Profile of Gelatinolytic Capacity of Raw Goat Milk and the Implications for Milk Quality

W. Y. Chen,* M. H. Weng,* S. E. Chen,* H. C. Peh,* W. B. Liu,* T. C. Yu,* M. C. Huang,* M. T. Chen,†
H. Nagahata,‡ and C. J. Chang*¹

*Department of Animal Science, National Chung Hsing University, Taichung 402, Taiwan, Republic of China

†Department of Bioindustry Technology, Da Yeh University, Chung Hwa 515, Taiwan, Republic of China

‡Department of Animal Health, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan

ABSTRACT

Both endogenous and exogenous proteinases occur in milk, and they can have beneficial or detrimental effects on dairy production. Because the lactation length of dairy goats is shorter and the somatic cell count (SCC) of goat milk is generally greater compared with dairy cows, the objectives of the present study were to investigate the prevalence of major proteinases in raw goat milk, their association with SCC and production stage, and their effects on milk quality. Milk samples were collected from individual goats in consecutive weeks for different durations, covering regular lactation, late lactation, and post-milk stasis. Long-term (monthly) or short-term (weekly) fluctuations of milk fibrinolytic and gelatinolytic capacities of individual goats were revealed chronologically on fibrin and gelatin zymograms, respectively. In a separate trial involving milk samples from 23 goats at random production stages, the percentage of ultracentrifuge force-precipitable casein of total milk protein was calculated to represent milk quality and was assessed to evaluate its correlation with the corresponding proteolytic capacities. The results for regular milk indicate that gelatinase B was more abundant than gelatinase A when they first appeared at SCC of $\sim 1 \times 10^6/\text{mL}$. During the last month before milk stasis, both gelatinases A and B were found to be prevalent and prominent in milk regardless of the broad SCC range recorded there. Fibrinolytic activity and the active form of gelatinase A were only regularly detected in post-stasis secretions and were scarce before stasis. The results of the milk quality trial indicate that milk of relatively high proteinase capacity tended to have a low casein ratio. Correlation analysis confirmed a significant relationship between gelatinase capacity of goat milk and production stage, SCC, or casein ratio. It is suggested that an elevation of gelatinolytic capac-

ity of goat milk coincides with an increase in somatic cell number accompanying the extension of lactation length, which is unfavorable for the production of a more desirable quality of goat milk.

Key words: goat milk, gelatinase, somatic cell, lactation stage

INTRODUCTION

In the Western world, goat milk is mostly processed for cheese manufacturing, which is a relatively small but growing industry. There are 1 million dairy goats in the United States producing 24,000 tonnes of fluid milk a year that goes to the manufacture of 600 tonnes of cheese (Van Hekken et al., 2004). The number of goats in Italy is slightly greater than that of the United States, approximately 1.2 million (Fantuz et al., 2001). Due to the increasing number of children suffering from intolerance to cow's milk, and because of the demand for natural and unprocessed food, the demand for unpasteurized goat milk by consumers is growing (Muehlherr et al., 2003). For Asian people, goat milk is a traditional nutraceutical usually consumed unprocessed. New evidence has evolved (Denhard et al., 2000; Prosser et al., 2004; Wu et al., 2006) supporting the description in an ancient Chinese medical text of goat milk as a tonic for the digestive system.

The distinctive properties of goat milk compared with those of cow milk have applications to the manufacturing of milk products. The smaller size of fat globule in goat milk affects the viscosity of milk (Attaie and Richter, 2000). Goat milk contains an α_{s2} - to β - to κ -casein ratio of approximately 1:3:1. Cow milk contains α_{s1} -casein as the major protein, whereas goat milk appears to be rather low in this casein subtype, which contributes to the lower heat stability of the latter (Mora-Gutierrez et al., 1991). The milk SCC standard for goat milk has been previously assigned as 1×10^6 cells/mL, in contrast to the 0.75×10^6 cells/mL standard for cow milk (Droke et al., 1993). Physiological, productive, and preservative factors have been related to the generally

Received May 17, 2007.

Accepted June 25, 2007.

¹Corresponding author: crchang@mail.nchu.edu.tw

greater milk SCC of goats compared with cows (Zeng et al., 1997; Paape et al., 2001; Sanchez et al., 2006). The microbiology count for bulk tank milk from goats was high (Muehlherr et al., 2003). However, unlike cow milk, which is subject to stringent hygiene and quality regulations, standards for production and distribution of goat milk are more relaxed.

