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Milk L-lactate concentration is increased during mastitis

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A study was undertaken in cattle to evaluate changes in milk L-lactate in relation to mastitis. A healthy, rear quarter of the udder of each of ten cows in mid-lactation was infused with 1000 colony-forming units (cfu) of *Streptococcus uberis* following an afternoon milking. Foremilk samples were taken at each milking from control and treated quarters and antibiotic treatment was applied following the onset of clinical mastitis or after 72 h. One cow did not become infected. Six quarters showed clinical symptoms of mastitis within 24–40 h and this was associated with a more than 30-fold increase in milk L-lactate (to 3·3 mM) and an increase in somatic cell count (SCC) from $4{\cdot}5\times10^3$ to 1×10^7 cells/ml. Three cows were subclinical, with cell counts ranging from $1{\cdot}5\times10^6$ to 1×10^7 cells/ml. In these animals, milk lactate ranged from 0·7 to 1·5 mM in the infected quarters up to 40 h post-infection, compared with less than 0·1 mM in control quarters. Milk was examined from 137 cows in mid-lactation which were known to have mastitis. Foremilk samples were taken aseptically from control and infected quarters of cows on commercial farms. Mean milk L-lactate concentrations and SCC were 0·14±0·02 mM and $1{\cdot}85\pm0{\cdot}3\times10^5$ cells/ml, respectively, in control (bacteriologically negative) samples. However, L-lactate concentrations exceeded 2·5 mM in the presence of some types of infection, the level of the lactate response being closely related to the impact of the infection on SCC. L-Lactate concentrations were relatively elevated in milk samples taken *post partum*, declining from 0·8 to 0·14 mM over the first few days of lactation. In conclusion, milk L-lactate has potential as an indicator of clinical and subclinical mastitis in dairy cows.

Keywords: Mastitis, milk lactic acid, lactate, somatic cell count, serum amyloid.

There are only two reports on the measurement of lactate in mammary secretions. Morr et al. (1957) determined the concentration of lactate to be around 0·1 mM in raw milk. Mackie et al. (1977) found a relatively slow but substantial increase in lactate in milk during mammary involution with concentrations reaching approximately 5 mM, 5 d after the last milking. The aim of the present study was to evaluate firstly, whether changes in milk L-lactate (hereafter referred to as lactate) concentration occurred during mastitis, and secondly, whether milk lactate measurement had any potential as a diagnostic of mastitis.

Mastitis is usually detected from clinical signs such as milk clots in foremilk, udder inflammation and from changes in somatic cell counts (SCC). Difficulty in detection of mastitis has led to the development of several manual, automated or semi-automated methods, alone or in combination, to detect infections (Douglas et al. 1997). Such methods can be as simple as clot detection on filters or can involve relatively complex systems measuring changes in electrical conductivity in milk (see for example, Hillerton & Walton, 1991; Milner et al. 1996; Woolford et al. 1998). In addition, there are a wide range of biochemical indicators of infection that have been evaluated, at least in research environments (Kitchen, 1981; Holdaway et al. 1996). Recent examples of these include nitric oxide (Bouchard et al. 1999) and the acute phase proteins, notably serum amyloid A (Hirvonen et al. 1999).

In the context of milk lactate being a potential diagnostic measurement for mastitis, Mayer et al. 1988 reported that milk oxygen concentrations were reduced during mastitis suggesting that, given a favourable supply of substrates, the metabolism of somatic cells in milk and/or the mammary epithelium may become anaerobic leading to release of lactate into milk. Others have reported that

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lactate dehydrogenase activity is elevated in mastitic milk. Such a change may or may not lead to enhanced lactate production depending upon substrate and co-factor availability and indeed could result in degradation of lactate.

The studies described below evaluated the time course of change in lactate concentrations in milk during the onset of mastitis and also examined the relationship between SCC and milk lactate in a range of samples collected from commercial dairy farms.

