EFFECT OF INCREASED MILKING FREQUENCY DURING EARLY LACTATION ON PERFORMANCE, METABOLISM, AND MAMMARY CELL PROLIFERATION OF DAIRY COWS

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ABSTRACT

Researchers have been interested in increased milking frequency (IMF) during early lactation only as a management practice to increase lactational milk yield. Initial work suggested that effects of early lactation IMF on milk yield persisted after cows returned to a normal milking frequency scheme, although milk yield responses have been variable among studies.

The first study (Chapter II) was designed to test the consistency of the milk yield responses to early lactation IMF under different management conditions on four commercial dairy farms. Cows (n=421) were enrolled in this study and assigned within farm at calving to either a 2x control treatment or a 4x IMF treatment for the first 21 d postpartum followed by a return to 2x milking. Cows milked 4x were milked at the beginning and end of each scheduled milking. This resulted in minimal milking intervals for the 4x cows of 3.5, 4, 5, 5.5, and 6 h for the four farms, respectively.

Milk yield and component responses were evaluated on three of the farms across the first 7 monthly test days. Early lactation IMF increased overall milk yield by 2.1 kg/d on these farms ($P < 0.01$). Analysis by farm suggested that the magnitude of the response was farm-dependent and ranged from 4 to 10%. Early lactation IMF generally decreased milk component percentages but increased component yields. The treatment had no effect on BCS on any of the farms; however, circulating NEFA in serum samples collected during

the period of IMF were increased in two farms and not affected in the other two, suggesting the influence of farm-specific factors.

In the second experiment (Chapter III), metabolic responses to early lactation IMF and mammary cell dynamics were evaluated. Primiparous (n=30) and multiparous (n=30) Holstein cows at the Cornell University Dairy Teaching and Research Center were assigned at calving to one of 2 treatments. The control group was milked 2x for 119 d while the IMF group was milked 4x from d 2 postcalving until d 21 and 2x from d 22 until d 119.

Overall responses of milk yield to early lactation IMF were not significant over the first 119 d postpartum; however, the interaction of treatment by week was significant in that IMF cows yielded 4.8 kg/d more milk than control cows during wk 2 and 3, but had comparable milk yields to controls thereafter. Milk component yields did not differ between treatments. Milk yield responses to IMF were apparent in primiparous cows when cows that did not receive mammary biopsies were analyzed separately. Early lactation IMF increased dry matter intake during the first 21 d postcalving but not the 119-d postpartum period. Early lactation IMF did not affect BCS or BW.

Concentrations of plasma NEFA were increased in multiparous but not primiparous cows during the period of IMF, suggesting that energy status of multiparous cows may have limited their responses to early lactation IMF. Concentrations of plasma BHBA were not affected by treatment. Mammary tissue was collected by biopsy in a subset of cows (n=8 cows per lactation group and treatment) at calving and at 21 and 75 d postpartum and used for immunohistochemical

localization of Ki-67. A treatment by day interaction existed for the percentage of labeled epithelial cells such that the IMF treated cows had a lower percentage of labeled epithelial cells on d 21 but a higher percentage at d 75. Further analysis is needed for determination of apoptosis rates to determine difference in cell turnover between treatments as well as cell activity in order to pinpoint possible mechanisms for the milk yield responses to early lactation IMF.

BIOGRAPHICAL SKETCH

Fernando Soberón was born on December 28, 1978 in México City, México to Fernando Soberón and María del Consuelo Sieiro de Soberón. Fernando is the firstborn of the family- he has one sister and two brothers: Mariana, Pablo and Gerardo from eldest to youngest. Fernando attended Instituto Cumbres, a Catholic school, from Kindergarten throughout high school. He studied sixth grade at Oaklawn Academy in Edgerton, Wisconsin. After graduating from high school, he spent six months working on a dairy farm in Torreón, México, where he developed a taste for dairy cattle management. He studied his undergraduate degree at Tecnológico de Monterrey Campus Querétaro in Querétaro, México. During his undergraduate studies he attended Iowa State University as an exchange student for one semester. He graduated in 2002 with the degree of Ingeniero Agrónomo Zootecnista. After graduation, Fernando started working for Elanco Animal Health as a sales representative, where he was promoted to territory manager, a job that he carried out until July of 2005 when he moved to Ithaca, New York to start his Master of Science degree program under the guidance of Dr. David M. Galton and Dr Thomas R. Overton. His thesis research was on the effects of frequent milking during early lactation on milk yield, carry over effect and mammary cell dynamics. During his Master's program, he married Melanie A. Schotthofer. After completion of his M.S. degree, he plans to continue his studies with a PhD degree in mammary development on dairy cows.