Both the native proteinases including plasmin (EC 3.4.21.7) and proteinases from leukocytes, and non-native proteinases from microorganisms infecting milk contribute to the overall proteolytic capacity of milk (Le Roux et al., 1995; Moussaoui et al., 2003; Larsen et al., 2004). Plasmin is converted from blood-borne plasminogen by regional plasminogen activator and is the predominant and most extensively studied protease in milk (Nielsen, 2002). Lysosome in somatic cells contains elastase (EC 3.4.21.37), cathepsin G (EC 3.4.21.20), and collagenases (EC 3.4.24.3; Le Roux et al., 2003). Gelatinase B (EC 3.4.24.35), however, is synthesized and stored in cytoplasmic granules by granulocytes and represents the major somatic cell-derived enzyme in mastitis milk (Faurischou and Borregaard, 2003). On the other hand, gelatinase A (EC 3.4.24.24) is mainly produced by the stromal cells (Donadio et al., 2005). Beyond plasmin, however, the implication of other milk proteinases to dairy production and milk quality has yet to be disclosed.

High levels of plasmin and plasminogen activator were found in goat milk during late lactation and subclinical mastitis, which correlated with the property changes of milk (Fantuz et al., 2001; Leitner et al., 2004; Weng et al., 2006). Inoculation with either endotoxin or the microorganism cell increased plasmin, gelatinase A, and gelatinase B in cow milk and induced the generation of γ -casein from β -casein (Raulo et al., 2002; Haddadi et al., 2005). Bacterial growth at refrigeration temperature induced the shift in plasmin from casein to the whey fraction and imposed potential impacts on its functionality as a food ingredient (Hayes and Nielsen, 2000). Alternatively, some traditional goat cheese is typically made from raw milk with microbial proteases responsible for enhancing the desirable flavor characteristics of the final product (Muehlherr et al., 2003). These efforts were exercised to reveal the properties of major milk proteinases under pathological or processing conditions. Basic information on the prevalence of proteinases spontaneously occurring in raw milk and their significance remains to be established. Proteinases of raw milk might contribute to quality variation of bulk tank milk and, ultimately, that of milk products. Because lactation length of dairy goats is shorter and SCC of goat milk is generally greater compared with dairy cows, this study used dairy goats as a model with the objectives of establishing a chronological profile of

major proteinases in raw goat milk and exploring to what extent somatic cell and lactation stage contribute the fluctuation.

MATERIALS AND METHODS

Reagents and Chemicals

Human plasmin was purchased from American Diagnostica (Greenwich, CT) and gelatinase zymography standard was purchased from Chemicon International (Temecula, CA). Thrombin and NP-40 were from Calbiochem EMD Biosciences (Darmstadt, Germany). Fibrinogen, gelatin, aprotinin, cystatin, leupeptin, pepstatin, and Tween 80 were products of Sigma-Aldrich (St. Louis, MO). Protein kit (dye-binding based), low range SDS-PAGE protein standards, and reagents for electrophoresis were from BioRad (Hercules, CA). Buffers and mediums of cell culture or analytical grade were supplied from Amersham-Pharmacia Biosciences (Uppsala, Sweden), Merck (Darmstadt, Germany), BDH (Poole, UK), and United States Biological (Swampscott, MA).

Animals and Sampling

Toggenberg and Alpine cross-breed goats were housed at the experimental farm facility of the Department of Animal Science, National Chung Hsing University, Taichung, Taiwan). Care of the animals complied with the guidelines of the Animal Welfare Committee of National Chung Hsing University. Lactating goats were fed and hand-milked twice a day as described previously (Weng et al., 2006). The drying-off scheme was introduced to individual goats as yield dropped to less than one-tenth of peak production, at which point milking frequency was decreased gradually from once per day to once every other day or less until complete stasis, within a period of 1 mo. Milk bacterial counts, SCC, and gross composition were measured for individual goats on a monthly basis (City Bureau of Animal Disease Prevention and Diagnosis, Taichung, Taiwan). Clinically healthy goats at 2 to 5 mo of the second to fourth lactation were selected for use in this study. The experimental protocols were approved by the National Science Council and Committee of Agriculture, Taiwan. Representative milk was sampled in consecutive weeks for up to 6 mo (7 animals) encompassing the month before and after milk stasis. An aseptic practice was applied for sampling and handling of milk sample. An adequate amount (30 to 50 mL) of raw milk was collected directly from the udders, transported on ice within 15 min, and skimmed at $2,000 \times g$ for 20 min at room temperature. Milk serum was recovered and stored in 1-mL eppendorf tubes at -70°C for protein

content determination and zymographic assay. The precipitated cell pellet was gently washed and counted in triplicate under microscope (Tian et al., 2005) to obtain microscopic SCC (MSCC).

Fibrin Zymography

The zymographic condition to estimate plasmin concentration in milk serum was a nonreducing, 10% SDS PAGE (Laemmli, 1970) containing 0.25 IU/mL thrombin and 1.5 mg/mL fibrinogen in the separating gel (Weng et al., 2006). Human plasmin and molecular weight markers were loaded in parallel in each round of zymography.

Gelatin Zymography

Estimation of gelatinase concentration in milk used a similar nonreducing, 10% SDS PAGE as above but containing 0.3% gelatin in the separating gel in place of thrombin and fibrinogen (Tian et al., 2005). After electrophoresis, gels were soaked in 2.5% Triton X-100 buffer for 30 min to renature proteinases, washed thoroughly with distilled H₂O, and then developed in 50 mM Tris buffer (pH 7.4) containing 200 mM NaCl, 0.02% Brij-35, and 5 mM CaCl₂ at 37°C for 16 h. Gelatinase zymography standard molecular weight markers and a volunteer's blood sample were loaded in parallel to validate the procedure and phenotyping (Makowski and Ramsby, 1996).

