Effect of somatic cell count and lactation stage on sheep milk quality

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Paper received October 16, 2003; accepted January 9, 2004

ABSTRACT

In order to evaluate the effects of mammary health status and lactation phase on the qualitative parameters of ovine milk, 213 individual milk samples were repeatedly collected from 40 primiparous Sarda ewes on a monthly basis. Yield, physico-chemical characteristics, casein fractions quantitative distribution, somatic cell count (SCC), cheese making properties and plasmin-plasminogen activity were determined on each sample. Repeated individual milk SCC were used as a marker of udder health status, allowing the definition of three classes: "Healthy" (H), "Infected" (I) or "Doubtful" (D). Samples were grouped into 4 classes of days in milk (DIM). To evaluate the influence of mammary health status and phase of lactation, a mixed model was performed using the ewe as random effect. Milk physico-chemical parameters were influenced both by the udder health status and by lactation phase. In particular, the udder health status adversely affected $\alpha_s$ and $\beta_1$-casein fractions (P<0.01) and curd firmness (P<0.05). Samples reactive to rennet were 92.73%, 70.65% and 64.60% in "H", "D" and "I", respectively. Lactation phase influenced the overall milk composition and technological characteristics. Plasmakin activity was higher in the "I" group than in the others (16.1 vs 11.8 and 11.2 U/ml; P<0.01) and it significantly (P<0.01) increased during lactation. In conclusion, both mastitis and mammary involution are proven to exert a detrimental effect on milk quality since they enhance its endogenous proteolytic activity.

Key words: Sheep milk, Somatic cell count (SCC), Lactation stage, Plasmin

RIASSUNTO

EFFETTO DELLA CONTA CELLULARE E DELLO STADIO DI LATTAZIONE SULLA QUALITÀ DEL LATTE OVINO

Allo scopo di valutare gli effetti che lo stato sanitario della mammella e la fase di lattazione esercitano sulle caratteristiche qualitative del latte ovino, sono stati eseguiti mensilmente prelievi individuali di latte da 40 pecore Sarde primipare, per un numero totale di 213 campioni. Ad ogni prelievo sono stati determinati la produzione giornaliera, le principali caratteristiche fisico-chimiche, le quantità delle diverse frazioni caseine, il numero di cellule somatiche espressi come "linear score" (LS), le caratteristiche reologiche del coagulo e le attività di plasmina e plasminogeno. La conta ripetuta delle cellule somatiche è stata utilizzata come indicatore dello stato sanitario della mammella, permettendo di definire tre classi: Sane (H), Dubbie (D) e Infette (I). I prelievi sono stati inoltre raggruppati in 4 classi di giorni di latt-
tazione (DIM). I dati così ottenuti sono stati elaborati secondo un modello misto che utilizzava la “pecora” come effettuo random. I risultati mostrano che sia lo stato sanitario sia la fase di lattazione influenzano le caratteristiche fisico chimiche e reologiche del latte. In particolare, si sono registrate differenze significative tra i gruppi “H” ed “I” per quanto riguarda i valori composizionali del latte (pH, Lattosio, CN/TN, αs1, β1 ed altre frazioni caseiniche) le caratteristiche reologiche (r e a 30) e l’attività enzimatica (PL) del latte, nel senso di un peggioramento della qualità del latte stesso nei soggetti infetti. Inoltre, l’infiammazione mammaria ha determinato una notevole riduzione dei campioni reattivi al caglio, che sono risultati pari al 92,73%, 70,65% e 64,60% rispettivamente nei gruppi “H”, “D” ed “I”.

