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From the Department of Clinical Veterinary Medicine, Hautjärvi and Helsinki, College of Veterinary Medicine, Helsinki, Finland.

Milk Plasmin, Antitrypsin, N-Acetyl-β-D-Glucosaminidase and Bacterial Growth in Lactoserum during the Early Post Partum Period

By *Satu Pyörälä* and *Liisa Kaartinen*

Pyörälä, S. and L. Kaartinen: Milk plasmin, antitrypsin, N-acetyl-β-D-glucosaminidase and bacterial growth in lactoserum during the early post partum period. Acta vet. scand. 1988, 29, 145–150. – The activities of plasmin, N-acetyl-β-d-glucosaminidase (NAGase) and antitrypsin in milk were determined during 1 week post partum. The concentration of NAGase and antitrypsin in milk decreased significantly during this period. A slight decrease in plasmin activity was also seen. Replication rates of E. coli and S. aureus in lactoserum were also determined. Both test bacteria showed a tendency for increasing growth rates towards the end of the period. Growth of E. coli was significantly ($p < 0.01$) enhanced in day 2 samples as compared with samples collected during the first day post partum.

cow; lactation; milk enzymes; S. aureus; E. coli.

Introduction

The composition of colostrum differs greatly from that of mature milk. The colostrum of the cow contains more mineral salts and protein and less lactose than normal milk (*Parrish et al.* 1950, *Jenness* 1986). The major proportion of proteins in colostrum is formed by immunoglobulins. This is due to the fact that the cow transfers passive immunity to the neonate via its colostrum. The increased permeability between milk and blood compartments causes leakage of small molecular weight plasma proteins, such as α_1-antitrypsin and bovine serum albumin into the milk. The high colostral antitrypsin activity is followed by a rapid decrease in activity in the first few days after calving (*Sandholm & Honkanen-Buzalski* 1979). The colostrum is known to contain a high number of somatic cells (SCC); this number then decreases for the next two weeks post partum (*Blackburn* 1966, *Cullen* 1968).

Recently, the milk content of the lysosomal enzyme N-acetyl-β-D-glucosaminidase (NAGase) during the peripartum period has been studied. Milk enzyme concentration declined rapidly in the first few days after calving and then gradually through the following four weeks (*Timms & Schultz* 1985). Both NAGase and antitrypsin have been shown to assist in the diagnosis of subclinical mastitis as indicators of inflammation (*Mattila et al.* 1986a). To these indicators belongs also plasmin, a proteolytic enzyme, which digests fibrin, the natural substrate for plasmin. Besides fibrin, β-casein has been seen to be a good substrate for plasmin (*Barry & Donnelly* 1981). Proteolytic splitting of casein seems to improve

its quality as growth medium for bacteria, which might be a factor tilting the host-microbe balance in favour of mastitis pathogens in mastitis (*Kaartinen & Sandholm* 1986).

Differences between bacterial growth in vitro in milk and in colostrum have been found by some authors (*Dutt et al.* 1986, *Oliver & Bushe* 1986, *Marshall et al.* 1986). Infection rates are known to be high in the immediate post partum period. There is currently interest in the identity of the factors responsible for lower resistance to infection observed during this period.

The purpose of the present study was to determine replication rates of mastitis pathogens in lactoserum collected during the early post partum period and to relate this with the milk levels of three inflammatory parameters, NAGase, antitrypsin and plasmin.

Materials and methods

Quarters from 9 Finnish Ayrshire cows were selected for the experiment. The samples from 2 quarters were excluded because of a present infection. Two of the cows were in their first lactation and the others had calved 2–6 times.

Milk samples were collected daily before morning milking during a seven-day period after calving (day 1 = calving day). On the first day milk was cultured using standard bacteriological procedures according the guidelines set down by the International Dairy Federation (*IDF* 1981).

Milk plasmin was determined using a fluorogenic coumaryl peptide substrate as described by *Richardson & Pearce* (1981), but on a microscale (*Mattila et al.* 1986b). The sample (300 µl) was mixed with 100 µl 0.4 mol/l trisodium citrate to dissociate casein micelles and to release the casein-bound plasmin. After centrifugation (10,000 × g, 2 min) fat was removed and a 20 µl aliquot was mixed with 220 µl of 0.05 mol/l Tris-HCl buffer, pH 7.4. To start the indicator reaction, 60 µl of substrate (1 mol/l N-succinyl-L-alanyl-L-phenylalanyl-L-lysyl-6-amino-4-methyl-coumarine, Serva No. 51156) was added. The plate was incubated at room temperature for 30 min and measured using a Fluoroskan fluorometer (Eflab, Helsinki) immediately after addition of the substrate and 30 min later.

