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Activities of indigenous proteolytic enzymes in caprine milk of different somatic cell counts

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ABSTRACT

Individual caprine milk with different somatic cell counts (SCC) were studied with the aim of investigating the percentage distribution of leukocyte cell types and the activities of indigenous proteolytic enzymes; proteolysis of casein was also studied in relation to cell type following recovery from milk. The experiment was conducted on 5 intensively managed dairy flocks of Garganica goats; on the basis of SCC, the experimental groups were denoted low (L-SCC; <700,000 cells/mL), medium (M-SCC; from 701,000 to 1,500,000 cells/mL), and high (H-SCC; >1,501,000 cells/mL) SCC. Leukocyte distribution differed between groups; polymorphonuclear neutrophilic leukocytes were higher in M-SCC and H-SCC milk samples, the percentage macrophages was the highest in H-SCC, and levels of nonviable cells significantly decreased with increasing SCC. Activities of all the main proteolytic enzymes were affected by SCC; plasmin activity was the highest in H-SCC milk and the lowest in L-SCC, and elastase and cathepsin D activities were the highest in M-SCC. Somatic cell count influenced casein hydrolysis patterns, with less intact α- and β-casein in H-SCC milk. Higher levels of low electrophoretic mobility peptides were detected in sodium caseinate incubated with leukocytes isolated from L-SCC milk, independent of cell type, whereas among cells recovered from M-SCC milk, macrophages yielded the highest levels of low electrophoretic mobility peptides from sodium caseinate. The level of high electrophoretic mobility peptides was higher in sodium caseinate incubated with polymorphonuclear neutrophilic leukocytes and macrophages isolated from M-SCC, whereas the same fraction of peptides was always the highest, independent of leukocyte type, for cells recovered from H-SCC milk. In caprine milk, a level of 700,000 cells/mL represented the threshold for changes in leukocyte distribution, which is presumably related to the immune status of the mammary gland. Differences in the profile of indigenous lysosomal proteolytic enzymes in caprine milk may influence the integrity of casein based on proteolysis patterns of sodium caseinate incubated with isolated and lysed leukocyte types.
Key words: caprine milk, plasmin, cathepsin D, elastase, leukocyte cell type

INTRODUCTION

In the European Union, Regulation 853/2004, which specifies hygiene rules for food of animal origin (EU, 2004), states that raw bovine milk must have a SCC lower than or equal to 400,000 cells/mL. The systematic extrapolation of findings from investigations on cows to small ruminants leads to errors in the application of discriminatory standards for sheep and caprine milk quality (Raynal-Ljutovac et al., 2007). Cows produce milk by the merocrine process, whereas goats secrete milk through the apocrine process; therefore, caprine milk typically has a naturally higher SCC than bovine milk. For ovine milk, Sevi et al. (1999) suggested a threshold of 700,000 cells/mL for bulk milk of satisfactory hygienic and processing quality; Albenzio et al. (2012b) found an impairment of mammary epithelium secretory efficiency above 300,000 SCC/mL in ovine milk. The Scientific Panel on Biological Hazards of the European Food Safety Authority concluded that high SCC cannot be used as a marker of udder infection in caprine milk (EFSA, 2005). In the United States, the maximum SCC for caprine milk for commercial use has been raised to 1,000,000 cells/mL (Park and Guo, 2006; Park, 2010).

Determination of differential milk leukocyte count, together with SCC, is a potentially useful approach for gaining information on the immune status of the mammary gland. In ovine and caprine milk samples, flow cytometry has been used to detect the percentages of PMNL, macrophages, and lymphocytes (Albenzio et al., 2009, 2011; Albenzio and Caroprese, 2011; Boulaaba et al., 2011).

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The principal indigenous proteolytic enzymes in milk consist of the plasmin-plasminogen system, cathepsin D, and elastase; these originate from blood plasma and leukocytes, and their activities are related to physiological aspects or external influences and affect milk cheesemaking properties (Albenzio et al., 2009; Moatsou, 2010). Plasmin is the predominant native proteinase in raw milk and is produced by hydrolysis of its inactive zymogen, plasminogen, by urokinase-type plasminogen activators, associated with somatic cells, or tissue-type activators, which are mainly associated with CN micelles (Politis, 1996). The second proteinase identified in milk is cathepsin D, and its activity is associated with somatic cells (Larsen et al., 1996). Somatic cells also contain other proteolytic enzymes such as cathepsins B, L, and G and elastase (Kelly and McSweeney, 2002), which are present in lysosomes of macrophages and PMNL (Albenzio et al., 2009).

