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Lactoferrin concentrations in bovine milk prior to dry-off

Kari A Newman[1], Päivi J Rajala-Schultz[1]*, Jeffrey Lakritz[2] and Fred J DeGraves[1]

[1] Department of Veterinary Preventive Medicine,
[2] Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Ohio State University, Columbus, Ohio 43210, USA

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Concentration of natural protective factors (NPFs) which have the ability to inhibit growth of mastitis-causing pathogens increase rapidly following the cessation of milking of dairy cows. One such NPF is lactoferrin, an iron-binding protein present in high concentrations in dry-cow secretions. Earlier studies have demonstrated that intermittent milking at the end of lactation increases levels of NPFs in milk and may decrease prevalence of intramammary infections at calving; however, most of these studies date back several decades and may not apply to current high-producing cows. The objective of this study was to assess whether an intermittent milking schedule prior to dry-off increases the concentration of lactoferrin in mammary secretions at the end of lactation and what other factors influence lactoferrin concentration at dry-off. One week prior to dry-off (pre-dry), cows were randomly assigned to an intermittent milking schedule or they continued to be milked twice daily. Duplicate quarter milk samples for microbiological culture were taken at pre-dry and at dry-off to determine infection status of quarters. Quarter somatic cell counts (SCC) were measured on the day of dry-off. Lactoferrin concentrations were quantified by ELISA. Intermittent milking, mean SCC for the last three months prior to dry-off, SCC at dry-off, lactoferrin concentration at pre-dry, quarter infection status at pre-dry and dry-off, days in milk at dry-off, breed, parity, cumulative milk yield for the final week of lactation and season were considered as potential explanatory variables. Their effect on lactoferrin concentration at dry-off was assessed using a mixed-effects linear regression model. Lactoferrin concentration increased significantly during the final week of lactation for cows on an intermittent milking schedule and was significantly associated with initial lactoferrin concentration and infection status at dry-off.

Keywords: Lactoferrin, dry-off, intermittent milking.

The dry period in the lactation cycle of a dairy cow is a critical time for maintenance of mammary health (Dingwell et al. 2003). Cows are highly susceptible to bacterial infections during the early dry period and in the last weeks prior to parturition (Smith et al. 1985; Oliver, 1987). After cessation of milking, as part of the involution process, composition of the mammary gland secretion changes to contain high concentrations of natural protective factors (NPFs) such as lactoferrin (Concha, 1986; Sordillo et al. 1987; Nickerson, 1989). Lactoferrin is considered a first line of defence against mastitis pathogens and it plays an important role in mammary gland immunology (Sordillo et al. 1997). Lactoferrin sequesters free ferric ion from its environment, rendering it unavailable to bacterial pathogens with iron requirements (Nonnecke & Smith, 1984b; Bushe & Oliver, 1987; Sordillo et al. 1997; Chaneton et al. 2008). It also has the ability to bind to the bacterial cell wall and cause rupture of Gram-negative outer membrane (Ellison et al. 1988). Thus, lactoferrin has both a bacteriocidal and a bacteriostatic effect in the mammary gland of the lactating cow. Lactoferrin concentrations in bovine milk have been shown to increase during times of immunological stress to the mammary gland and during the dry period and they are also negatively correlated with milk yield (Gaunt et al. 1980; Hagiwara et al. 2003; Cheng et al. 2008).

The method of drying cows off influences the involution process and can affect natural defence systems during the dry period (Natzke et al. 1974; Oliver & Sordillo, 1989). Most studies evaluating different drying-off methods date back several decades (Oliver et al. 1956; Natzke et al. 1974; Bushe & Oliver, 1987) and results from these studies may no longer be applicable to the high-producing, modern dairy cow. The current recommendations for drying-off cows by the National Mastitis Council (NMC) include

*For correspondence; e-mail: paivi.rajala-schultz@cvm.osu.edu

abrupt cessation of milking (*Recommended Mastitis Control Program*, www.nmconline.org/docs/NMCchecklistNA.pdf, accessed 15 May 2009). In a high-producing dairy cow, this can cause substantial pressure on the teat canals until the milk that accumulates in the udder is reabsorbed. In fact, increasing milk yield at dry-off has been shown in recent studies to be significantly associated with prevalence of intramammary infections during the dry period and after calving (Dingwell et al. 2004; Rajala-Schultz et al. 2005; Green et al. 2007). An intermittent milking schedule prior to dry-off, on the other hand, has been shown to effectively reduce milk yield at dry-off in older studies (Natzke et al. 1974; Oliver et al. 1990).

