Research Note

Assessment of β-Glucuronidase Levels in Goat’s Milk as an Indicator of Mastitis: Comparison with Other Mastitis Detection Methods

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ABSTRACT

The use of somatic cell counts (SCCs) for the diagnosis of mastitis is not a well-established procedure for the caprine species, because nonleucocytic cell-like particles are normally observed as a result of the apocrine secretion process of the goat mammary gland. The infection levels of 124 goats were measured by the β-glucuronidase test, which was compared with the SCC method and the California mastitis test (CMT). Seventy-nine of 124 samples (63.7%) showed SCCs lower than $1.3 \times 10^3$ cells/μl. Of these samples, 93% showed low levels of β-glucuronidase activity (<15 U/ml). In the remaining 36.3% of the samples, SCCs were higher than $1.3 \times 10^3$ cells/μl. Of these samples, 88% showed high levels of β-glucuronidase activity (15 to 100 U/ml). The CMT gave similar results. In this study, the β-glucuronidase test was standardized for goat milk and shown to be reliable, enabling one to count only the somatic enzyme cells in milk and avoiding the interference encountered with the SCC method.

Mastitis is an illness that is characterized by inflammation of the mammary gland. It is the most common illness among goats on dairy farms. The main cause of mastitis is infection of the teat brought about by unregulated and contaminated milking machines, insufficient sanitary conditions, failure to properly seal teats after milking, failure to isolate chronic sick animals, bad treatment of goats, etc. Mastitis is infectious and highly contagious, with considerable morbidity and low mortality. Gross changes in milk composition induced by this illness are reflected in physical, chemical, and bacteriological changes, such as milk jellification; a decrease in important technological components such as lactose, casein, fats, and minerals such as calcium, phosphorus, and potassium; and increases in other unimportant technological components, such as serum proteins and chlorides. All of these changes affect cheese efficiency and starter culture action.

The presence of pathogens, somatic cells, and changes in sensory properties are all important factors that should be analyzed when studying the microbiological and hygienic quality of raw milk (5). The indicator that is most used for mastitis detection is the somatic cell count (SCC). Recently, interest in mastitis prevention and in research on milk cell counting for goats has increased; however, complete information on the causes of variation in cell counts and on their applicability to the diagnosis of subclinical mastitis in these species is still not available (4).

Application of the SCC is the traditional mastitis assay method. This test performs well for cow’s milk, but its results are difficult to interpret for goat’s milk. This difficulty is mainly due to the fact that nonleucocytic cell-like particles can normally be seen as a result of the apocrine secretion process of the goat mammary gland. These particles are large fragments of cytoplasm originating from the distal portion of alveolar secretory cells and are of a size similar to that of milk leucocytes (5 to 30 μm in diameter). They contain abundant RNA-positive granular material (associated with dilated cisternae of the rough endoplasmic reticulum), large amounts of protein, and some lipid, but no DNA (2, 14). It is very important to use techniques that avoid such interference and allow the counting of only the somatic cells. The pyronin Y-methyl green stain direct microscopic method is DNA-specific and is able to differentiate between leukocytes and cytoplasmic particles, separating cytoplasmic particles from actual somatic cells (2, 15).

The production of polymorph nuclear leukocytes and macrophages is one of the essential body defenses against clinical and subclinical mastitis. The SCC method involves counting the number of these cells. During the inflammatory process, these cells also secrete hydrolytic enzymes and other products. These enzymes can be nonlysosomal enzymes, such as lactate dehydrogenase and leucine 2-naphthylamylase, or lysosomal enzymes, such as β-galactosidase, N-acetyl-β-glucosaminidase, alpha mannosidase, and β-glucuronidase (9). β-Glucuronidase has proved to be
TABLE 1. Relationship between SCC and percentage of samples containing β-glucuronidase

<table>
<thead>
<tr>
<th>SCC (×10³/ml)</th>
<th>% of samples with β-glucuronidase levels of &gt;15 U</th>
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<tbody>
<tr>
<td>&lt;1,300</td>
<td>100</td>
</tr>
<tr>
<td>&gt;1,300</td>
<td>100</td>
</tr>
</tbody>
</table>

The most significant selectively released enzyme in this process (11, 12).

The purpose of this work was to study mastitis prevalence in goats via the β-glucuronidase test and to compare this test with the SCC method and the California mastitis test (CMT).

MATERIALS AND METHODS

Collection of milk samples. One hundred twenty-four samples were collected from local goat dairy farms. Female goats aged between 12 and 60 months were milked for this study. After discarding the first few streams, lacteal secretions were collected at the morning milking in sterile test tubes as mixed samples of the two halves of the udder; the udders had previously been washed with water. The samples were immediately cooled to 4°C, transported to our laboratory, and processed within 4 h.

SCC. The SCC analysis was performed by using the technique described by the International Dairy Federation (6). The sample was heated in a water bath to 30 to 40°C, and then it was mixed carefully and cooled to the temperature at which the microsyringe had been calibrated. A 0.01-ml portion of the milk sample was placed on a clean slide in a 1-cm² area. It was then dried and dipped in dye solution for 30 min, dried again, dipped in tap water until all surplus dye was washed away, and dried again. The dye solution used was pyronin Y-methyl green stain. The counting of cells was carried out with a Zeiss microscope.