Milk Quality vs. Proteinase Capacity of Milk

Fifteen twice-milking goats as well as 8 dried goats within 1 mo following milk stasis were selected for use in the milk quality trial. Fresh udder samples were collected and immediately skimmed as described above. A portion of skimmed serum was stored as aliquots for later analyses, the remainder was centrifuged at 100,000 × *g* for 1 h at 4°C. Protein content of the original skimmed serum and the supernatant after ultracentrifugation was determined and precipitable casein was calculated as the difference. The percentages of precipitable casein of total milk protein were used to estimate milk quality.

After gel scanning, quantification of the photographic negative band on zymogram was performed by using TotalLab software (v. 1.11, Ultra Lum Inc., Claremont, CA). Calibration with reference to gelatinase standards was conducted.

Statistical Analyses

Statistical analysis was performed with the Proc GLM and Proc CORR procedures (SAS Institute, 2003).

This study tested 2 models. The first model was as follows:

$$Y_{ij} = \mu + L_i + M_j + L_iM_j + e_{ij} \quad [1]$$

where the dependent variables (Y_{ij}) were corrected zymogram data of gelatinase B, gelatinase A, and active gelatinase A, respectively; μ = overall mean; L_i = lactation stage; M_j = MSCC; L_iM_j = lactation stage × MSCC interactions; and e_{ij} = error term (experimental variation among goats, parity, milk yield, DIM, milk frequency, weeks after stasis, etc.). In the second model:

$$Y_{ijk} = \mu + B_i + A_j + M_k + B_iM_k + A_jM_k + B_iA_jM_k + e_{ijk} \quad [2]$$

the dependent variable (Y_{ijk}) was casein ratio, B_i = gelatinase B capacity in milk, A_j = gelatinase A capacity in milk, M_k = MSCC, B_iM_k = gelatinase B capacity × MSCC interactions, A_jM_k = gelatinase A capacity × MSCC interactions, and $B_iA_jM_k$ = gelatinase B × gelatinase A capacity × MSCC interactions.

Linear, logarithmic, polynomial, or exponential regression was analyzed between pairs of variables. A *P*-value < 0.05 was considered significant in each analysis.

RESULTS

Validation of Zymography and Proteinase Phenotyping

Fibrin and gelatin zymographic methods were applied, respectively, to reveal the chronological fluctuation of fibrinolytic and gelatinolytic capacities of goat milk during various phases of lactation. Proteinases were characterized by zymographic technique based on substrate specificity and by molecular weight at the same time. Under zymographic conditions, protease is separated from potentially coexisting inhibitors and the hindered catalytic site of latent protease is exposed. Therefore, the density of digest band on zymograms indicates the overall capacity rather than the actual *in vivo* activity of the proteinase of interest.

Human plasmin in the range of 10 to 80 ng of protein for fibrin zymography shows apparent semiquantitative characteristics (Figure 1A). The semiquantitative trend for gelatinase standard on gelatin zymography was observed from 0.2 to 1.4 ng of protein (Figure 1B). In contrast to the gelatinase phenotypes of human blood, commercial gelatinase zymography standards contained both latent and active forms of gelatinase A and a small amount of gelatinase B. The SDS-PAGE (Figure 1C) and gelatin zymography (Figure 1D) of a representative milk sample confirmed the semiquanti-

Figure 1. Semiquantitative zymography for human plasmin standard on fibrin SDS-PAGE (A) and human gelatinase standard on gelatin SDS-PAGE (B) loaded at the indicated nanogram levels. Panels C and D show a representative goat milk sample on regular SDS-PAGE (C) and on gelatin SDS-PAGE (D) loaded at the indicated microgram protein levels. LF = lactoferrin, IGh = immunoglobulin heavy chain.

tative aspects for the 92-kDa gelatinase B from 40 to 100 μ g of loaded protein, and in smaller amounts, the 72-kDa gelatinase A. Caseins bands (21 to 24 kDa) of this representative milk sample were visible on both SDS-PAGE and gelatin zymography under the loading level, establishing it as a reliable internal control. No fibrinolytic capacity was detected for the same representative goat milk sample with the same loading range

as gelatin zymography (data not shown). Based on these observations, throughout the study, the amount of skimmed milk serum containing 80 and 60 μ g protein, respectively, was used in fibrin and gelatin zymography.

