Effects of Twice-Daily Nursing on Milk Ejection and Milk Yield During Nursing and Milking in Dairy Cows

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ABSTRACT

Milk production and hormonal responses to milking in Holstein cows that were milked twice daily, and that either also nursed calves twice daily 2 h after milking for 9 wk after calving ($n = 10$) or that served as nonnursing controls ($n = 8$) were examined to assess how nursing affected responses to machine milking. Milk yield at milking during the 9 wk of nursing was lower in nursing cows compared with control cows (26.1 ± 1.0 vs. 35.5 ± 1.1 kg) that were only machine milked. During nursing, the amount drunk by calves increased from 6.5 ± 0.7 kg/d on wk 1 to 12.5 ± 1.4 kg/d on wk 9. When this was added to the amount of milk obtained at milking, nursing cows did not differ from control cows in total milk produced (35.5 ± 1.0 vs. 35.5 ± 1.0 kg). Residual milk yield, after i.v. injection of oxytocin after milking, was higher in nursing cows than in control cows (8.7 ± 0.8 vs. 3.2 ± 0.8 kg). During the 6 wk after weaning, milk production was the same for the nursing and control cows (34.0 ± 1.35 vs. 34.7 ± 1.42 kg). Plasma oxytocin levels during milking were greater for control cows than for nursing cows (31.7 ± 5.4 vs. 18.0 ± 2.8 pg/mL), but were equivalent to concentrations in nursing cows during nursing (35.5 ± 7.5 pg/mL). Plasma concentrations of prolactin and cortisol increased after both milking (control vs. nursing: prolactin: 40.2 ± 6.8 vs. 32.9 ± 6.1 ng/mL; cortisol: 6.4 ± 1.23 vs. 7.4 ± 1.10 ng/mL) and nursing (control vs. nursing: prolactin: 18.6 ± 7.3 vs. 38.9 ± 6.6 ng/mL; cortisol: 2.34 ± 1.15 vs. 7.37 ± 1.04 ng/mL). In contrast to previous studies, there was no obvious advantage for milk production by keeping a calf with the cow. This appears to result from the reduced oxytocin secretion during milking for the nursing cows.

Key words: dairy cow, nursing, milk ejection, endocrine response

INTRODUCTION

Early separation of the calf from the cow is a keystone of the modern dairy industry, but there is renewed interest in cow-calf rearing systems in dairy production (von Keyserlingk and Weary, 2007) following reports of improved cow and calf health and production (Fröberg et al., 2007) and because of the growing interest in ecological or organic milk, in which calves are sometimes kept with their dams for a period of time. Furthermore, mixed nursing and milking regimens remain popular in many parts of the world (Negrão and Marnet, 2002; Combellas et al., 2003; Hernández et al., 2006). Cows that nurse calves had increased overall milk production (Margerison et al., 2002; Combellas et al., 2003; Fröberg et al., 2007) and nursed calves had higher weight gains during the period of nursing than bucket-fed calves (Metz, 1987; Bar-Peled et al., 1997; Flower and Weary, 2001), which may improve later milk production and reproductive efficiency (Bar-Peled et al., 1997; Shamay et al., 2005).

The increased milk production of cows nursing a calf may result from hormonal changes in the cow. Bar-Peled et al. (1998) found greater plasma concentrations of growth hormone (GH), prolactin, and IGF, and lower insulin concentrations in nursing cows. Generally, the oxytocin response to nursing was greater than that to milking (Akers and Lefcourt, 1984; Lupoli et al., 2001), and when nursing is ad libitum, the cows may have reduced oxytocin and prolactin release at milking (Akers and Lefcourt, 1984). Yet reducing the frequency of nursing may limit the suppressive effects of nursing on milk ejection during machine milking (Bar-Peled et al., 1998; Lupoli et al., 2001).

The aim of this study was to examine the effects on milk production and hormonal changes associated with milk ejection and synthesis, of allowing cows to nurse twice daily. Allowing nursing to occur after milking rather than before milking was examined to assess whether it would prevent the apparent reduction in oxytocin secretion during machine milking.

Received July 5, 2007.

Accepted December 14, 2007.

