Effects of Increasing Milking Frequency During the Last 28 Days of Gestation on Milk Production, Dry Matter Intake, and Energy Balance in Dairy Cows

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ABSTRACT

Forty-eight Holstein cows were used in a randomized block design to evaluate different dry period lengths and prepartum milking frequencies (MF) on subsequent milk production, milk composition, solids-corrected milk production, dry matter intake (DMI), and energy balance. Lactating cows, milked 2 times/d, began a 7-d covariate period 35 d prior to the expected calving date. Cows were milked 0 times/d (0×), 1 time/d (1×), and 4 times/d (4×) for the last 28 d of gestation. If milk production decreased to less than 0.5 kg/milking or 1 kg/d, milking via machine ceased; however, teat stimulation continued 1 or 4 times/d according to the treatment assignment. All cows were milked 2 times/d postpartum (wk 1 to 10). Prepartum DMI tended to be greater for 1× and 4× compared with 0×. Prepartum, cows milked 1× produced 17% less milk than cows milked 4× (5.9 and 7.1 kg/d, respectively). There were no differences in prepartum and postpartum body condition scores, body weights, and DMI. Postpartum milk production by cows following their third or greater gestation was greater for 0× and 4× compared with 1×. Postpartum milk production by cows following their second gestation was significantly decreased with increased MF (0× vs. 1× and 4×). Regardless of parity, postpartum solids-corrected milk was greater for 0× compared with 1× and 4×. Postpartum fat yield was greater for 0× vs. 4×, with 1× being intermediate. Postpartum protein yield was greater for 0× vs. 4×, whereas 0× tended to have greater protein yield than 1×. Postpartum energy balance was greater for 1× and 4× relative to 0×. Continuous milking (1× and 4×) resulted in a loss of milk production in the subsequent lactation for cows following their

second gestation; however, for cows following their third or greater gestation, increasing the MF from 1× to 4× in the last 28 d of gestation alleviated the loss in milk production.

Key words: continuous milking, energy balance, milking frequency, transition period

INTRODUCTION

The benefits derived from continuous milking are greater energy balance (**EB**) postpartum, reduced postpartum plasma NEFA concentrations, reduced postpartum liver triglyceride concentrations (Rastani et al., 2005), and earlier first postpartum ovulation (Gümen et al., 2005). However, a 12 to 25% loss in milk production in the subsequent lactation has consistently been reported in cows that were continuously milked compared with cows given a 56- to 60-d dry period (**DP**) without supplemental bST (Swanson, 1965; Smith et al., 1967; Remond et al., 1992, 1997; Annen et al., 2004a; Rastani et al., 2005). A recent experiment (Annen et al., 2004a) indicated no loss of milk production in cows that were continuously milked in their third or greater gestation, when bST was given either according to label instructions (no bST between parturition and 63 DIM) or continuously (off-label; bST given in late gestation and early lactation), compared with milk production in cows with a 60-d DP that received bST according to label instructions. Annen et al. (2004b) randomly assigned cows in their second gestation to a control or bST treatment (bST given continuously from late gestation through early lactation) and used the half-udder model to impose a 60-d DP or continuous milking. Continuous milking reduced milk production by 52% for the first 30 d postpartum; however, bST administration did not alleviate the decline. These results suggest that it is possible to achieve similar milk production in cows that are milked continuously during their third or greater gestation relative to those with a 60-d DP. However, whether it is possible to achieve these results without the use of bST prepartum is unclear.

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If continuous milking is to become a viable management practice, it is necessary to find ways to reduce the loss in postpartum milk production. Increasing milking frequency (**MF**) during lactation is known to result in increased milk production (Pearson et al., 1979; Poole, 1982; DePeters et al., 1985; Hillerton et al., 1990). Furthermore, increasing MF in early lactation results in greater milk production for the entire lactation (Bar-Peled et al., 1995; Hale et al., 2003; Dahl et al., 2004b). Increasing MF also results in increased mammary gland DNA synthesis in cows (Hillerton et al., 1990) and goats (Wilde et al., 1987; Boutinaud et al., 2003), as well as increased mammary gland RNA synthesis in goats (Wilde et al., 1987; Boutinaud et al., 2003). Thus, increasing MF prepartum may prevent cell loss and maintain cell activity in the mammary gland. Additionally, increasing MF results in reduced concentrations and activities of plasmin and plasminogen (Stelwagen et al., 1994; Kelly et al., 1998; Sorensen et al., 2001), which are associated with gradual involution of the mammary gland in dairy cows (Politis et al., 1989; Politis et al., 1990).