Le dedico esta tesis a mi familia, especialmente a mis padres y a mis abuelos por siempre creer en mí. Su constante apoyo ha sido vital para la culminación de este proyecto. También quiero dedicar esta tesis a mi esposa, quien ha cuidado y alimentado mis sueños.

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This project wouldn't be possible without the constant love and support from my family and friends that have backed me up from the beginning. I'm especially blessed by the love and support of my wife, Melanie Soberón, who among other countless contributions spent many hours correcting my English.

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LIST OF ABBREVIATIONS

2x = Milked two times per day

3x = Milked three times per day

4x = Milked four times per day

6x = Milked six times per day

BCS = Body Condition Score

BHBA = β-hydroxybutyrate

BrdU = Bromodeoxyuridine

BW = Body Weight

CI = Calving Interval

CM = Continuous Milking

cwt= Hundred weight

d = Day(s)

DD = Days Dry

DHI = Dairy Herd Improvement

DIM = Days in Milk

DMI = Dry Matter Intake

DNA = Deoxyribonucleic acid

FM = Frequent Milking

GH = Growth Hormone

IGF-I = Insulin-like Growth Factor-I

IMF = Increased Milking Frequency

NEFA = Nonesterified fatty acids

PL = Placental Lactogen

PRL = Prolactin

rbST = Recombinant Bovine Somatotropin

RNA = Ribonucleic acid

SCC = Somatic Cell Count

TDM = Test Day Model

TUNEL = Terminal deoxynucleotidyl transferase dUTP nick end labeling

wk = Week(s)

CHAPTER I

Literature Review:

Regulation of Milk Synthesis and Secretion in Dairy Cows.

Introduction

Mammals are differentiated from all other species by their ability to synthesize milk in their mammary glands to nurse their young. The importance of milk as a component of human nutrition has been acknowledged for centuries. Several breeds of dairy cattle and small ruminant species have been bred for milk production beyond that required to feed their young, and as a result these are known for their high capability for milk production.

Milk synthesis and secretion has been studied for over 100 years. Milk synthesis is carefully regulated by the endocrine system. There are three groups of hormones that have been identified to have an effect on the mammary gland. The reproductive hormones (estrogen, progesterone, placental lactogen, prolactin, and oxytocin) have a direct impact on the mammary gland; estrogen is responsible for prepubertal mammary growth, prolactin is a lactogenic hormone needed for lobuloalveolar growth, and oxytocin is required during milking for complete milk letdown (Byatt et al., 1994; Meyer, 2005). The metabolic hormones (growth hormone, glucocorticoids, thyroid hormone, and insulin) act indirectly on milk secretion by affecting the responsiveness of the mammary gland to the reproductive hormones and by affecting nutrient flux to the mammary gland. Lastly, the mammary gland itself is an endocrine organ that produces growth

hormone, leptin, prolactin, and parathyroid hormone-related peptide (Neville et al., 2002; Svennersten and Olsson, 2005).

The mammary gland grows in accordance to the rest of the body with the exception of two stages of allometric growth. The first stage of allometric growth occurs prior to puberty and the second stage occurs after conception (Meyer, 2005). Lactogenesis and galactopoiesis are the two metabolic processes that initiate milk synthesis. Lactogenesis was described by Neville et al. (2002) as mammary differentiation and it consists of the set of processes that lead to the beginning of a full lactation. Galactopoiesis was described by Akers (2006) as the maintenance of milk secretion.