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MATERIALS AND METHODS

Animals and Housing

Animals were housed, fed, and treated according to the appropriate guidelines (Agriculture Canada, 1990). Multiparous Holstein cows ($n = 20$) were housed in individual tie stalls, in a single barn that was artificially ventilated with lights on from 0600 to 2200 h (>80 lx). The cows were fed a TMR for ad libitum intake, with food delivered once daily and water available ad libitum from float-activated water bowls shared by adjacent cows. Milking was performed twice daily (average times 0600 and 1800 h) in a milking parlor. Individual milk yields were recorded electronically by Metatron milk meters (Westfalia, Elk Grove Village, IL).

Experimental Procedures

All experimental procedures were approved by the institutional animal care committee, complying with the requirements of the Canadian Council for Animal Care. Two weeks before expected calving date, cows were brought into individual calving pens, where they remained until 5 d after calving. The 20 cows were divided into 2 equally sized groups: nursing cows (nursing group) and cows that had calves removed soon after birth (control group), but because of technical problems, data from 2 control cows were eliminated. The cows were semirandomly allocated to the groups while balancing groups for milk production in the previous lactation (nursing: 7,781 kg; control: 7,609 kg), sex of calf, date of calving, and parity (2 to 6). Calves from the control cows were left with their dams for 6 to 20 h and then moved to the nursery, where they were kept in individual pens and fed milk at the level of 10% BW up to 4 wk, when they were gradually weaned off milk and onto a grain diet. At the time of separation, the cow was milked and colostrum was offered to the calf by bucket. Calves from the nursing cows were left with their dams for 5 d. On d 6, all cows were moved to a tie stall after the morning milking, and nursing calves remained in their calving pen situated immediately behind their dam's tie stall. Each day for 9 wk, the nursing cows were placed in their calves' pens 2 h (± 5 min) after the morning and evening milkings, and were left there until the calf stopped sucking and remained away from the udder for more than 10 s. At 9 wk of age, the calves were removed to individual pens in the nursery. After d 6, all calves had ad libitum access to calf starter and water.

Measures

Milk Production and Intake. The milk yield of cows was measured at each milking for the first 16 wk

of lactation. Residual milk was measured after milking and after nursing on 2 different days, separated by a 2-d interval at wk 8 of lactation. For both groups of cows, residual milk after milking was measured by injecting 10 IU of oxytocin in the tail vein 3 min after the teat cups had been removed automatically by the milking system, and then remilking the cow 15 to 30 s after the injection. For nursing cows, residual milk after a nursing was measured by taking the cows to the milking parlor immediately after nursing and following the same procedure. At the same time, residual milk in the control cows was measured the same way.

Calf milk intake at nursing was measured 4 times weekly by using the weigh-suckle-weigh technique. Calves were equipped with a harness to collect urine and feces and were brought to the weigh scale immediately before and after nursing.

Calf Growth. Calves were weighed at birth and at 9 wk (before weaning) and 16 wk (after weaning) of age.

Blood Sampling. Blood samples were taken during 1 milking and 1 nursing at wk 8 of lactation. Cows were fitted with indwelling jugular cannulas 2 d before sampling. For 3 of the 5 d before blood sampling, the cows were habituated to a specific position in the milking parlor and the presence of the blood sampler during milking. One blood sample was taken 3 h before milking to habituate the animal and check the cannula. Sampling began 2 h before milking, with samples taken at -120, -90, -60, -45, -30, -15, and -10 min (just before the cow was moved to the milking parlor), -6, -4, and -2 min (when the cow was in the parlor), 0 min (when teat cups were attached), and then every 2 min during milking. After milking was finished ($t = 0$ min), a further series of samples were taken at 2 min after the teat cups were removed and then at 4, 6, 10, and 15 min and then every 15 min up to 110 min postmilking. For nursing cows, a third series of samples were then taken every 2 min up to the beginning of nursing ($t = 0$ min at 120 min after milking), every 2 min until 4 min after the end of nursing (or 10 min for control cows), and then at 10, 15, 30, 45, and 60 min postnursing. Blood samples were taken at equivalent times in control cows, with 5 samples taken over 10 min during the period when nursing cows were nursing. Blood was put immediately on ice and centrifuged no later than 20 min after collection. Plasma was stored at -20°C until assayed.