Materials and Methods

Experiment 1

Ten healthy, Friesian or Jersey cows in mid-lactation (121±5 d in milk) were used. Average milk yield was 10·9±0·8 l/d, SCC was <50 000 cells/ml and there was no recorded history of mastitis from clinical observation or monthly monitoring of SCC. Cows were in their first or second lactation, grazed on ryegrass and white clover pasture and were milked twice daily (at 08.00 and 16.00) throughout the study. Milk yelds were normal for young cows in New Zealand on summer pasture. Foremilk conductivity was measured and composite milk samples were taken for SCC measurement 5 d before the trial began. On the two days before intramammary infusion of the pathogen, electrical conductivity (Technipharm Digital Mastitis Checker, Rotorua, New Zealand) was measured in foremilk from each hind quarter, prior to cows being foremilk-sampled. Foremilk samples from the first milking were taken aseptically from all quarters for bacteriology and all pre-infusion samples had SCC measured to ensure quarters were clear of infection before infusion of the pathogen. Cows were infused with approximately 1000 colony-forming units (cfu) of a strain of *Streptococcus uberis* isolated from a cow with acute mastitis. This strain was shown *in vitro* (and *in vivo* following treatment) to be highly sensitive to cloxacillin. After overnight culture in Todd-Hewitt broth, the inoculum was serially diluted in quarter-strength Ringers solution (Fort Richard Laboratories, Auckland, New Zealand) then 0·1 ml was spread-plated and incubated at 37 °C overnight on Columbia blood agar containing 5% whole bovine blood and 0·1% aesculin (Fort Richard Laboratories, Auckland, New Zealand). The inoculum was diluted to give 1000 cfu/ml and loaded into 1-ml disposable syringes with attached infusion cannulas (Interlink™ Syringe Cannula, Becton Dickenson & Co., NY) and stored at 4 °C.

After the afternoon milking on the second day of the experiment, a single hind teat of each cow was swabbed with ethanol (70% v/v), 1 ml of inoculum was infused via the teat and gently massaged up into the gland for 2–3 min. At all milkings post-infusion, cows were observed for visual signs of infection such as redness, tenderness or swelling of the udder and/or the appearance of clots in the foremilk. Conductivity measurements were made on foremilk from all quarters during the observation period. The inter-quarter ratio of electrical conductivity (IQR) was calculated as described by Woolford et al. (1998):

$$IQR=(Q_x-Q_{low})/Q_{low}$$

where Q_x is the conductivity in the quarter being examined and Q_{low} is the lowest conductivity among the quarters.

Foremilks from the two hind quarters were sampled at normal milking times for 3 d after infusion. Infused quarters were treated with a course of intramammary antibiotics (sodium cloxacillin as Orbenin LA, Beecham Veterinary Products, Auckland, New Zealand; 200 mg/quarter at 2-d intervals over 4 d) when clinical mastitis was observed ($n=6$ at 24 h) or after 3 d ($n=3$). After intramammary antibiotic treatment was complete, both hind quarters were sampled on days 10, 14 and 36. Foremilk samples were taken aseptically for bacteriology from the two hind quarters at the first milking after the infusion, and again immediately prior to intramammary antibiotic treatment. Further samples for bacteriological examination were taken on days 10, 14 and 36 after infection, to ensure complete recovery. Milk yield was recorded and quarter and composite milk samples were taken at each milking throughout the experimental period.

Milk analyses

SCC was determined in both composite and foremilk samples using a cell counter (Fossomatic 450; Foss Electric). No discrimination was possible among cell counts >10^7 cells/ml. Milk [Na^+] and [K^+] were determined by plasma emission spectrometry (Varian Vista, ICP-OES; Varian Australia Pty Ltd, Victoria, Australia). A separate sample of skim milk was taken for determination of L-lactate (see below). A subset of the foremilk samples (pre-infection and 24 h and 64 h post-infection) were assayed for serum amyloid A (SAA) by ELISA (Tridelta Development Ltd, Greystones, Co Wicklow, Ireland).