Monthly Profile of Proteinase in Goat Milk and the Concurrent SCC

The full 6-mo weekly sampling scheme was completed in 2 goats (no. 75 and 439), and sampling during the months immediately before and after milk stasis was successful in 5 goats (no. 86, 284, 10, 59, and 80). The long-term profile of proteinase capacity of goat milk was displayed on separate zymograms at monthly intervals for individual goats (Figure 2) with the first sample collected after milk stasis serving as a positive control. The results show that fibrinolytic and gelatinolytic capacities were detected in milk of goat no. 75 only during the last 2 mo in lactation, whereas gelatinolytic capacity, but not fibrinolytic capacity, was detected in milk of goat no. 439 throughout the 6-mo sampling period. The casein bands seen in gelatin zymograms in parallel with gelatinase bands for each time point demonstrate the reliability of the protease profile.

The corresponding profile of MSCC indicate a stable and relatively low level, mostly less than 1×10^6 /mL milk, for goat no. 75 for the entire duration, whereas that for goat no. 439 rose abruptly in the last 2 mo to exceed 4×10^6 /mL.

Weekly Profile of Proteinase in Milk During Late Lactation and the Concurrent SCC

The short-term and close-up profile of proteinase capacity of goat milk during the last month of lactation was displayed at weekly intervals on separate zymograms for each goat with the first post-stasis sample serving as a positive control (Figure 3B). Fibrinolytic capacity was not detected on any zymograms (data not shown). Prominent gelatinolytic capacity was observed in 3 out of the 4 goats where the density of the gelatinase B band overrode that of gelatinase A.

In the corresponding profile of MSCC, 2 types of patterns were determined. The MSCC level of goats 86 and 284 were relatively stable and not much different from the 1×10^6 /mL level except for the last time-point of goat 284. The MSCC level of goats 10 and 59, on the other hand, increased abruptly in the middle of the last month of lactation to 5×10^6 /mL and then continued to increase (Figure 3A).

Profile of Proteinases and MSCC after Milk Stasis

The profile of proteinases of mammary secretion of goats 59 and 80 following stasis at weekly intervals

Figure 2. Monthly profiles of SCC and proteases in milk from 2 individual goats: A) Microscopic SCC, B) fibrin SDS-PAGE, and C) gelatin SDS-PAGE were displayed as times relative to milk stasis.

on separate zymograms indicated that both fibrinolytic and gelatinolytic capacity were prominent compared with that of the last month's milk (Figure 4). Moreover, the active form of gelatinase A appeared after milk stasis. Meanwhile, MSCC level after milk stasis was extreme and beyond $10 \times 10^6/\text{mL}$.

Correlation Analysis of Gelatinase Capacity of Goat Milk with Lactation Stage and MSCC

The share of lactation stage and somatic cell in contributing to the fluctuation of proteinase capacity in goat milk was estimated by correlation analysis. Zymograms of each individual goat were quantified using the photographic negative bands and its own background, adjusted for standards, and expressed in arbitrary units. Pooled results of all tested goats were correlated against the corresponding time relative to milk stasis (Figure 5) and MSCC (Figure 6). An increasing trend of capacities in goat milk of all 3 gelatinases detected was observed with the advancement of lactation and drying off and was more so for MSCC (Figure 5). On

the other hand, when the capacities of gelatinase of goat milk were correlated against the corresponding MSCC (Figure 6), gelatinase B seemed to be more scattered around than the rest of the gelatinases. The correlation coefficient (r) (Table 1) shows that the association between gelatinase B capacity of goat milk and time in lactation or MSCC was marginal, whereas those of gelatinase A were mostly significant ($P < 0.05$ and $R^2 > 0.5$) depending on the mode of regression.

Milk Quality as Related to Proteinase Capacity of Milk

Proteinase capacity of milk from the 23 goats was aligned as histograms in descending order of their corresponding casein ratio (Figure 7). The results revealed that udder secretions of the dried-off goats had lower casein ratio and greater proteinase capacity when compared with most lactating goats. Goats 14 and 15, however, belonged to the lactating group but were high in milk proteinase capacity and low in casein ratio. On the other hand, milk of goats 7 to 13 had low proteinase

Figure 3. Weekly profiles of somatic cell counts and proteases in the last-month milk of 4 individual goats. A) Microscopic SCC and B) gelatin SDS-PAGE were displayed as times relative to milk stasis.

capacity despite the relatively low casein ratio compared with goats 1 to 6 (Figure 7).

Correlation Analysis Between Proteinase Capacity of Goat Milk and Milk Quality

To reveal the association between proteinase capacity of milk and milk quality, capacity data were correlated against the corresponding casein ratio for the 23 goats tested (Figure 8) and the correlation coefficient analyzed (Table 1). The results show that the relationship between proteinase capacity of goat milk and casein ratio was apparently negative. The association was significant for gelatinase A and gelatinase B using exponential mode of regression, but was not significant for plasmin.