Hormonal Assays. Cortisol assays were performed in the manner described in Rushen et al. (2001). Intra- and interassays coefficients of variation (CV) were 7 and 11%, respectively. Oxytocin assays were performed in duplicate by means of an enzyme immunoassay technique (Marnet et al., 1994) after an extraction step on C₁₈ cartridges, vacuum drying, and concentration ($\times 4$). Intraassay CV was 6% at a concentration of 106.7 pg/

mL, and interassay CV was 12%. Samples were re-assayed if the duplicates differed by more than 10%. Double-antibody RIA was used to determine concentrations of prolactin and GH as described by Lapierre et al. (1990 and 2000, respectively). Interassay and intraassay CV were 5.4 and 3.8% for prolactin and 2.1 and 4.8% for GH, respectively.

Statistical Analyses

Milk yield at milking, milk consumed by the calf, and total milk production (adding the last 2 together) were analyzed with a mixed model (PROC MIXED, SAS Institute Inc., Cary, NC) that included type of cow (control or nursing) as the main factor, week of lactation as a repeated-measures factor, and the interaction between week of lactation and type of cow. Average daily gain for calves was calculated during the 9-wk period of nursing, for 7 wk after weaning, and for the full 16-wk period and was analyzed with a mixed model that included sex of calf and treatment.

Mean hormone concentrations were calculated for the 2 final samples taken before the cows were moved into the milking parlor (baseline before milking), for the 4 samples when the cows were in the milking parlor but before the milking machine was started (preparation), for all samples when the cows were actually milked, and for the first 2 samples taken after the cows had been returned to their stalls (postmilking). Analysis was performed in a mixed model, which included type of cow as a main factor (control or nursing), phase of milking (baseline before milking, preparation, milking, and postmilking) as a repeated-measures factor, and the interaction between type of cow and phase of nursing.

Mean hormone concentrations were calculated for the 3 samples taken when the cow was in the nursing pen but before nursing began (prenursing baseline), for all samples taken when the cow was nursing the calf (nursing), and for the first 2 samples taken when the calf had been removed but before the cow had been returned to its stall. Analysis was performed in a mixed model, which included type of cow as a main factor (control or nursing), phase of nursing (baseline before nursing, nursing, and postnursing) as a repeated-measures factor, and the interaction between type of cow and phase of nursing.

RESULTS

Milk Production

Figure 1 shows the daily milk yield at milking during each week for the 2 groups of cows and the amount of milk consumed by the calves. There was a significant

Figure 1. Mean daily milk yield at milking of control and nursing cows, milk drunk by calves of nursing cows (vertical bars), and total milk yield (milk yield at milking + milk drunk by calf) of nursing cows at each week.

interaction between week of lactation and type of cow ($P < 0.0001$) for the milk obtained during milking. During wk 1 to 9, the milk yield of nursing cows during milking (26.1 ± 1.0 kg) was lower ($P < 0.01$) than that for the control cows (35.5 ± 1.06 kg). After wk 9, there was no significant difference ($P = 0.72$) between the 2 groups (control vs. nursing cows: 34.7 ± 1.42 vs. 34.0 ± 1.35 kg). Nevertheless, when the amount of milk consumed by the calves was added to the amount of milk obtained during milking, the total milk yield during wk 1 to 9 did not differ ($P = 0.98$) between the 2 groups (control vs. nursing cows: 35.5 ± 1.04 vs. 35.5 ± 1.00 kg). Total milk yield over the full 15 wk did not differ ($P = 0.85$) between the 2 groups of cows (control vs. nursing cows: 35.2 ± 1.14 vs. 34.9 ± 1.09 kg).

On the day that residual milk was measured, milk yield during the milking was significantly ($P < 0.001$) lower for nursing cows than control cows (18.7 ± 1.0 vs. 12.0 ± 1.0 kg, respectively), whereas residual milk was significantly ($P < 0.001$) greater in nursing cows (3.2 ± 0.84 vs. 8.7 ± 0.8 kg). When total milk was calculated by adding milk obtained at milking with residual milk, there was no significant ($P = 0.31$) difference between the 2 groups of cows (21.9 ± 0.79 vs. 20.7 ± 0.79 kg).