Increasing MF is associated with an increased prolactin (**PRL**) concentration (Bar-Peled et al., 1995; Dahl et al., 2004a) and increased expression of prolactin receptor (**PRL-R**; Dahl et al., 2002). Cows that are milked more frequently may be more sensitive to PRL, which is key to the differentiation of mammary epithelial cells. This management practice may prevent the decrease in milk production experienced by continuously milked cows (i.e., cows with no DP) in the subsequent lactation.

The objective of this experiment was to evaluate different DP lengths and prepartum MF on subsequent milk production, DMI, and EB. We hypothesized that increasing MF in the last 28 d of gestation would increase the PRL concentration and PRL-R expression, leading to greater mammary gland differentiation, and would improve milk production in the subsequent lactation in cows that were milked continuously relative to those that were not milked.

MATERIALS AND METHODS

Cows and Treatments

Forty-eight Holstein cows (30 second gestation, and 18 third or greater gestation) were selected from the university dairy herd for this experiment. All cows had to fulfill the following selection criteria: 1) carrying a single fetus, 2) less than 400 DIM at 60 d prior to expected calving date (**ECD**) or have a BCS <4.0 if over 400 DIM, 3) producing at least 15 kg of milk/d at 60 d prior to ECD, and (4) producing at least 10 kg of milk/d at 35 d prior to ECD. In addition, if cows were receiving bST, it was ceased before 120 d prior to ECD. From 60 d prior to ECD through 29 d prior to ECD, all cows were

Table 1. Ingredient composition of diets

Ingredient	Prepartum	Postpartum
	(% of DM)	
Corn silage	42.0	31.5
Alfalfa silage	15.0	15.5
Ground corn	18.0	26.8
Straw	10.0	0.0
Cottonseed, whole linted	4.0	9.0
Roasted soybeans, whole	3.0	7.8
Soybean meal (48%)	4.0	2.8
Corn gluten meal	2.0	3.3
Blood meal	0.5	0.8
Trace-mineralized salt ¹	0.5	0.6
Dicalcium phosphate	0.2	0.4
Calcium carbonate	0.3	0.4
Vitamin mix ²	0.3	0.3
Magnesium oxide	0.2	0.2
Sodium bicarbonate	0.0	0.6

¹Contains 0.55% Mn, 0.55% Zn, 0.35% Fe, 0.14% Cu, 0.008% I, 0.006% Se, and 0.002% Co.

²Contains 3,304 IU/g of DM of vitamin A, 1,101 IU/g of DM of vitamin D, and 55 IU/g of DM of vitamin E.

milked 2 times/d. From 35 to 29 d prior to ECD, cows were fed the same diet (Table 1). Additionally, DMI and milk production were measured daily, milk composition and SCS were measured at 4 consecutive milkings (i.e., 2 consecutive days), and BW and BCS were measured once during the covariate week; the weekly averages for the previously mentioned variables were used as covariate measurements. The University of Wisconsin, College of Agriculture and Life Sciences, Animal Care and Use Committee approved all procedures.

Cows were blocked by parity (second or third and greater gestation) and average daily milk production between 35 and 29 d prior to ECD, and were randomly assigned to 1 of 3 treatments before the first milking 28 d prior to ECD: milking 0 times/d (0×), 1 time/d (1×), or 4 times/d (4×) during the last 28 d of gestation. Because SCM production with a 56- and 28-d DP was similar in a previous experiment (Rastani et al., 2005), we used a 28-d DP with cows milked 0× as the control treatment. Cows milked 1× were milked at approximately 330 h, and cows milked 4× were milked at approximately 330, 600, 1530, and 1800 h. If milk production decreased to less than 0.5 kg/milking or 1 kg/d, milking ceased; however, teat stimulation continued 1 or 4 times/d according to the treatment assignment. Teat stimulation results in a PRL release similar to milking (Reinhardt and Schams, 1974; Akers and Lefcourt, 1983). After parturition, all cows were milked 2× at approximately 400 and 1600 h.

All cows were fed the same prepartum diet from 35 d prior to ECD through parturition and the same postpartum diet from parturition through 70 DIM (Table 1). Cows were fed ad libitum throughout the entire experi-

Table 2. Nutrient composition of diets

Composition ¹	Prepartum	Postpartum
DM, %	45.6	53.6
NE _L , ² Mcal/kg	1.58	1.69
	(% DM)	
CP	13.4	16.0
NDF	38.2	30.5
ADF	23.7	18.9
NFC	38.4	40.7
Fatty acids	3.4	6.4
Lignin	4.6	4.2
NDICP	1.3	2.0
ADICP	0.7	0.9
Ash	6.9	7.4

¹Nonfiber carbohydrates calculated by the difference $100 - [(\%NDF - \%NDICP) + \%CP + (\%FA + 1) + \%ash]$. NDICP = neutral detergent insoluble CP; ADICP = acid detergent insoluble CP.

