TOTAL AND DIFFERENTIAL CELL COUNTS AND N-ACETYL β-D-GLUCOSAMINIDASE ACTIVITY IN SOW MILK DURING LACTATION

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ABSTRACT

Colostrum and milk collected from 11 sows throughout lactation were used to estimate total and differential cell counts and N-acetyl-β-D-glucosaminidase activity (NAGase). The mean log10 cell counts did not change significantly through the four weeks of lactation, ranging between 250 000 and 750 000 cells/ml. Polymorphonuclear neutrophils (PMN) decreased from about 56% of total leukocytes at day 1 (colostrum) to 12-14% at day 14 and day 21. Macrophages were 35% at day 1 and were the predominant cell type throughout the remainder of lactation, peaking at 77-80% at day 14 and 21. The PMN were again increased on day 28 (44% PMN vs 52% macrophage). The mean lymphocyte proportions ranged between 7.0 and 11.3% during the first two weeks of lactation and were decreased to 4.6-5.6% in the second two weeks of lactation. The activity of NAGase declined 9.5 fold (p<0.0001) between day 1 and day 14 with the greatest decline between day 1 and day 3. The activity of NAGase remained constant through the last two weeks of lactation. NAGase activity was significantly correlated with log10 of cell counts in sow milk (r = 0.42).

INTRODUCTION
The presence of N-acetyl-β-D-glucosaminidase (NAGase, EC 3.2.1.30) in cow milk has received particular attention because of the close correlation between the activity of that glycosidic enzyme in milk and the somatic cell count of milk during lactation (Kitchen et al., 1980; Obara & Komatsu, 1984). The NAGase activity of mammary secretions from cows is increased during intramammary infection (Kitchen et al., 1980) and during mammary involution (Timms & Schultz, 1985; Hurley, 1987). The NAGase activity of colostrum declines rapidly after parturition (Timms & Schultz, 1985; Hurley, 1987) and is low in milk from non-infected cows.

Changes in NAGase activity have not been reported in sow milk during lactation. This report describes changes in NAGase activities and total and differential leukocyte counts of sow milk during lactation.

MATERIALS AND METHODS
Six primiparous and five multiparous sows, of crossbred origin from a rotational mating scheme involving Yorkshire, Landrace, Hampshire and Duroc breeds, were used in this study. Colostrum and milk samples were collected by hand-stripping from the right anterior thoracic mammary gland after subcutaneous injection of 20 iu oxytocin. Samples were collected within 16 hr of the birth of the first piglet (day 1) and on days 3, 7, 14, 21 and 28 of lactation. Piglets were weaned on day 28 of lactation. An aliquot of whole milk was frozen at -20 °C for NAGase and protein assays. Ten ml of the fresh sample was...
diluted with one volume of PBS (13 mM potassium phosphate, 150 mM sodium chloride, pH 7.5) and centrifuged at 800 × g for 15 min at 4 °C. The cream layer was removed, resuspended in 20 ml PBS and centrifuged as before. Cell pellets from the first and second centrifugations were combined and resuspended to about 50 × 10⁶ cells/ml with PBS. Total cell counts were estimated by direct microscopic cell counting using a hemocytometer and crystal violet stained cells (Bryant et al., 1958). Differential counts were made on cytocentrifuge preparations of cells stained by Wright's method. The protein concentration of milk was measured according to Lowry et al. (1951) with bovine serum albumin as standard.

The assay for NAGase was essentially as described previously (Hurley, 1987). Colostrum or milk samples (150 µl, diluted 1:10 with distilled water) were added to 50 µl 1 M sodium citrate (pH 4.5) containing 1.5% Triton X-100, and 50 µl water. To this was added 500 µl 3.3 mM p-nitophenyl-N-acetyl-β-D-glucosaminide (Sigma Chemical Co, St Louis, MO) in 0.2 M sodium citrate (pH 4.5). The tubes were mixed and incubated at 37 °C for 30–60 min. The reaction was stopped by adding 1.5 ml 1 M glycine (pH 10.5). Two ml of chloroform (water saturated) were added, the tubes mixed and the phases separated by centrifugation at 800 × g for 15 min. The aqueous layer was filtered through QS-P polypropylene columns containing filter paper discs (ISOLAB, Inc, Akron, OH) and the absorbance of nitrophenol at 410 nM was determined on the aqueous layer. Filtering the aqueous layer before determining absorbance greatly increased the repeatability and reliability of this assay on sow milk.

Data were analysed statistically as a split-plot design accounting for sow parity (primiparous vs multiparous), sow nested within parity and repeated measures over time. Effects of parity were tested with sums of squares for sows nested within parity. A conservative F-test was used for changes over time of lactation (Gill & Hafs, 1971).

RESULTS

The log₁₀ mean cell numbers in sow milk did not significantly change (p>0.05) during lactation (Table I). Mean milk cell numbers ranged from about 250 000 cells/ml (day 7) to about 750 000 cells/ml (day 21).

The mean concentration of NAGase activity was significantly changed over time (p<0.0001), declining during the first week of lactation and remaining constant throughout the rest of lactation (Table I). Sow colostrum contained nearly 9.5 fold more NAGase activity than day 14 milk. A similar decline in NAGase activity (p<0.001) occurred when NAGase was expressed as a proportion of total protein (Table I); colostrum had four fold more NAGase activity/µg protein than day 14 milk.

Polymorphonuclear neutrophils (PMN) were proportionately the most abundant cell type in colostrum (Table II) but declined in proportion after day 1 (p<0.05). Macrophages were the predominant cell type in milk through the remainder of lactation and were increased after day 1 (p<0.05). The proportion of PMN in milk was higher (p<0.01) in primiparous sows (36.4±3.9, mean ± standard error) compared with multiparous sows (17.5±4.5). The proportion of lymphocytes and macrophages in milk were lower (p<0.02 and p<0.02, respectively) in primiparous sows (6.4±1.1 and 57.1±3.8, respectively) than in multiparous sows (9.7±1.2 and 72.9±4.3, respectively). Macrophages often contained large vacuoles presumably filled with ingested milk fat droplets. Lymphocytes were present throughout lactation but the proportions of lymphocytes did not change during lactation (p>0.05). Numbers (log₁₀) of PMN, macrophages and lymphocytes were unchanged (p>0.05) during lactation. Few cells were observed that were considered to be mammary epithelial cells.