Caprine milk is largely processed into cheese, and production of such cheese is increasing; caprine milk has also been recommended as an ideal substitute for cow milk for infants and patients who suffer from cow milk allergy (Bevilacqua et al., 2001; Albenzio et al., 2012). In the dairy industry, fresh raw milk is usually stored at refrigeration temperatures before cheesemaking, and during this time, indigenous and microbial enzymes may hydrolyze CN and negatively affect the ability of milk to be processed into cheese. Few studies have focused on indigenous enzymes in caprine milk (Santillo et al., 2009), and to the best of our knowledge, no studies have been performed on the role of different leukocyte types on proteolysis in such milk.

The present study examined individual caprine milk samples with different levels of SCC with the aim of investigating the percentage distribution of leukocyte types and the activities of indigenous proteolytic enzymes. In particular, CN proteolysis patterns were studied in relation to leukocyte cell types isolated from caprine milk samples with different somatic cell counts.

MATERIALS AND METHODS

Experimental Design and Milk Sampling Collection

The experiment was conducted in 5 intensively managed dairy flocks of Garganica goats, located in Apulia region (southern Italy). The goats were milked twice daily in a parlor using a pipeline milking machine. Before milk collection, goats were carefully examined by a veterinarian to confirm the absence of any sign of clinical mastitis such as fever, pain, or gland swelling, and a small quantity of milk was checked visually for signs of mastitis. Animal handling followed the recommendations of European Union directive 86/609/EEC concerning animal care. Milk SCC was determined using a Fossomatic Minor (Foss Electric, Hillerød, Denmark) according to the International Dairy Federation standard (IDF, 1995). Based on SCC, samples were classified as low SCC (**L-SCC**; <700,000 cells/mL); medium SCC (**M-SCC**; from 701,000 to 1,500,000 cells/mL); and high SCC (**H-SCC**; >1,501,000 cells/mL).

Four sampling cycles were performed over a 6-mo period; each sampling cycle was conducted over a 3-wk period. At each sampling time, individual milk samples were analyzed for SCC over 3 consecutive days to verify the level of SCC over time for the same goat; on the fourth sampling day, a milk sample with similar SCC to that measured on the 3 previous days was included in the study and assigned to 1 of the 3 SCC classes.

A total of 1,600 individual caprine milk samples were randomly selected from the pool of the samples collected during milk sampling and used for the study; the number of individual caprine milk samples was 525 in the L-SCC group, 560 in the M-SCC group, and 515 in the H-SCC group. The mean SCC for each group tested was 316,234 ± 43,000 cells/mL in L-SCC; 1,066,835 ± 52,000 cells/mL in M-SCC; and 2,282,348 ± 53,000 cells/mL in H-SCC.

Enzymatic Assays, Differential Leukocyte Count, and Cell Isolation from Individual Caprine Milk Samples

Plasmin and plasminogen, elastase, and cathepsin D activities were determined according to the procedures described by Albenzio et al. (2009). Cell pellets from milk samples were obtained and treated as reported by Albenzio et al. (2009). Lymphocytes, macrophages, and PMNL were selected for analysis by gating on the forward scatter and side scatter. Fluorescein isothiocyanate and R-phycoerythrin fluorescence were measured at 519 nm (FL1) and 578 nm (FL2), respectively, and fluorescence FL1 versus FL2 was used to determine the proportions of CD14/CD11b and CD14/CD5. The proportion of nonviable milk cells was determined by staining the cell pellet, resuspending in 200 μL of PBS (D8537, Sigma-Aldrich, Buchs, Switzerland), with 50 μL of propidium iodide (P4864, Sigma-Aldrich; 4 μL/mL), and incubating for 15 min. Samples were analyzed by flow cytometry (Cell Lab Quanta SC, Beckman Coulter Inc., Fullerton, CA), and fluorescence was measured at 617 nm (flow cytometric method).

Separation of macrophages, PMNL, and lymphocytes from milk somatic cells was performed according to the method of Caroprese et al. (2008) using positive magnetic separation (EasySep, StemCell Technolo-

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