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Influence of feeding level after drying off on incidence of mastitis and keratin plug formation in dairy cows

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ABSTRACT

Two groups of 70 cows, including 51 identical twin sets, were grazed at either an unrestricted (14 kg dry matter intake/day; DMI/d) or restricted (6 kg DMI/d) daily pasture allowance for two weeks after drying off (DO). Incidence of new intramammary infection (IMI) and teat open/closed status was assessed at 7, 14 and 21 days after DO. Keratin plug formation was determined on a subset of 40 cows at 7 and 14 days after DO. Number of clinical mastitis (CM) cases and total new IMI did not differ between cows maintained on unrestricted or restricted DMI. However *Streptococcus uberis* was isolated from more new IMI in unrestricted animals than restricted animals (11.7% vs. 4.6 % of quarters respectively; $P < 0.01$). At 7 and 14 days after DO, more teats were classified as open in the unrestricted group compared to the restricted animals (57% vs. 43% of quarters respectively; $P < 0.01$). However weight of keratin collected from teat canals did not differ between nutritional treatments. Results suggest that unrestricted nutrition after DO did not increase risk of CM but does increase risk of *S. uberis* infection.

Keywords: drying off; mastitis; keratin; DMI; nutrition.

INTRODUCTION

The bovine mammary gland is highly susceptible to mastitis during the early dry period (Cousins *et al.*, 1980, Williamson *et al.*, 1995). New infection rates during the dry period exceed those during lactation (Cousins *et al.*, 1980, Nickerson, 1990) and these infections can persist into the next lactation, causing somatic cell count (SCC) increases and clinical mastitis (CM) (Williamson *et al.*, 1995).

The mammary gland's first line of defence is the teat canal, which appears to act primarily as a physical barrier, preventing entry of bacterial pathogens (Comalli *et al.*, 1984). Previous research has identified that closed or sealed teats (quarters that retain secretion when gentle pressure is applied to the teat sinus in a milking motion) are less susceptible to dry period mastitis compared to open teats (Williamson *et al.*, 1995).

Rapid formation of a keratin plug after dry off (DO) is considered important in preventing new intramammary infection (IMI) (Lacy-Hulbert *et al.*, 1999a, Nickerson, 1990). This plug acts as a physical barrier, preventing microbial access to the teat sinus (Bright *et al.*, 1990, Comalli *et al.*, 1984). The teat canal of closed teats has been found to contain significantly more keratin than open teats (Lacy-Hulbert *et al.*, 1999a).

It is often recommended that feed intake be restricted during the drying off process to "switch off" milk production. However, increased susceptibility to mastitis has been linked to reduced or poor nutritional status (Erskine, 1993). Previous work conducted in the United States (Bushe & Oliver, 1987) recommended restricted nutrition prior to DO to reduce milk yields. In New Zealand however, cows produce considerably less milk than US animals. Our research suggests that the

absence of a feed restriction prior to DO does not adversely affect susceptibility to mastitis (Lacy-Hulbert & Woolford, 1999). However, the ideal level of nutrition in the period immediately following DO remains unclear.

This experiment compared the effect of a restricted or unrestricted level of feeding for two weeks immediately after DO on keratin plug formation, and the incidence of clinical mastitis and IMI in the early dry period.

MATERIALS AND METHODS

Experimental Design

A total of 63 primiparous and 77 multiparous Friesian, Jersey and Friesian-Jersey crossbred cows, including 51 sets of identical twins, were selected from the Dexcel Lye Farm research herd. Cows were divided into two groups of 70 animals, balanced for predicted calving date, udder health status, peak milk flow rate and average daily milk yields. Prior to DO, all cows were grazed together on perennial ryegrass/white clover pasture and offered sufficient pasture to allow an intake of 16 kg dry matter (DM) per day. After the last milking, the groups were randomly assigned to either a restricted or unrestricted level of feeding.

Restricted animals were fed pasture to achieve the recommended DMI (6 kg DM/day) to maintain current live weight (450 kg) and body condition score (BCS) at this stage of lactation and gestation (Holmes *et al.*, 2002). Unrestricted cows were fed at a higher intake (14 kg DM/day), so as to gain one BCS in a 30-day period. Cows had access to a fresh allocation of pasture daily. Treatments were maintained for 14 days post DO, following which both groups were fed at a DMI of 12 kg DM/day for the next 7 days which then completed their

involvement in the trial. Composition of pasture fed to restricted and unrestricted groups was similar.