In dairy cattle the persistency of lactation directly impacts profitability. Maintaining the number and activity of the epithelial cells is crucial to sustaining a persistent lactation (Capuco et al., 2003). After calving, dairy animals have a rapid increase in milk production. This increase in production is mainly driven by an increase in epithelial cell activity (Capuco et al., 2003). The factors affecting the decline in milk production after peak lactation are species-dependent; in dairy cows and goats the decrease in production after peak lactation is mainly due to the loss of secretory cells by apoptosis (Knight and Wilde, 1987; Stefanon et al., 2002; Capuco et al., 2003; Boutinaud et al., 2004).

During lactation of dairy cows, there is constant turnover of epithelial cells. The persistency of the lactation curve depends on the ratio of the rates of epithelial cell proliferation and apoptosis as components of mammary cell turnover (Capuco et al., 2003). There are

many husbandry practices that will modify the turnover ratio of the epithelial cells in the mammary gland such as milking frequency and the use of growth hormone (Boutinaud et al., 2004). The reproductive status of the animal will also affect the turnover ratio on the mammary gland (Stefanon et al., 2002).

After lactation, the mammary gland goes through an involution process as a preparation for subsequent lactations. This process is characterized by the loss of epithelial cells due to apoptosis (Accorsi et al., 2002). Recent research has suggested that the mammary gland in the dairy cow does not involute in the same manner as other species, rather it remodels and the dry period is used by the mammary gland to replace senescent epithelial cells with new secretory tissue (Capuco et al., 1997). The importance of this dry period has been extensively researched and demonstrated. In dairy cows, the absence of a dry period may reduce milk production up to 20% in the subsequent lactation of multiparous and primiparous cows (Capuco et al., 1995; Annen et al., 2004; Andersen et al., 2005).

The profitability of dairy farms is tightly related to the total milk sold and the expenses they incur to produce it. Production per cow has been a major driving force in the economic equation of profitability (Bauman, 1992). Greater milk production can be achieved by increasing peak milk yield and by increasing lactation persistency. Many management practices have been studied throughout the years to help producers enhance milk production and increase their profitability. The application of rbST, exogenous oxytocin, milking frequency, photoperiod length, dry period length, pre-calving milking,

housing and facilities, cooling systems, and animal comfort are just some of the different management avenues that have been pursued in order to improve milk production (Hale et al., 2003; Annen et al., 2004; Collier et al., 2006).

Biology of milk secretion

Dynamics of cell number

Milk production is a function of the number of differentiated epithelial cells present in the mammary gland as well as the activity level of those cells. The interaction between the number and activity of cells determines the shape of the lactation curve and total milk production. The determinant factor for the increase or decrease of milk yield throughout the lactation differs among species (Knight and Wilde, 1987; Capuco et al., 2001; Stefanon et al., 2002; Boutinaud et al., 2004; Miller et al., 2006).

The number of epithelial cells in the mammary gland depends on the rate of proliferation of new cells and the rate of apoptosis (cell death). When the rate of cell apoptosis is less than the rate of cell proliferation, the mammary gland grows and likewise, when the rate of apoptosis is greater than the rate of cell proliferation, the mammary gland regresses in productive capacity. In order to better understand the dynamics of the cell numbers in the mammary gland, research emphasis is placed on the rate of cell turnover. The overall rate of turnover is defined by the relationship between cell proliferation and cell apoptosis (Capuco et al., 2001).