²Net energy for lactation calculated according to NRC (2001), based on actual DMI.

mental period. Diets were fed as a TMR at 1030 and 1630 h, and were formulated to meet nutrient requirements according to the NRC (2001) and predicted DMI. The nutrient composition of the diets is presented in Table 2.

Sampling and Analyses

Dry matter intake was measured daily. Forages and concentrate samples were obtained weekly and dried for 48 h to 60°C to determine DM; results were used to adjust the forage-to-concentrate ratio. The DM of Orts was assumed to be equal to the DM of feed offered. Weekly forage samples were composited monthly for analysis; samples of concentrates were taken monthly for analysis. Samples were dried in a forced-air oven at 60°C for 72 h and ground in a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for CP (method 2001.11; AOAC, 2005) with the following modifications: 0.3 g of sample was analyzed; 15 mL of H₂SO₄, 2.87 g of Na₂SO₄, and 0.13 g of CuSO₄ were added to each tube for digestion; digestion time was 90 min; 40 mL of water was added to the tubes after digestion; samples underwent steam distillation into a boric acid solution (4% wt/vol) containing 0.20% methyl red indicator solution and 0.98% bromocresol green solution; and 0.1 N H₂SO₄ was used to titrate the distillate. Samples were also analyzed for fatty acids (Sukhija and Palmquist, 1988), NDF (Van Soest et al., 1991; Mertens, 1999), ADF, and lignin (Van Soest et al., 1991). Neutral detergent fiber and ADF residues were analyzed for CP [neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP), respectively; Goering et al., 1972]. The NFC component was calculated as $100 - [(\%NDF - \%NDICP) + (\%FA + 1) + \%CP + \%ash]$.

Milk yield was recorded at each milking. Milk samples were obtained from consecutive milkings on 2 d every

week and analyzed for fat, protein, lactose, TS, and SCC using infrared spectrometry (AgSource Milk Analysis Laboratory, Menomonie, WI), and weighted means were computed according to milk production. Body weight was recorded weekly for each cow, and calf BW was measured within 24 h of calving. The BCS was recorded weekly for each cow by 3 individuals (Wildman et al., 1982).

Blood samples were taken from the coccygeal vein or artery at 300 and 1500 h on d 30, 21, and 14 prior to ECD, daily from 10 d prior to ECD to 3 d postpartum, and on d 7 and 14 postpartum in Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin. Samples were centrifuged at $915 \times g$, 4°C, for 15 min immediately after sample collection, and plasma was decanted and stored at -20°C until analysis. Plasma PRL was determined in samples obtained from 108 h prior to calving to 24 h after calving by RIA, as described by Miller et al. (1999). Mean intra- and interassay coefficients of variation were 8.6 and 6.0%, respectively. Assay sensitivity averaged 1.8 ng/mL.

Total RNA was isolated from lymphocytes, as described by Auchtung et al. (2003). A subset of samples (8 blocks) was analyzed for PRL-R. Expression of PRL-R mRNA was analyzed from samples obtained at -7, -1, 1, and +7 d relative to calving. Purification of RNA was conducted using the RNeasy mini kit (Qiagen, Valencia, CA), and residual DNA was removed using the DNA-free procedure (Ambion, Austin, TX). Total RNA (2.5 µg) was reverse transcribed to 21.5 µL of cDNA using the StrataScript First-Strand synthesis system (Stratagene, La Jolla, CA). Real-time PCR was performed using an ABI Prism 7900 HT (Applied Biosystems, Foster City, CA). Primers and probes used for 18S were as described by Auchtung et al. (2003), whereas primers and probes to determine total PRL-R (long and short forms combined) were determined using Primer Express (Applied Biosystems). Sequences of the PRL-R probe, forward primer, and reverse primer were 5'-TGGAAGGCAAAATCCCCTA-3', 5'-AGGCCCTCAGAGCACAAG-3', and 5'-TGGGTCCATTGGCCAGAA-3', respectively. The probe was labeled at the 5'-end with the reporter FAM and at the 3'-end with the quencher MGBNFQ. The PCR product length was 60 base pairs. Amplification mixes (20 µL) contained 10 µL of TaqMan universal PCR master mix (Applied Biosystems), 2 µL of cDNA, 40 pmol of each primer, and 4 pmol of probe. Reactions were run in triplicate, with PRL-R and 18S run in separate wells. The relative standard curve included 7 dilutions of cDNA derived from heifer mammary tissue, and calculations were as described by Auchtung et al. (2003).