DISCUSSION

Proteinases of raw goat milk are not only responsible for the undergoing milk protein modification after col-

lection, but also represent a physiologically meaningful bioactive component. Plasmin can associate with target proteins such as fibrin and casein micelles through its kringle domain to facilitate efficient proteolysis (Politis, 1996). Plasmin-initiated proteolysis also involves in a variety of normal and pathological processes that require cell migration and tissue remodeling. High levels of plasmin and plasminogen activator were reported in goat milk during late lactation and subclinical mastitis and were attributed to the changes of milk properties (Fantuz et al., 2001; Leitner et al., 2004). Our previous study demonstrated the occurrence of plasmin in goat milk during regular lactation and its close association with casein ratio (Weng et al., 2006). Similar to the report of plasmin in goat milk, the current results indicated that gelatinolytic capacity was apparently more prevalent in goat milk during late lactation than during regular lactation. Because the zymographic analysis was conducted by loading the same amount of protein (but not same volume of milk serum); that is, 80 and 60 μ g protein equivalent of skimmed milk serum for

Figure 4. Weekly profiles of SCC and proteases in udder secretions of two dried goats. A) Microscopic SCC, B) fibrin SDS-PAGE, and C) gelatin SDS-PAGE were displayed as times relative to milk stasis.

fibrin and gelatin zymography, respectively, condensation caused by decreased milk yield accompanying the regression of mammary function was eliminated. In addition, despite the semiquantitative nature of the assay, because proteinase capacity was displayed in consecutive months or weeks on each zymogram for a single goat, the unique profile of proteinase capacity on a specific zymogram represents the individuality of goat, whereas distinctive profiles on different zymograms reliably reflect variation among the population. Both individuality and variation in milk traits exert effects on bulk tank milk quality, and was especially so for goats compared with cows.

The present study, based on zymographic results, shows that more prominent gelatinolytic capacity was found in raw goat milk compared with the homologous fibrinolytic capacity. It was pointed out in our previous study that plasmin activity was more readily detected by a chromogenic method than by fibrin zymography (Weng et al., 2006). Although a direct comparison between the abundance of gelatinase and plasmin in milk is not realistic, their relative levels might be physiologically meaningful. Gelatinases are members of the matrix metalloproteinase (MMP) family, which are named after the zinc ion and the conserved methionine residue

at the active site. Gelatinase B (MMP9) represents the largest and most complex member. Both gelatinase A (MMP2) and gelatinase B have fibronectin type II repeats to mediate binding to collagens and inserting into the catalytic domain (Page-McCaw et al., 2007). There is complex relationship between MMP and pathology and relatively little understanding of the normal, *in vivo* function of MMP in development. Historically, gelatinases are thought to function mainly as enzymes that degrade structural components of the extracellular matrix (ECM). The cleavage of collagen IV by gelatinase results in the exposure of cryptic sites that promote migration. An array of functions has been reported for the broader term of collagenase; they produce specific substrate-cleavage fragments with biological activity, activate, deactivate or modify signaling molecules, regulate the interaction of ECM with cells, and release ECM-bound growth factors and gene regulation (Page-McCaw et al., 2007). The regulation of gelatinase activity is highly complex and is established at 5 different levels (Van den Steen et al., 2002). Transcription of the gelatinase gene depends on a large variety of soluble factors, including cytokines, growth factors, and hormones and specific signaling pathways that induce or repress its gene promoter. The specific regulation of its

Figure 5. Distribution of gelatinolytic capacities of goat milk, as well as microscopic somatic cell counts (MSCC), for experimental goats according to the corresponding time relative to milk stasis.

secretion occurs in cells storing gelatinase in granules. After secretion, latent gelatinase must be activated through an activation network. The enzyme activity is further regulated by inhibition and by other mechanisms, such as fine-tuning and stabilization by glycosylation. Plasmin as the key initiator of the activation cascade of gelatinase has long been acknowledged

Figure 6. Distribution of gelatinolytic capacities of goat milk for experimental goats according to the corresponding microscopic somatic cell counts (MSCC).

(Cuzner and Opdenakker, 1999). It is reasonable to assume that only a minor increase in the plasmin system is required to bring about greater gelatinase capacity. The presence of active form of gelatinase B or gelatinase A was found in some infected biological fluids (Jouglin et al., 2000; Raulo et al., 2002; Makowski and Ramsby, 2003). We observed a consistent appearance of a 62-kDa active gelatinase A and simultaneously more prominent fibrinolytic capacity in dry secretion; both were barely detected otherwise, suggesting plasmin-initiated activation of gelatinase A in dried glands. However, due to the few numbers of observations, no promising correlation results are available currently to solidify their association.

Table 1. Correlation coefficients between proteinase capacity of goat milk and various parameters of milk production

	Gelatinase B	Gelatinase A (inactive)	Gelatinase A (active)	Plasmin	MSCC
Time in production cycle (n = 37)					
Linear	0.5305	0.5746	0.4964	NA ¹	0.5794
Logarithmic	0.4899	0.4512	0.3695	NA	0.459
Polynomial	0.5397	0.8003*	0.7927*	NA	0.8082*
Exponential	0.0458	0.0283	0.3548	NA	0.695
Microscopic SCC (n = 37)					
Linear	0.5207	0.8184*	0.8704*	NA	1
Logarithmic	0.6796	0.7241*	0.6461	NA	1
Polynomial	0.6544	0.8217*	0.879*	NA	1
Exponential	0.3665	0.4138	0.6166	NA	1
Casein ratio (n = 23)					
Linear	0.5096	0.4466	NA	0.3165	NA
Logarithmic	0.6052	0.7429*	NA	NA	NA
Polynomial	0.6927	0.6098	NA	0.4169	NA
Exponential	0.7146*	0.7863*	NA	0.376	NA

¹NA = not available.**P* < 0.05.

