Effect of *Streptococcus uberis* infection on milk characteristics of individual quarters

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ABSTRACT

Composite 24 h milk samples were collected for detailed milk analysis from each quarter of ten cows known to be free of intramammary infection. Milk yield and concentration of lactose and serum albumin (BSA) were significantly higher (P<0.001), and chloride ions significantly lower (P<0.001), in hind compared with fore quarters. Four cows developed clinical intramammary infection when subsequently challenged in the left hind quarter with *Streptococcus uberis*. For all parameters measured, i.e. protein, lactose, somatic cell count (SCC), BSA and immunoglobulins (IgG), milk from the infected quarter differed significantly from the uninfected quarters. No effect of infection was observed in quarters adjacent to the infected quarter, except for SCC, which increased significantly (P<0.001) in the uninfected hind quarter. Milk proteolytic activity in the infected quarter increased significantly (P<0.001) due to increased conversion of plasmin to plasminogen.

Keywords: milk-composition; mastitis; Streptococcus-uberis; quarter-milk.

INTRODUCTION

Mastitis causes complex and costly changes to milk quality and its processing characteristics (Munro *et al.*, 1984). Numerous pathogens can cause mastitis and each induces an inflammatory response of differing severity. Most changes in milk composition are as a result of this inflammation, involving a massive ingression of active, phagocytic leukocytes which reduces milk secretion by the epithelium and increases permeability of the epithelium. Lipolytic and proteolytic changes in milk may also be attributed directly to the mastitis pathogens since the two most common pathogens in New Zealand, *Staphylococcus aureus* and *Streptococcus uberis*, can release lipases and plasminogen activators respectively (Saggers & Stewart, 1968; Leigh, 1993 & 1994), when grown in milk.

Studies of the effects of mastitis on milk composition either compare milk collected from infected and uninfected cows, of similar breed, age and yield etc. (Natzke *et al.*, 1972) or from infected and uninfected quarters, usually the contralateral pair within a cow (LeVan *et al.*, 1985) or compare milk from the same quarter before, during and after an infection (Anderson & Andrews, 1977). Each design has various limitations and the purpose of this study was to determine whether a within-cow model would be suitable for identifying the less conspicuous, pathogen-related changes in milk composition. It was first determined whether, in the absence of infection, different quarters within a cow produce milk of the same composition and secondly, whether a *Str. uberis* infection, induced experimentally in one hind quarter, affected the milk composition of adjacent, uninfected quarters. Changes in milk proteolytic activity were also determined for quarters infected with *Str. uberis.*

METHODS

A composite 24 h milk sample was collected from each quarter of 10 Friesian x Jersey cows and analysed for milkfat, protein and lactose (Milkoscan, Foss Electric, Hillerood, Denmark), for somatic cell count (SCC; Fossomatic, Foss Electric), bovine serum albumin (BSA) and immunoglobulins (IgG; Radial Immunodiffusion, Binding Site, Birmingham, UK) and chloride concentration (atomic absorption spectra). Cows were of mixed parity and between 60-113 days in milk and had remained free of intramammary infection since calving as determined by monthly bacteriological analysis of the milk. Of six animals challenged, four cows subsequently developed a clinical infection following infusion of 500 cfu of *Str. uberis* directly into the teat sinus of the left hind (LH) quarter (Hill, 1988). These cows were of mixed parity and included one first lactation heifer. Clinical symptoms, i.e. milk clots and swelling of the gland, were visible by d 3 after infusion and intramammary antibiotics were given from d 4 and d 11 onwards (1 tube at 24 h intervals for 3 d). Composite 24 h milk samples were collected from each quarter on days 3, 9 and post-infection, on d 23 for detailed analysis. Additional samples were collected on days -2, 1 through to 11 and 23 after infusion for analysis of milkfat, protein, lactose and SCC. Plasmin (PN) and plasminogen (PGN) activity were measured (Stelwagen *et al.*, 1994b) in am milk samples only, collected 2 d prior to infusion and on days 1, 2, 3, 5, 8, 10, 11 and 23 after infusion. Effects of quarter location within cow on milk composition were determined by ANOVA and paired t-test. Changes in quarter milk composition, during infection in the LH, were determined by paired t-test using pre-infection values as the covariate (Minitab Inc., Penn State College, PA 16801, USA).

