J. Dairy Sci. 98:1–8
http://dx.doi.org/10.3168/jds.2015-9772

Suspension of milking in dairy cows produces a transient increase in milk lactoferrin concentration and yield after resumption of milking

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ABSTRACT

Lactoferrin is a multifunctional glycoprotein with a range of antimicrobial and immune-related properties that is found at >10-fold higher concentration in human milk (~1.7 g/L) relative to bovine milk (~0.15 g/L). Consumer demand is increasing for bovine lactoferrin through a wide range of nutritional and cosmetic consumer products. Increasing lactoferrin yield and concentration in bovine milk could assist in satisfying this increasing demand and may also help in increasing resistance to bovine mammary infection. Two experiments with cows in mid and late lactation were carried out to examine milking strategies to increase milk lactoferrin concentration and yield. Milking was suspended in cows normally milked twice daily, for periods of 2, 4, or 7 d (mid lactation) or 2 or 4 d (late lactation) after which cows were milked out and twice-daily milking resumed for 4 d. In all groups, lactoferrin concentration was significantly increased during the remilking period, approaching concentrations similar to those found in human milk (~1 g/L). Lactoferrin yields were significantly higher in all treatment groups, although increasing the nonmilking period beyond 2 d offered no advantage. Milk yield was lower initially after resumption of milking but recovered to preexperimental values by the fourth day of remilking in all groups, except the 4-d nonmilking group in late lactation. Milk somatic cell count was significantly elevated in all groups at the start of remilking but had substantially reduced by d 4 and reached a preexperimental level in the 2-d nonmilking group of mid-lactation cows. In summary, extended milking intervals can be used as a tool to produce a short-term increase in the concentration and yield of lactoferrin from bovine milk during established lactation, without any apparent long-term effects on milk yield and quality.

Key words: lactation, mammary, milk synthesis, epithelium

INTRODUCTION

Lactoferrin (**LF**), also sometimes referred to as lactotransferrin, is an 80-kDa glycoprotein, best known for its iron-binding and antibacterial properties (Adlerova et al., 2008). Its biological role appears, however, to be much wider than that of simply being an iron ligand, and it is implicated in playing a role in wider host defense, exerting antiviral, antioxidant, and immunomodulating activities (El-Loly and Mahfouz, 2011). In mammals, LF is present in all biological fluids, such as tears, sweat, saliva, and milk. Neutrophils are an important source of LF, with most of the LF in blood plasma coming from neutrophils (Adlerova et al., 2008). The LF in milk and colostrum is likely also, at least partly, to originate from neutrophils, but the mammary epithelium itself can also synthesize LF (Sanchez et al., 1992; Molenaar et al., 1996).

In milk, LF is associated with the whey fraction, and in human milk, it is the second most abundant whey protein (Vegarud et al., 2000) at a concentration of ~1.7 g/L. Because of the importance of LF in human milk, its role in host defense, and the fact that there is cross-reactivity and structural homology between human and bovine LF (Magnuson et al., 1990), significant interest has arisen to commercially exploit the bioactivity of bovine milk LF. Bovine LF can now be found in a range of commercial consumer products ranging from infant formulas and other nutritional products for humans and pet animals, to skin and oral-care products (Wakabayashi et al., 2006; Stelwagen et al., 2009; García-Montoya et al., 2012). Furthermore, bovine LF has also been proposed as a therapeutic for bovine mastitis and as a synergistic adjunct to penicillin treatment (Lacasse et al., 2008). Increasing consumer demand for LF for both human and animal applications may necessitate increasing the availability of LF for extraction of LF from milk, which is the context for the current study. However, a competitive approach also exists through the production of recombinant bovine and human LF for therapeutic purposes (Weinberg, 2007).