Whether high SCC is a cause, an accompanying event, or a result of greater proteinase capacity of goat milk cannot be answered in confidence by this study. A survey in the United States indicated that the average SCC for goat milk was 1.32×10^6 cells/mL, where 8.6% of the producers had SCC $< 0.75 \times 10^6$ cells/mL and 34.5% had $< 1 \times 10^6$ cells/mL (Droke et al., 1993). Therefore, during the long-term survey in this study, milk SCC of goat no. 75 was within the acceptable range for goats but was only so in earlier months for goat no. 439. The SCC of noninfectious goats varies with factors such as individual and daily variation (Zeng et al., 1997), duration of lactation (Luengo et al., 2004), or even estrus cycle (Moroni et al., 2007). The level of SCC in goat was reported as high as $7.2 \pm 0.7 \times 10^6$ cells/mL at estrus and declined toward $1.7 \pm 0.7 \times 10^6$ cells/mL at the luteal phase, suggesting single digital variation of SCC might not be uncommon during the relatively shorter production interval of dairy goats compared with dairy cows. Consequently, premature regression of mammary production might be one of the causes of high milk SCC in goat no. 439. Moreover, when short-term profile of proteinase capacity was contrasted with those of MSCC during late lactation, the 2 goats (no. 86 and 284) with relatively similarly low MSCC expressed greatly different proteinases capacity in their milk. Furthermore, despite of several-fold difference in MSCC, prominent proteinase capacity was observed for goats 284, 10, and 59. The correlation analysis conducted after pooling data of all stages did suggest that both time in production and MSCC only moderately contribute to gelatinase B capacity in goat milk, and were more significantly to that of gelatinase A. It is suspected that if more observations had been accumu-

lated, different mode of correlations might be elucidated for different stages of production.

Regarding the origin of milk gelatinase other than somatic cells, bacterial proteases might be considered one of the possibilities. All goats used in this study complied with the 5 log cfu/mL tentative national standard. A recent survey in Switzerland including 8% of the country's dairy goats pointed out the median standard plate count for bulk tank goat milk was 4.70 log cfu/mL with a minimum of 2.00 log cfu/mL and a maximum of 8.64 log cfu/mL (Muehlherr et al., 2003) suggesting an broad allowable range of bacterial count in goat milk. Despite the fact that the level of gelatinase B in *Escherichia coli*-inoculated milk was reported to be increased after 24 h of incubation (Haddadi et al., 2005), bacterial proteinases can be disregarded in our study because all measurements were conducted in raw milk and the involved animals were free of infection according to regular screen standards. As for endogenous sources, greater expression of matrix-degrading proteinases in developing or involuted mammary tissue compared with lactating cows was recently confirmed (Rabot et al., 2007). The increase of percentage of gelatinase B-secreting leukocytes in somatic cells during involution of mammary gland might contribute to a part (Sordillo and Nickerson, 1988).

Casein ratio of goat milk was negatively correlated to the capacities of gelatinases B and A of goat milk, each contributing about 50% of the fluctuation described in exponential mode, although both gelatinases were apparently intercorrelated. Nevertheless, there were several out-skirted goats with abnormally low casein ratio while lactating. It was our original presumption that proteolytic modification of the casein micelle

Figure 7. Aligned histograms of percentages of ultracentrifuge-precipitable casein of total milk protein (upper panel) and proteinase capacity (lower panel) of raw milk collected from 23 goats.

increases soluble casein and, therefore, decreases ultracentrifuge-precipitable casein. High plasmin level has been shown to relate to the decrease of casein to protein ratio during late lactation of the goat (Fantuz et al., 2001). A correlation between gelatinase level and the extent of caseinolysis has not been noticed before. Goat milk casein is relatively less homogeneous in micelle size and therefore inherently expresses lower and variable casein ratios (Renner, 1982). It is also well known that the functionality of mammary epithelial cells af-

fects casein synthesis. Furthermore, it is widely acknowledged that the length of lactation of dairy goats varies significantly among individuals, breeds, seasons, and kidding frequency (Salama et al., 2005); that is, the rate of natural regression of mammary gland presumably varies greatly among goats. We presume that the preterm low casein ratio observed in regular milk might be partially related to early regression of mammary function. Because the MSCC level was not monitored accordingly in the present milk quality trial, the

Figure 8. Distribution of casein ratio of total milk protein of experimental goats according to the respective capacity of proteinases of goat milk.

contribution of somatic cell-derived proteinases to quality modification of raw milk (Faurschou, and Borregaard, 2003; Sanchez et al., 2006) might have differed greatly.