RESULTS

Milk yields were 40% higher in the hind pair of quarters (HQ) compared with the front pair of quarters (FQ) (means ± sed.: 6.21 vs. 4.46 ± 0.24 l/d, P<0.001; n=20). Despite this difference, the concentration of BSA, IgG and lactose were all significantly higher in HQ com-

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TABLE 1: Milk composition of the infected LH (n = 4), pre infection (d -2), during the clinical episodes (d 3 & 9) and post infection (d 23).

Milk Constituent mean (± SEM)	Milk Yield (l)	SCC ($x10^3$/ml)	Milkfat (g/100g)	Protein (g/100g)	Lactose (g/100g)	BSA (mg/l)	IgG (mg/l)	Chloride (mg/l)
Pre Infection	5.98 (1.02)	32 (12)	4.51 (0.41)	3.56 (0.15)	4.89 (0.06)	253 (32)	632 (197)	1415 (79)
Clin. Infection 1	3.31 (0.83)*	7014(2332)*	5.08 (0.23)(+)	3.62 (0.09)^{NS}	4.33 (0.39)^{NS}	1552 (683)$^{(+)}$	1409 (304)$^{(+)}$	2254 (492)$^{(+)}$
Clin. Infection 2	3.49 (0.53)*	5707(1360)*	4.94 (0.37)*	4.02 (0.17)*	4.24 (0.16)*	1015 (433)$^{(+)}$	1769 (410)*	1994 (159)**
Post Infection	3.58 (0.70)*	384 (199)$^{(+)}$	4.66 (0.35)^{NS}	3.61 (0.19)^{NS}	4.64 (0.13)$^{(+)}$	380 (45)**	792 (169)^{NS}	1876 (210)*

^{NS} P>0.10, $^{(+)}$ P<0.10, * P<0.05, ** P<0.01, *** P<0.001

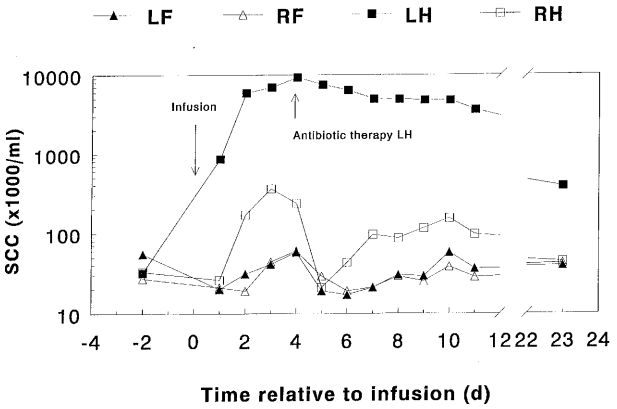
pared with FQ (BSA: 234.8 vs. 208.3 ± 4.5mg/l, P<0.001; IgG: 507.7 vs. 486.7 ± 11.0 mg/l, P<0.05; Lactose: 4.95 vs. 4.92 ± 0.01%, P<0.01). In contrast chloride concentration was significantly lower in HQ compared with FQ (1415 vs. 1475 ± 9.1 mg/l, P<0.001). The left pair of quarters showed a significantly higher BSA concentration (225.9 vs. 217.3 ± 3.9 mg/l, P<0.05) and a lower chloride concentration (1437 vs. 1453 ± 8.3, P<0.05) compared with the right quarters.

Changes in milk composition for the infected LH quarters are summarised in Table 1. During infection milk yields dropped significantly (P<0.05), as did the concentration of lactose. Concentration of BSA, IgG, SCC and chloride all increased significantly in the infected quarter and remained high when clinical symptoms had subsided and milk appeared visibly normal (d 23).

