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Effects of glandular bacterial infection and stage of lactation on milk clotting parameters: Comparison among cows, goats and sheep

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ABSTRACT

Effects of glandular bacterial infection and stage of lactation on milk quality were compared among cows, goats and sheep. These parameters affected milk quality in all three species: sheep were most affected by bacterial infection and goats by stage (particularly end) of lactation. The study highlighted the effectiveness of lactose level as a predictor of milk quality; in all three species, the correlation between lactose level and curd firmness (CF) was higher than those between casein as a percentage of total protein and CF, or between somatic cell count and CF. In all three species, lactose concentrations $\leq$4% were associated with non-clotting milk. A model that describes the simultaneous and close association between reductions in lactose concentration and milk yield, on the one hand, and reductions in lactose concentration and milk quality on the other hand, is presented.

1. Introduction

High-quality raw milk constituents are required to obtain high yields and good quality of dairy products such as yoghurt and cheese. However, there are variations in milk yield and composition, not only between, but also within species, because of diversity among genotypes, management practices, stage of lactation, etc., and also interactions among these factors. One of the most common problem influencing production in dairy animals is mastitis, in both its clinical and its subclinical forms (Halasa, Huijps, Osteras, & Hogeveen, 2007; Leitner et al., 2004a; Leitner, Krifucks, Merin, Lavi, & Silanikove, 2006; Leitner, Merin, & Silanikove, 2004b). Subclinical mastitis, which is generally unnoted, results in impaired quality of milk intended for yoghurt and cheese production (Forsback, Lindmark-Mansson, Andren, Akerstedt, & Svennersten-Sjaunja, 2009; Merin et al., 2008). Nevertheless, cows with subclinical mastitis are frequently associated with moderate increase in somatic cell counts (SCCs) (Auldist, Coats, Sutherland, Mayes, & McDowell, 1996; Lindmark-Mansson, Branning, Alden, & Paulsson, 2006) and thus may contribute to overall lower quality of bulk tank milk, which indicates that SCC level is not a predictive factor for quality in milk with low SCC (Forsback, Lindmark-Mansson, Andren, & Svennersten-Sjaunja, 2010; Leitner et al., 2008b). It is rare that all the glands of a given animal are subclinically infected with bacteria. Therefore, it could be worthwhile to separate the low-quality milk obtained from individual infected glands, because this may be of economic importance in modern dairy farming, in which milk payment is dependent on bulk milk SCC (Forsback et al., 2010).

Another major factor that affects milk quality is stage of lactation: milk composition changes markedly during lactation, with regard to its basic components, micelle structure, and salt equilibrium and, consequently, its technological and physicochemical properties (Coulon, 1994; Lucey & Fox, 1992). Thus, these changes affect the yield and quality of the resulting cheese (Kefford, Christian, Sutherland, Mayes, & Grainger, 1995; Lucey, 1996). Early-lactation milk tends to have good coagulability by rennet (White & Davies, 1958) whereas, in contrast, late-lactation milk is considered less suitable for cheese manufacture, mainly because of defects in syneresis of the curd (O'Keeffe, 1984).

Many of the animals that carry subclinical chronic infections or are close to the end of the lactation are not identified because there are no recognizable symptoms and the milk appears normal. Routine milk testing, such as California mastitis test (CMT) on the farm or more advanced laboratory techniques such as use of sophisticated cell counters enable identification of subclinically infected animals soon after they acquire the infection. However, these methods are laborious and/or require special equipment; also, in many cases, they identify the infected animals long after they become infected. Thus, the possibility of taking a real-time

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rational decision to separate low-quality milk would depend on availability of on-line technology to recognize such changes.

Modern dairy farms are characterized by a high level of computerized data acquisition, which provides on-line information on each cow's milk yield, milk composition etc. (Katz et al., 2007). In particular, the AfiLab milk analyzer provides information on SCC, lactose, and total protein. This information has been found to be relevant in classifying the quality of milk at the individual gland and the whole animal udder level, with regard to milk clotting properties and cheese production (Leitner et al., 2004a, 2004b; Merin et al., 2008).

The aim of the present study was to evaluate the influence of the stage of lactation and udder condition on milk quality by defining the influence of SCC and milk constituents on milk clotting parameters. It comprised a comparative study that addressed cows, goats, and sheep.

2. Materials and methods

2.1. Animals

Animals entered the study were at two stages in their lactation: mid-lactation (ML) and late-lactation (LL). Of the ML animals, ~50% were infected (ML-I) at least in one gland by various coagulase-negative staphylococci (CNS) while the other were uninfected (ML-F). LL animals were always free of infection. Milk from each gland was tested for SCC and bacteria three times, at 1-week intervals.