Received May 3, 2015.
Accepted July 9, 2015.
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In bovine milk, LF is considered a minor whey protein, with its concentration being much lower at 0.1 to 0.2 g/L (Vegarud et al., 2000; Farr et al., 2002) than that of human milk. However, the concentration of LF in milk varies significantly between individual cows in the same herd (Stelwagen et al., 2009) and is affected by the physiological status of the cow, specifically stage of lactation, udder health (i.e., milk SCC score), and daily milk yield (Cheng et al., 2008). In addition, reducing milking frequency from twice daily to once daily increases the concentration of LF in milk (Farr et al., 2002; Stelwagen et al., 2011), demonstrating that milking management can be a tool to increase milk LF concentration.

The objectives of the present study were to examine the effect of an extended period without milking during mid and late lactation on the concentration and yield of LF in bovine milk and to determine whether management of milking intervals could be used to increase LF yield for commercial use. The underlying hypothesis was that suspension of milking for more than 24 h would increase the concentration and yield of LF in bovine milk during recovery and that the effect would be more pronounced during late lactation.

MATERIALS AND METHODS

Experimental Protocol

Lactating dairy cows, grazing rye grass–white clover pasture, were subjected to extended periods (up to 7 d) without milking at 2 stages of lactation (mid and late). Individual milk yields were recorded at each milking before and after treatment.

In the first experiment, 18 pregnant, mid-lactation Friesian, Jersey, and Friesian × Jersey cross cows (116 ± 16 DIM; 15.4 ± 0.7 kg/d of milk) were allocated to 1 of 3 groups (n = 6 per group, balanced for age, breed, and milk yield). Immediately before the trial, the milk of all cows was tested bacteriologically to ensure udders were free of infection. Subsequently, cows were not milked for 2 (2-d group), 4 (4-d group), or 7 d (7-d group), after which twice-daily milking of all cows resumed (d 0) and the production of cows followed for a further 4 d.

Experiment 2 was conducted in a similar manner to experiment 1, except that 20 cows in late lactation were used (240 ± 15 DIM; 9.3 ± 0.5 kg/d of milk). Prior to the experiment the milk of all cows was tested bacteriologically to ensure udders were free of infection. Then, cows were not milked for 2 (2-d group) and 4 d (4-d group), after which twice-daily milking was resumed (d 0) and milk yield recorded at each milking for a further 4 d.

All animal procedures were carried out in compliance with the animal welfare guidelines and the approval of the Ruakura Animal Ethics Committee (Hamilton, New Zealand).

Milk Sampling and Analyses

In experiments 1 and 2, milk samples were obtained at each milking during 2 d before trial and during the first milking and the following 4 d of remilking.

Milk samples were analyzed by infrared spectrometry for fat, protein, and lactose content (Milkoscan Foss Electric A/S, Hillerød, Denmark), and SCC was determined using a fluorometric cell-counting technique (Fossomatic; Foss Electric A/S).

Lactoferrin concentrations in milk were measured by ELISA kit, following the manufacturer's instructions (Bethyl Laboratories, Montgomery, TX)

Statistical Analyses

Statistical analyses were conducted using ProStat (Version 6.5; Poly Software International, Pearl River, NY). Comparisons with preexperimental data were analyzed by ANOVA. During the remilking period, treatment differences were analyzed by multivariate ANOVA, because of the repeated measures nature of the data at different days. In case of an overall treatment effect, individual time points were then analyzed by ANOVA. Means were considered significantly different at $P < 0.05$.

RESULTS

Milk composition was normal for the breeds and stage of lactation before the suspension of milking and did not differ among any of the groups during mid or late lactation (Table 1). Milk-composition data (i.e., fat, protein, lactose) for both experiments during the remilking period are only shown when concentrations had returned to normal, i.e., when they were no longer statistically different from pretrial values (Table 1). Initial milk-composition data following remilking are not reported, because they were outside of the normal calibration range used for mid-infrared spectroscopy or an insufficient sample was obtained. Interestingly, on d 0 a marked increase in yellow color of the secretion (most notably in the 4-d and 7-d groups) was observed, giving the secretion an appearance resembling colostrum.

Experiment 1

Milk-yield response data of the cows in mid lactation are shown in Figure 1. Preexperiment milk yield