Experiment 2

The relationship between milk L-lactate concentration and SCC was studied using milks from cows with intramammary infections (clinical and subclinical) in eight commercial herds. Cows were selected in the first instance on elevated SCC (>500 000 cells/ml) in composite milk samples. Foremilk from individual quarters of selected cows was then taken aseptically. Samples were collected from infected ($n=137$) and control ($n=48$) quarters. Wherever possible, control samples were taken from an uninfected (bacteriologically negative and low SCC) quarter from the same cow. L-Lactate concentration, SCC and bacteriology were measured.

Experiment 3

To test the utility of lactate measurements for the identification of cows with intramammary infections during very

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early lactation, 13 cows were sampled over the colostral period. Foremilk samples were taken from all four quarters from each of 13 cows beginning immediately before the first machine milking (day 1) after calving and then daily, before the afternoon milking, for 7 d or until a clinical infection developed. Separate foremilk quarter samples were taken aseptically for bacteriology at the first milking and again on day 5 after calving. L-Lactate concentration was determined in all milk samples. SCC was not measured before day 2 because of difficulties with sample viscosity with early colostrum.

Bacteriology

Str. uberis infections were identified from a subsample (10 μl loop) of the foremilk which was streaked onto a quarter-plate of Columbia blood agar containing 5% whole bovine blood and 0·1% aesculin. Plates were incubated at 37 °C and examined after 24 and 48 h. Presumptive identification of isolates was from colony morphology, Gram stain, haemolysis, aesculin reaction, catalase production and tube coagulase reaction. Identification of causative organisms in other infections was made according to methods described by Hogan et al. (1990).

L-Lactate analyses

L-Lactate concentration was measured in deproteinized milk using Boehringer analytical kits (Scientific Supplies Ltd, Panmure, Auckland, New Zealand). The method measured the changes in absorbance at 340 nm with change in NADH concentration when, in the presence of L-lactate dehydrogenase and NAD^+, L-lactate is oxidized, yielding NADH and pyruvate. To scale down the assay to a microplate format, the volume of the reaction mixture was reduced to one-tenth of that recommended in the kit. Milk and colostrum samples were prepared initially by centrifuging at 500 ***g*** for 10 min and the skimmed sample collected and stored frozen at −20 °C for later analysis. Defatted samples (400 μl) were deproteinized with 150 μl of 0·5 M-zinc sulphate, followed by the addition of 150 μl of sodium hydroxide (approximately 0·75 M). The concentration of the latter was determined by previous titration against 0·5 M-zinc sulphate so that one volume of sodium hydroxide neutralized one volume of zinc sulphate. The mixture was centrifuged at 12 500 ***g*** for 10 min, 210 μl of supernatant removed, mixed with 90 μl of 0·33 M-potassium phosphate buffer, pH 7·1 and re-centrifuged. The supernatant was used directly in the assay. Recovery of lactate in spiked samples of milk was 100%. Coefficient of variation was 2·3% and minimum detectable concentration was 0·01 mM. The assay was linear at least in the range 0 to 10 mM. Lactate concentrations in milk were stable at 4 °C for at least 24 h.

Ethical approvals

All animal studies were undertaken with the approval of the Ruakura Animal Ethics Committee (Hamilton, New Zealand).

Statistical analysis

Treatment effects were evaluated by comparison of pre-treatment means with post-treatment means and between control and treated quarters using paired *t* tests. Differences between means were considered significant when $P<0·05$.

Results

Experiment 1

Bacteriological examination of foremilk samples taken at the first milking after infusion of the pathogen revealed that *Str. uberis* was present in milk from infused quarters (only) of all cows. Six of the ten cows developed clinical mastitis in the infused quarter, as indicated by the appearance of clots in the foremilk and three cows remained subclinical (no visible signs). In one cow, the infection failed to establish, with only minor changes in cell count and lactate being apparent. There were no changes in other markers of mastitis, i.e., foremilk SCC, conductivity, SAA or Na^+/K^+ for this cow and her data have been excluded from the results shown below.