Oxytocin

Figure 2 shows oxytocin concentrations for the 2 groups of cows. Marked increases were apparent for the control cows during milking and for the nursing cows during nursing. There was a trend for an interaction between the type of cow and phases of milking ($P = 0.06$). There were no significant differences between the

Figure 2. Mean plasma oxytocin concentrations at each sampling time of control and nursing cows. The time of milking is indicated by a filled vertical bar and the time of nursing by a hatched vertical bar.

Figure 3. Mean plasma prolactin concentrations at each sampling time of control and nursing cows. The time of milking is indicated by a filled vertical bar and the time of nursing by a hatched vertical bar.

2 groups of cows before nursing (control vs. nursing: 17.3 ± 3.4 vs. 16.4 ± 3.1 pg/mL; $P = 0.85$), during udder preparation (17.0 ± 4.2 vs. 13.2 ± 2.3 pg/mL; $P = 0.41$), or after milking (25.1 ± 4.5 vs. 17.9 ± 4.3 pg/mL; $P = 0.28$). Nevertheless, during the period of milking, oxytocin levels were higher ($P = 0.03$) in the control cows (31.7 ± 5.4 pg/mL) compared with the nursing cows (18.0 ± 2.8 pg/mL).

There was a significant interaction between the type of cow and the phases of nursing ($P = 0.003$). Before nursing, oxytocin levels did not differ ($P = 0.23$) between control and nursing cows (10.9 ± 3.8 vs. 17.4 ± 3.4 pg/mL), but during nursing, oxytocin levels rose markedly in the nursing cows (35.5 ± 7.5 pg/mL) until they were higher ($P = 0.04$) than in the control cows (11.0 ± 1.0 pg/mL).

Prolactin

Figure 3 shows the plasma prolactin concentrations for the 2 groups of cows. Marked rises were apparent at both nursing and milking. There was no significant interaction between the type of cow and phases of milking ($P = 0.36$). Before milking, prolactin concentrations did not differ between the 2 groups of cows (19.6 ± 4.2 vs. 16.4 ± 3.8 ng/mL; $P = 0.85$). Prolactin concentrations did not change during udder preparation ($P = 0.23$), and there was no difference between the 2 groups of cows (21.4 ± 4.6 vs. 17.2 ± 4.1 ng/mL; $P = 0.41$). Prolactin levels rose during milking ($P < 0.001$), but there was no difference between the 2 groups of cows (40.2 ± 6.8 vs. 32.9 ± 6.1 ng/mL; $P = 0.413$). Concentrations continued to rise after milking ($P < 0.001$), but there were no

differences between the 2 groups (80.2 ± 10.2 vs. 61.5 ± 9.2 ng/mL; $P = 0.43$).

There was a significant interaction between the type of cow and phases of nursing ($P = 0.003$). Before nursing, there were no differences between the 2 groups of cows ($P = 0.23$; 24.4 ± 5.3 vs. 15.9 ± 4.7 ng/mL). During nursing, prolactin concentrations rose and were higher ($P = 0.02$) in the nursing cows (38.9 ± 6.6 ng/mL) than in the control cows (18.6 ± 7.3 ng/mL). After nursing, prolactin levels remained higher ($P = 0.009$) in the nursing cows than in the control cows (17.8 ± 7.6 vs. 43.6 ± 6.9 ng/mL).

GH

Growth hormone concentrations throughout the day are shown in Figure 4. Overall, there was a tendency for lower GH concentrations in the nursing cows ($P = 0.06$), but concentrations varied greatly throughout the day, and there was an interaction between the type of cow and time of day ($P < 0.001$), although the pattern was not very clear. No clear changes were associated with either milking or nursing. During the milking procedure, there were no significant changes in concentrations with time ($P = 0.11$), but there were overall differences between the 2 groups ($P = 0.03$). Growth hormone concentrations were lower ($P < 0.05$) in nursing cows compared with control cows before milking (8.6 ± 1.73 vs. 3.6 ± 1.54 ng/mL), during udder preparation (7.8 ± 1.54 vs. 3.1 ± 1.38 ng/mL), during milking (6.7 ± 1.15 vs. 3.3 ± 1.03 ng/mL), and after milking (6.9 ± 1.05 vs. 3.4 ± 0.94 ng/mL). During nursing, there were no overall differences between the groups ($P = 0.25$) and no change