Previous reports (Harmon et al. 1975; Hurley 1989; Hagiwara et al. 2003; Cheng et al. 2008) determined lactoferrin concentrations in the secretions of healthy cows and cows with clinical mastitis during mid lactation and during the dry period, but little research has been done recently regarding the lactoferrin content of milk at the end of lactation, prior to dry-off. In the 1980s, Bushe & Oliver (1987) showed that the secretions of cows enrolled in an intermittent milking schedule not only had higher lactoferrin concentrations, but higher concentrations of serum albumin and immunoglobulin G, in addition to lower citrate levels. This suggests that intermittent milking at the end of lactation may have a positive effect on NPFs in the milk (Bushe & Oliver, 1987). The goal of this study was to determine milk lactoferrin concentrations one week prior to dry-off and on the day of dry-off, in cows dried off by abrupt cessation of milking and in cows milked intermittently for a week prior to dry-off and to assess which other factors are associated with lactoferrin concentrations at the end of lactation.

Materials and Methods

Study population

Milk samples were collected aseptically at the end of lactation for microbiological culture and determination of lactoferrin concentration from 87 cows from the Ohio State University Dairy Research Herd between June 2006 and December 2007. Because the dairy has a seasonal calving schedule, all samples were taken in the latter halves of both years. Cows were chosen to participate in the study based on confirmed pregnancy during their current lactation and enrollment in no other research trials during the period in which the study took place.

The study herd comprised of Holstein and Jersey cows. Cows were blocked by breed and then randomly assigned to an intermittent milking group ($n=40$) or a control group ($n=47$) one week prior to dry-off (pre-dry). During the first year of the study, cows in the intermittent milking group were housed in tie stalls beginning one week prior to dry-off and only allowed into the milking parlour during scheduled milkings. Control cows were housed in a free stall barn with the rest of the milking herd and were milked twice daily until the day of dry-off. During the second year of the study, however, owing to management changes in the herd, intermittently milked cows were housed with control cows in the free stall barn. Intermittently milked cows were milked once a day for a period of 4 d, were not milked for a day, were milked once the following day, were left unmilked for one more day, and were milked the morning of the last day and then immediately dried off following the morning milking. The identification numbers of the intermittently milked cows were programmed into the computer (S.A.E. AFIKIM, Kibbutz Afikim, Israel) to alert milking personnel not to milk these cows except on the predetermined schedule. These cows entered the parlour with the rest of the herd but were milked only on their intermittent milking schedule. Control cows were milked twice daily as usual until the day of dry-off, when they were milked once in the morning and immediately dried off. Milk yield was recorded on the day of dry-off as well as for the nine days prior to dry-off. All quarters of all cows were treated with cephapirin benzathine-containing dry cow product (Cefa-Dri®, Fort Dodge Animal Health) on the day of dry-off, following sample collection and the final milking.

Sample collection and microbiological culture

Duplicate quarter milk samples were collected aseptically according to NMC guidelines (Oliver et al. 2004) one week prior to dry-off and on the day of dry-off. An additional 45-ml milk sample was also obtained from each quarter on the day of dry-off and these samples were transported to Dairy Herd Improvement Association (DHI) on the day of collection for somatic cell count (SCC) determination. The other samples were immediately cooled for transport to the laboratory and were then frozen for at least 24 h prior to culturing. Samples were examined by plating 0·01 ml milk on tryptic soy agar (TSA) with 5% sheeps' blood and MacConkey agar (Remel Inc., Lenexa KS, USA) using sterile disposable calibrated loops. Plates were incubated for 48 h at 37 °C, and bacterial growth was recorded at 24 h and 48 h of incubation. Bacterial species were identified following NMC guidelines (Oliver et al. 2004). Colonies on blood agar with similar morphology were counted and recorded as colony forming units (cfu)/ml of milk. The quarter infection status was determined using a single milk sample, applying the criteria proposed by Torres et al. (2009). Briefly, if a sample contained at least 100 cfu/ml of contagious pathogens or 1000 cfu/ml or more of any other pathogens, a quarter was considered infected. A quarter sample was considered contaminated if it contained three or more unique isolates (Oliver et al. 2004). If the first sample was contaminated, the second sample was used instead. If both samples from a quarter were contaminated, infection status of that quarter was considered unknown. After plating, samples were immediately frozen to −70 °C and were thawed prior to dilution for lactoferrin ELISA. For lactoferrin quantification,