Bacteriological status of the foremilk was determined for quarters of all cows at approximately 14 and 7 days prior to DO and at DO. Individual quarters identified as infected with major mastitis pathogens received dry cow antibiotic therapy (DCT) (Dryclox Xtra, Bomac Laboratories Ltd, Manakau City, NZ) at DO. All quarters were resampled for bacteriology once only after DO, at either day 7, 14 or 21 to determine incidence of new IMI.

A subset of 40 cows, comprising 20 uninfected twin sets, was used to assess keratin growth and plug formation after DO. On day 15 before DO, all teat canals were sampled for keratin and then on day 7 and 14 after DO the left and the right quarters were sampled, respectively. Mammary secretion samples were collected after keratin sampling, and DCT administered. These quarters were not resampled at day 21.

On days 7, 14 and 21 after DO, the open/closed status of the teat was assessed for all cows using the method described by Williamson *et al.* (1995). Udders were manually palpated to check for CM and if detected, were sampled aseptically and a course of lactating cow intramammary antibiotic administered (Orbenin LA, Pfizer Animal Health Group, Mt Eden, Auckland, NZ).

Cow live weight and BCS of all cows were assessed weekly for 2 weeks before DO, on the day of DO and weekly for 3 weeks following DO, at approximately 0900 hrs. The BCS was assessed using a 1 to 10 scale described by MacDonald & MacMillan (1993).

Sample Collection and Analysis

Bacteriological samples were collected following aseptic preparation of the teat end and analysed according to standard microbiological procedures (National Mastitis Council, 1999). Somatic cell count (SCC) was analysed by a Fossomatic automated cell counter (Foss Electric, DK-3400, Hillerod, Denmark). Samples collected after DO or from CM cases were diluted 1:10 with sterile phosphate buffered saline prior to measurement. Clinical mastitis was diagnosed by the presence of clinical signs i.e. clots or discolouration of the udder secretion, heat, swelling or pain in the udder.

Keratin was collected within the eye of a sterile, preweighed, size 14G tapestry needle (Bright *et al.*, 1990), gently inserted into the teat canal to a depth of 10 mm and rotated. Keratin wet weight was measured within one hour of sampling.

Pasture height was assessed pre and postgrazing three times a week using a rising plate meter (Farmworks, Palmerston North, NZ). Pasture mass was assessed visually using calibrated assessors and the DMI

calculated from pre and postgrazing differences (Roche *et al.*, 2002).

Statistical Analysis

All SCC data were log base 10 transformed prior to analysis. Effect of nutritional treatment on proportion of infected quarters and open teats was analysed using generalised linear models, with logit link and binomial error structure. Effect of nutrition on keratin weight, SCC, cow live weight and BCS was analysed using Residual Maximum Likelihood (REML, Genstat, 2002). Keratin weights were further analysed with teat open or closed status included as a fixed effect in the model to estimate differences between these states. For cows sampled for keratin, we also investigated the relationship between average milk flow rate (FR) and % teats open using REML analysis.

RESULTS

Intramammary Infection and Bacterial Isolates

A total of 89 quarters developed new IMI between DO and the end of the study. Clinical mastitis occurred in 37 of these quarters. Nine quarters infected with *S. uberis*, *S. dysgalactiae* or *Staph. aureus* prior to DO were treated with dry cow antibiotic and not included in subsequent analysis. Unrestricted nutrition had no effect on proportion of quarters that became infected after DO (20%), compared to restricted nutrition (16.4%), or on the proportion that developed CM (7.6% vs. 8.1% of quarters respectively). However more subclinical infections were observed in the unrestricted group (17.9% vs. 10.4% of quarters; $P < 0.05$) compared to the restricted group.

