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## Sealing of the bovine teat canal after drying off

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### ABSTRACT

Closed teats have proven more resistant to dry period mastitis than open teats. Natural rate of keratin plug formation within closed and open teats, and consequences of bacterial colonisation of the teat canal, were examined using 67 low somatic cell count cows. Cows were dried off without antibiotic therapy and teats assessed for closure status at 5, 10, 15 and 20 d after drying off. Weight and bacteriological status of the keratin plug was determined by reaming teats with a sterile pre-weighed tapestry needle 7 d prior to drying off and on one occasion post drying off. Initially (d 5), keratin weight for all teats increased by 100% compared to lactating teats (4.89 mg/qtr). Between d 10-20, keratin weight from open teats (29% of teats) was 34-55% less than for closed teats. Subclinical infections in the teat canal significantly reduced likelihood of teats closing after drying off.

**Keywords:** teat closure; drying off; keratin plug.

### INTRODUCTION

The bovine mammary gland is highly susceptible to mastitis during the early dry period when the udder is undergoing involution (Cousins *et al.*, 1980). New infections that occur at this time can persist into the subsequent lactation and are often responsible for clinical mastitis and increased somatic cell counts (SCC) in early lactation. Any factors that increase milk SCC in early lactation are of major concern to the New Zealand dairy industry. Previous research has identified that closed or sealed teats are less susceptible to dry period mastitis (Williamson *et al.*, 1995). Closed teats are those from which no fluid can be expressed when a mild squeezing action is applied to the teat barrel. Intramammary antibiotics administered at drying off can aid teat closure, but the actual mechanism responsible for teat closure is unclear. Simple, effective, non-antibiotic strategies that can accelerate or improve the natural closure process would be extremely beneficial to dairy farmers.

The closure mechanism is likely to involve tightening of the smooth muscle fibres surrounding the teat canal as well as formation of a keratin 'plug' within the lumen of the teat canal. Keratin is a lipid-rich material derived from the sloughed, keratinised cells of the epithelial lining of the canal (Williams, 1984). Colonisation of the teat canal by bacterial pathogens may hinder formation of the keratin plug. Experiments were conducted to establish the natural size and rate of formation of the keratin plug within closed and open teats, and to determine the consequences of teat canal colonisation by bacteria.

### MATERIALS AND METHODS

A total of 67 cows with low SCC (<150,000/ml) were selected from DRC herds and managed in three groups (19 Friesian, 28 Jersey and 20 Friesian and Friesian x Jersey crossbreds). Bacteriological status of the foremilk was determined 7 d prior to drying off and again at drying off. All cows were dried off by abrupt cessation of milking, with no

administration of dry cow antibiotic therapy. For the 19 Friesian and 28 Jersey cows, all teats were assessed for open or closed status by manual manipulation and attempted expression of mammary secretion (Williamson *et al.*, 1995) at 5, 10, 15 and 20 d after drying off. For the remaining group of 20 cows, all teats were assessed once only, at 15 d after drying off.

At 7 d prior to drying off, all teats of the 19 Friesian and 28 Jersey cows were sampled for keratin, using aseptic technique, by reaming the teat canal with the eye of a sterile, pre-weighed tapestry needle. This method removes approximately 70% of available keratin from the teat canal during lactation and 85% during the dry period (Bright *et al.*, 1990). During lactation, keratin is replaced and regenerated on a daily basis, so keratin removed by reaming is replaced within 2-3 d (Capuco *et al.*, 1990). During the dry period however, any removal of keratin will stimulate growth rate of the keratin. Therefore to estimate the natural rate of plug formation, teats were sampled only once after drying off. For the 47 cows previously sampled when lactating, 25% of teats were sampled at 5, 10, 15 and 20 d after drying off. For the remaining 20 cows, all teats were sampled once only at 15 d after drying off. After reaming, samples of gland secretion were collected aseptically from all teats. To all non lactating teats a tube of intramammary antibiotics was infused after sampling to reduce the risk of mastitis. Any clinical infections were treated with a course of lactating cow intramammary antibiotics.

Wet weight of the keratin removed was determined and then a sub-sample of keratin was removed from each needle for determination of bacteriological status. Samples of keratin, milk or dry cow secretion were streaked onto quarter-plates of Columbia blood agar containing 5% whole bovine blood and 0.1% aesculin and incubated at 37°C for 48 h. Presumptive identification of isolates was by colony morphology, Gram's stain, haemolysis, catalase production, aesculin reaction and tube coagulase reaction. Statistical analysis of keratin wet weight was performed using the mixed model procedure (SAS, 1994).

### RESULTS

Before drying off, teats sampled for keratin (n=188) yielded  $4.89 \pm 0.15$  mg ( $\pm$  SEM) wet weight of keratin. By d 5 after drying off, the amount of keratin harvested had more than doubled ( $P < 0.001$ ), averaging  $11.20 \pm 0.70$  mg keratin, with no difference apparent between open and closed teats (Table 1). By d 10 after drying off however, closed and open teats differed in the amount of keratin that could be harvested. Between d 10 and d 20 open teats yielded 34-55% less keratin ( $P < 0.001$ ) than closed teats (Table 1).