Energy Calculations

Net energy intake (NE_I) was determined by multiplying DMI by the calculated energy density of the diet.

Energy density of the diet was calculated according to NRC (2001) considering a discount factor based on TDN intake above maintenance. Energy required for body maintenance (NE_M) was computed using the equation NE_M (Mcal/d) = $BW^{0.75} \times 0.08$ (NRC, 2001). Pregnancy requirements (NE_P) were computed using the equation NE_P (Mcal/d) = $\{[(2 \times 0.00159 \times \text{days pregnant} - 0.0353) \times (\text{calf BW}/45)]/0.14\} \times 0.64$ (NRC, 2001). Milk energy was calculated using the equation NE_L (Mcal/d) = $MP \times [(0.0929 \times F) + (0.0563 \times P) + (0.0395 \times L)]$, where MP is milk production (kg), F is fat percentage in the milk, P is true protein percentage in the milk, and L is lactose percentage in the milk (NRC, 2001). Estimated EB prepartum was calculated on a weekly basis using the equation EB (Mcal/d) = $NE_I - (NE_M + NE_P + NE_L)$. Estimated EB postpartum was calculated on a weekly basis using the equation EB (Mcal/d) = $NE_I - (NE_M + NE_L)$.

Statistical Analyses

Data were analyzed as repeated measures using the mixed models procedure of SAS (SAS Institute, 1999). For all parameters except plasma PRL and PRL-R mRNA expression, pre- and postpartum data were analyzed separately. Three cows (third lactation or greater, one from each treatment) were eliminated from the postpartum analysis; reasons included toxic mastitis (2 cows) and severe enteritis (one cow). All measurements, except PRL and PRL-R, were reduced to weekly means before statistical analysis. For prepartum milk composition, continuously milked treatments (1× and 4×) were compared, because no samples were taken from the other treatment prepartum (0×). The statistical model used for analysis of all measurements included treatment effect, parity, time, interaction of parity and treatment, and interaction of treatment and time. The terms specified for the random statement were cow and block nested within parity. The covariance structure used to best fit the model was selected based on Akaike's information criterion (SAS Institute, 1999). All data were covariately adjusted. If treatment was significant in the model, differences between treatments were determined using the PDIFF option (SAS Institute, 1999). If the parity × treatment interaction was significant in the model, parity × treatment × week was added to the model. Least squares means and standard errors of the means are reported. Significance was declared at $P < 0.05$, and trends were discussed at $P < 0.15$.

RESULTS AND DISCUSSION

DMI

Mean prepartum DMI tended to be greater for 4× and 1× compared with 0× (13.3 and 13.4 vs. 12.3 kg/d, respec-

tively; $P < 0.07$). Prepartum DMI was greater at 3 wk prepartum for 1× and 4× relative to 0× ($P < 0.03$; Figure 1). Because the cows were all fed the same diet, the increased DMI was likely due to the increased milk production of the cows milked continuously prepartum, and consequently their need to consume more feed to meet their requirements, as previously reported by Rastani et al. (2005). Many researchers have found that DMI is similar for cows milked 2 and 3 times/d postpartum (Pearson et al., 1979; Poole, 1982; DePeters et al., 1985; Barnes et al., 1990), as was the case for the 1× and 4× cows prepartum.

Mean postpartum DMI was similar for all treatments and averaged 22.7 kg/d. In contrast, in a previous study, cows with a 0-d DP that were milked 2 times/d during the last 28 d of gestation tended to have greater DMI in early lactation compared with cows with a 28-d DP (i.e., cows milked 0 times/d; Rastani et al., 2005).

Milk Production

Prepartum, 17% less milk was produced by 1× than 4× cows (5.9 and 7.1 kg/d, respectively), but because of high variability among cows, these differences were not statistically significant (Figure 2A). There was a significant treatment × time interaction, with milk production decreasing for 1× and 4× cows over the last 28 d of gestation, whereas 0× cows did not produce any milk during this time ($P < 0.001$; Figure 2A). There was also a tendency for a parity × treatment interaction ($P < 0.08$; Figure 2 and Table 3), with continuously milked cows (1× and 4×) following their second gestation producing more milk prepartum than did their older counterparts. To our knowledge, this is the first experiment evaluating the use of altering prepartum MF during the last 28 d of gestation. Increased MF postpartum has resulted in increased milk production (Pearson et al., 1979; Poole, 1982; DePeters et al., 1985; Hillerton et al., 1990; Stelwagen et al., 1994; Bar-Peled et al., 1995; Stelwagen and Knight, 1997; Hale et al., 2003; Dahl et al., 2004b).