2.1.1. Cows

Israeli Holsteins cows in mid-lactation (MLC), with milk yields over 35.0 L d^{-1}, or late-lactation cows (LLC), i.e., ~90 days before the next parturition, with milk yields of 6.0–14.0 L d^{-1}, were studied. Among the MLCs, 45 glands were free of bacteria (MLC-F) and 51 glands were subclinically infected (MLC-I) mainly with *Staphylococcus chromogenes*. Among the LLCs, only the milk from 58 glands that were free of bacteria was studied. The cows were milked thrice daily (at 0500, 1200, and 2000) and were fed a typical Israeli total mixed ration containing 65% concentrate (17% protein) and 35% forage. Cows were provided with confined shelters that provided 10 m^2 of shaded slatted floor and 10 m^2 of concrete-surfaced yard for each cow. Food was offered ad libitum in mangers located in the sheds.

2.1.2. Goats

Crossbreeds of Alpine, Saanen and Shami dairy goats, with milk yields over 3.0 L d^{-1} (MLG), and 0.3–1.0 L d^{-1} at late lactation (LLG), i.e., ~70 days before the next parturition, were studied. Among the MLGs, 37 glands were free of bacteria (MLG-F) and 66 were subclinically infected (MLG-I), mainly with *Staphylococcus epidermidis* or *Staphylococcus simulans*. Of the LLGs, only the milk of 96 glands that were bacteria-free was studied. The goats' accommodation included 4 m^2 in closed sheds plus 4 m^2 of open yard for each animal. Food was offered in mangers located in free-stall barns, consisting of approximately 1.8 kg of 16% protein concentrates and 1.0–1.2 kg of hay.

2.1.3. Sheep

Assaf dairy sheep with milk yields >2.5 L d^{-1} (MLS), and ~70 days before the next parturition, with yields of 0.5–1.0 L d^{-1} (LLS), were studied. Among the MLS, 47 glands were free of bacteria (MLS-F) and 56 glands were subclinically infected (MLS-I) mainly with *S. epidermidis* or *S. chromogenes*. Among the LLS, only the milk of 30 bacteria-free glands was studied. The sheep had 4 m^2 in closed sheds plus 4 m^2 of open yard for each animal. Food was offered in mangers located in free-stall barns, total mixed ration containing 70% concentrate (17% protein) and 30% forage.

2.2. Sample collection and analyses

Milk was sampled from each gland during the morning milking. For the bacteriological tests, the teats were cleaned and disinfected and 5-mL samples of foremilk were taken. Bacteriological analysis was performed according to accepted microbiological procedures of the US National Mastitis Council (Oliver, Gonzalez, Hogan, Jayarao, & Owens, 2004). On the test days, an additional sample (100–300 mL of mixture of the whole udder yield) was taken from each quarter for analysis as follows: SCC with the Fossomatic 360 (Foss Electric, Hillerød, Denmark) and gross milk composition, i.e., protein, fat and lactose contents, with the Milkoscan FT6000 (Foss Electric). These analyses were performed at the Israel Cattle Breeders' Association Laboratory (Caesarea, Israel). Casein content was determined according to standard methods (Marshall, 1992). Curd firmness (CF) and rennet clotting time (RCT) were determined with the Optigraph (Ysebaert, Frepillon, France). Samples (10 mL) were placed in the wells and equilibrated at 30 °C. Coagulating enzyme was Fromase 15 TL (0.5 mL, Gist-Brocades nv, Delft, The Netherlands), diluted (1:100) to achieve clotting within about 900 s in bovine milk. In order to be consistent in interpretation of the results of the 3 animal species it was decided to use equal amounts of coagulating enzyme, and therefore, time to obtain best CF was different among the species, i.e., 90 min for cows, 60 min for goats and 40 min for sheep.

2.3. Statistical analyses

All statistical analyses were carried out with JMP software (SAS Institute, 2000). The experimental unit was animal. The main parameters analyzed were: milk yield (L d^{-1}), SCC (×1000) and log SCC, fat (g L^{-1}), protein (g L^{-1}), casein (g L^{-1}), casein as percentage of total protein (% casein), lactose (g L^{-1}), RCT (s) and CF (V). For each animal species, the effects of Group (i.e., mid-lactation, infection-free, ML-F; mid-lactation, infected, ML-I; and late-lactation, LL) and Lactation number (i.e., 1st lactation, 2nd lactation, and 3rd lactation or more) on the analyzed parameters were determined by two-way ANOVA in a random design. The statistical model was:

Model [1]: $Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}$

where: μ = mean of all data, α_i = difference between the mean of Group i and the trial mean (fixed effect), β_j = difference between the mean of lactation j and the trial mean (Fixed effect), e_{ijk} = residual variance between measurements (Random error).