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only the first sample from each quarter was used. Individual cow SCC and milk yield records for the months prior to dry-off were obtained from DHI herd records.

Lactoferrin quantification

Lactoferrin concentration was determined using ELISA analysis. Procedures for lactoferrin quantification were performed following the instructions of the Bethyl Bovine Lactoferrin Quantitation Kit (Bethyl Labs, Inc., Montgomery TX, USA) with the following modifications: Tween 20 was not added to the sample diluant and PBS with a pH of 7·3 was used rather than 50 mm-Tris and 0·05 M-carbonate–bicarbonate. Between-plate variation was assessed using duplicate readings of seven plates over a period of 5 d. Within-plate variation was calculated over six plates on three separate days.

Ninety-six-well microtitre plates were coated with 10 µg/ml of goat anti-bovine lactoferrin affinity purified antibody. Serial dilutions of whole milk at a ratio of 1 : 10 000 in a 1% bovine serum albumin/PBS solution were used for the ELISA. Goat anti-bovine lactoferrin horseradish peroxidase (HRP) conjugate antibody was used as the detection antibody at a dilution of 1 : 10 000. Standards were designed through serial dilution using the Bovine Lactoferrin Calibrator. A standard curve was generated for each plate (7·8–2000 ng/ml) and plates were read at 450 nm absorbance values. Individual samples were analysed and read in duplicate and each plate was read twice by a Labsystems Multiscan plate reader (Labsystems and Life Sciences Ltd. UK). Absorbance values for each sample were averaged over the four readings. The number of micrograms per millilitre was calculated based on the absorbance value and the slope and intercept of the standard curve.

Owing to variability in the literature regarding the use and preparation of skimmed or whole milk for lactoferrin ELISA analysis (Soyeurt et al. 2007; Chaneton et al. 2008; Cheng et al. 2008) a portion of the samples were initially analysed using both whole and skimmed milk. Samples were skimmed at 3000 rpm in a Sorvall Legend RT centrifuge (Thermo Scientific, Waltham MA, USA) at 4 °C for 45 min. Milk samples were then drawn from below the fat layer and diluted in an identical fashion to samples taken from whole milk. Duplicate whole milk and skimmed milk samples were placed side-by-side on a 96-well microtitre plate for concentration comparison.

Data analysis

Infection status at dry-off was categorized based on the type of organism isolated, i.e., uninfected, infected with major (e.g. *Staphylococcus aureus, Streptococcus* spp., *Escherichia coli*, other Gram-negative organisms) or infected with minor pathogens [coagulase negative staphylococci (CNS), *Corynebacterium* spp.]. In a case of mixed infections, a sample was considered infected with major pathogens if culture results included both a major and a minor pathogen or two major pathogens and infected with minor pathogens if both isolates were minor pathogens.