There was a significant parity effect, a parity × treatment interaction ($P < 0.05$), and a tendency for a parity × treatment × week interaction for postpartum milk production ($P < 0.15$; Figure 2). Postpartum milk production for cows following their second gestation was greater for 0× (40.4 kg/d) compared with 1× and 4× cows (34.1 and 30.3 kg/d, respectively; $P < 0.05$; Figure 2B and Table 3). Differences existed at wk 3 through 6 and wk 8 through 10 postpartum for the 0× vs. 4× treatment and at wk 4 through 6, 9, and 10 postpartum for the 0× vs. 1× treatment ($P < 0.05$; Figure 2B). Our data support previous research (Annen et al., 2004a; Collier et al., 2004) indicating that a DP is necessary for cows in their second gestation to obtain maximal milk production in

Figure 1. Dry matter intake (kg/d) of cows with different prepartum milking frequencies. Treatments were 0× (■), 1× (□), and 4× (▲) during the last 28 d of gestation. Values are means $\pm$ SE. Prepartum: treatment, $P < 0.10$ (SE = 0.48). Postpartum: (SE = 0.79). Time points with asterisks (*) indicate that DMI differed ($P < 0.05$) between the 0× and 1× treatments, and plus signs (+) indicate that DMI differed ($P < 0.05$) between the 0× and 4× treatments.

the subsequent lactation. Prepartum milking of cows in their second gestation, independent of frequency, impedes continued mammary development, whereas older animals no longer have a mammary growth requirement (Collier et al., 2004).

Postpartum milk production for cows in their third or greater lactation was greater for 0× and 4× compared with 1× (43.3 and 41.9 kg/d vs. 32.7 kg/d; $P < 0.01$; Figure 2C and Table 3). Differences existed at wk 1 through 6 and wk 8 through 10 postpartum for 0× vs. 1× treatments and at wk 3 through 5 and wk 9 postpartum for 4× vs. 1× treatments ($P < 0.05$; Figure 2C). In a previous study, cows that were continuously milked (2 times/d in the last 28 d of gestation) had a 12% decrease in milk production in the subsequent lactation relative to cows with a 28-d DP (Rastani et al., 2005). Increasing MF to 4× during the last 28 d of gestation resulted in similar milk production as cows with a 28-d DP (i.e., cows on the 0× treatment).

Milk Composition and SCM Production

Prepartum, the milk protein percentage differed by treatment (6.16, and 4.87% for the 1× and 4× treatments, respectively; $P < 0.02$). The milk fat percentage prepartum was similar among the continuous milking treatments (4.75 and 4.29% for the 1× and 4× treatments, respectively). Cows on the 1× treatment produced 20%

less SCM than cows on the 4× treatment (7.7 and 9.7 kg/d of SCM, respectively), but that difference was not statistically significant.

Although there was no difference in milk fat percentage postpartum, milk fat yield was greater for 0× compared with 4× ($P < 0.001$), with 1× being intermediate (Table 4). Previous research showed no difference between milk fat percentage for cows with no DP and cows with a 28-d DP (Annen et al., 2004a; Rastani et al., 2005), whereas milk fat yield was greater for cows with a 28-d DP compared with those that were continuously milked (Rastani et al., 2005). Milk protein percentage was greater for 1× and 4× cows compared with 0× cows (3.24 and 3.20% vs. 2.94%; $P < 0.02$; Table 4). The 4× treatment had a reduced milk protein yield compared with the 0× treatment (1.16 vs. 1.27 kg/d; $P < 0.03$; Table 4), and there was a tendency for 1× cows to have reduced protein yield compared with 0× cows (1.12 vs. 1.27 kg/d; $P < 0.12$; Table 4). There was no parity $\times$ treatment interaction for any of the milk composition variables. These data agree with a previous study in which the milk protein percentage was greater and milk protein yield was reduced for cows with no DP relative to cows with a 28-d DP (Rastani et al., 2005). In contrast to our data, Annen et al. (2004a) reported no difference in milk protein percentage between cows with either a 30-d DP or no DP.

