Why does the increase of plasmin worsen the coagulation properties of milk in dairy sheep?

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RIASSUNTO – Perché il maggiore contenuto in plasmina peggiora la coagulabilità del latte ovino? *Sono stati analizzati 448 campioni individuali di latte ovino prelevati da 105 pecore di razza Sarda. I campioni di latte sono stati suddivisi in classi rispetto al contenuto in plasmina (PL). Nei campioni di latte con tempo di coagulazione <30 min sono state osservate relazioni positive fra PL, NCN, SCC e pH, mentre sono risultate negative le relazioni fra PL e lattosio. Il contenuto di caseina non è stato influenzato dalla PL. Questo andamento è stato osservato anche per i campioni di latte che non hanno coagulato entro 30 minuti. I parametri di coagulazione sono peggiorati all'aumentare del contenuto in PL nel latte. In conclusione i risultati confermano che il contenuto in caseina non è un valido indicatore per valutare la coagulabilità del latte. Il peggioramento dei parametri di coagulazione è fortemente influenzato dagli indici di alterazione della permeabilità dell'epitelio secretivo quali l'aumento di pH e NCN e la riduzione del lattosio.*

Key words: plasmin, milk, coagulation properties, sheep.

INTRODUCTION – The concentrations of whole casein and fat are the factors which play the most important role in determining the cheese-making qualities of milk. Plasmin (PL) is the main proteolytic enzyme in milk and has been found to be associated with enhanced casein hydrolysation. ß-casein is the most susceptible to breakdown by this enzyme. Most of the enzyme is in the form of the inactive zymogene plasminogen (PG), which is converted to PL by the action of PG activators. PL activity is also associated with alteration of mammary epithelium permeability and a subsequent increase in paracellular flow. These proteolytic activities worsen the milk quality, coagulation properties and cheese yield. The level of PL in milk increases towards the end of lactation, with mastitis, and with the increasing age of the animals. At present the relationship between PL, SCC and casein are not clearly defined. Some authors found positive relationships between PL and SCC with CN (Baldi *et al.*, 1996; Bianchi *et al.*, 2004), others negative (Fantuz *et al.*, 2001), and some have found no relationship (Albenzio *et al.*, 2004). The aim of this work was to study why milk with higher PL content has worse coagulation properties.

MATERIALS AND METHODS – Individual milk samples (No. 448) were collected in two flocks from 105 Sarda ewes during whole lactation. Milk samples were analysed for fat, total protein and lactose content with a Milkoscan 6000, and for SCC with a Fossomatic 360. Non-casein nitrogen (NCN) and casein were determined according to the FIL-IDF standards. The pH of each milk sample was measured with a potentiometer. Rennet clotting time (r), rate of firming at 20 mm (k_{20}) and curd firmness at 30 min (a_{30}) were measured using a Formagraph.

Plasmin and PG concentrations in milk were determined following the method described by Ballou *et al.* (1995) with slight modifications. A standard curve was prepared to convert PL (Sigma Chemical, St. Louis, MO) activity to PL concentration by plotting changes in absorbance against concentrations of PL over a range from 0 to 10 mg/l. The data were grouped into five classes of PL concentration: [PL]<2 mg/l; 2<[PL]<5 mg/l; 5<[PL]<10 mg/l; 10<[PL]<15 mg/l and [PL]>15 mg/l. Data were analysed with one-way ANOVA to assess differences between PL classes for milk samples that did not coagulate in 30 min (group NC) and for milk samples with r<30 min (group C) separately. Correlation coefficients were calculated to assess the relationships between variables.

RESULTS AND CONCLUSIONS – About 20% of milk samples did not coagulate during the 30 min test period. Composition and coagulation properties of milk in the different plasmin classes are reported in Table 1 for group C. With the increase of PL concentration, the SCC and pH values increased significantly (P<0.001). Total protein and NCN increased and lactose decreased with the PL content. Those findings agree with those of some authors (Fantuz *et al.*, 2001; Bianchi *et al.*, 2004). No differences were found in the casein content among the different PL classes. This result agrees with that of Albenzio *et al.* (2004), who did not find any relationship between PL and CN. The PG content was always higher than the PL content and there was no defined trend. The ratio PG/PL decrease to PL increase indicated enhanced conversion of PG to PL in the mammary gland, as was reported by Politis *et al.* (1996) during late lactation. The r increases and a_{30} decreases with PL content, as was expected. The worsening of milk coagulation properties could not be explained by the reduction of casein but seemed to be mainly related to alterations in the blood-milk barrier, as can be seen from the results for pH, NCN and lactose.