Data analysis was performed using Statistical Analysis System, SAS v.9.1.3 (SAS Inst. Inc., Cary NC, USA). Within-and between-plate variation in lactoferrin concentrations was assessed by calculating the coefficient of variation (CV). Descriptive statistics (mean and 95% confidence intervals or median and 10th and 90th percentiles) were calculated for all continuous variables to assess differences between the treatment and control group. The proportions of quarters infected at pre-dry and at dry-off between the groups were calculated and compared using Chi-square test of independence. Analysis of quarter-level lactoferrin concentration at dry-off was done using a mixed effects linear regression. Initially, all potential explanatory variables were individually regressed on lactoferrin concentration at dry-off. Interdependence of quarters within a cow was accounted for by using compound symmetry covariance structure during the modelling. Treatment group status, cumulative milk yield for the final week of lactation, breed, parity (lactations 1, 2, 3+), season (summer, June–September inclusive; and autumn, October–January inclusive) and days in milk at dry-off were considered for the model. Mean of the DHI SCC and daily milk yield for the last three months prior to dry-off, quarter-level SCC at dry-off, lactoferrin concentration (mg/ml) at pre-dry, and infection status based on bacterial culture at pre-dry and at dry-off were also included in the analysis. Infection status of quarters with two contaminated samples remained unknown and thus, these observations were not included in the modelling. SCC were log-transformed (logSCC) for the data analysis. Continuous variables were centered at their median, based on the data available. All variables associated with lactoferrin with a P value of $<0{\cdot}20$ in the initial screening were included in a multivariate model. Once the initial multivariate model was established, the least significant variables were dropped one at a time based on Wald chi square P values until only significant variables remained in the model.

Results

Descriptive statistics

Data from 40 cows in the treatment group and 47 cows in the control group were included in the analysis. Because some cows had fewer than four functional quarters, data from 155 treatment quarters and 181 control quarters were available. Owing to contaminated samples, infection status of 3 treatment quarters and 5 control quarters remained unknown either at pre-dry or at dry-off.

Descriptive statistics on the treatment and control cows are given in Table 1. Lactation number, days in milk at dry-off, milk yield, or SCC between control and treatment cows prior to enrolment in the study did not differ. Owing to the different milking schedules, the actual milk yield on

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Table 1. Descriptive statistics for treatment (intermittently milked) and control cows [median, 10th and 90th percentile (in parenthesis)] for somatic cell count and mean and 95% confidence interval (in parenthesis) for other continuous variables; number and percentage of quarters infected for the infection status). Pre-dry (one week prior to dry-off) refers to the enrolment of cows to the study. Overlapping confidence intervals imply no statistical difference, $P>0{\cdot}05$

	Treatment cows† ($n=40$)	Control cows‡ ($n=47$)
Milk at predry, kg/d (mean over the last 3 months)	25·7 (23·7, 27·7)	25·6 (23·7, 27·5)
Cumulative milk yield, kg (for the last week of lactation)	78·7 (69·0, 88·5)	125·8 (107·3, 144·2)
SCC ($\times 10^{-3}$)/ml at pre-dry, (median over the last 3 months)	188 (39, 1625)	157 (32, 884)
Lactation number	1·8 (1·5, 2·2)	1·6 (1·4, 1·9)
Days in milk at dry-off	334 (318, 367)	343 (317, 511)
Quarter infection status§	$n=155$	$n=181$
Infected at predry, total number and %	40 (25·8%)	40 (22·1%)
infected with major pathogens	19 (12·3%)	12 (6·6%)
infected with minor pathogens	21 (13·6%)	28 (15·5%)
Infected at dry-off, total # and %	43 (27·7%)	38 (21·0%)
infected with major pathogens	20 (12·9%)	11 (6·1%)
infected with minor pathogens	23 (14·8%)	27 (14·9%)

† Cows were on an intermittent milking schedule for the final week of lactation
‡ Cows were milked twice daily and dried off by abrupt cessation of milking
§ Major pathogens included *Staphylococcus aureus*, *Streptococcus dysgalactiae*, coliforms, and *Nocardia spp.*, minor pathogens included coagulase-negative staphylococci and *Corynebacterium spp.*

Table 2. Quarter level lactoferrin concentrations (mg/ml, mean and 95% confidence interval) and somatic cell counts (SCC) (median and 10th and 90th percentile) by quarter infection status one week prior to dry-off (pre-dry) and at dry-off for cows milked intermittently during the final week of lactation (treatment group) and for cows dried off by abrupt cessation of milking (control group). Non-overlapping confidence intervals imply statistical difference at $P<0{\cdot}05$ level