Figure 2. (A) Milk production (kg/d) of all cows with different prepartum milking frequencies. Prepartum: treatment, $P < 0.001$; parity $\times$ treatment, $P < 0.08$; treatment $\times$ time, $P < 0.001$ (SE = 0.88). Postpartum: treatment, $P < 0.004$; parity, $P < 0.05$; parity $\times$ treatment, $P < 0.03$; treatment $\times$ time, $P < 0.08$ (SE = 1.9). (B) Milk production (kg/d) of cows in and following their second gestation with different prepartum milking frequencies. (C) Milk production (kg/d) of cows in and following their third or greater gestation with different prepartum milking frequencies. Treatments were 0x (■), 1x (□), and 4x (▲) during the last 28 d of gestation. Values are means $\pm$ SE. Time points with asterisks (*) indicate that milk production differed ($P < 0.05$) between the 0x and 1x treatments, plus signs (+) indicate that milk production differed ($P < 0.05$) between the 0x and 4x treatments, and caret signs (^) indicate that milk production differed ($P < 0.05$) between the 1x and 4x treatments.

Table 3. Effect of parity and prepartum milking frequency on pre- and postpartum milk yield

Item	Treatment ¹			SE ²
	0×	1×	4×	
Cows following their second gestation				
Prepartum ³ milk yield, kg/d	0.0 ^a	7.1 ^b	9.0 ^b	1.0
Postpartum ⁴ milk yield, kg/d	40.4 ^a	34.1 ^b	30.3 ^b	1.9
Cows following their third or greater gestation				
Prepartum milk yield, kg/d	0.0 ^a	5.0 ^b	5.5 ^b	1.4
Postpartum milk yield, kg/d	43.3 ^a	32.7 ^b	41.9 ^a	3.3

^{a,b}Differ at $P < 0.05$.

¹Treatments were milking 0, 1, or 4 times/d prepartum.

²Pooled standard error of least squares means.

³Prepartum period is -28 to -1 d relative to calving.

⁴Postpartum period is +1 to +70 d relative to calving.

Postpartum SCM production was greater for 0× (40.3 kg/d) compared with 1× and 4× cows (40.3 kg/d vs. 34.6 and 32.2 kg/d; $P < 0.01$; Figure 3). Differences existed at wk 1 through 10 postpartum for the 0× vs. 1× treatment; at wk 1 through 4 and at wk 10 postpartum for the 0× vs. 4× treatment; and at wk 7 postpartum for the 1× vs. 4× treatment ($P < 0.05$; Figure 3). These results are due to the greater yield of fat and protein from 0× cows compared with 1× and 4× cows. Postpartum milk composition did not differ by parity, resulting in no parity × treatment interaction in SCM production.

The SCS was not affected by treatment (Table 4). This is in agreement with previous studies, in which SCS did not differ between cows with a 0-d DP and those with a 28- or 30-d DP (Annen et al., 2004a; Rastani et al., 2005).

PRL Concentration Around Calving

Prolactin concentration around the time of calving was not affected by treatment (Figure 4). However, there was a treatment × time interaction in plasma PRL concentra-

tions. The difference in plasma PRL concentration was due to differences in the time cows reached the peak of the periparturient PRL surge as well as the time to return to baseline. The plasma PRL concentration peaked at -12 h prepartum for 0× cows, whereas cows that were milked continuously (1× and 4×) peaked at -24 h prepartum. No difference was detected in the magnitude of the surge, as measured by the area under the curve from -60 to 24 h relative to calving, or in PRL-R mRNA expression (data not shown). The earlier and greater PRL surge for cows milked prepartum was reported previously (Malven et al., 1987).

Our initial hypothesis was that increasing MF in continuously milked cows would increase the plasma PRL concentration and up-regulate PRL-R mRNA expression during the time around calving. This hypothesis was based on previous research studies in which the postpartum increase in MF was associated with a greater PRL concentration (Bar-Peled et al., 1995; Dahl et al., 2004a) and greater expression of PRL-R (Dahl et al., 2002). Additionally, prepartum milking of cows was shown to result in an abrupt increase in secretion of PRL into the blood (Malven et al., 1987). Therefore, in the present study, cows milked more frequently in the last 28 d of gestation were also expected to respond with an increase in the number of mammary cells that differentiate, leading to an increase in milk production.

