fthenakis, G. C., E. T. El-Masannat, J. M. Booth, and J. E. T. Jones. 1991. Somatic cell count of ewes' milk. *Br. Vet. J.* 147:575–581.
- Gonzalez-Rodriguez, C. M., C. Gonzalo, F. S. Primitivo, and P. Carmenes. 1995. Relationship between somatic cell count and intramammary infection of the half udder in dairy ewes. *J. Dairy Sci.* 78:2753–2759.
- Heegaard, C. W., T. Christensen, M. D. Rasmussen, C. Benfeldt, N. E. Jensen, K. Sejrsen, T. E. Petersen, and P. A. Andreasen. 1994. Plasminogen activators in bovine-milk during mastitis, an inflammatory disease. *Fibrinolysis* 8:22–30.
- Hogan, S. J., N. R. Gonzales, J. R. Harmon, S. C. Nickerson, P. S. Oliver, J. W. Pankey, and K. L. Smith. 1999. *Laboratory Handbook on Bovine Mastitis*. rev. ed. National Mastitis Council, Inc., Madison, WI.
- Las Heras, A., L. Dominguez, and J. F. Fernandez-Garayzabal. 1999. Prevalence and aetiology of subclinical mastitis in dairy ewes of the Madrid region. *Small Rumin. Res.* 32:21–29.
- Le Roux, Y., O. Colin, and F. Laurent. 1995. Proteolysis in samples of quarter milk with varying somatic cell counts: 1. Comparison of some indicators of endogenous proteolysis in milk. *J. Dairy Sci.* 78:1289–1297.
- Leitner, G., M. Chaffer, Y. Caraso, E. Ezra, D. Kababea, M. Winkler, and A. Saran. 2003a. Udder infection and milk somatic cell count, NAGase activity and milk composition—fat, protein and lactose—in Israeli Assaf and Awassi sheep. *Small Rumin. Res.* 49:157–164.
- Leitner, G., M. Chaffer, S. Zamir, T. Mor, A. Glickman, M. Winkler, L. Weisblit, and A. Saran. 2001. Udder disease etiology, milk somatic cell count and NAGase activity in Israeli Assaf sheep throughout lactation. *Small Rumin. Res.* 39:107–112.
- Leitner, G., U. Merin, N. Silanikove, E. Ezra, M. Chaffer, N. Gollop, M. Winkler, A. Glickman, and A. Saran. 2003b. Effect of subclinical bacterial contaminations on somatic cell counts, NAGase activity and gross composition of goat's milk. *J. Dairy Res.* (accepted)
- McCarthy, F. D., J. B. Lyndsey, M. T. Gore, and D. R. Notter. 1988. Incidence and control of sub-clinical mastitis in the intensively managed ewes. *J. Anim. Sci.* 66:2715–2721.
- Politis, I. 1996. Plasminogen activator system: Implication for mammary cell growth and involution. *J. Dairy Sci.* 79:1097–1107.
- Politis, I., I. Bizelis, and E. Rogdakis. 2002. The urokinase-plasminogen activator system in ovine macrophages and neutrophils. *Small Rumin. Res.* 44:17–23.
- Politis, I., E. Block, and J. D. Turner. 1990. Effect of somatotropin on the plasminogen and plasmin system in the mammary-gland—proposed mechanism of action for somatotropin on the mammary-gland. *J. Dairy Sci.* 73:1494–1499.
- Politis, I., B. Zavizion, F. Cheli, and A. Baldi. 2002. Expression of urokinase plasminogen activator receptor in resting and activated bovine neutrophils. *J. Dairy Res.* 69:195–204.
- Schaar, J., and H. Funke. 1986. Effect of subclinical mastitis on milk plasminogen and plasmin compared with that on sodium, antitrypsin and N-acetyl-D-glucosaminidase. *J. Dairy Res.* 53:515–528.
- Shamay, A., S. J. Mabjeesh, and N. Silanikove. 2002. Casein-derived phosphopeptides disrupt tight junction integrity, and precipitously dry up milk secretion in goats. *Life Sci.* 70:2707–2719.
- Shamay, A., F. Shapiro, H. Barash, I. Bruckental, and N. Silanikove. 2000. Effect of dexamethasone on milk yield and composition in dairy cows. *Ann. Zootech.* 49:343–352.
- Shamay, A., F. Shapiro, G. Leitner, and N. Silanikove. 2003. Infusion of casein hydrolyzates into the mammary gland disrupt tight junction integrity and induce involution in cows. *J. Dairy Sci.* 86:1250–1258.
- Silanikove, N., A. Shamay, D. Sinder, and A. Moran. 2000. Stress down regulates milk yield in cows by plasmin induced  $\beta$ -casein product that blocks K<sup>+</sup> channels on the apical membranes. *Life Sci.* 67:2201–2212.
- Silanikove, N., F. Shapiro, and A. Shamay. 2003. Use of an ion-selective electrode to determine free Ca ion concentration in the milk of various mammals. *J. Dairy Res.* 70:241–243.
- Urech, E., Z. Puhon, and M. S. Schalibaum. 1999. Changes in milk protein fraction as affected by subclinical mastitis. *J. Dairy Sci.* 82:2402–2411.
- Watson, D. J., and J. F. Buswell. 1984. Modern aspects of sheep mastitis. *Br. Vet. J.* 140:529–534.
- Zachos, T., I. Politis, R. C. Gorewit, and D. M. Barbano. 1992. Effect of mastitis on plasminogen-activator activity of milk somatic-cells. *J. Dairy Res.* 59:461–467.