The high gelatinolytic capacity of milk is a common phenomenon of the declining phase of goat lactation occurring to facilitate somatic cell transmigration and mammary tissue remodeling. Due to the lack of standards for goat milk and various kidding regimens practiced by dairy goat farmers, the risk of compromised quality of bulk tank goat milk would be suspected to increase with the extension of length of goat lactation, which is frequently recommended for simpler management and less postpartum stress.

ACKNOWLEDGMENTS

This work was supported by grants of National Science Council, Taiwan, ROC (NSC91-2313-B005-128 and NSC92-2313-B005-121).

REFERENCES

- Attaie, R., and R. L. Richter. 2000. Size distribution of fat globules in goat milk. *J. Dairy Sci.* 83:940–944.
- Cuzner, M. L., and G. Opdenakker. 1999. Plasminogen activators and matrix metalloproteinases, mediators of extracellular proteolysis in inflammatory demyelination of the central nervous system. *J. Neuroimmunol.* 94:1–14.
- Denhard, M., R. Claus, O. Munz, and U. Weiler. 2000. Course of epidermal growth factor (EGF) and insulin-like growth factor I (IGF-I) in mammary secretion of goat during end-pregnancy and early lactation. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* 47:533–540.
- Donadio, A. C., S. Durand, M. M. Remedi, S. Frede, D. G. Ceschin, S. Genti-Raimondi, and G. A. Chiabrando. 2005. Evaluation of stromal metalloproteinases and vascular endothelial growth factors in a spontaneous metastasis model. *Exp. Mol. Pathol.* 79:259–264.
- Droke, E. A., M. J. Paape, and A. L. Di Carlo. 1993. Prevalence of high somatic cell counts in bulk tank goat milk. *J. Dairy Sci.* 76:1035–1039.
- Fantuz, F., F. Polidori, F. Cheli, and A. Baldi. 2001. Plasminogen activation system in goat milk and its relation with composition and coagulation properties. *J. Dairy Sci.* 84:1786–1790.
- Faurschou, M., and N. Borregaard. 2003. Neutrophil granules and secretory vesicles in inflammation. *Microbes Infect.* 14:1317–1327.
- Haddadi, K., F. Moussaoui, I. Hebia, F. Laurent, and Y. Le Roux. 2005. *E. coli* proteolytic activity in milk and casein breakdown. *Reprod. Nutr. Dev.* 45:485–496.
- Hayes, K. D., and S. S. Nielsen. 2000. Plasmin levels in fresh milk whey and commercial whey protein products. *J. Dairy Sci.* 83:387–394.
- Jouglin, M., C. Robert, J. Valette, F. Gavard, F. Quintin-Colonna, and J. Denoix. 2000. Metalloproteinases and tumor necrosis factor- α activities in synovial fluids of horses: Correlation with articular cartilage alterations. *Vet. Res.* 31:507–515.
- Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head bacteriophage T4. *Nature* 227:680–685.
- Larsen, L. B., M. D. Rasmussen, M. Bjerring, and J. H. Nielson. 2004. Proteases and protein degradation in milk from cows infected with *Streptococcus uberis*. *Int. Dairy J.* 14:899–907.
- Leitner, G., U. Merin, and N. Silanikove. 2004. Changes in milk composition as affected by subclinical mastitis in goats. *J. Dairy Sci.* 87:1719–1726.
- Le Roux, Y., O. Colin, and F. Laurent. 1995. Proteolysis in samples of quarter milk with varying somatic cell counts. 1. Comparison of some indicators of endogenous proteolysis in milk. *J. Dairy Sci.* 78:1289–1297.
- Le Roux, Y., F. Laurent, and F. Moussaoui. 2003. Polymorphonuclear proteolytic activity and milk composition change. *Vet. Res.* 34:9–45.
- Luengo, C., A. Sanchez, J. C. Corrales, C. Fernandez, and A. Contreras. 2004. Influence of intramammary infection and non-infection factors on somatic cell counts in dairy goats. *J. Dairy Res.* 71:169–174.
- Makowski, G. S., and M. L. Ramsby. 1996. Calibrating gelatin zymograms with human gelatinase standards. *Anal. Biochem.* 236:353–356.
- Makowski, G. S., and M. L. Ramsby. 2003. Zymographic analysis of latent and activated forms of matrix metalloproteinase-2 and -9 in synovial fluid: Correlation to polymorphonuclear leukocyte infiltration and in response to infection. *Clin. Chim. Acta* 329:77–81.