Multiple comparisons between groups were made applying the Tukey–Kramer HSD *t*-test.

The effects of group (i.e., ML-F, ML-I, or LL) and animal (i.e., cow, goat, or sheep) on the analyzed parameters were determined with a three-way ANOVA model, in a random design. The statistical model was:

Model [1]: $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk}$

where: μ = mean of all data, α_i = difference between the mean of group i and the trial mean (fixed effect), β_j = difference between the mean of animal j and the trial mean (fixed effect), $\alpha\beta_{ij}$ = interaction between group and animal (fixed effect), e_{ijk} = residual variance between measurements (Random error).

Multiple comparisons between Group × Animal combinations were done with Student's *t*-test. Correlations between CF and the

parameters protein (g L^{-1}), casein (g L^{-1}), % casein, protein + fat (g L^{-1}), SCC (×1000), SCC (log), and lactose (g L^{-1}) were determined for each animal separately.

3. Results

Tables 1–3 present the variables measured for each of the animal species (cows, goats and sheep, respectively) and results of statistical analysis of the data. For all three species, lactation number had no significant effect on any of the parameters tested. No correlations were found between CF and level of protein, casein, or protein + fat, for the three species (Fig. 1). However, % casein and lactose showed significant positive correlations with CF, while SCC and log SCC showed significant negative correlation, but with lower *r* values.

In cows, the SCC was significantly higher in the LLC milk than in that of the MLC-F, and that of MLC-I had significantly higher SCC than both other groups. Fat, protein and casein levels were significantly higher in the LLC milk, with no difference between those of MLC-F and MLC-I. However, the percentage of casein in total protein was significantly lower in milk from MLC-I and LLC than in that from MLC-F. Similarly, lactose level was significantly lower in MLC-I and LLC milk than in MLC-F milk. RCT was significantly longer in both MLC-I and LLC than in MLC-F; consequently, CF-90 was significantly lower in both MLC-I and LLC milk than in MLC-F (Table 1).

In goats, the SCC was significantly higher in MLG-I milk than in that of MLG-F, and that of LLG had significantly higher SCC than that of both other groups. Fat, protein and casein levels were significantly higher in the LLG milk than in that of MLG-F and MLG-I, which did not differ. However, the percentage of casein in total protein was significantly higher in MLG-F milk than LLG milk, and both were higher than in MLG-I milk. Lactose level was significantly higher in MLG-F milk than in that of MLG-I and both was significantly higher than in that of LLG. The RCT was significantly longer in LLG milk than in that of MLG-I and MLG-F, and significantly longer in MLG-I than MLG-F. The CF 60 min after enzyme addition (CF-60) was significantly lower in MLG-I and LLG milk than in that of MLG-F (Table 2).

In ovine milk, SCC was significantly higher in MLS-I milk than in those of MLS-F and LLS, which did not differ significantly. Fat content was higher in the LLS milk than in that of MLS-F and MLS-I, but significantly so only with respect to that of MLS-I. No significant differences were found among the groups in protein and casein content, but the percentage of casein in total protein was significantly lower in MLS-I milk than in that of the others. Lactose content was significantly lower in MLS-I milk than in that of MLS-F milk. The RCT was significantly longer in both MLS-I and LLS milk, and twice as long as that of MLS-F. The CF 40 min after enzyme addition (CF-40) was significantly lower in MLS-I and LLS milk than in that of MLS-F, and MLS-I was significantly lower than LLS (Table 3).

The effects of lactation stage and infection status (LS–IS, i.e., ML-F, ML-I and LL) and species (i.e., cow, goat, and sheep) on the analyzed parameters were determined by using a three-way ANOVA model, in a random design. As expected, the effects of species and LS–IS status on all the tested parameters were found significant, and there were significant interactions (species × LS–IS status), therefore, multiple comparisons between (species × LS–IS) status combinations were done by applying Student's *t*-test separately for each parameter (not shown).