	Treatment cows†		Control cows‡	
	Pre-dry	Dry-off	Pre-dry	Dry-off
Lactoferrin, mg/ml, mean (95% CI§)				
All quarters	0·62 (0·53,0·71)	1·10 (0·99, 1·22)	0·57 (0·50, 0·64)	0·55 (0·48, 0·62)
Uninfected	0·68 (0·56, 0·80)	1·17 (1·03, 1·32)	0·55 (0·46, 0·63)	0·49 (0·42, 0·56)
Minor pathogens¶	0·39 (0·26, 0·51)	0·84 (0·92, 1·06)	0·57 (0·39, 0·76)	0·60 (0·44, 0·77)
Major pathogens††	0·51 (0·37, 0·64)	1·04 (0·72, 1·35)	0·86 (0·43, 1·29)	1·09 (0·68, 1·50)
SCC, ($\times 10^{-3}$)/ml, median (10th, 90th percentile)				
All quarters	ND	623 (51, 3555)	ND	172 (5, 1633)
Uninfected	ND	407 (23, 1989)	ND	124 (5, 1329)
Minor pathogens¶	ND	1117 (176, 3230)	ND	287 (93, 1633)
Major pathogens††	ND	3613 (442, 8433)	ND	909 (65, 5784)

† Cows milked on an intermittent milking schedule for one week prior to dry-off
‡ Cows milked twice daily and dried off by abrupt cessation of milking
§ 95% confidence interval for the mean
¶ Minor pathogens included coagulase-negative staphylococci and *Corynebacterium spp.*
†† Major pathogens included *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *coliforms*, and *Nocardia spp.*
ND, not determined

the day of dry-off was not considered comparable between the treatment groups. Therefore, cumulative milk yield for the final week of lactation was calculated and used in the analysis instead of the last-day milk yield. Cumulative milk yield for the cows in the intermittently milked group (78·7 kg) was significantly lower than the milk yield for cows in the control group (125·8 kg) ($P<0{\cdot}001$). While SCC during the last three months of lactation did not differ significantly between the groups (Table 1), on the day of dry-off, quarters of the treatment cows had significantly higher SCC than those of the control cows (Table 2).

On the day of enrollment (pre-dry), the proportion of quarters infected in the treatment (25·8%) and control (22·1%) group did not differ significantly. Among the

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treatment cows, 19 (12·3%) quarters were infected with major pathogens and 21 (13·6%) with minor pathogens, whereas control cows had 12 (6·6%) quarters infected with major pathogens and 28 (15·5%) with minor pathogens. Two quarters (one in each group) had a mixed infection with both a major and minor pathogen and these were classified as infected with major pathogens. The overall proportion of quarters infected in the two groups was also similar at dry-off (27·7% of quarters infected in the treatment group and 21·0% in the control group) (Table 1).

Lactoferrin ELISA

Within-plate CV of the lactoferrin concentration for the subset of skimmed samples was 5%, while within-plate CV for the standards was 6%. Because the mean concentrations of the skimmed samples (0·72 mg/ml) and the whole milk samples (0·71 mg/ml) were similar and the variability in the samples was small, it was concluded that the lactoferrin antibodies reacted specifically to lactoferrin and not to other milk components in whole milk samples compared with skimmed. Therefore, whole milk samples were used for the remainder of the analysis.

The overall quarter-level lactoferrin concentration one week prior to dry-off (pre-dry) was 0·62 mg/ml in the treatment and 0·57 mg/ml in the control group ($P>0·05$) (Table 2). By the day of dry-off, the mean lactoferrin concentration in the treatment cows had increased to 1·10 mg/ml, making the difference between pre-dry and dry-off concentrations significant in the treatment group (non-overlapping 95% confidence intervals, Table 2). In the control group, however, the lactoferrin concentration had not increased (0·55 mg/ml at dry-off) and this made the difference between the treatment and the control group also significant at dry-off ($P<0·0001$).

Lactoferrin concentrations did not differ significantly based on the infection status of the quarters at pre-dry between the treatment groups (Table 2). In the treatment group, concentrations significantly increased from pre-dry to dry-off in all infection categories and uninfected quarters had the highest concentrations both at pre-dry (0·68 mg/ml) and at dry-off (1·17 mg/ml). In the control group, however, concentrations at pre-dry and dry-off were not significantly different in any infection category and quarters infected with major pathogens had the highest lactoferrin levels at both time points (0·86 mg/ml and 1·09 mg/ml, respectively) and uninfected quarters had the lowest concentrations.