Individual cow, foremilk SCC and lactate concentrations for the six cows with clinical mastitis are shown in Fig. 1a and for the subclinical cows ($n=3$) in Fig. 1b, L-lactate in foremilk from the infused quarters from all clinical cows increased more than 30-fold above that in control quarters (3·27±0·61 *v.* 0·10±0·02 mM, $P<0·01$, $n=6$) and pre-infusion values of 0·08±0·01 mM ($P<0·01$, $n=6$) within 24 h of bacterial infusion (Fig. 1a). In the subclinical group, foremilk L-lactate concentration was elevated in the infused gland of two cows at 24 h and the third at 40 h post infusion (Fig. 1b). Peak milk lactate concentrations in these animals were 1·5, 1·5, and 0·9 mM, 24–40 h after infusion and had increased significantly over pre-infusion values by 40 h after infusion (1·3±0·23 *v.* 0·05±0·01 mM, $P<0·05$).

Foremilk SCC from the infused quarters of the clinical group were also greater than control (9160±540 *v.* $246±87\times10^3$ cells/ml, $P<0·01$) and pre-infusion values ($45±11\times10^3$ cells/ml, $P<0·01$) within 24 h of infusion (Fig. 1a). While there was an increase in SCC in all the subclinical cows, this was not statistically significant owing to the small number of animals ($n=3$) and the variability in response among the cows (peak 1536, 8020 and $10\,000\times10^3$ cells/ml, Fig. 1b).

Five of the six clinical cows received the first dose of intramammary antibiotic treatment, after milking, 24 h after infusion, the other clinical cow at 40 h after infusion.

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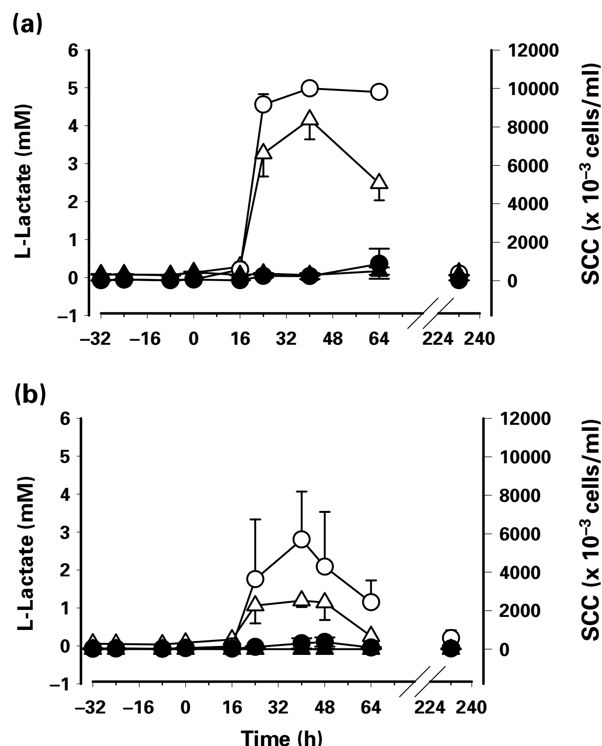

Fig. 1. Changes in lactate concentration (△, infected; ▲, control) and somatic cell count (○, infected; ●, control) in foremilk from udder quarters infected with *Streptococcus uberis* at time zero. (a) Responses in cows showing clinical mastitis ($n=6$). (b) Responses in cows with subclinical infections ($n=3$). Note that clinical cases were treated with antibiotic 24 ($n=5$) or 40 h post infection. Subclinical cows were treated with antibiotic at 72 h post infection.