Table 4 summarizes the measured results and the significant values (*P*) for the parameters tested (SCC, % casein, lactose, and CF) of the three animal species, as obtained for mid-lactation, free (ML-F); mid-lactation, infected (ML-I); and late-lactation (LL) animals. The SCCs of the three species tested were significantly lower in ML-F, in the range ~100–300 × 10^3 cells, with no significant difference between species, whereas infection significantly increased SCC in all species, with the highest response in sheep, in which SCC was significantly higher than in infected cows and goats. Among the LL animals, only moderate increases in SCC were recorded in cows and

Table 1
Days in milk (DIM), milk yield, SCC, gross milk composition (fat, protein, casein, lactose), rennet clotting time (RCT) and curd firmness (CF) of cows' milk for mid-lactation, bacteria-free (MLC-F), mid-lactating, infected (MLC-I), and late-lactation, bacteria-free (LLC).[a]

Parameter	MLC-F[b]	MLC-I[b]	LLC[b]	R^2	*P* [F]
DIM	173 ± 14.7^b	165 ± 12.4^b	426 ± 11.3^a	0.524	<0.001
Milk (L d^{-1})	39.04 ± 1.46^a	38.59 ± 1.50^a	12.88 ± 1.4^b	0.606	<0.001
SCC (×1000)	99 ± 19^c	1541 ± 103^a	498 ± 97^b	0.422	<0.001
Fat (g L^{-1})	37.0 ± 1.8^b	38.3 ± 0.8^b	46.8 ± 1.7^a	0.164	0.002
Protein (g L^{-1})	33.9 ± 0.7^b	33.7 ± 0.7^b	43.7 ± 0.7^a	0.504	<0.001
Casein (g L^{-1})	26.9 ± 0.6^b	25.6 ± 0.6^b	35.3 ± 0.5^a	0.417	<0.001
%Casein[c]	78.72 ± 0.69^a	75.85 ± 0.71^b	73.78 ± 1.33^b	0.165	0.001
Lactose (g L^{-1})	49.8 ± 1.2^a	42.1 ± 1.4^b	43.5 ± 0.9^b	0.226	<0.001
RCT (s)	930 ± 220^b	2394 ± 238^a	3025 ± 197^a	0.308	<0.001
CF-90 (V)	11.57 ± 1.03^a	4.71 ± 1.18^b	6.78 ± 0.82^b	0.177	<0.001

[a] Results are presented as mean ± SE; values within rows with no common superscript differ significantly ($P < 0.05$).
[b] The number of quarters were: MLC-F, 45; MLC-I, 51; LLC, 58.
[c] %Casein = (casein/protein) × 100.

Table 2
Days in milk (DIM), milk yield, SCC, gross milk composition (fat, protein, casein, lactose), rennet clotting time (RCT) and curd firmness (CF) of goats' milk for mid-lactation, bacteria-free (MLG-F), mid-lactating, infected (MLG-I), and late-lactation, bacteria-free (LLG)[a].

Parameter	MLG-F[b]	MLG-I[b]	LLG[b]	R^2	*P* [F]
DIM	129 ± 15.9^b	121 ± 6.3^b	269 ± 4.8^a	0.476	<0.001
Milk (L d^{-1})	3.07 ± 0.2^a	2.64 ± 0.16^b	0.80 ± 0.2^c	0.542	<0.001
SCC (×1000)	292 ± 46^c	2861 ± 642^b	6274 ± 710^a	0.253	<0.001
Fat (g L^{-1})	33.2 ± 2.4^b	36.0 ± 1.9^b	50.5 ± 1.9^a	0.303	<0.001
Protein (g L^{-1})	32.0 ± 2.8^b	34.7 ± 2.2^b	56.6 ± 2.2^a	0.380	<0.001
Casein (g L^{-1})	23.1 ± 1.9^b	23.3 ± 1.6^b	40.3 ± 1.5^a	0.423	<0.001
%Casein[c]	74.16 ± 0.6^a	68.26 ± 0.84^c	70.59 ± 0.80^b	0.157	<0.001
Lactose (g L^{-1})	47.2 ± 0.4^a	44.1 ± 1.0^b	37.0 ± 1.0^c	0.266	<0.001
RCT (s)	443 ± 50^c	621 ± 30^b	2107 ± 230^a	0.236	<0.001
CF-60 (V)	8.57 ± 1.91^a	1.26 ± 2.77^b	5.2 ± 1.30^b	0.094	0.038

[a] Results are presented as mean ± SE; values within rows with no common superscript differ significantly ($P < 0.05$).
[b] The number of quarters were: MLG-F, 37; MLG-I, 66; LLG, 96.
[c] %Casein = (casein/protein) × 100.