Figure 4. Mean plasma growth hormone concentrations at each sampling time of control and nursing cows. The time of milking is indicated by a filled vertical bar and the time of nursing by a hatched vertical bar.

with nursing ($P = 0.81$). There was a significant interaction between type of cow and phase of nursing ($P = 0.01$), mainly because of a higher GH concentration in the nursing calves ($P = 0.02$) before nursing occurred, but not at other times (Figure 4).

Cortisol

Figure 5 shows cortisol concentrations for both groups of cows. Cortisol concentrations showed marked rises during both milking and nursing, and in the nurs-

Figure 5. Mean plasma cortisol concentrations at each sampling time of control and nursing cows. The time of milking is indicated by a filled vertical bar and the time of nursing by a hatched vertical bar.

ing cows concentrations began increasing before nursing. There was no significant interaction between the type of cow and the phases of milking ($P = 0.63$). The rise in cortisol concentrations during milking was significant ($P < 0.0001$), but there were no differences between the 2 groups of cows, either before milking (control vs. nursing cows: 5.1 ± 0.95 vs. 4.6 ± 0.85 ng/mL; $P = 0.69$), during udder preparation (4.3 ± 0.95 vs. 5.1 ± 0.85 ng/mL; $P = 0.33$), during milking (6.4 ± 1.23 vs. 7.4 ± 1.10 ng/mL; $P = 0.56$), or after milking (9.7 ± 1.89 vs. 11.5 ± 1.70 ng/mL; $P = 0.50$). There was no interaction between the type of cow and phases of nursing ($P = 0.19$).

Calf Growth

Average daily gain during the 9-wk nursing period was greater ($P < 0.001$) for nursing calves (0.98 ± 0.05 kg/d) than for control calves (0.48 ± 0.05 kg/d). Average daily gain after weaning was lower ($P = 0.044$) for nursing calves (0.53 ± 0.09 kg/d) than for control calves (0.82 ± 0.10 kg/d), yet average daily gain over the full 16 wk did not differ between treatments ($P = 0.23$).

DISCUSSION

The results showed a clear suppression of oxytocin secretion at milking among cows that nursed a calf, supporting previous findings (Akers and Lefcourt, 1984; Lupoli et al., 2001). Thus, limiting the nursing to 1 period after each milking did not prevent the nursing-induced reduction of oxytocin secretion during machine milking. Despite this suppression of oxytocin secretion, the secretion of both cortisol and prolactin during milking were unaffected. Growth hormone appeared to be reduced in nursing cows. The reduced oxytocin secretion led to increased residual milk and reduced milk yield at milking. Nonetheless, recovery of milk yield was rapid once the calf was weaned, although nursing did not increase overall milk production.

Milk yield at milking can be reduced when nursing is ad libitum (Metz, 1987), although previous studies found that when the nursing was restricted to twice daily, milk yield at milking could be reduced, but by a smaller amount than the calves drink (Bar-Peled et al., 1998). In tropical cross-bred cattle, twice daily nursing did not reduce milk yield at milking, indicating an overall increase in milk production (Margerison et al., 2002). In contrast to these findings, we found no evidence that nursing after twice daily milking led to any overall increase in milk production in our higher producing Holsteins. The milk yield at milking was reduced by almost exactly the amount that the calves consumed; this is interesting given that nursing occurred after

milking. Nevertheless, once the calves were weaned, milk yields quickly returned to normal levels.