Regression analysis

Treatment group (i.e. intermittent *v.* regular milking schedule), cumulative milk yield during the last week of lactation, lactoferrin concentration at pre-dry, dry-off SCC, and infection status at dry-off ($P<0·05$ for all) and SCC during the last three months of lactation ($P<0·20$) were associated with lactoferrin concentration at dry-off in the initial screening and were included in the multivariate model. Increasing SCC levels were associated with increasing lactoferrin concentrations at dry-off and higher milk yield was associated with lower lactoferrin concentrations. Season of dry-off, parity and breed of cow, days in milk at dry-off and pre-dry infection status, on the other hand, were not associated with lactoferrin concentrations at dry-off. On the final multivariate model, intermittent milking, lactoferrin concentration at pre-dry, and infection status at dry-off remained significantly associated with lactoferrin concentration at dry-off (Table 3). Consistent with the descriptive data, intermittently milked cows had significantly higher lactoferrin concentrations at dry-off ($P<0·001$), even after adjusting for quarter infection status and lactoferrin concentration a week earlier. Higher lactoferrin concentrations at pre-dry were significantly ($P<0·001$) associated with increasing lactoferrin levels at dry-off: for every 1-mg increase at pre-dry above the median value (0·46 mg/ml) the concentration at dry-off increased by 0·55 mg. Quarters infected with major pathogens had significantly higher lactoferrin concentrations at dry-off than uninfected quarters ($P=0·0121$).

Table 3. Mixed effects linear regression model explaining lactoferrin concentrations (mg/ml) in quarter milk samples at dry-off from cows milked intermittently for one week prior to dry-off and from cows dried off abruptly

Variable	Estimate	SE	*P*
Intercept	0·48	0·06	<0·001
Treatment cows†	0·51	0·09	<0·001
Predry lactoferrin‡	0·55	0·05	<0·001
Infection status at dry-off§			
Major pathogens	0·14	0·06	0·0121
Minor pathogens	0·02	0·05	0·685

† Treatment cows were milked intermittently for one week prior to dry-off. Cows milked twice daily until the day of dry-off were the reference group
‡ Centered on the median (0·46 mg/ml)
§ Reference group was uninfected cows. Major pathogens included *Staphylococcus aureus, Streptococcus dysgalactiae,* coliforms, and *Nocardia spp.*, minor pathogens included coagulase-negative staphylococci and *Corynebacterium spp.*

Discussion

Mean lactoferrin concentrations for clinically healthy cows in mid lactation have been reported at 0·01–0·35 mg/ml, with a lot of variability, however, between cows and studies (Harmon et al. 1975; Gaunt et al. 1980; Schanbacher et al. 1993; Chaneton et al. 2008). With clinical or subclinical mastitis, lactoferrin concentrations increase significantly and may be as high as 3·6 mg/ml (Harmon et al. 1975; Gaunt et al. 1980; Chaneton et al. 2008). It has also been shown that as lactation progresses, the mean lactoferrin concentration in bovine milk increases (Gaunt et al. 1980; Cheng et al. 2008). Neither parity nor breed of cow

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has been associated with milk lactoferrin concentrations in previous studies (Kutila et al. 2003; Cheng et al. 2008) and the results from the present study were consistent with those observations. Bushe & Oliver (1987) reported that mean lactoferrin concentrations one week prior to dry-off ranged from 0·4 mg/ml to 1·05 mg/ml and Nonnecke & Smith (1984a) recorded average lactoferrin concentrations of 0·76 mg/ml on the day of dry-off. Quarter level lactoferrin concentrations in the present study one week prior to dry-off and the concentrations in the control cows at dry-off were consistent with those previously reported values at the end of lactation. The intermittently milked cows, however, had significantly higher lactoferrin concentrations on the day of dry-off.