Total new *S. uberis* intramammary infections (clinical and sub-clinical) were higher ($P < 0.01$) in the unrestricted cows (Table 1), while clinical cases where no pathogen was isolated (sterile mastitis) were higher ($P < 0.05$) in the restricted intake group. There was no difference in the total number of new coagulase negative staphylococci (CNS) infections between treatments

Teat Open and Closed Status

More teats ($P < 0.01$) were classified as open in cows with unrestricted intake at day 7 and 14 after DO (Figure 1) but by day 21 no difference was evident between nutritional treatments. There were twice as many new infections in open teats compared to closed teats at day 21 after DO ($P < 0.001$) and there was a positive association ($P < 0.001$) between percent open teats within a cow and average milk FR prior to DO.

TABLE 1: Pathogens isolated from clinical and subclinical infections detected in quarters on either a restricted (R) or unrestricted (U) intake after dry off.

	Clinical Mastitis		Subclinical Mastitis		Total		Total P value
	R	U	R	U	R	U	
N Total	20	17	22	30	42	47	NS ¹
% SU²	30	53	23	50	26	51	**
% CNS³	0	0	73	47	38	30	NS
% Other⁴	0	6	5	3	2	4	-
% Sterile⁵	70	41	-	-	33	15	*

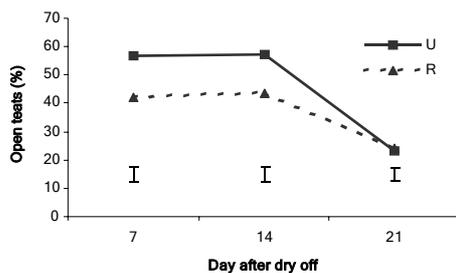
¹NS = not significant; * P <0.05; ** P <0.01; - = not analysed

²SU = *S. uberis*

³CNS = coagulase negative staphylococci

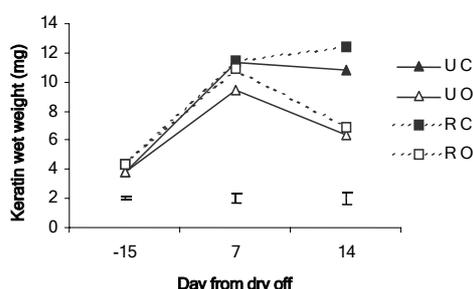
⁴Other = *S. aureus*, *C. bovis*, Bacillus and other species

⁵Sterile = no pathogen isolated

FIGURE 1: The effect of time after dry off on the proportion of open teats in cows on unrestricted (U) or restricted (R) nutritional treatments (SED shown by bars).

Teat Canal Keratin

Approximately 2.5 times as much keratin was collected from teats at day 14 after DO compared with pre DO but no effect of nutrition was observed (Figure 2). The wet weight of keratin reamed did not differ between open or closed teats at day 7 after DO. However by day 14, there was 70% more keratin (P <0.001) reamed from closed teats compared to open teats for both treatment groups (11.6 vs. 6.6 mg for closed and open teats, respectively).

FIGURE 2: Amount of teat canal keratin (mg) collected from quarters of cows on restricted (R) or unrestricted (U) nutrition treatments, with open (O) or closed (C) teats (SED shown by bars).

Somatic Cell Count

Average SCC of uninfected quarters increased from $0.21 \pm 0.42 \times 10^6$ cells/ml (\pm SD) at DO to approximately $11.5 \pm 7.5 \times 10^6$ cells/ml (\pm SD) at 7 days after DO. For cows on restricted nutrition, the SCC was higher at day 7 (11.2 vs. 6.8×10^6 cells/ml, P <0.01) and day 14 (13.5 vs. 7.6×10^6 cells/ml, P <0.001) compared with unrestricted cows. The average SCC at day 21 was similar between nutritional treatments (7.2×10^6 cells/ml).

Live weight and BCS

Cows on restricted nutrition consumed 6 ± 0.2 kg DM/d compared with 14 ± 0.6 kg DM/d by unrestricted cows. By day 14 after DO, restricted cows were lighter (446 vs. 458 kg, P <0.001) than the unrestricted group but no difference was measured at day 21 after DO (463 kg). The BCS after DO was higher (P <0.001) in unrestricted cows compared to restricted cows, at day 14 (Table 2) and a difference in BCS (P <0.01) was still detectable 7 days after treatments ceased, although cows previously restricted had gained condition.