Others have reported that a lower frequency of nursing reduces the suppression of oxytocin release during machine milking (Lupoli et al., 2001; Negrão and Marnet, 2002). Indeed, Bar-Peled et al. (1997) reported higher oxytocin concentrations in cows that suckled 2 adopted calves 3 times daily compared with machine-milked cows, but their sampling schedule made it difficult to determine whether these higher levels reflected oxytocin secretion during milking. Our results indicate that restricting nursing to 2 periods after milking was not sufficient to completely overcome this block in high-producing Holstein cattle. Schams et al. (1984) showed that only small amounts of oxytocin (5 pg/mL) are sufficient to trigger some milk ejection in dairy cows. Although we noted much lower oxytocin concentrations in nursing cows during machine milking than in control cows, the concentrations still appeared sufficient to trigger milk ejection, because we obtained close to 80% of the normal yield, which corresponds to our previous findings (Rushen et al., 2001). Bruckmaier and Blum (1998) obtained only 20% of normal milk yield when milk ejection was blocked, which was the amount stored as cisternal milk.

Because nursing occurred after milking, the block on oxytocin secretion was likely due to the establishment of a bond between the mother and the calf (von Keyserlingk and Weary, 2007). Possibly, Bar-Peled et al. (1997) did not find a block on oxytocin secretion because the cows did not form such bonds with the 2 adopted calves. Although we have no data on the likely physiological mechanisms underlying this block, centrally acting opioids were implicated in the establishment of maternal bonds in a number of species (Panksepp et al., 1980), and there is evidence of opioid inhibition of oxytocin secretion in cattle (Tancin et al., 2000).

Because milk yield at milking returned to control levels during the week that the calves were weaned from the cows, we conclude that the secretion of oxytocin during milking returned quickly to normal once the cows were prevented from nursing their calves. Oxytocin concentrations markedly increased at milking for control cows and at nursing for nursing cows. There is evidence that nursing can be more effective than machine milking in stimulating oxytocin secretion (Lupoli et al., 2001; Negrão and Marnet, 2002); however, we found no evidence that oxytocin concentrations during nursing differed from those during milking. These differences may reflect the different nursing regimens: our calves nursed 2 h after milking, whereas those of Lupoli et al. (2001) nursed 1 h before.

Prolactin and cortisol concentrations increased during both nursing and milking, as has been noted pre-

viously (Tancin et al., 2000; Negrão and Marnet, 2002). This is in contrast with the results of Lupoli et al. (2001), who found a rise in cortisol only during milking and not during nursing. Johansson et al. (1999) reported a reduction in somatostatin concentrations, which is one of the principal factors inhibiting secretion of GH, in cows after milking. Despite this, we found no evidence that concentrations of GH were increased after either milking or nursing.

Bar-Peled et al. (1998) reported that concentrations of both prolactin and GH were higher in cows milked and nursing compared with cows that were milked an equivalent number of times. Nevertheless, we found no evidence that either cortisol or prolactin concentration differed between nursing and milking. Furthermore, in contrast to Bar-Peled et al. (1998), we found that nursing cows appeared to have overall lower concentrations of GH compared with control cows. Despite evidence that milk yield tends to be correlated with circulating GH levels in cattle (Auchtung et al., 2001), these lower GH levels did not lead to reduced production, although this might explain why we did not find higher overall milk production in nursing cows.

Nursed calves gain significantly more weight during the period they were nursed, supporting the findings of Flower and Weary (2001), no doubt because of the higher milk intake. The milk intakes were similar to those reported by de Passillé and Rushen (2006) in a similar experiment. Nonetheless, once weaned, nursed calves gained significantly less weight than control calves, perhaps because of a lower intake of calf starter (Jasper and Weary, 2002). Although there were no significant differences in final weight, nursed calves remained slightly heavier. Bar-Peled et al. (1997) similarly reported higher calf weight gains during a 6-wk nursing period, but lower BW 6 wk after weaning. This may reflect the greater number of nursing events (3 vs. 2) allowed by Bar-Peled et al. (1997). Increased weight gain of heifers can be an advantage in reducing age at first calving (Shamay et al., 2005), and high growth rates attributable to nursing that are restricted to the early period of growth may lead to improvement in milk production (Bar-Peled et al., 1998).

ACKNOWLEDGMENTS

We thank Isabelle Blanchet, Marjolaine St. Louis, Mario Léonard, Keith Carter, and the barn staff at Lennoxville for their help. Funding was supplied by a Natural Sciences and Engineering Research Council grant to A. M. de Passillé.

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