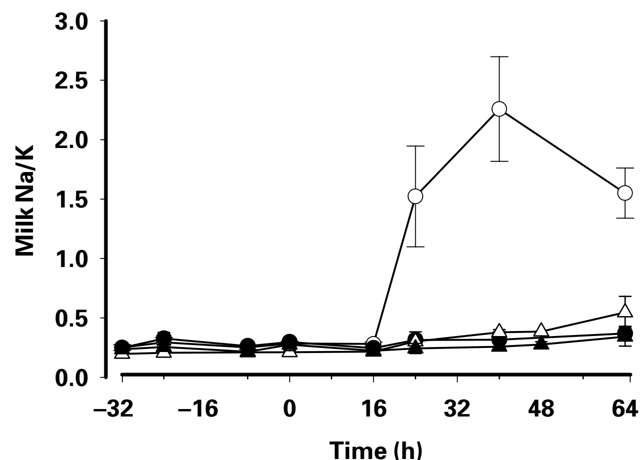

Fig. 2. Changes in milk Na^+/K^+ ratio (±SEM) with time before and after (time 0) infection with *Streptococcus uberis*. Results are shown for clinical infections ($n=6$; ○), adjacent control quarters ($n=6$; ●), subclinical infections ($n=3$; △), and adjacent control quarters ($n=3$; ▲).

Both lactate and SCC were still elevated at 64 h post infection but had declined to control values by day 10. The subclinical cows were not treated until 72 h after infusion of the pathogen. Both lactate and SCC had begun to decline before antibiotic treatment began.

Interquarter conductivity ratio (IQR). The IQR of the infused gland of the clinical cows increased significantly above pre-infusion values within 24 h of infusion (1·02±0·02 *v.* 1·72±0·14, $P<0·01$). There was no change in IQR in the subclinical cows, as a group, post infusion (maximum at 64 h post infusion, 1·21±0·11 *v.* pre-infusion 1·02±0·01). However, one of the three subclinical cows, 6503, would have been identified as having an infected quarter using conductivity measurements, showing an increase in IQR of more than 15% (Woolford et al. 1998) within 24 h of infusion (1·38 *v.* 1·03 before infusion).

Sodium to potassium ratio. Na^+/K^+ was significantly higher after infusion in the foremilk of the infused gland of the clinical cows (Fig. 2). Ratios were higher than before infusion (1·52±0·42 *v.* 0·27±0·02, $P<0·05$) and the Na^+/K^+ ratio in the control gland (0·31±0·07, $P<0·05$) at 24 h after infusion. There was no change in the mean Na^+/K^+ ratio of the foremilk of the subclinical cows as a group. However, foremilk from the infused gland of cow 6503 (which showed a change in IQR) increased after infusion to reach a peak at 64 h (0·79 *v.* 0·18 before infusion; control quarter, 0·50).

Serum amyloid A. Concentration of SAA in the infused gland of the clinical cows was higher than that in milk from the control gland (24·6±5·1 *v.* 9·8±0·2 μg/ml, $P<0·05$) and in the pre-infusion milk samples (9·7±0·05 μg/ml, $P<0·05$) within 24 h of infusion. SAA increased in concentration to 108·1±28·2 μg/ml by 64 h after infusion in milks from clinical quarters. At 24 h after bacterial infusion SAA in foremilk from cows in the subclinical group had not increased over control or pre-infusion concentrations, but was significantly higher 64 h after infusion relative to control quarters (24·3±3·5 *v.* 9·6±0·1 μg/ml, $P<0·05$) and pre-infusion samples (10·0±0·3 μg/ml, $P<0·05$).

Experiment 2

Milk lactate concentrations in foremilk samples collected from infected ($n=137$) and control ($n=48$) quarters are shown in Fig. 3. Lactate concentration in these samples began to increase above a (nominal) threshold of 0·5 mM when quarter SCC was above 1–2 × 10^6 cells/ml. Lactate concentrations in milk were increased by all types of infections (Table 1) with the lactate concentration being more related to the SCC induced by the infection than the type of infection *per se*. Thus, in *Corynebacterium bovis* infections, where SCC did not rise above 10^6/ml, milk lactate concentration was also low (0·25 mM).