Our results indicate that increasing the MF caused different responses, depending on the physiological stage of the dairy cow (i.e., prepartum vs. postpartum). Prolactin is not the only hormone regulated by postpartum MF. Also, IGF-I concentrations are increased postpartum in cows milked more frequently, as previously reported (Hale et al., 2003). Thus, one could speculate that IGF-I may be increased with prepartum MF, and the increased IGF-I concentration may act on the mammary gland, resulting in increased milk production. Nevertheless, the observed interaction of treatment × parity on milk pro-

Table 4. Effect of prepartum milking frequency on postpartum milk yield, SCM yield, and milk components from 1 to 70 DIM

Item	Treatment ¹			Statistic	
	0×	1×	4×	SE ²	$P <$
Milk yield, kg/d	41.8 ^a	33.4 ^b	36.1 ^b	1.4	0.01
SCM yield, kg/d	40.3 ^a	34.6 ^b	32.2 ^b	2.1	0.01
Fat, %	3.49	3.54	3.33	0.13	NS ³
Fat yield, kg/d	1.49 ^a	1.31 ^{ab}	1.19 ^b	0.08	0.005
Protein, %	2.94 ^a	3.24 ^b	3.20 ^b	0.08	0.02
Protein yield, kg/d	1.27 ^a	1.16 ^{ab}	1.12 ^b	0.06	0.08
SCS ⁴	3.06	3.74	3.96	0.46	NS

^{a,b}Differ at $P < 0.05$.

¹Treatments were milking 0, 1, or 4 times/d prepartum.

²Pooled standard errors of least squares means.

³ $P > 0.15$.

⁴SCS = $\log_2(\text{SCC}/100,000) + 3$.

Figure 3. Solids-corrected milk production (kg/d) of cows with different prepartum milking frequencies. Treatments were 0x (■), 1x (□), and 4x (▲) during the last 28 d of gestation. Values are means $\pm$ SE. Postpartum: treatment, $P < 0.01$ (SE = 2.1). Time points with asterisks (*) indicate that SCM differed ($P < 0.05$) between the 0x and 1x treatments, plus signs (+) indicate that SCM differed ($P < 0.05$) between the 0x and 4x treatments, and caret signs (^) indicate that SCM differed ($P < 0.05$) between the 1x and 4x treatments.

duction indicates that if the IGF-I concentration is increased, then it results in a different response based on parity; cows in their third or greater lactation have increased milk production with increased milking fre-

quency from 1x to 4x, whereas cows in their second lactation do not. This interaction between parity and DP length on subsequent milk production was previously reported by Annen et al. (2004a). They observed no loss

Figure 4. Plasma prolactin concentration (ng/mL) of cows with different prepartum milking frequencies. Treatments were 0x (■), 1x (□), and 4x (▲) during the last 28 d of gestation. Values are means $\pm$ SE. Time, $P < 0.001$; treatment $\times$ time, $P < 0.02$ (SE = 5.6). Time points with asterisks (*) indicate that plasma prolactin concentrations differed ($P < 0.05$) between the 0x and 1x treatments, plus signs (+) indicate that plasma prolactin concentrations differed ($P < 0.05$) between the 0x and 4x treatments, and caret signs (^) indicate that plasma prolactin concentrations differed ($P < 0.05$) between the 1x and 4x treatments.

Figure 5. Energy balance (Mcal/d) of cows with different prepartum milking frequencies. Treatments were 0× (■), 1× (□), and 4× (▲) during the last 28 d of gestation. Values are means $\pm$ SE. Prepartum: treatment, $P < 0.01$; treatment $\times$ time, $P < 0.002$ (SE = 0.97). Postpartum: treatment, $P < 0.01$ (SE = 1.4). Time points with asterisks (*) indicate that energy balance differed ($P < 0.05$) between the 0× and 1× treatments, and plus signs (+) indicate that energy balance differed ($P < 0.05$) between the 0× and 4× treatments.

in milk production by cows that were milked continuously in their third or greater gestation when bST was given either according to label instructions (no bST between parturition and 63 DIM) or continuously (off-label), compared with milk production from cows with a 60-d DP that received bST according to label instructions. However, there was a loss of milk production in cows milked continuously in their second gestation. Because supplemental bST also increases IGF-I concentrations (Peel and Bauman, 1987), it is possible that the same mechanism of action occurred in continuously milked cows in the study by Annen et al. (2004a).

EB, BW, and BCS

Mean prepartum EB was greater for 0× compared with 1× and 4× cows (4.7 Mcal/d vs. 0.9 and 0.7 Mcal/d; $P < 0.01$; Figure 5). Differences existed at wk 4 and 3 prepartum for 0× compared with 1× or 4× cows ($P < 0.05$; Figure 5). This is in contrast to results from a previous experiment in which cows with a 28-d DP had greater prepartum EB than did cows milked continuously (i.e., no DP, milked 2 times/d) at wk 4 and 3 prepartum (Rastani et al., 2005). In this experiment, the EB difference between 0× and 1× or 4× cows was primarily due to differences in milk production during this time.