Introduction

Sheep milk is almost totally processed in large or small scale cheese factories, hence, the study of factors potentially affecting its cheese-making properties is needed. The presence of mastitis in the flock and its control represent a major interest in dairy ewe farming. The main concern for udder inflammation lies in the potential economic losses for the producer (increased culling rate, increased ewe and lamb mortality, lower production, treatment costs and, in certain areas, reduced milk payment) and on the health risk for the consumer (presence of pathogenic microflora in milk and cheese). In the European Union, these considerations led to the approval of Directives 92/46 and 94/71, that state bacteriological and cellular acceptability thresholds for milk. While aetiology, detection and control of mastitis are a major research topic resulting in a vast bibliographic production, the relationships between clinical and/or subclinical mastitis and sheep milk quality still need to be investigated. In order to better understand these aspects, Bergonier et al. (1999) suggest a decision rule for detecting clinical and subclinical mastitis in the dairy flock. It must be noted, however, that SCC thresholds are still a debated tool in the diagnosis of mastitis, as recently reviewed by the same author (Bergonier and Berthelot, 2003). With regard to Sarda sheep, a recent work by Nudda et al. (2003) showed the major effect of SCC on milk quality whereas its value rose above 1,000,000 cells/ml. The aim of the present work is to point out the mechanism by which udder health status and lactation phase, one of the main factors of variability, affect milk production.

Material and methods

Animals and samplings

The trial was carried out in a flock of Sarda ewes reared in the Apennine area of Central Italy. Animals were housed in the same conditions and were fed the same pasture, alfalfa hay and received 400 g/d of concentrate (33% barley, 33% corn and 33% oat grain). From the group of ewes that had lambed during late January/early February, 40 primiparous ones were selected. A total amount of 213 individual milk samples was collected from the selected ewes on a monthly basis, at the morning milking, over the period from weaning to the dry period (March - August).

Laboratory analyses

Yield and physico-chemical characteristics: at the time of sampling, milk was weighed and pH was recorded. Lactose and fat content were determined on a Milkoscan apparatus (Foss Electric, Hillerød, Denmark); dry matter (DM), ash (Ash), calcium (Ca), phosphorus (P), chloride (Cl), total...
nitrogen x 6.38 (TN), non protein nitrogen x 6.38 (NPN) and non caseinic nitrogen x 6.38 (NCN) were assessed according to ASPA (1995). In addition, casein content (CN = TN-NCN), total whey protein (WP = NCN-NPN) and casein index (CN/TN*100) were calculated.

Casein fractions: $\alpha_{s1}$, $\beta_1$, $\beta_2$, $\beta_3$, $\beta_4$, $\kappa^+$ and $\alpha_{s2}$ and $\gamma$-casein were identified by isoelectric focusing, as described by Caroli et al. (1998) and subsequent quantitative estimation was performed using TDS Quantity One software (Biorad, Philadelphia, Pennsylvania). The ratios of some fractions to others were also calculated.

Technological properties: lactodynamographic parameters, expressed as clotting time ($r$), firming time ($k_{20}$) and curd firmness ($a_{30}$), were determined using a Formagraph apparatus (Foss Electric) (ASPA, 1995).

Enzymatic activity: plasmin (PL) and plasminogen (PG) activities were colorimetrically determined, as described by Baldi et al. (1997), using d-Val-Leu-Lys p-Nitroanilide·2HCl (Sigma Chemical Co, St. Louis, MO) as chromogenic substrate; plasminogen to plasmin ratio (PG:PL) was also calculated.

Cellular count: SCC was performed using a Fossomatic 90 cell counter (Foss Electric) and expressed as linear score (LS), as suggested by Ali and Shook (1980).

**Statistical Analysis**

Repeated individual milk somatic cell counts (SCC) were used as a marker of udder health status, making it possible to define three classes (healthy, doubtful and infected). An udder was considered healthy (H) if every month control SCC was below 500,000 cells/ml, infected (I) when at least two SCC were over one million per ml and doubtful (D) in the other cases (Bergonier et al., 1999).

On the basis of days in milk (DIM), samples were then grouped into four classes: 1; 2; 3; 4 (being <70, 70 - 140, 140 - 200 and >200 DIM, respectively).