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Table 1. SCC and milk lactate concentrations (±SEM) in milk samples from udder quarters infected with different microorganisms or uninfected (control). Samples were collected from cows in commercial herds

Organism (no. of samples)	SCC ($\times 10^{-3}$ cells/ml (±SEM))	Milk lactate (mM±SEM)
Streptococcus dysgalactiae (3)	7140±2860	2·62±1·03
Unidentified (20)	6980±740	2·59±0·36
Staphylococcus aureus (21)	6550±810	1·76±0·35
Escherichia coli (8)	6360±1520	2·90±0·64
Streptococcus uberis (20)	5750±900	2·66±0·50
Yeast (7)	2150±1600	0·98±0·43
Corynebacterium bovis (39)	960±280	0·25±0·07
Coagulase neg. staphylococci	410±64	0·18±0·03
Control quarters (48)	185±31	0·14±0·02

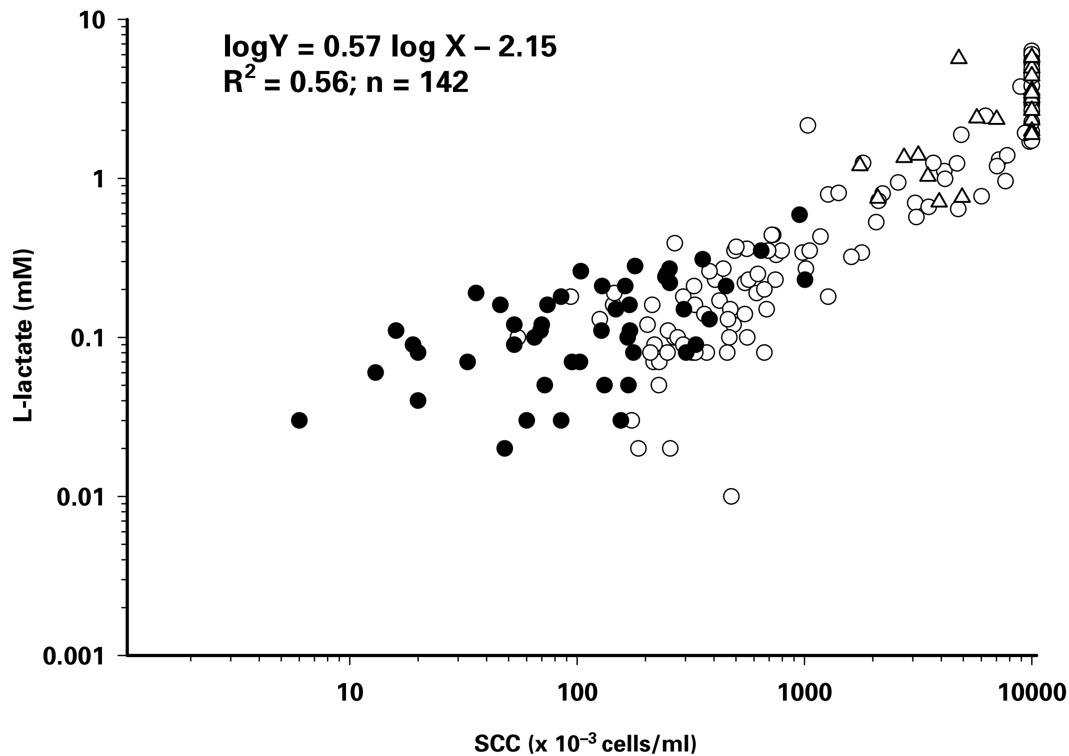

Fig. 3. Log–log plot of milk lactate in infected ($n=137$; ○) and uninfected ($n=48$; ●) glands relative to SCC. Note that for cell count, the maximum measurement possible was 10^7 cells/ml. Samples were taken from quarters of cows in commercial herds with no recent antibiotic treatment but with infections of unknown duration. Results are categorized by infective organism in Table 1. Unidentified infections ($n=20$) are shown by △.

