

Evaluation of the biological activation of plasmin plasminogen system in sheep and goat milk

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RIASSUNTO – Valutazione dell'attivazione biologica del sistema plasmina plasminogeno nel latte ovicaprino. *La qualità del latte risulta influenzata da diversi fattori quali lo stadio di lattazione e lo stato sanitario della ghiandola mammaria. L'obiettivo del presente lavoro è stato quello di valutare l'interazione fra lo stato sanitario della ghiandola mammaria e alcuni indici della qualità del latte quali le cellule somatiche e il sistema plasmina-plasminogeno nei piccoli ruminanti. Dai dati ottenuti è emerso che esiste una stretta correlazione fra la conta delle cellule somatiche e l'attività dell'attivatore del plasminogeno valutata nel latte di capra come in quello di pecora, indicando come l'attività proteolitica del latte possa essere ritenuta indice dello stato di salute della ghiandola mammaria.*

Key words: plasmin, plasminogen activator, milk, small ruminants.

INTRODUCTION – Milk composition and quality are affected by several factors of which stage of lactation and mammary gland health status represent the main ones. These two factors become more effective in small dairy ruminants, for which stage of lactation is synchronized as result of the seasonal breeding cycle. As a consequence in dairy sheep and goats milk quality and cheese making properties tend to decline. Most of these effects on milk quality are also associated with an increase in the somatic cell count (SCC), which is in turn related with the gradual involution of mammary gland with the lactation progress (Fantuz *et al.*, 2001; Baldi *et al.*, 2002). However, high SCC in milk could be related to intramammary infections (IMI), sub-clinical and chronic mastitis in both sheep and goats. Stage of lactation and SCC are also correlated with the activity of plasmin and plasminogen activator system, which participates to several enzymatic cascade involved in both mammary gland involution and inflammation processes. It is known that plasmin (PL) is the predominant native protease in milk (Politis *et al.*, 1989). Plasmin occurs in milk together with its inactive zymogen, plasminogen (PG). A complex of molecular interactions between plasminogen activators (PA) and PL and PG, underlies the activation of this system. The activity of plasmin-plasminogen system, the ratio between its components, the number of somatic cells and the bacteria presence could be considered in the principle parameters for the determination of milk quality. The aim of this study was to evaluate these parameters in sheep and goat milk coming from late lactation animals in relation to the health status of mammary gland.

MATERIALS AND METHODS – Milk samples obtained from late lactating (140±10 days) Alpine goats (29 samples) and Comisana sheep (25 samples) were collected. The herds were free from brucellosis, tuberculosis and mycoplasmosis. 50 ml of milk were collected aseptically from each goat and sheep and were kept at 4°C until the SCC and bacteriological tests were performed. The SCC was determined for each milk sample by an automated fluorescent microscopic somatic cell counter (Bentley Somacount 150, Bentley Instrument,

USA). Groups were defined as LSCC (low SCC) when the SCC content was lower than 1000000/ml otherwise HSCC (high SCC). Log transformation was applied to SCC before analysis. Milk samples (10 µl) were spread on blood agar plates (5% defibrinated bovine blood). The plates were incubated aerobically at 37°C and examined at 24 hours post seeding. The colonies were provisionally identified by morphology, haemolysis pattern and Gram stain. The representative colonies were subcultured on blood agar plate and incubated aerobically at 37°C to obtain pure colonies. Gram positive cocci were tested for catalase and coagulase production. Milk samples were frozen and stored at -20°C for the determination of the activity of the plasmin-plasminogen system. Activities of PL, PG and PA were determined according to the colorimetric method described by Baldi *et al.* (1996). PL, PG and PA activities are expressed as Units/ml; one unit being the amount of the enzyme that produce the change in absorbance at 405 nm of 0.1 in 60 minutes. Values are expressed as means ± SE (SAS, 1999). Means comparison were done utilizing Duncan's multiple range test with P<0.001 selected as the level of significance. Correlation coefficients were calculated to investigate the relationships between variables.

RESULTS AND CONCLUSION – Of 29 goat milk samples screened, 12 (41.4%) resulted negative and 17 positive (58.6%) for bacteriological findings. Within the positive ones the most prevalent (n=10) mastitis agents were coagulase-negative Staphylococci (CNS). *Staphylococcus aureus* accounted for 7 of milk samples examined and was the second most common pathogen isolated. Bacteriological findings in sheep milk, screened in 25 milk samples, demonstrated as the most prevalent mastitis agents were *Staphylococcus spp.* 60% (n=15). and *Streptococcus spp.* accounted for 12% (n=3) of milk samples examined. The 28% of samples were negative. The activities of plasmin-plasminogen and plasminogen activator system in goat and sheep milk are summarized in the Table 1.

Table 1. The activity of plasmin-plasminogen and plasminogen activator system in goat and sheep milk.

	LSCC	HSCC	P
Goat			
PL, U/ml	29.56±5.93	40.24±4.64	0.15
PG, U/ml	20.73±3.01	5.68±2.66	<0.001
PA, U/ml	1897.4±314.76	4001.55±234.6	<0.001
PG/PL	1.06±0.19	0.20±0.17	<0.05
Sheep			
PL, U/ml	24.97±7.2	39.46±5.69	NS
PG, U/ml	53.71±7.21	45.24±5.88	NS
PA, U/ml	553±510.73	2018.66±372.98	<0.05
PG/PL	2.92±0.98	2.1±0.67	NS

In goat milk with HSCC content the PL activity tended (P=0.15) to be higher than in milk with LSCC content, while the PG activity was significantly (P<0.001) lower than that recorded in LSCC milk samples. The PA activity was significantly different between the two SCC classes with 2-fold increase in HSCC. PG/PL ratio was reduced (P<0.05) in HSCC group compare to LSCC one. These results could indicate an accelerated conversion of PG to PL, due to the increase of PA activity.

SCC was positive and significantly correlated with PA activity ($r^2=0.74$; P<0.001) and to bacteriological findings ($r^2=0.35$; P<0.05) confirming that the proteolytic activities in the milk is also related to the health status of mammary gland. As a consequence a negative correlation between SCC and PG/PL ($r^2=-0.43$; P<0.05) was obtained. Data here reported indicate that SCC represents a major factor in determining PL-PG-PA activities. PL and PG activity in sheep milk in both somatic cell group did not show any significant difference. In sheep milk with HSCC content, the PA activity was significantly (P<0.05) 3.5-fold higher than in milk samples with

LSCC (Table 1). Positive and significant correlations between SCC and PL ($r^2=0.45$; $P<0.05$) and SCC and PA ($r^2=0.46$; $P<0.05$) were evidenced. Although several studies have documented that plasmin activity in milk increases with the rise in SCC and the advancement of lactation (Albenzio *et al.*, 2004) in relation to an higher conversion of PG, this seems to be not the case for dairy sheep.

In the present study, when bacteriological findings were considered, no effect was observed on PL PG and PA and PG/PL ratio (data not shown), for both goat and sheep milk. As a consequence SCC was more effective than presence of pathogen agents in milk on the activation of plasmin system. In dairy cows, Politis (1996) found higher levels of plasmin and plasminogen activator in milk obtained from mastitic quarters compared to healthy ones. Therefore, during mastitis the increase activity of PL PG PA system observed was mainly due to an increase transfer of PG from blood to milk rather than an accelerate conversion of PG to PL (Politis, 1996). Our results in small ruminant are not consistent indicating that relationships between SCC, bacteriological finding and IMI have to be further investigated in dairy sheep and goats.

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