All data were processed using the PROC GLM from SAS (1990), according to the following linear model:

\[ Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha \beta)_i + \delta_{ ij} + \varepsilon_{ij} \]

where: $Y_{ij}$ = dependent variable, $\mu$ = overall mean, $\alpha_i$ = udder status (H; D; I), $\beta_j$ = DIM (1; 2; 3; 4), $(\alpha \beta)_i$ = interaction between udder status and DIM, $\delta_{ ij}$ = random effect of ewe within udder status, $\varepsilon_{ij}$ = error term.

**Results and discussion**

As a first result, it was observed that the frequencies of udders classed “H”, “D” or “I” were 25, 40 and 35% and those of samples within the four lactation stages were 17.4, 37.5, 28.2 and 16.9 %, respectively. Also worth noting is that interaction between udder status and lactation phase was significant only for pH and CN/TN ratio.

Results in Table 1 show that the effect of the udder health status was not significant on daily yield. In a recent work, Leitner et al. (2003) suggest that quanti-qualitative effect of udder infection could be less evident whereas only one half is mastitic, in fact the healthy one might compensate by producing more milk. DIM significantly affected milk production, since such parameter decreased from 1108 to 455 g, with the most marked decline occurring between the third and the fourth lactation phase.

Both health status and DIM influenced pH, in particular “I” and “D” values significantly differed from the “H” ones, and a marked, though not significant, increase in this value was observed at the last DIM class (from 6.58 up to 6.79). Other parameters were generally affected by udder health status and DIM, in particular, DM and fat tended to increase while lactose markedly decreased in late lactation, in coincidence with the highest LS values for the “I” subjects, as will be discussed afterwards. Due to its osmotic effect, lactose is responsible for drawing water into the milk and thus for milk production (Alais, 1984). The interaction of the two considered factors resulted in a markedly different pH trend between the three classes (Figure 1). An increase was apparent at the end of lactation for the “I” and “D” groups, while pH values remained rather constant in the “H” group.

In Table 2 the content of the main nitrogen compounds is reported. Milk from “I” and “D” udders had higher TN and CN contents than those
from “H”; nevertheless, CN/TN value was significantly higher in the “H” group in agreement with the observations of Duranti and Casoli (1991).

During lactation an overall increase in milk concentration of nitrogen compounds was found, whereas CN/TN showed a significant (P<0.01) decrease in the last lactation stage, meaning a remarkable shift from CN to other nitrogen compounds having less (WP) or no (NPN) cheese making value. When considering the interaction of health status and DIM (Figure 2), CN/TN showed an evident drop at the end of lactation in “I” and “D” samples, while it remained almost unvaried in the “H” group. Other authors (Pauselli et al., 1992; Casoli et al., 1994; Sordillo et al., 1997; Urech et al., 1999; Casoli et al., 2002; Pauselli et al., 2003) have pointed out the detrimental effect of mammary gland inflammation and mammary involution on milk cheese making properties.

Mammary health status affected some casein fraction percentages. Table 3 shows that $\alpha_{s1}$ and $\beta_1$-CN significantly differed between the three groups, being higher in “H” groups; on the contrary, $\beta$, $\beta_s$, and $\kappa+\alpha_{s2}$-CN were significantly

