J. Dairy Sci. 90:3263–3269
doi:10.3168/jds.2006-780

Factors Affecting the Plasmin-Plasminogen System in Milk Obtained from Three Greek Dairy Sheep Breeds with Major Differences in Milk Production Capacity

G. Theodorou, A. Kominakis, E. Rogdakis, and I. Politis[1]
Department of Animal Science, Agricultural University of Athens, 11855 Athens, Greece

ABSTRACT

The purpose of this study was to evaluate the effect of breed, stage of lactation, and health status of the udder on the plasmin-plasminogen system in ovine milk. A total of 38 ewes were used from 3 breeds [Boutsiko (n = 12), Chios (n = 12), and a synthetic breed (50% Boutsiko, 25% Arta, and 25% Chios, n = 14)] with major differences in their genetic potential with respect to milk yield. Milk samples were collected every 2 wk throughout the lactation period and were analyzed for fat, protein, lactose, and somatic cell count (SCC). In addition, milk plasmin (PL), plasminogen (PG), and plasminogen activator (PA) activities were determined. The Chios breed had the greatest average daily milk yield, the synthetic breed had an intermediate milk yield, and ewes of the Boutsiko breed had the lowest milk yield. Milk samples obtained from the Boutsiko breed had similar PL and PA activities, compared with those obtained from the other 2 breeds. The ratio of PG:PL was less in milk samples from the Boutsiko breed compared with the other 2 breeds, indicative of an increased rate of conversion of PG to PL for this breed. There was no correlation between PL activity and daily milk yield in ewes from all 3 breeds. Activities of PL, PG, and PA were greater in ovine milk with elevated SCC (>300,000/mL) compared with activities in milk with low SCC (<300,000/mL). The ratio of PG:PL was less in the high-SCC group compared with the low-SCC group, which indicates an increased rate of conversion of PG to PL for the high-SCC group. There was a decrease in PG and PA activities as well as in the PG:PL ratio in late lactation milk (mo 5 to 6) when compared with early or mid lactation milk (mo 1 to 4). Thus, the PL-PG system is affected by breed, stage of lactation, and the health status of the udder. No relationship was found between PL activity and daily milk yield in the 3 Greek dairy sheep breeds. Plasmin is not a marker for gradual involution in the Greek sheep breeds studied.
Key words: plasmin, plasminogen, plasminogen activator, ovine milk

INTRODUCTION

Several serine proteinases are present in milk. Plasmin (**PL**), which occurs in milk together with its inactive zymogen plasminogen (**PG**), is the most significant protease contributing to total proteolytic activity (Politis et al., 1989; Politis, 1996). Plasmin hydrolyzes α_s-CN and β-CN in milk and decreases milk's ability to withstand processing. Increased activities of PL and plasminogen activator (**PA**) have been associated with a deterioration of the coagulation properties of milk because of proteolysis of CN by PL (Srinivasan and Lucey, 2002).

A number of studies have examined the importance of the PL-PG system in the bovine mammary gland. Politis et al. (1989) provided data indicating that enhanced conversion of PG to PL is correlated with gradual involution (the declining phase of lactation). Plasmin might be a marker for gradual involution. Milk obtained during the first 5 to 6 mo of lactation contains low amounts of PL and PG; plasmin and PG increase in late lactation milk. However, the PG:PL ratio, an index independent of changes in milk volume, declines as lactation advances, indicating enhanced conversion of PG to PL during late lactation. Gilmore et al. (1995) found that late lactation milk contained more PA than did early lactation milk. Stage of lactation also affects the PL-PG system in ovine milk, but the results are inconclusive. Late lactation was associated with higher concentrations of milk PL and PA in Sardinian ewes (Bianchi et al., 2004). On the contrary, Albenzio et al. (2004, 2005) reported that the highest activities of PL and PG were observed in early lactation milk and the lowest in late lactation milk obtained from Comisana ewes.

The PL-PG system in ovine milk is affected by the health status of the udder. More specifically, PL and

Received November 22, 2006.
Accepted March 9, 2007.
[1]Corresponding author: i.politis@aua.gr

PA activities were greater by 74 and 139%, respectively, in infected vs. noninfected glands (Leitner et al., 2004). Furthermore, Bianchi et al. (2004) reported that elevated SCC was associated with increased PL activity (18%), and decreased PA activity (23%), whereas PG activity did not vary with SCC. Albenzio et al. (2004) reported increased PL activity for milk samples with high SCC (>1,000,000/mL) compared with those with low SCC (<500,000/mL) throughout lactation. Furthermore, there was an increase in PG activity for milk samples with high SCC compared with low SCC only during mid lactation (110 to 130 d of lactation).