Table 1. Composition and coagulation properties with different plasmin content (in mg/l) of ovine milk with rennet clotting time ≤ 30 min (Group C).

	plasmin class					P
	PL<2	2<PL<5	5<PL<10	10<PL<15	PL>15	
n.	71	77	107	58	45	
plasmin, mg/l	1.04	3.48	7.31	12.12	20.41	
log SCC	2.22A	2.56B	2.74BC	2.95CD	3.02D	**
pH	6.59A	6.56A	6.59A	6.62AB	6.68B	**
fat, %	6.85	6.91	6.97	6.78	6.85	NS
protein, %	5.47A	5.67AB	5.81CB	5.93CD	6.04D	**
casein, %	4.19	4.33	4.24	4.20	4.33	NS
NCN, %	0.18A	0.19AB	0.20BC	0.22CD	0.23D	**
lactose, %	4.92A	4.70AB	4.64B	4.60B	4.30C	**
casein/protein	0.80A	0.82AB	0.81AB	0.80A	0.79A	**
plasminogen, mg/l	13.06A	25.69BC	28.23B	26.11BC	21.54C	**
plasminogen/plasmin	15.70A	7.63B	4.02BC	2.20C	1.13C	**
r, min	16.96A	16.05A	17.73A	18.02A	20.87B	**
k_{20}, min	2.13A	2.35AB	2.68BC	2.71C	3.00C	**
a_{30}, mm	44.80AB	51.66C	48.55BC	47.77BC	39.54A	**

Values in the same line with different superscript letters were significantly different.
** P<0.001; NS = not significant.

The composition and coagulation properties of NC milk in the different plasmin classes are reported in Table 2. Only the SCC and lactose content showed a defined trend: the SCC increased and lactose decreased as PL rose. The pH did not vary significantly between the different PL classes, but its values are higher than those observed in all PL classes of group C. The protein and CN content did not vary between the PL classes.
The trends of pH, NCN, and lactose in PL classes of group C mirrored the PL classes of the NC group. Thus the aggregate of these variables should enable us to discriminate between milk with good or bad coagulation properties.

Table 2. Composition and coagulation properties with different plasmin content (in mg/l) of ovine milk with rennet clotting time > 30 min (Group NC).

	plasmin class					P
	PL<2	2<PL<5	5<PL<10	10<PL<15	PL>15	
n.	18	4	6	15	47	
plasmin, mg/l	1.07	2.63	7.34	12.69	25.50	
log SCC	2.87a	2.62a	3.34ab	3.78b	3.70b	*
pH	6.74	6.93	6.76	6.83	6.90	NS
fat, %	8.10a	6.55b	7.47b	7.11b	7.01b	*
protein, %	6.00	6.11	6.42	6.14	6.26	NS
casein, %	4.33	4.10	4.55	4.10	4.04	NS
NCN, %	0.24a	0.27ab	0.24a	0.27ab	0.29b	*
lactose, %	4.17a	3.79ab	3.77ab	3.67ab	3.50b	*
casein/protein	0.74	0.71	0.78	0.74	0.72	NS
plasminogen, mg/l	4.01a	8.38a	28.58c	21.61b	21.05b	*
plasminogen/plasmin	5.00a	2.77ab	3.92ab	1.71bc	0.90c	*

Values in the same line with different superscript letters were significantly different.
* P<0.05; NS = not significant.

The correlation analyses (Table 3) showed a medium relationship of R with pH, PL and SCC. No relationships were detected between casein and PL and SCC. These results confirm that the worsening of milk coagulation parameters is due to alteration of the indices of mammary permeability (PL, pH and SCC).

Table 3. Matrix of co-relationships between plasmin and certain milk components and coagulation parameters.

	R	k_{20}	a_{30}	pH	PL	log SCC
k_{20}	0.55					
a_{30}	-0.81	-0.61				
pH	0.40	0.21	-0.34			
PL	0.30	0.33	-0.17	0.28		
log SCC	0.30	0.38	-0.17	0.31	0.49	
casein	0.17	-0.05*	0.04*	0.12	0.07*	0.08*

* not significantly different from zero.

In conclusion, the results confirm that the CN content is not a valuable indicator when assessing the coagulation properties of milk. The worsening of milk coagulation properties are closely related to the indices of permeability of mammary epithelium, expressed by the high values of pH and NCN and the lower values of lactose.

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