**Table 1.** Yield and physico-chemical characteristics

| Udder health status | Lactation stage | SED |
|---------------------|-----------------|-----|
|                      | H | D | I | 1° | 2° | 3° | 4° |
| Yield g/d           | 843| 854| 859|1108C|962 B|895 B|455 A|237,4|
| pH                  | 6.58 Aa | 6.65 AbB | 6.68 Bb | 6.58 | 6.56 | 6.62 | 6.79 | 0.132 |
| DM %                | 17.32 B | 17.08 A | 18.16 B | 15.90 A | 17.17 B | 18.45 C | 18.56 C | 1.329 |
| Fat                 | 6.52 | 6.75 | 6.75 | 5.34 A | 6.26 B | 6.96 C | 8.13 D | 1.077 |
| Lactose             | 4.90 B | 4.73 B | 4.45 A | 4.89 B | 5.00 B | 5.06 B | 3.85 A | 0.463 |
| Ash                 | 0.97 A | 1.01 B | 1.03 B | 1.03 B | 0.99 A | 0.99 A | 1.01 AB | 0.062 |
| Ca                  | 0.217 a | 0.220 ab | 0.228 b | 0.220 ab | 0.222 ab | 0.229 b | 0.215 a | 0.021 |
| P                   | 0.148 | 0.147 | 0.147 | 0.156 B | 0.154 B | 0.140 A | 0.139 A | 0.018 |
| Cl                  | 0.089 | 0.205 | 0.192 | 0.036 | 0.057 | 0.119 | 0.436 | 0.098 |

A, B, C, D: P<0.01; a, b: P<0.05

Figure 1. pH values in the three classes during lactation
(P<0.01) lower in this group than in the “I” one, while “D” group showed intermediate values. Among the ratios between the casein fractions, only $\beta_2/\beta_1$ and $(\kappa+\alpha_\text{S}_2)/\alpha_\text{S}_1$ were significantly higher in the “I” group than in the “H” (P<0.01) and in the “D” (P<0.05) ones.

The trend of casein fractions during lactation was consistent with previous work by Caroli et al. (2000) in the ovine species. The $\alpha_\text{S}_1/\beta_2$ ratio tended to increase throughout lactation while $(\kappa+\alpha_\text{S}_2)/\alpha_\text{S}_1$ showed an opposite trend. After an initial increase, the values of $\alpha_\text{S}_1/\beta_1$ and $\beta_2/\beta_1$ tended to decrease in the last phase of lactation. The use of the ratios between particular casein fractions was an attempt at identifying biological synthetic indexes suitable for milk quality evaluation.

The linear increasing trend of $\alpha_\text{S}_1/\beta_2$ ratio throughout lactation is an example of the possible efficiency of such indexes for the evaluation of physiological aspects related to milk samples. Nevertheless, as regards the udder health status, more efficient information can be drawn from the analysis of the single fractions, or sum of fractions, instead of from the evaluation of particular ratios between them.

Since animals had been classed on the basis of SCC, LS significantly differed between the 3 health status groups (Table 4).

It was interesting to evaluate cellular count trends during lactation for the 3 classes (Figure 3), though this interaction was not significant. LS value was not only constantly different between

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**Table 2. Nitrogen compounds**

| Udder health status | Lactation stage |
|---------------------|----------------|
|                     | H             | D             | I             |
| TNx6.38 %           | 5.66 A        | 6.16 B        | 6.13 B        |
| CN                  | 4.40 A        | 4.65 B        | 4.54 AB       |
| WP                  | 1.03 A        | 1.26 B        | 1.36 B        |
| NPN                 | 0.23          | 0.27          | 0.22          |
| CN/TN               | 77.6 Bb       | 75.7ABa       | 74.5 Aa       |

| Lactation stage | 1° | 2° | 3° | 4° |
|----------------|----|----|----|----|
| TNx6.38 %      | 5.47 A | 5.82 B | 6.23 C | 6.40 C |
| CN             | 4.47 Bb | 4.80 Cc | 4.67 BCc |
| WP             | 1.15 A | 1.19 A | 1.46 B |
| NPN            | 0.20 a | 0.24 ab | 0.28 b |
| CN/TN          | 76.9 B | 77.0 B | 73.3 A |