NAGase was determined using the fluorometric method described by *Kitchen* (1978) with a commercially available kit (Milk NAGase Test, Eflab) (*Mattila & Sandholm* 1985) Trypsin-inhibitor capacity (antitrypsin) was measured using the Milk Antitrypsin Test (Eflab) (*Sandholm et al.* 1984, *Mattila & Sandholm* 1985).

Bacterial growth in milk lactoserum was investigated by microturbidometry. Strains of Escherichia coli and Staphylococcus aureus were used, both originating from clinical cases of mastitis. Milk lactoserum (100 µl) prepared from skimmed milk by high-speed centrifugation (20 000 g, 60 min, + 4°C) was diluted with 100 µl 0.9 % saline and inoculated with 50 µl of bacterial suspension in 0.9 % saline (10^7 CFU/ml). Samples were incubated for 20 h at + 37°C and shaken for 15 min hourly. The increase in turbidity was measured at 620 nm once per hour with a Multiskan MCC (Labsystems, Helsinki). The analyser was interfaced to a micro-computer to calculate delta absorbances, store them and draw the growth curves. The generation time for test bacteria in each sample was defined from the tangent of the growth curve at the logarithmic phase (*Mattila et al.* 1986b).

A kinetic analysis of the decrease in milk levels of the indicators (NAGase, antitrypsin) was carried out using the least squares fitting method for a two-compartment first-order kinetic model. Mann-Whitney and

Kruskall-Wallis tests (*STSC* 1986) were used in the statistical analysis.

Results

No significant differences between days were seen in terms of milk plasmin activity. However, there was a slight tendency towards a decrease in activity during the last days. Both NAGase and antitrypsin activities decreased gradually after parturition (Fig. 1). the decrease was statistically significant between days 1 and 2, and days 2 and 3 ($p < 0.01$). the half-lives of both activities were about the same, NAGase 9.0 and antitrypsin 8.4 h.

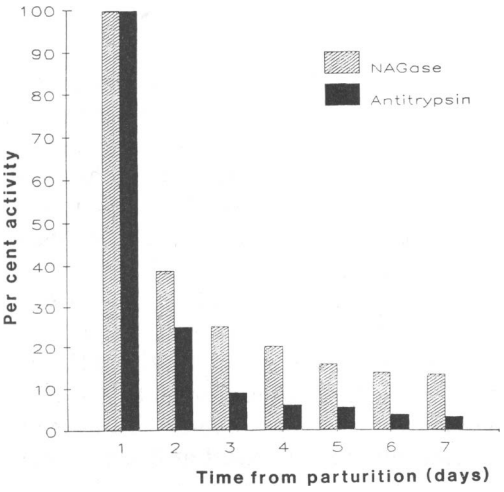

Figure 1. Relative decrease in N-acetyl-β-D-glucosaminidase and antitrypsin in milk samples collected after parturition.

In the bacterial growth studies both E. coli and S. aureus showed low growth rates just after parturition, as indicated by longer generation times of both test bacteria. The replications rates increased gradually during the follow-up period, but the only significant difference found was in the growth of E. coli between days 2 and 2 ($p < 0.01$). Fig. 2

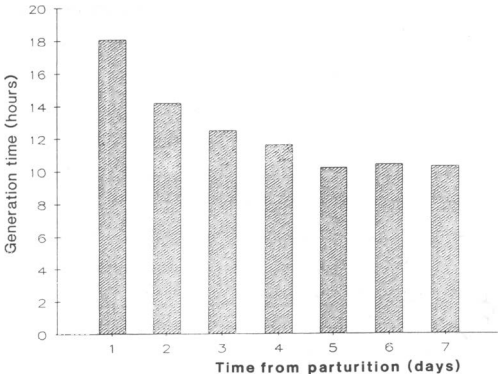

Figure 2. Mean generation time of Escherichia coli in lactoserum collected through 1 week post partum.

shows the mean generation times of E. coli for consecutive days post partum.

Discussion

Higher milk plasmin activities have been recorded in the first lactation month than in milk from later lactation (*Mattila et al.* 1986b). In the present study no clear differences were seen in milk plasmin content between the days of the first week after calving. Blood plasma is rich in plasminogen, the inactive precursor of plasmin. *Schaar & Funke* (1986) found higher plasminogen and plasmin concentrations in milk samples taken from inflammed quarters than in normal milk. Plasminogen requires activation by specific activators to become the active protease plasmin. Activation is determined by the activator/inhibitor balance and by plasmin inhibitors. This study did not reveal any significant difference between colostrum and milk in this respect.