Table 3
Days in milk (DIM), milk yield, SCC, gross milk composition (fat, protein, casein, lactose), rennet clotting time (RCT) and curd firmness (CF) of sheep milk for mid-lactation, bacteria-free (MLS-F), mid-lactating, infected (MLS-I), and late-lactation, bacteria-free (LLS)[a].

Parameter	MLS-F[b]	MLS-I[b]	LLS[b]	R^2	*P* [F]
DIM	99 ± 9.2^b	119 ± 8.5^b	204 ± 4.9^a	0.452	<0.001
Milk (L d^{-1})	2.28 ± 0.16^a	1.57 ± 0.18^b	0.99 ± 0.18^c	0.333	<0.001
SCC (×1000)	129 ± 48^b	7211 ± 1197^a	403 ± 94^b	0.299	<0.001
Fat (g L^{-1})	72.7 ± 0.32^{ab}	68.7 ± 0.32^b	76.1 ± 0.34^a	0.062	<0.001
Protein (g L^{-1})	47.74 ± 0.17	50.1 ± 0.17	51.8 ± 0.18	0.059	NS
Casein (g L^{-1})	35.8 ± 0.11	34.1 ± 0.11	36.3 ± 0.16	0.051	NS
%Casein[c]	76.39 ± 1.43^a	67.94 ± 1.44^b	74.57 ± 0.5^a	0.294	<0.001
Lactose (g L^{-1})	47.9 ± 0.12^a	40.5 ± 0.1^c	43.8 ± 0.13^b	0.205	<0.001
RCT (s)	547 ± 22^c	1820 ± 205^a	802 ± 37^b	0.255	<0.001
CF-40 (V)	18.27 ± 1.10^a	8.22 ± 0.98^c	14.54 ± 1.27^b	0.301	<0.001

[a] The results are presented as mean ± SE; values within rows with no common superscript differ significantly ($P < 0.05$).
[b] The number of quarters were: MLS-F, 41; MLS-I, 56; LLS, 39.
[c] %Casein = (casein/protein) × 100.

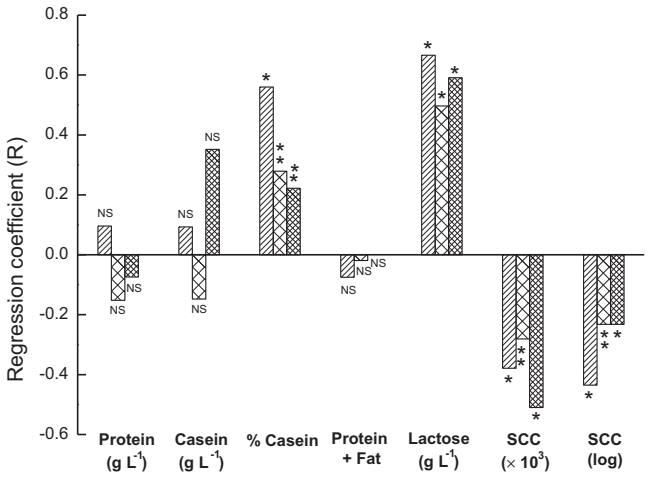

Fig. 1. Correlations and respective probability (P[r]) for (▨) cows, (▩) goats, and (▩) sheep between levels of protein, casein, (protein + fat), % casein and lactose, SCC, and log SCC, and curd firmness (CF) of milk: * – $P > 0.001$; ** – $P > 0.05$; NS – not significant.

sheep, whereas for goats the SCC was significantly higher than in ML-I animals. For all three species, % casein was significantly higher in ML-F than in ML-I or LL, with no significant differences among species. In contrast, infection significantly decreased % casein in all three species, the greatest decrease being in sheep. The decrease in % casein in the LLS was moderate and significantly lower than in the MLS-I, whereas in goats and cows the decreases in % casein were similar and did not differ from those in ML-I animals. In all three species, lactose level was significantly higher in ML-F than in ML-I animals, with no significant difference between species. Infection significantly decreased lactose level in all species, with the highest decrease being recorded in sheep. The decreases in lactose level in late lactation in sheep and cows were moderate and significantly lower than that in goats. Curd firmness was significantly higher in milk from ML-F animals than in that from ML-I and LL samples of all three species, with significant differences among species: CF was significantly higher in sheep than in cows and goats. Within each of the three species, CF in infected animals was significantly lower than in infection-free ones. At late lactation, CF in all animals of the three species was lower than that in ML-F animals, but higher than that in ML-I animals.