A, B, C: P<0.01; a, b, c: P<0.05
Table 3. Casein fractions

| Udder health status | Lactation stage |
|---------------------|-----------------|
|                      | H | D | I | 1° | 2° | 3° | 4° | SED |
| αs1 %               | 38.6 ± 37.3 ± 33.6 | 29.5 ± 32.0 ± 41.0 | 40.0 ± 5.80 |
| βtot                | 42.7 ± 42.3 ± 41.9 | 45.8 ± 40.8 ± 40.5 | 41.2 ± 5.82 |
| − β1                | 22.7 ± 21.7 ± 19.1 | 22.5 ± 16.3 ± 18.9 | 25.1 ± 3.68 |
| − β2                | 14.0 ± 13.8 ± 13.8 | 16.0 ± 13.9 ± 15.0 | 11.0 ± 1.95 |
| − β3                | 3.7 ± 4.2 ± 5.2 | 4.6 ± 6.3 ± 4.1 | 3.2 ± 2.09 |
| − β4                | 2.2 ± 2.7 ± 3.7 | 2.3 ± 4.6 ± 2.5 | 2.1 ± 1.61 |
| κ+αs2 %             | 15.2 ± 16.6 ± 20.3 | 20.7 ± 20.7 ± 11.6 | 14.2 ± 4.81 |
| γ                  | 3.5 ± 3.9 ± 4.9 | 4.1 ± 4.0 ± 5.3 | 2.8 ± 2.94 |

Table 4. LS and technological parameters

| Udder health status | Lactation stage |
|---------------------|-----------------|
|                      | H | D | I | 1° | 2° | 3° | 4° | SED |
| LS                  | 3.3 ± 4.8 ± 6.6 | 4.5 ± 4.4 ± 5.2 | 5.6 ± 2.01 |
| r min               | 16'55'' ± 20'20'' ± 21'31'' | 22'14'' ± 19'56'' ± 12'58'' | 23'16'' ± 6'28'' |
| κ20                 | 02'38'' ± 03'03'' ± 03'16'' | 03'05'' ± 02'49'' ± 01'53'' | 04'08'' ± 1'13'' |
| κ30 mm              | 41.3 ± 37.4 ± 35.3 | 42.5 ± 41.6 ± 37.7 | 30.0 ± 9.60 |

Table 5. Enzymatic activity

| Udder health status | Lactation stage |
|---------------------|-----------------|
|                      | H | D | I | 1° | 2° | 3° | 4° | SED |
| PL U/ml             | 11.2 ± 11.8 ± 16.1 | 11.0 ± 10.1 ± 13.0 | 17.3 ± 3.67 |
| PG                  | 27.5 ± 27.8 ± 26.1 | 28.4 ± 27.8 ± 28.3 | 24.0 ± 3.89 |
| PG/PL               | 2.85 ± 2.92 ± 2.30 | 3.71 ± 3.98 ± 2.66 | 0.46 ± 1.435 |

A, B, C: P<0.01; a, b, c: P<0.05
groups, but it was also markedly increasing for the “I” group, reaching LS = 8.1 at end lactation, while the others remained rather unvaried.

Udder health status had a remarkable effect on milk rennet reactivity, in fact, “H”, “D” and “I” groups showed 92.73, 70.65 and 64.60% reactive samples, respectively. Milk from “H” group exhibited significantly lower $r$ and higher $a_{30}$. Moreover $k_{20}$ values tended to be higher in the “I” and “D” though not significantly different from “H” (Table 5). These results are in agreement with those reported by other authors with regard to cow (Politis and Kwai-Hang, 1988) and in sheep milk (Duranti et al., 1990; Pauselli et al., 1992; Casoli et al., 1992). While for $a_{30}$ a linear trend was detected, with a constant decrease of the parameter throughout lactation, for $r$ and $k_{20}$ a non linear trend was found, with the least values in the third lactation stage. This result could be due to the relationships with other compositional and biochemical parameters, reducing the clotting and the syneresis times as well as the coagulum strength and thus influencing both favorably and unfavorably the clotting aptitude of milk.