Experiment 3

Nine of the thirteen cows were free of infection over the collection period *post partum*. The other four cows were either infected at calving or developed infections during the study. L-Lactate concentrations and SCC were elevated in colostrum from uninfected quarters above that found in milk (Fig. 4). Lactate declined from an average of 0·8 mM on the first day of lactation to a nadir of 0·13 mM by day 5. SCC was still relatively elevated at this time at an average of approximately 4×10^5 cells/ml (Fig. 4). Concentrations of L-lactate in the foremilk samples from the infected quarters ('natural' infections, all *Str. uberis*) of four cows were all greater than 2·0 mM and provided valuable diagnostic information as early as day 1 *post partum* (results not shown).

Discussion

Lactate concentration in milk responded rapidly to mammary infection, increasing more than 30-fold in milk from quarters with clinical mastitis and around 10-fold in milk from quarters with subclinical infections and a lower SCC.

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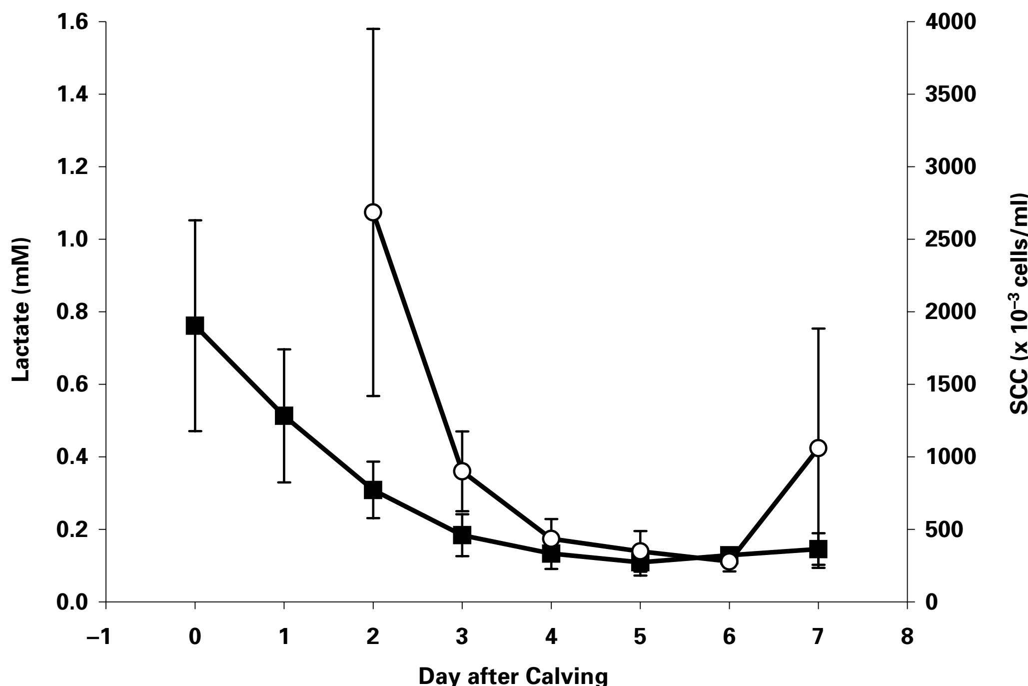

Fig. 4. Mean SCC (○; $\times 10^{-3}$ cells/ml) and lactate (■; mM) in foremilk samples taken in the first 7 d *post partum* from nine cows. SCC were unmeasurable in early colostrum samples.

While the current results are from a small number of animals, the magnitude of the responses is encouraging with regard to the potential utility of lactate as a marker of intramammary infection. While the milk yield of the animals used in Experiment 1 might be regarded as relatively low, it was within the normal range for animals grazing summer pastures in New Zealand and we would not expect lactate or SCC responses to differ markedly in similar experiments with higher-producing cows.