4. Discussion

4.1. General responses

Within each species, except sheep, there was no correlation between the levels of fat, protein, and casein, and CF, which is consistent with previous findings (Leitner, Merin, Lavi, Egber, & Silanikove, 2007; Leitner, Silanikove, & Merin, 2008a). Nonetheless, the yield of curd from ovine milk is typically significantly higher than those from goat and bovine milk (Leitner et al., 2008a), because the levels of fat, protein and casein are typically higher in ovine milk. In all three species, a positive relationship between % casein, lactose, and CF and a negative relationship between SCC and CF were found, which is consistent with previous findings (Auldist et al., 1996; Forsback et al., 2009; Leitner et al., 2004a,2004b; 2006; 2007; Le Roux, Colin, & Laurent, 1995; Lindmark-Mansson et al., 2006). The present study highlighted the effectiveness of lactose as a predictor of milk quality: in all three species the correlation between lactose and CF was higher than those for % casein and SCC. The present study also indicated significant inter-species differences in responses to intramammary infection and late lactation.

4.2. Effect of sub-clinical infection at mid-lactation

In milk of all three species, subclinical intramammary infection with coagulase-negative staphylococci (CNS) at mid-lactation simultaneously affected milk quality, clotting time, and curd firmness. Milk yield was least affected, and not significantly so, in cows. Previously, Leitner et al. (2004a, 2008a) found that in goats and sheep, when only one gland was infected with CNS, there was compensatory increase of milk yield from the uninfected second gland, so that the loss in yield for the whole animal level was greatly reduced in sheep and attenuated in goats (Leitner et al., 2004a, 2008a). Thus, the quadratic arrangement of the mammary glands in cows is advantageous in this regard, as it offers higher capability for compensation. Nevertheless, it should also be borne in mind that deterioration in milk quality is almost certainly associated with loss of curd yield (Leitner et al., 2008a, 2008b). Furthermore, when milk is used for cheese production, when only one gland is infected, the negative effect of CNS infection in terms of losses attributed to reduce curd yield is greater than the effect of reduced milk yield (Leitner et al., 2008a). Thus, although milk yield is not greatly affected in subclinically infected cows, this level of infection still significantly affects milk quality and curdling efficiency (Leitner et al., 2006, 2008b, and present results). This effect was found to depend on bacterial species, with subclinical infections by some species such as *Staphylococcus dysgalactiae* and *Escherichia coli* being particularly devastating, although the effect of CNS was still notable (Leitner et al., 2006, 2008b). Whereas the effects of CNS infection at mid-lactation on milk yield and quality were significant in goats, the parallel responses in sheep were much greater. These results are consistent with previous results and conclusions (Leitner et al., 2004a, 2004b, 2008a; Merin, Silanikove, Shapiro, Bernstein, & Leitner, 2004).

During the last decade our laboratories extensively studied the physiological basis for the reductions in milk yield and quality under exposure to intramammary infection and stress (Leitner et al., 2004a, 2004b, 2007, 2008a, 2008b; Silanikove, Leitner, Merin, & Prosser, 2010; Silanikove, Merin, & Leitner, 2006; Silanikove, Shapiro, Shamay, & Leitner, 2005; Silanikove, Shapiro, & Shinder, 2009). Collectively, these studies have shown that enzymatic hydrolysis of CN by plasmin liberates peptides that serve as local regulators of mammary gland functions. In particular,

Table 4
SCC, % Casein, Lactose (Lac.) and curd firmness (CF) in milk of the three animal species, divided into mid-lactation, free (ML-F); mid-lactation, infected (ML-I); and late-lactation (LL) animals and the significance value for statistical comparison by ANOVA (P).

Animal	ML-F				ML-I				LL			
Parameter	SCC	% Casein	Lac.	CF	SCC	% Casein	Lac.	CF	SCC	% Casein	Lac.	CF
Cow	99	78.7	49.8	11.6	1541	75.8	42.1	4.71	498	73.8	43.5	6.78
Goat	292	74.2	47.2	8.6	2861	69.1	44.1	1.26	6274	70.6	37.0	5.20
Sheep	129	76.4	47.9	18.3	7211	67.9	40.5	8.22	403	74.6	43.8	14.54
P value	NS	0.06	0.08		0.001	0.001	0.003		0.001	0.001	0.001	

a peptide that is formed by the activity of plasmin on β-CN (β-CN f1-28) down-regulates milk secretion in cows and goats; it reduces the output of lactose and other osmotic components from the alveoli into the gland lumen. The negative effect of the plasmin system on milk quality has been known for a long time (Bastian & Brown, 1996; Politis, 1996) and is well established (Silanikove et al., 2006, 2010). An updated version of the above-described model is depicted in Fig. 2.