Milk composition of the uninfected quarters within each udder did not change significantly during the infection period except for the contralateral, uninfected hind quarter. In this quarter, the RH, a significant increase in SCC (P<0.01) was observed when clinical signs were apparent in the LH (Figure 1). A reduction in SCC of the RH coincided with the first course of antibiotic treatment given to the LH although no reduction in SCC was observed for the LH. A second increase in SCC was observed for the RH (P<0.01) when antibiotic therapy ceased (d 7).

No change in proteolytic activity was observed for milk from RH quarters compared with pre-infusion values (data not shown). However, for milk from the LH quarter, PN activity began to increase on d 2 after infection and peaked on d 5 (pre-inf. vs. d 5: 1.9 vs. 59.6 ± 2.9 units/ml; P< 0.001), whereas PGN activity was at its lowest level on d 5, although the decline was not statistically significant (pre-inf. vs. d 5: 58.9 vs. 29.5 ± 16.7 units/ml; P> 0.10). As a result of the changes in PN and PGN activity, the PN:PGN ratio declined sharply on d 2 and reached its lowest point on d 5 (pre-inf. vs. d 5: 39.8 vs. 0.50 ± 11.8; P<0.10).

FIGURE 1: Milk SCC for infected LH and adjacent, uninfected quarters (n=4).



DISCUSSION

In the absence of infection, compositional differences between front and hind quarters were unexpected. An increased milk BSA, IgG and chloride concentration and a reduced milk lactose content are changes normally indicative of increased epithelial leakage, allowing mixing of the blood and milk components (Stelwagen *et al.*, 1994a). The lower milk lactose and higher chloride content observed for the front quarters might suggest that these quarters were experiencing greater vascular leakage across the epithelial membrane but this cannot account for the higher BSA content of the hind quarters. Further work is required to determine whether these differences between quarters are consistent for other animals and identify factors which could account for them.

The course and nature of the clinical symptoms observed during the four infections were typical of those observed during naturally occurring infections by *Str. uberis.* Significant increases in milk BSA, chloride and IgG content, and a decrease in milk lactose, were consistent with increased vascular leakage through the epithelial membrane, induced by the inflammatory response.

Increases in the SCC of adjoining RH quarter for all four infected cows were unexpected. A similar SCC response to a major inflammatory event was described previously for hind quarters, following infusion of *Escherichia coli* endotoxin into homolateral front quarters (Schultze & Bramley, 1978). This response however, was of only 24 h duration and was attributed to lymphatic clearance of the endotoxin. To our knowledge, the significant and prolonged inflammatory response by the contralateral, uninfected hind quarter has not previously been reported. No reduction in milk production was observed for this quarter so the increase in SCC cannot be attributed to a simple concentration effect of reduced milk volumes. The SCC responses may be typical of changes which normally occur during a clinical episode of mastitis but have not been described previously due to lack of SCC analysis on a quarter basis. Early anatomical studies indicated the

existence of a small number of lymphatic and arterial connections between the two sides of the udder (El Hagri, 1945) which, during an infection, could allow movement of fluids and inflammatory products into the neighbouring, contralateral quarters.

This inflammatory response requires further investigation if the complete effects of mastitis on milk composition are to be determined. This result may preclude the use of the contralateral quarter as a suitable within-udder control for more detailed mastitis studies. Compositional differences between fore and hind quarters, in the absence of infection may also preclude use of the homolateral quarter for a within udder control.

Significant changes in the proteolytic activity of milk occurred in response to *Str. uberis* infection and these changes were confined to the infected quarter. The decrease in the PN to PGN ratio indicated that the inactive zymogen (PGN) was converted to its active form (PN). Because this conversion requires the presence of plasminogen activators (PA), an increase in PA activity must have occurred. It is not clear at this stage if the increase in PA activity was due to increased expression of PA in mammary tissue (Stelwagen *et al.*, 1994a; Ravn *et al.*, 1995) or that the increase was due, at least in part, to production of PA by *Str. uberis* (Leigh, 1993, 1994). Clearly the latter warrants further investigation.

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