Statistically significant changes in milk lactate occurred by 24 h post infection in cows exhibiting clinical and subclinical symptoms. A (nominal) threshold concentration of 0·5 mM for lactate would have successfully diagnosed all infections at 40 h and 8/9 at 24 h. Electrical conductivity and visual inspections indicated the infections in the six clinical cases and in one of the subclinical cases, only at one time-point.

The advantage of lactate over other markers of mastitis is the extent and speed of the change in its concentration, with a substantial increase in concentration occurring within 24 h of infection. The rise in lactate concentration coincides with the invasion of leucocytes and the associated decline in intramammary oxygen tension (Mayer et al. 1988). Whether the increase in lactate is caused by leucocyte metabolism, or is a response to it, remains to be established. Association of leucocyte count with lactate in mammary secretions during involution suggests that leucocytes could be a major source of lactate (Mackie et al. 1977). Further, the milk concentration of D-lactate in normal and mastitic milk was negligible. D-Lactate is a common product of bacterial metabolism but is not produced by mammalian cells.

In the majority of samples of mastitic milks obtained from 'natural' infections, lactate concentrations were elevated. However, in two samples obtained by us from cows where infections were advanced and red blood cells were present, lactate concentrations were unexpectedly low (<0·5 mM). In samples of milk from clinical cases of mastitis, without red blood cells present, lactate was stable at 4 °C for at least 24 h and showed only minor changes at room temperature over the same period.

The rapid response of milk lactate to infection makes it potentially a very useful marker of mastitis, particularly in situations where automated recognition of infection is required, for example with robotic milking systems. Direct comparison with conductivity measurements was not possible over an extended range of samples from naturally infected animals. However, lactate was able to detect infections in samples from cows where conductivity was unchanged, suggesting that lactate might be a more sensitive measure. Further, when milk lactate was elevated, it remained so throughout milking. Serial samples from mastitic quarters showed a 20% decline in lactate from foremilk to main flow but a high concentration (1·5 mM) was sustained throughout milking (results not shown).

The degree of change in milk lactate was most closely correlated with SCC and any differentiation in the lactate response with the type of infection appeared to relate more closely to the SCC response to the infection than to the infective organism *per se*. Thus the utility of lactate as

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a diagnostic test for mastitis would appear to rely on an SCC response occurring. Further, the sensitivity of a lactate test would markedly improve when SCC rose above 10^6 cells/ml when milk lactate increases markedly in concentration above a nominal threshold of 0·3–0·5 mM (see Table 1 and Fig. 3).

Limited results for very early colostrum/milk indicate that milk lactate also has potential value as a diagnostic measure of mastitis over the first few days of lactation, when the frequency of mastitis tends to be highest.

SAA analyses are available commercially as effective diagnostic tests for mastitis. Certainly, milk SAA shows a several-fold increase with mastitis over the initial days of infection. Relative to lactate, however, SAA poses greater technological hurdles for in-line detection procedures.

Conductivity measurements in their own right have problems of sensitivity and specificity in mastitis detection, requiring the use of conductivity ratios among foremilk samples from quarters for optimal performance (Woolford et al. 1998). We have not examined the utility of lactate measurement on composite as opposed to quarter-milk samples where dilution would compromise the accuracy and precision of mastitis diagnosis. Whether it is possible to detect mastitis using in-line biosensors on a per cow basis will depend in large part on the performance of such sensors. Certainly, detection of mastitis in quarter milk through lactate measurement would require a much less precise sensor than that needed for composite milk.

The lactate assay used here is specific and amenable to semi-automation in a microtitre plate format. However, there has been considerable investment in technology for lactate measurement as a human medical diagnostic. Further, hand-held lactate testing devices are available and used by athletes to monitor fitness. This technology has been developed using thick film, disposable sensors for the measurement of lactate in blood serum and similar technology has been shown to be effective for lactate analysis in dairy products (Collier et al. 1998). Thus, available technology is amenable to the practical development of automated tests for use both as a cow-side and as an in-line diagnostic for mastitis.

Lactate measurements in milk apparently offer an opportunity for accurate, automated early detection of mastitis.

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