Our results on NAGase activity in milk during consecutive days after calving agreed with those of *Timms & Schultz* (1985). NAGase activity was highest on day 1, and a rapid fall was seen thereafter. On day 7 post partum the levels were still higher than the

mean milk level recorded in later lactation (*Mattila et al.* 1986a).

Milk antitrypsin activity decreased gradually during the first week after parturition, as also reported by *Honkanen-Buzalski & Sandholm* (1981). In colostrum, antitrypsin is strongly affected by the presence of colostral trypsin inhibitor and by the increase in vascular permeability in the udder. The function of colostral inhibitors is to protect immunoglobulins from proteolytic damage during the colostral-intestinal transfusion to the newborn.

The generation times of pathogens in vitro have been shown to be shorter in early lactation and decrease again uniformly towards late lactation (*Mattila et al.* 1986b). In the present material, bacterial generation times in vitro were longer on day 1 than on the following days. Consequently, lactoserum from the days towards the end of the first week would seem to be a better growth medium for bacteria. The level to which generation times of E. coli declined on day 7 was somewhat lower than the mean level recorded in the first month (*Mattila et al.* 1986b). The growth rate on day 1 was lower than the mean level during mid-lactation, indicating a slight growth inhibitory effect in colostrum.

It is well known that mastitic infections caused by gram positive bacteria may persist within the mammary gland from one lactation period to another, while infections caused by gram negative bacteria may not. This can be predicted by in vitro growth of various strains of bacteria in dry cow secretions. Reports concerning bacterial in vitro growth rates in lacteal secretions collected during different stages of lactation seem therefore to be conflicting. Our results from bacterial growth studies with E. coli agreed with those of *Oliver & Bushe* (1986), who found a growth inhibition of E. coli and K. pneumoniae especially during the dry period, but also at parturition. One week later the growth was clearly enhanced. According to *Dutt et al.* (1986), the in vitro growth of E. coli was generally higher in milk than in secretions of the dry period, but again significantly lower on calving day than 2 weeks later. The same difference was seen in the growth of Streptococcus uberis, but at a non-significant level. Opposite results were obtained in a study on in vitro growth of streptococci in mammary secretions: all strains examined grew better on calving day than on day 7 post partum (*Todhunter et al.* 1985).

Colostrum and mastitic milk have some similar properties. The levels of inflammatory markers are high both in colostrum and mastitic milk, though in colostrum bacterial growth seems to be concomitantly inhibited. Mastitic milk and whey have been shown to support bacterial growth in vitro and bacterial replication rates to have a strong positive correlation with concentrations of inflammatory markers in the milk. It has been suggested that casein degradation products contribute as bacterial nutrients to the enhanced growth in mastitic milk (*Mattila et al.* 1986b). One growth limiting factor for bacteria may be the lack of a suitable nitrogenous substrate due to the low proteinase contents of milk. As the amount of active plasmin in colostrum was even slightly higher than later, this explanation for lower replication rates seen on day 1 is not likely.

At parturition the mammary gland secretion contains high concentrations of immunoglobulins, various bacteriostatic nonspecific agents and a great number of leucocytes (*Reiter* 1978). These features are also found to some extent in mastitic milk. The lower growth rate of bacteria in colostrum may be due to complement-mediated bacterial activity; however, no really marked growth in-

hibition was seen. It is well known that cows are highly susceptible to mastitis during the peripartum period. Despite its high potential at that time, the defence system of the gland seems to be less able to prevent mastitis.

As regards the bacterial growth studies, this study is merely preliminary. It would be of interest to complement bacterial growth studies with more species of bacteria during a longer follow-up period.

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Sammanfattning

Mjölk plasmin, antitrypsin, N-acetyl-β-D-glucosaminidase och bakterietillväxt i lactoserum den första perioden postpartum.

Aktiviteten av plasmin, N-acetyl-β-D-glucosaminidase (NAGase) och antitrypsin i mjölk bestämdes under 1 vecka efter kalvningen. Mjölkkoncentrationen av NAGase och antitrypsin minskade signifikant under denna period. En liten minskning i plasminaktiviteten kunde också ses. Replikationsgraden av E. coli och S. aureus i laktoserum bestämdes även. Båda testbakterierna visade en tendens till ökad replikation mot slutet av perioden. Tillväxten av E. coli var signifikant ($p < 0.01$) förhöjd i proven från dag 2 jämfört med proven som togs den första dagen postpartum.

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Reprints may be requested from: Satu Pyörälä, SF-04840 Hautjärvi, Finland.