**TABLE 1:** Wet weight of keratin harvested from teats at 7 d before drying off and from open and closed teats at d 5, 10, 15 and 20 after drying off.

Days pre/post drying off	Wet Weight Keratin (mg/qtr)		SED <sup>1</sup>	% open	n
	Closed	Open			
-7	4.89		-	-	188
5	11.76	11.76	1.14 <sup>NS</sup>	36%	47
10	12.11	5.99	1.51 <sup>***</sup>	32%	47
15	13.34	9.30	1.07 <sup>***</sup>	29%	127
20	11.06	7.22	1.56 <sup>*</sup>	26%	47

<sup>1</sup> NS  $P > 0.05$ ; \*  $P < 0.05$ ; \*\*\*  $P < 0.001$

Clinical mastitis developed in 10/268 (3.7%) of quarters after drying off, although approximately 50% of quarters were found to harbour subclinical infections (131/268). The same pathogen was isolated from both keratin and gland secretion for 66% (89/131) of cases; some pathogens were isolated from the keratin only (34% of cases). Coagulase negative staphylococci (CNS) were the most common pathogens isolated (82% of cases), followed by *Corynebacterium bovis* (13%), *Streptococcus uberis* (11.5%) and *Staphylococcus aureus* (4%).

Of the 222 teats that entered the dry period with no infection, 135 remained uninfected and 23% of these teats were deemed to be open. However, for the 87 teats that gained an infection, the percentage of open teats was significantly higher, at 40% ( $P < 0.01$ ; Table 2).

**TABLE 2:** Association between the change in bacteriological status of teat canal keratin, determined before and after drying off, and the percentage of teats which were deemed open when keratin was sampled, post drying off.

Bacteriological status of keratin		Number of teats		$\chi^2$ test
Before drying off	After drying off	n	% Open	P value
Uninfected	Uninfected	135	23%	0.006
Uninfected	New Infection	87	40%	
Infected	Uninfected	6	17%	NS
Infected	Infected	40	45%	

### DISCUSSION

Sealing of the teat after drying off appears to be an important component of the natural resistance of a cow to mastitis (Williamson *et al.*, 1995). This experiment demonstrated that closed teats yielded a larger plug of keratin, although differences between open and closed teats were only apparent from d 10 after drying off. This would suggest that factors other than keratin mass, are responsible

for teat closure prior to this time. Increasing muscle tone around the teat canal may be important at this time.

The amount of keratin recovered from open teats reduced significantly between d 5 and d 10 after drying off. The lower recovery of keratin from open teats, from d 10 onwards, may be an anomaly of the closure assessment technique, since by definition, open teats are those from which fluid can be expressed. It is entirely possible that some of the 'loose' keratin may be removed from open teats during this process. However this does not appear to occur at d 5, when open and closed teats yielded very similar amounts of keratin. Indeed, the flushing effect of the expressed fluid would be most powerful at this time due to the hydrostatic pressure of fluids accumulating within a gland that has not been milked for 5 days.

An alternative hypothesis might be that the rate of growth and regeneration of keratin may require at least 5 days to respond to the absence of milking. During lactation, the epithelium of the canal must maintain a rapid regeneration rate since up to 40% of the keratin layer is removed at each milking (Capuco *et al.*, 1990). Previous experiments have demonstrated that changes in growth rate are apparent for at least 4 d after imposing treatments that reduce keratin removal from the teat canal (Lacy-Hulbert *et al.*, 1996). Significant changes in the microscopic anatomy of the teat canal have been observed between d 0 and d 7 after drying off (Comalli *et al.*, 1984), including a reduction in the mitotic activity of the basal cells of the epithelium. Further work is required to determine why open and closed teats might respond differently to the cessation of milking.

The significance of bacterial populations within the keratin and dry cow secretion is unknown. The results suggest that teats that remain uninfected after drying off have an increased chance of closure. Development of an infection after drying off, particularly within the keratin plug, may increase the chances of a teat remaining open after drying off.

Practical measures are needed which will encourage teat closure, particularly during the first 10 d of the dry period. Such measures would promote keratin plug formation or minimise the risk of bacterial colonisation of the teat canal. Stimulation of keratin growth could involve increasing the daily rate of keratin sloughing immediately prior to drying off, such as by more frequent milking or by reaming, or could involve topical application to the teat canal of substances which directly stimulate keratin growth. Infusion of a very small amount of intramammary antibiotics into the teat canal may also provide an alternative, highly localised protection to the mammary gland, which may help to cure existing infections within the teat canal or reduce the likelihood of new infections. Both steps may increase the likelihood of a teat canal closing soon after drying off.

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