Dairy sheep farming is a sector of major economic importance in Greece. Two typical indigenous breeds, Chios and Boutsiko, along with a synthetic breed were used in this study. The Chios breed is characterized by high milk yield and litter size, whereas the less-productive Boutsiko breed is known for its adaptability under harsh environmental conditions and its reduced susceptibility to mastitis (Hatziminaoglou et al., 1990; Simos et al., 1996; Kominakis et al., 1998; Ploumi et al., 1998). The synthetic breed (50% Boutsiko, 25% Arta, and 25% Chios) has been recently formed in an attempt to upgrade the Boutsiko breed by combining the high productivity of the Chios and Arta breeds with the robustness of the Boutsiko breed. The PL-PG system has not been investigated extensively in dairy sheep breeds with substantial differences in their genetic potential with respect to milk production.

The objective of the present study was to develop a model describing the PL-PG system in ovine milk. Relationships between several factors (breed, stage of lactation, and health status of the udder) and activities of milk PL, PG, and PA were evaluated. Important relationships were then further assessed as to their causative involvement in the PL-PG system. Moreover, correlations among PL, PG, and PA with all major milk components were determined. The significance (or lack of it) of the PL-PG system for the phenomenon of gradual involution in sheep was examined.

MATERIALS AND METHODS

Animals and Milk Sampling

Thirty-eight ewes were used in this study; 12 from the Boutsiko breed, 14 from a synthetic breed (50% Boutsiko, 25% Arta and 25% Chios), and 12 from the Chios breed. Animals were housed within the premises of the experimental farm of the Agricultural University of Athens. The ewes lambed between November 10, 2005, and January 10, 2006. All ewes that lambed outside of this period, independently of their lambing number, were excluded from the experiment. It was essential that all animals completed their lactations by the first week of July to avoid the extremely hot conditions that occur in Greece during July and August. Ewes of the Boutsiko and the synthetic breeds were in their first lambing, whereas ewes of the Chios breed were in their second or third lambing. After weaning (45 d after lambing), the ewes were milked twice daily at 0500 and 1700 h using a milking machine. Individual milk yield was recorded every 2 wk and milk samples were collected during the morning and evening milking on the same dates. Milk samples were divided into 2 aliquots; the first was immediately analyzed for major milk components and the second aliquot was frozen and stored at –20°C for PL, PG, and PA analysis. Samples were collected for 6 mo after weaning (entire lactation).

Milk samples were analyzed for fat, protein, and lactose by the infrared method using a Milkoscan 133 (Foss Electric, Hillerød, Denmark) calibrated against the Mojonnier method for fat, Kjeldahl method for protein, and the polarimetric method for lactose according to official methods (AOAC, 1980). Somatic cell count was determined with a Fossomatic cell counter (Foss Electric).

Determination of PL, PG, and PA Activities

Activities of PL and PG (also called plasminogen-derived activity) in milk were determined by a combination of the methods described by Politis et al. (1989) and Politis and Ng Kwai Hang, 1989). In detail, milk (3 mL) was mixed with 1 mL of 0.4 *M* sodium citrate and centrifuged at 27,000 × *g* for 20 min. The supernatant was recovered and assayed for PL and PG. Plasminogen-derived activity is defined as the PL activity generated after addition of urokinase. The sum of PL+PG was calculated by adding the activity of PL plus the activity of PG. Both assays were performed in 250 μL of 0.1 *M* Tris-HCl buffer (pH 7.4) containing 0.6 m*M* Val-Leu-Lys-p-nitroanilide (V7127; Sigma Chemical Co., St Louis, MO), 30 Plough units (2.5 μL) of urokinase (U0633; Sigma), and 30 μL of the milk supernatant. All assays were duplicated. The reaction mixture was incubated at 37°C and absorbance at 405 nm was recorded at hourly intervals. A sample without supernatant served as a control for the detection of spontaneous breakdown of the substrate. In all cases, spontaneous hydrolysis was negligible. Plasmin activity was measured in the same reaction mixture without added urokinase. Plasmin and PG activities were determined from the linear part of the absorbance vs. time curve. One unit of PL was defined as the amount of enzyme that produced a change in absorbance of 0.1 at 405 nm in 60 min.

A colorimetric assay was used to measure PA activity in the CN fraction (Gilmore et al., 1995). The principle of