Previous research has also shown that intermittent milking increases NPFs in milk. Bushe & Oliver (1987) demonstrated that intermittently milked cows had consistently higher levels of lactoferrin and other NPFs at dry-off than cows dried-off abruptly; however, the differences were not significant. Only when intermittent milking was combined with restricted feeding regimen (hay only) levels of lactoferrin were significantly higher than in cows dried-off abruptly. Kutila et al. (2003) showed that in 48 clinically healthy cows that were intermittently milked for two weeks prior to dry-off, lactoferrin concentrations at dry-off averaged 5·29 mg/ml and within 2 d of dry-off, lactoferrin concentrations increased to an average of 8·09 mg/ml. They did not, however, record lactoferrin concentrations prior to the intermittent milking schedule, but hypothesized that levels at dry-off increased due to the two-week intermittent milking schedule. Earlier, Welty et al. (1976) demonstrated that within 2–4 d after dry-off, lactoferrin concentrations of bovine dry secretions increased from 0·25 mg/ml to 1·57 mg/ml, showing that cows that are not milked for one or more days have an increase in lactoferrin concentration. Lactoferrin concentrations peaked at 30 d after dry-off and have been reported to be as high as 118·5 mg/ml in individual cows (Welty et al. 1976). They increased an average of 1·15 mg/ml per day during the first week of involution and were closely associated with the onset of involution (Welty et al. 1976). It is likely that cows that have been intermittently milked prior to dry-off have begun the initial process of involution. Gaunt et al. (1980) reported that mean dry period lactoferrin concentrations 30 d after the cessation of milking can be 15-times greater than the average at the end of lactation and 50-times greater than the average for the entire lactation.

Milk lactoferrin concentrations varied by mammary quarter, in agreement with previous studies (Welty et al. 1976; Kutila et al. 2003; Chaneton et al. 2008). Major pathogens such as *Esch. coli*, *Staph. aureus*, and *Str. uberis* have been reported to increase lactoferrin concentrations in bovine milk above the levels associated with CNS and *Corynebacterium* spp. (Harmon et al. 1975; Hagiwara et al. 2003; Chaneton et al. 2008). Also in the present study, lactoferrin concentrations at dry-off in milk from quarters infected with major pathogens were significantly higher than in milk from uninfected quarters and also higher than in milk from quarters infected with minor pathogens. Similarly, quarters infected with minor pathogens had higher levels of lactoferrin (even though not significantly) at dry-off than uninfected quarters after adjusting for the treatment group and predry lactoferrin concentration (Table 3). These observations agree with other reports that lactoferrin concentration increases in response to intramammary infection (Hagiwara et al. 2003; Chaneton et al. 2008; Cheng et al. 2008). On the other hand, Sordillo et al. (1987) reported that quarters infected with major pathogens had significantly lower concentrations of lactoferrin, and the authors suggested that lower levels of this antibacterial component may have contributed to the reduced natural defence against these pathogens, thus resulting in infection.

In the present study, a significant relationship between lactoferrin concentration and SCC at dry-off was found, which suggests that both increased simultaneously, probably due to the onset of the involution process, combined with consequently decreasing milk volume. Both infection status and quarter level SCC at dry-off were significantly associated with lactoferrin concentrations at dry-off in the initial univariate screening. However, when entered in the model simultaneously, only infection status at dry-off, but not SCC, remained significantly associated with the lactoferrin concentrations, suggesting that they reflect the same phenomenon. Lactoferrin concentration has also previously been reported to have a significant positive association with SCC and in clinical cases of mastitis, both lactoferrin concentration and SCC have been shown to increase, indicating that both are associated with intramammary infections (Harmon et al. 1975; Hagiwara et al. 2003; Kutila et al. 2003; Cheng et al. 2008). The results from the present study also show that both infection status and SCC were associated with lactoferrin concentrations at the end of lactation.

Conclusion

The present study confirmed results from earlier studies conducted at considerably lower milk production levels, that intermittent milking prior to dry-off successfully decreased milk yield and more importantly, increased lactoferrin concentrations before dry-off in modern high-producing dairy cows. Moreover SCC and infection status were associated with lactoferrin concentrations at the end of lactation. Whether the increase in lactoferrin is linked to maintaining good udder health during the dry period needs to be investigated further.

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