- Mora-Gutierrez, A., T. F. Kumosinski, and H. M. Farrell, Jr. 1991. Quantification of α_{s1} -casein in goat milk from French-Alpine and Anglo-Nubian breeds using reversed-phase high performance liquid chromatography. *J. Dairy Sci.* 74:3303–3307.
- Moroni, P., G. Pisoni, G. Savoini, E. van Lier, S. Acuña, J. P. Damián, and A. Meikle. 2007. Influence of estrus of dairy goats on somatic cell count, milk traits, and sex steroid receptors in the mammary gland. *J. Dairy Sci.* 90:790–797.
- Moussaoui, F., F. Laurent, J. M. Girardet, G. Humbert, J. L. Gaillard, and Y. Le Roux. 2003. Characterization and proteolytic origins of specific peptides appearing during lipopolysaccharide experimental mastitis. *J. Dairy Sci.* 86:1163–1170.
- Muehlherr, F. E., C. Zweifel, S. Corti, J. E. Blanco, and R. Stephan. 2003. Microbiological quality of raw goat's and ewe's bulk-tank milk in Switzerland. *J. Dairy Sci.* 86:3849–3856.
- Nielsen, S. S. 2002. Plasmin system and microbial proteases in milk: Characteristics, roles, and relationship. *J. Agric. Food Chem.* 50:6628–6634.
- Paape, M. J., B. Poutrel, A. Contreras, J. C. Marco, and A. V. Capuco. 2001. Milk somatic cells and lactation in small ruminants. *J. Dairy Sci.* 84(E Suppl.):E237–E244.
- Page-McCaw, A., A. J. Ewald, and Z. Werb. 2007. Matrix metalloproteinases and the regulation of tissue remodeling. *Nat. Rev. Mol. Cell Biol.* 8:221–233.
- Politis, I. 1996. Plasminogen activator system: Implications for mammary cell growth and involution. *J. Dairy Sci.* 79:1097–1107.
- Prosser, C., K. Steiwagen, R. Cummins, P. Guerin, N. Gill, and C. Milne. 2004. Reduction of heat-induced gastrointestinal hyperpermeability in rats by bovine colostrums and goat milk powders. *J. Appl. Physiol.* 96:650–654.
- Rabot, A., F. Sinowatz, B. Berisha, H. H. D. Meyer, and D. Schams. 2007. Expression and localization of extracellular matrix-degrading proteinases and their inhibitors in the bovine mammary gland during development, function, and involution. *J. Dairy Sci.* 90:740–748.
- Raulo, S. M., T. Sorsa, T. Tervahartiala, T. Latvanen, E. Pirila, J. Hirvonen, and P. Maisi. 2002. Increase in milk metalloproteinase activity and vascular permeability in bovine endotoxin-induced and naturally occurring *Escherichia coli* mastitis. *Vet. Immunol. Immunopathol.* 85:137–145.
- Renner, M. 1982. Milk and Milk Products in Human Nutrition, Vdkswirtschaft, Verlag GmbH, Munich, Germany.
- Salama, A. A. K., G. Caja, X. Such, R. Casals, and E. Albanell. 2005. Effect of pregnancy and extended lactation on milk production in dairy goats milked once daily. *J. Dairy Sci.* 88:3894–3904.
- Sanchez, A., D. Sierra, C. Luengo, J. C. Corrales, C. T. Morales, A. Contreras, and C. Gonzalo. 2006. Influence of storage and preservation on Fossomatic cell count and composition of goat milk. *J. Dairy Sci.* 88:3095–3100.
- SAS Institute. 2003. SAS/STAT User's Guide. Release 6.12. SAS Institute Inc., Cary, NC.
- Sordillo, L. M., and S. C. Nickerson. 1988. Morphologic changes in the bovine mammary gland during involution and lactogenesis. *Am. J. Vet. Res.* 49:1112–1120.
- Tian, S. Z., C. J. Chang, C. C. Chiang, H. C. Peh, M. C. Huang, J. W. Lee, and X. Zhao. 2005. Comparison of morphology, viability and function between blood and milk neutrophils from peak lactating goats. *Can. J. Vet. Res.* 69:39–45.
- Van den Steen, P. E., B. Dubois, I. Nelissen, M. P. Rudd, R. A. Dwek, and G. Opdenakker. 2002. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). *Crit. Rev. Biochem. Mol. Biol.* 37:375–536.
- Van Hekken, D. L., M. H. Tunick, and Y. W. Park. 2004. Rheological and proteolytic properties of Monterey Jack goat's milk cheese during aging. *J. Agric. Food Chem.* 52:5372–5377.
- Weng, M. H., C. J. Chang, Y. W. Chen, W. K. Chou, H. C. Peh, M. C. Huang, M. T. Chen, and H. Nagahata. 2006. Contribution of somatic cell-associated activation of plasminogen to caseinolysis within the goat mammary gland. *J. Dairy Sci.* 89:2025–2037.
- Wu, F. Y., P. H. Tsao, D. C. Wang, S. Lin, J. S. Wu, and Y. K. Cheng. 2006. Factors affecting growth factor activity in goat milk. *J. Dairy Sci.* 89:1951–1955.
- Zeng, S. S., E. N. Escobar, and T. Popham. 1997. Daily variations in somatic cell count, composition, and production of Alpine goat milk. *Small Rumin. Res.* 26:253–260.