TABLE 2: Effect of unrestricted (U) and restricted (R) pasture intake after dry off (DO \pm n days) on cow body condition score (BCS).

Treatment	DO -13	DO	DO+14	DO+21
U	4.2	4.1	4.6	4.7
R	4.1	4.1	4.2	4.5
SED	0.1	0.1	0.1	0.1
P value	NS ¹	NS	***	**

¹NS = not significant; ** P <0.01; *** P <0.001

DISCUSSION

There is increasing concern that feed restriction may reduce mammary resistance to infection (Erskine, 1993). In the present study contrasting feeding levels immediately after DO did not influence the incidence of total new IMI, although subclinical infections were higher in unrestricted cows. An increase in the number of new infections with *S. uberis* during the dry period was observed and this agrees with Neave *et al.* (1950).

The number of *S. uberis* infections and proportion of open teats were higher in unrestricted cows, suggesting that more open teats may lead to an increase in susceptibility to *S. uberis* mastitis in the first 14 days after DO. The results of this experiment suggest that an unrestricted diet immediately after DO may predispose cows to *S. uberis* infection.

The number of sterile mastitis cases observed after DO was unexpectedly high, particularly among the restricted animals. Previous reports on dry period clinical mastitis rarely report sterile cases (Williamson *et al.*, 1995) whilst in early lactation cows, the incidence of sterile or aseptic mastitis has been reported as averaging 22% but ranging from 0-54% (McDougall, 1999). It is possible that these cases were not true clinical mastitis cases, but were simply unusual udder secretions. Further studies are needed to determine whether sterile mastitis is accurately identifiable after DO.

The higher SCC levels in restricted intake animals supports earlier findings of Lacy-Hulbert *et al.* (1999b), who found that a feed restriction applied prior to DO increased milk SCC. It is possible that the increased SCC is a result of a reduced secretory volume but, without the measurement of secretion volume, no conclusion can be drawn. Higher SCC levels in the restricted cows, with a greater concentration of phagocytotic cells present in the teat and gland sinus, may have prevented bacterial colonisation inside the teat (Nickerson, 1990) and may have contributed to the lower incidence of *S. uberis* mastitis.

It was hypothesised that restricted nutrition may adversely affect keratin growth however no effect was observed. Quantities of keratin reamed from dry and lactating cows were comparable to those found by Bright *et al.* (1990), who also found that twice as much keratin was obtained from the teat canal of dry cows. A difference between open and closed teats, in terms of size of keratin plug was only visible at day 14 after DO, suggesting that up to 14 days is required for a competent keratin plug to form. Previous studies (Lacy-Hulbert *et al.*, 1999a) found larger keratin plugs in closed teats from day 10 onwards.

Over the 21 days after DO, the number of closed teats increased to 76%, which is greater than that reported by Williamson *et al.* (1995), who found 50% of quarters were closed by 40 days after DO. During the first 14 days after DO the likelihood of teat openness was positively correlated with milk flow rate and with nutrition, with cows on unrestricted intake having a greater proportion of open teats. Teat openness in the unrestricted cows may be affected by the greater secretory volume, which would increase intramammary milk pressure and increase potential of leakage. In addition, the diameter of the teat canal is related to milk flow rate (Lacy-Hulbert & Hillerton, 1995) and it is possible that cows with a high milk flow rate require a longer period of time for the keratin plug to form.

During early involution the mammary gland may be more susceptible to infection due to the termination

of teat spraying and flushing of bacteria from the teat (Nickerson, 1990), causing higher levels of exposure to bacteria, and to changes in udder secretion composition (Cousins *et al.*, 1980). In conclusion, the present study examined if the level of feeding immediately after DO impacts on natural infection rates and found that an unrestricted intake in the first 14 days after DO increased susceptibility of cows to *S. uberis* infection. In practical terms, if farmers allow their cows an unrestricted intake to gain condition, additional mastitis control measures such as regular teat spraying or use of dry cow antibiotic therapy, will be required to prevent new *S. uberis* infections. Alternatively, cows should be restricted for up to 14 days after DO to reduce the risk of *S. uberis* infection and then be allowed to gain condition. Weekly checks for clinical mastitis are recommended for the first 21 days after DO.

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