Thus, reduction in milk yield due to bacterial infection (Leitner et al., 2008b; Merin et al., 2008) and stress (Silanikove, Shamay, Sinder, & Moran, 2000; Silanikove et al., 2009) would always be associated with reduced milk quality, because of the pivotal role of plasmin in these processes and because increased plasmin activity is associated with CN breakdown. Furthermore, our most recent findings in this field have shown that casein hydrolysates not merely cause disappearance of CN, which is thus not available for curdling but play an active role in delaying coagulation and impairing curd quality (Merin et al., 2008). This insight accounts for the coordination between the acute reductions in milk yield and milk quality in response to subclinical infection in the three species and the more acute response in sheep in comparison with goats and cows. The evolutionary physiological basis that underlies the reduction in milk clotting properties under conditions associated with milk stasis is prevention of formation of coagulates that might obstruct the evacuation of secretions from the mammary glands and thus, in turn, lead to complications such as necrosis and uncontrolled inflammation (Heegaard et al., 1994).

4.3. Effect of late-lactation

According to the model depicted in Fig. 2, in all three animal species, late-lactation negatively affected milk yield and milk quality in a coordinated manner. The simultaneous reduction in milk yield and milk quality was notably greater in goats than in cows and sheep. In keeping with this model, end of lactation in goats is characterized by a particular sharp increase in plasmin activity (Fantuz, Polidori, Cheli, & Baldi, 2001; Leitner et al., 2004a, 2004b) and consequently accelerated CN breakdown. Because CN hydrolysates contain pro-inflammatory components, the sharp increase in plasmin activity at the end of lactation in goats is associated with a sharp increase in SCC, which comprise mainly leukocytes, which results in a sterile (bacteria-free) inflammatory response (Shamay, Leitner, Shapiro, & Silanikove, 2003; Shamay, Mabjeesh, & Silanikove, 2002; Silanikove et al., 2005).

The inflammatory response at the end of lactation may be interpreted as a pre-adaptive response to the forthcoming involution stage, because the acute inflammatory response will aid clearance of existing bacterial infection, and thereby help to prevent the transmission of infection from one lactation to the next. Among the three species studied, goats are the best adapted to a natural environment (Silanikove et al., 2000, 2010). Thus, whereas selection for high milk yield and persistency diminished the pre-involution adaptive response in cows and sheep, this archaic natural adaptation is expressed more vigorously in goats.

4.4. Significance of lactose level as an indication of infection and milk quality

It is well known that lactose concentration is reduced during clinical (Werner-Misof, Pfaffl, & Bruckmaier, 2007) and subclinical mastitis (Leitner et al., 2004a, 2004b, 2007). The reduction in lactose concentration in subclinically infected animals in which tight junction integrity is partially or wholly maintained indicates that the reduction is related to reduced secretion of lactose by