Enzyme assay procedure. The β-glucuronidase test (9) was first applied to goat’s milk using the synthetic substrate 5-nitrophenyl β-d-glucuronide (pnPG; Sigma Chemical Co., St. Louis, Mo.). The samples were skimmed by centrifugation at 0°C and 10,000 × g. The assay was performed with 0.4 ml of the skimmed milk, 0.2 ml of 40 mM pnPG (1.5 g of pnPG in 100 ml of distilled water), and 0.4 ml of 1 M acetate buffer (pH 4). The mixture was incubated for 4 h at 50°C. The reaction was stopped with 4 ml of 0.5 M carbonate buffer (pH 10.0) and centrifuged for 20 min at 3,000 × g. The p-nitrophenol liberated by the substrate in the supernatant was measured against a blank (containing distilled water instead of substrate solution) with a spectrophotometer (410 nm, Bausch and Lomb). A standard curve was prepared with 1 mM p-nitrophenol in 100 ml of acetate buffer; 0.01 to 0.25 ml of a standard solution of 5-nitrophenol was added to 1 ml of whole milk, and the mixture was brought up to a final volume of 4 ml with 0.5 M carbonate buffer. After centrifuging, the extinction coefficient was determined at 410 nm, and optical density was plotted against nanomoles of 5-nitrophenol per liter. One unit of β-glucuronidase was defined as the number of nanomoles of 5-nitrophenol per liter released from the substrate per milliliter of milk per min.

CMT. The CMT was used for all of the samples. The reaction involved in the CMT is the disintegration of leukocytes when milk is mixed with the reagent (NaOH and an anionic surface active agent) (11). In a negative sample (score of 0), the mixture of milk and reagent remains liquid and produces no precipitate. As the score increases, the degree of precipitation increases, and when the CMT score reaches 4, a distinct gel with a central peak is formed. The CMT score is based on the number of leukocytes in milk (1). The infection level was measured by a CMT reactive agent (Leuko-Test, Tecnofarm) on a scale of 0 to 4 (for goats, 0 to 1 = negative reaction; 2 = slightly positive reaction; 3 = positive reaction; 4 = highly positive reaction).

TABLE 2. Comparison of SCC results, CMT results, and β-glucuronidase activity for 124 samples of goat milk

<table>
<thead>
<tr>
<th>β-glucuronidase activity range</th>
<th>No. of samples with SCC levels (×10³/ml) of:</th>
<th>No. of samples with CMT scores of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples with</td>
<td>&lt;1,300</td>
<td>0–1</td>
</tr>
<tr>
<td>&lt;1,300</td>
<td>4</td>
<td>2–4</td>
</tr>
<tr>
<td>&gt;1,300</td>
<td>5</td>
<td></td>
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</table>

Comparison of SCC results, CMT results, and β-glucuronidase activity for 124 samples of goat milk

RESULTS

The levels of β-glucuronidase in the examined milk were found to range from 0 to 100 U. Seventy-nine of 124 samples (63.7%) showed SCCs lower than 1.3 × 10³ cells per ml. Of these samples, 93% showed low levels of β-glucuronidase activity (<15 U/ml). In the remaining 36.3% of the samples, SCCs were higher than 1.3 × 10³ cells per ml. Of these samples, 88% showed high levels of β-glucuronidase activity (15 to 100 U/ml). The relationship between SCC and the percentage of β-glucuronidase levels of >150 U/ml is shown in Table 1. The CMT gave similar results: 62.5% of the samples had negative CMT levels and low levels of β-glucuronidase activity, and 37.5% of the samples had positive CMT levels and high levels of β-glucuronidase activity. A comparison between the SCC results, the CMT results, and the enzyme levels is shown in Table 2.

DISCUSSION

The levels of β-glucuronidase in the examined milk were found to range from 0 to 100 U. Based on values taken from the results of different studies (3, 8, 10, 13, 16), a count of 1.3 × 10³ cells per ml was accepted as the value at which milk was considered contaminated. β-Glucuronidase activity is an important gauge of the inflammatory process (11, 12). However, under normal conditions, macrophages and polymorphonuclear leukocytes release β-glucuronidase at low levels (<15 U). The observation of low SCC levels along with β-glucuronidase activity at low levels (~15 U). The observation of low SCC levels along with β-glucuronidase activity at low levels (~15 U) may be due to false-negative results. On the other hand, the observation of high SCC levels along with β-glucuronidase levels of >15 U for some samples (6%) may be due to false-positive results. In the remaining 36.3% of the samples, SCCs were higher than 1.3 × 10³ cells per ml.

The samples with high SCCs but with normal levels of β-glucuronidase may contain milk from cows in a different lactation stage, subjected to stress, or having undergone recent parturition, factors which may increase the

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number of somatic cells (7). The samples with low levels of β-glucuronidase could be interpreted as diagnostic of subclinical mastitis. The interpretation of SCC as a mastitis indicator for goats is difficult because the secretion of the goat's mammary gland produces high epithelial desquamation, leading to frequent counting mistakes.

In this work, the β-glucuronidase test was standardized for goat's milk and was shown to be reliable, measuring only the enzyme in milk and avoiding the interference encountered with the SCC method. Diagnostic techniques for mastitis in goat's milk are very important in the goat dairy industry, which is constantly expanding.

ACKNOWLEDGMENTS

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REFERENCES