Postpartum mean EB was 4.5 Mcal/d greater for 1× vs. 0× cows (2.3 Mcal/d vs. -2.2 Mcal/d; $P < 0.001$) and

5.0 Mcal/d greater for 4× vs. 0× cows (2.8 Mcal/d vs. -2.2 Mcal/d; $P < 0.001$). Differences existed during wk 3, 4, and during wk 2, 4, 5, and 10 postpartum for 0× compared with 1× cows, and during wk 2, 4, 5, and 10 postpartum for 0× compared with 4× cows ($P < 0.05$; Figure 5). At wk 10, 0× cows return to negative EB, which was the result of an increase in SCM milk production. Cows milked 0× had a more severe negative EB postpartum compared with their continuously milked counterparts (1× and 4×). This is in agreement with previous research in which cows with a 28-d DP had a greater negative EB postpartum compared with cows that were milked continuously (Rastani et al., 2005). The more severe negative EB for 0× cows compared with 1× and 4× was due to increased postpartum SCM production.

Despite the differences in prepartum EB (Figure 5), calf BW was not affected by treatment; mean calf BW were between 42.9 and 45.7 kg. The BCS at calving ranged from 3.21 to 3.40 and was not affected by treatment. Postpartum BCS loss and postpartum BW loss were not affected by treatment (data not shown). Previous research has shown that the postpartum BCS loss and BW loss are greater in cows with a 28-d DP compared with a 0-d DP (0.25 units and 29 kg, respectively; Rastani et al., 2005). Based on this, 0× cows should have lost more BCS units and BW than 1× or 4× cows. However, the differences in postpartum EB were not as great in

this experiment, which may have prevented detection of changes in BW and BCS.

The improvement in postpartum EB for cows milked continuously (1× and 4×) during the last 28 d of gestation is intriguing. Alleviation of negative EB is key to improving the metabolic status of dairy cows. Once cows are in negative EB, their metabolism shifts from anabolism to catabolism. This consequently leads to a predisposition to metabolic disorders, such as hepatic lipidosis and ketosis (Collard et al., 2000; Hayirli and Grummer, 2004).

Improvements in energy status with continuously milked cows (1× and 4×) may result in a decrease in postpartum metabolic disorders (Collard et al., 2000; Hayirli and Grummer, 2004). Unfortunately, there were not enough cows in this experiment to determine statistically whether the incidence of metabolic disorders around calving differed because of treatments. The incidences of disorders for cows in the 0×, 1×, and 4× treatments were displaced abomasum (1/15, 0/15, 0/15), clinical milk fever (0/15, 0/15, 1/15), ketosis (0/15, 2/15, 0/15), mastitis (2/15, 3/15, 1/15), metritis (0/15, 1/15, 0/15), and retained placenta (0/15, 1/15, 2/15). Definitions of the above disorders are given in LeBlanc et al. (2002).

Energy balance in early lactation has been correlated with resumption of ovarian activity (Butler et al., 1981). In a previous study, continuous milking of cows resulted in a more rapid resumption of ovarian activity, greater first-service conception rate, fewer services per conception, and fewer days open (Gümen et al., 2005). Although EB has a negative relationship with neutrophil function (Stabel et al., 2003), it has a positive relationship with lymphocyte response to mitogens (Ropstad et al., 1989). Goff (2006) hypothesized that negative EB leads to immune suppression in dairy cows. Thus, elimination of the period of negative EB in early lactation through the practice of continuous milking may alleviate immune suppression, enhance reproductive inefficiency, and prevent metabolic disorders in dairy cows. Future experiments using large numbers of animals are needed to test this hypothesis adequately.

CONCLUSIONS

Increasing prepartum MF (0× vs. 1× and 4×) during the last 28 d of gestation resulted in a loss of milk production in the subsequent lactation for cows in their second lactation. However, cows in their third or greater lactation had a similar milk yield in the subsequent lactation when they were milked 0× or 4× during the last 28 d of gestation. Data from cows in their third or greater lactation were in agreement with our initial hypothesis that increasing prepartum MF would improve milk production in the subsequent lactation for cows that were denied a DP. However, it did not appear to be mediated

through changes in PRL concentration as originally hypothesized. Additionally, cows that were milked continuously (4× and 1×) had a less severe negative EB postpartum compared with a 28-d DP (0×). Future research using large numbers of animals is needed to examine the effects of continuous milking and reduced negative EB on the incidence of metabolic disorders and reproductive performance.

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