As expected, PL was higher in the “I” group than in the “D” and “H” ones (16.1 vs 11.8 and 11.2 U/ml; P<0.01), while PG showed significant differences only between the “I” and “D” groups (27.8 vs 26.1 U/ml; P<0.05). According to the observations of Casoli et al. (1999) and Caroli et al. (2000) in sheep and Baldi et al. (1997) in cow milk, PL activity markedly increased along with lactation while that of PG slightly decreased. The trend of PG/PL showed a progressively more efficient conversion of the inactive precursor to the active protease. Since PL activity was constantly higher in the “I” group than in the other ones reaching its peak at end of lactation (Figure 4), a relationship between SCC and PL is strongly suggested, in agreement with other studies in cow (Politis and Ng Kwai Hang, 1989; Ballou et al., 1995) and sheep (Casoli et al., 1999; 2002) milk.

The results point out the role of PL and SCC as the major proteolytic factors in milk. Plasmin is known to hydrolyse $\beta$- and $\alpha$-caseins with the production of protease-peptone and $\gamma$-casein (Eigel, 1997; Le Roux et al., 1995; Bastian and Brown, 1996; Cocker et al., 1999; Mariani et al., 1999) with detriment to technological properties of milk (Ng-Kwai-Hang et al., 1984; Duranti and Casoli, 1991; Pirisi et al., 1994; Pulina et al., 1996; Mara et al., 1998). Bastian and Brown (1996) report that $\kappa$-casein is thought to be resistant to the action of PL.

Since polymorphonuclear neutrophil cells (PMN) are known to be the major component of SCC in mastitic milk (Kehrli and Shuster, 1994;
Morgante et al., 1996), it can be assumed that the proteolytic activity of SCC lies mainly on the presence of PMN-derived elastase. However, though this enzyme can hydrolyze $\alpha_s$1-CN (Considine et al., 2000), $\beta$-CN (Considine et al., 1999) and whey proteins (Jakobsson et al., 1983), PL should be considered as the main proteolytic agent in milk, in agreement with other authors. In fact, Urech et al. (1999), in the case of mild subclinical mastitis, have demonstrated PL to be active in milk in the intramammary environment even between milkings. Moussaoui et al. (2002) observed, in an experimentally induced acute inflammation, that PL was the main proteolytic agent in milk with SCC < 2,000,000, while higher SCC resulted in an interaction between PL and PMN-derived proteases (elastase and cathepsin D). Furthermore, Bastian and Brown (1996) reported a role of $\kappa+\alpha_s$-CN fraction in the activation of plasminogen. In the present work this casein fraction was significantly higher in the “I” group, so that a direct influence of SCC on plasmin can be suggested.

Conclusions

The given results confirm the remarkable influence that the two considered variability factors had on milk quality. In particular, a detriment to its physico-chemical and cheese-making characteristics was observed in the infected subjects and in late lactation.

With regard to the udder health status, it must be noted that the majority of animals should be considered as potentially affected by mastitis. However, when considering its effect on milk characteristics, the most significant differences were found between the “H” and “I” groups, as was highlighted by milk compositional values (pH, Lactose, CN/TN, $\alpha_s$, $\beta$; and other casein fractions), rheological characteristics ($r$ and $\alpha_3$) and enzymatic activity (PL). All results obtained for these parameters pointed out a marked detriment in quality and rennet coagulation for the “I” group. Doubtful health status of udders resulted in somewhat intermediate properties which could hardly be discriminated from healthy ones. For this purpose, further investigation on the variation in total and differential cell count could contribute to detect the presence of mammary pathology due to its strict relationship with the increased concentration of leucocytes.

As expected, during lactation a decrease in milk yield and Lactose content was observed, while DM, Fat and TNx6.38 significantly increased. The quality of milk proteins was nega-
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The authors would like to thank Mr. Luigi Brachelente for his technical support.

Research supported by MURST contract year 2001, prot. 2001077279.

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