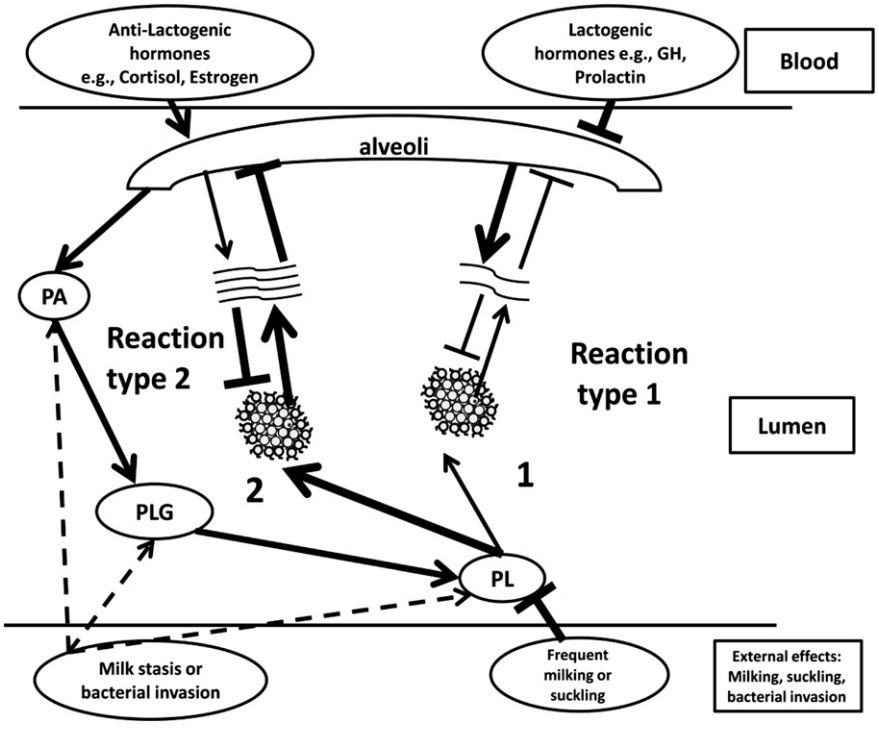

Fig. 2. Overview of the plasminogen activator–plasminogen–plasmin negative feedback mechanism that down-regulates milk secretion and simultaneously impairs milk quality (clotting parameters). The basic model is derived from Silanikove et al. (2006). Mild activation of the plasmin system (reaction type 1), which is a natural process, results in milk-borne regulatory elements that block apically located K^+-channels, thus affecting the secretion of lactose into the milk and decreasing milk clotting qualities. Stronger activation of the plasmin system activity is associated with acute decrease in lactose concentration (reaction type 2), to below ~4%, and with secretion of milk with impaired clotting parameters. Bold arrows indicate flow of signal along the feedback loop. Dashed arrows represent amplifying effects on the plasmin system. Casein derived active peptides, type 1: ≈; casein derived active peptides, type 2: ≈; casein micelle: ●.

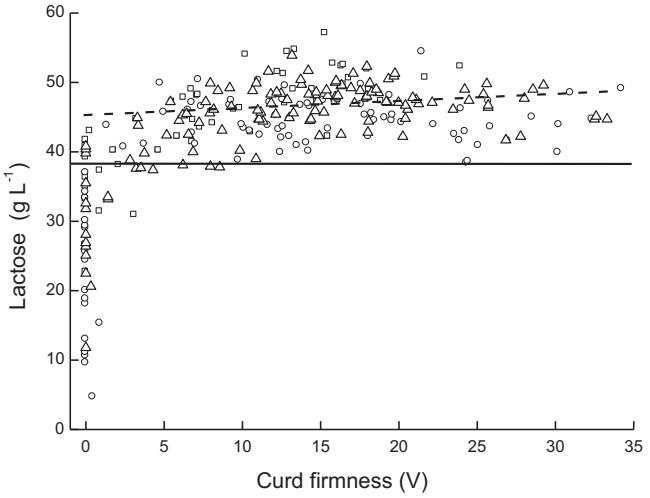

Fig. 3. Relationship between lactose concentration in milk of the three animal species (□, cows; ○, goats; Δ, sheep) and curd firmness. Regression line: $y = 0.009x + 4.52$; $R^2 = 0.0352$.

mammary gland cells (Leitner et al., 2004a, 2004b, 2007). Indeed, the milk-borne regulatory element (i.e., β-CN f1–28), which blocks apically located K^+-channels (Silanikove et al., 2000), affects the secretion of lactose into the milk (Silanikove et al., 2000, 2009). Thus, in accordance with the scheme depicted in Fig. 2, activation of the plasmin system will result in reduction of lactose secretion. Lactose concentration, therefore, can serve as a marker for inflammation and deterioration in milk clotting parameters. Interestingly, in all three species, reduction of lactose content to ~4% characterized milk that would not coagulate and, therefore, would be of no value for cheese production (Fig. 3).

5. Conclusions

The present results provide dairies that process milk into cheese with new criteria that will enable them to identify and isolate milk that will not coagulate. Such milk might still meet the criteria for drinking milk; therefore, farmers will be able to exploit the milk they produce more economically. The effectiveness of lactose, % casein, and SCC as predictors of milk quality for cheese production is impaired at the dairy tank level because of dilution of milk from subclinically infected glands with good-quality milk. Thus, the effect of subclinical mastitis and late lactation on milk quality remained significant (Leitner et al., 2007), and this consideration highlighted the importance of future development of new techniques (see: Akerstedt, Waller, & Sternesjo, 2009, for a first effort in this direction) that will be sensitive to milk quality on the tank level, and therefore will enable large dairies to pay farmers for milk according to its designated quality (i.e., for drinking or cheese manufacture). Individual on-line measurements of milk-quality parameters, particularly lactose level, will enable producers to identify animals that yield low-quality milk, and thereby meet the dairies' top price-quality standards by separating milk according to its best properties, for cheese production or drinking, and thus to maximize their profit from the milk they sell.

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