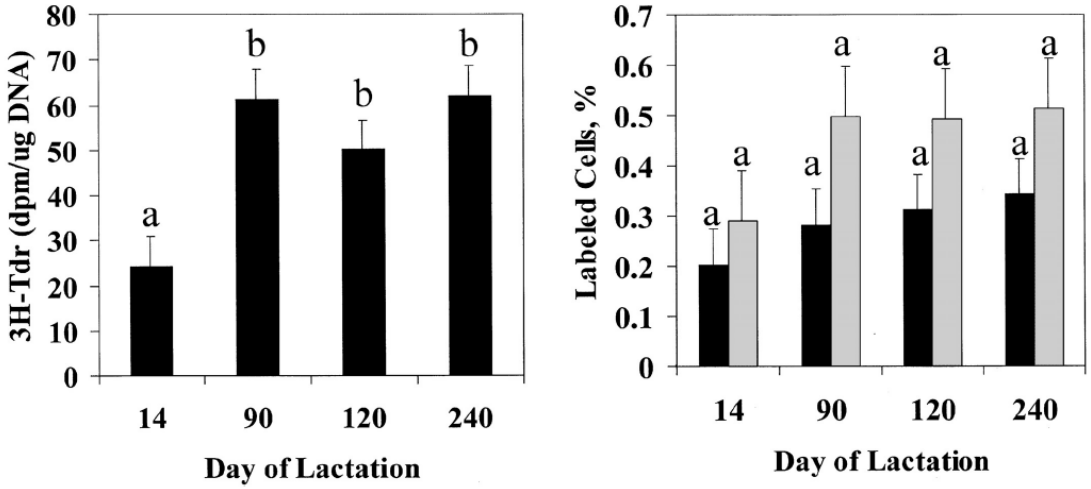
To determine the dynamics of cell number and activity during lactation in dairy cows, Capuco et al. (2001) conducted an experiment in which cows were slaughtered at four different stages of lactation. Cows were slaughtered at 14, 90, 120, and 240 days in milk (DIM); 24 h before slaughter the cows were treated with bromodeoxyuridine (BrdU), a fluorescent marker used to identify proliferating cells. The cows averaged three lactations and 11,876 kg of milk during their lactation (305-d). The total number of proliferating cells peaked at 14 d post-calving and declined to the lowest number of cells at 240 d. With the exception of d 14, where milk yield per cell was at its lowest, the relationship between number of epithelial cells and milk yield did not differ significantly during the rest of the lactation (Capuco et al., 2001).

Based on the results of cell number and milk yield, Capuco et al. (2001) concluded that the increase in milk yield from calving to the peak of lactation is due to the increased differentiation of mammary epithelium. The decrease in milk yield after peak lactation is due to the loss of epithelial cells and is not attributed to a decrease in cell activity. The cell activity was determined as the volume of milk per unit of mammary DNA. The percentage of epithelial cells in the mammary tissue was found to be influenced by the stage of lactation (Capuco et al., 2001). These conclusions are similar to findings in goats (Knight and Peaker, 1984). Sorensen et al. (2006) analyzed the RNA to DNA ratio in the mammary gland as a measure of the synthetic capacity of mammary cells. The ratio increased between 14 and 88 d

postcalving and remained constant for the rest of lactation (Sorensen et al., 2006).

Mammary cell proliferation was measured by Capuco et al. (2001) using three different methods. The three methods used were BrdU, Ki67 antigen, and DNA and RNA analyses. Out of the three methods, only BrdU offered an estimation of cell proliferation rate. However, the three methods showed comparable results. The results for Ki67 antigen, a marker for cell proliferation, and BrdU incorporation are shown in Figure 1.1. At 14 d postpartum, cell proliferation was lower compared to the rest of the lactation. Even though the number of cells labeled with Ki67 and BrdU were not influenced by days in milk, the arithmetic means were lower at 14 d postpartum than for those at timepoints measured subsequently during lactation.

These data, in conjunction with apoptotic rates measured by TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling (Figure 1.2)), suggest that mammary growth does not extend from pregnancy into lactation; although measurements were not recorded before d 14 postpartum and mammary growth may have occurred during the first 14 d of lactation (Capuco et al., 2001).



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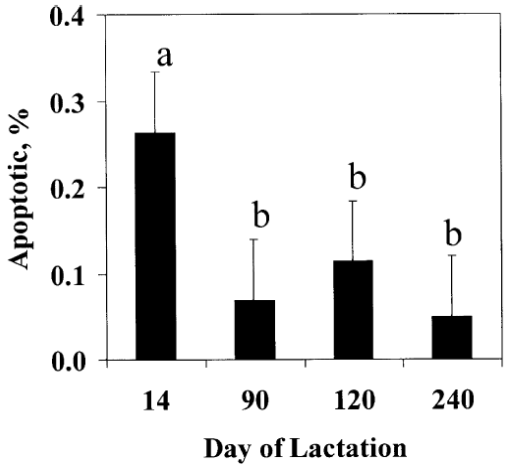

Figure 1.2. Mammary cell apoptotic index during lactation. Data are expressed as a percentage of total cells. Each bar represents the mean + SE for four to six cows. Apoptotic index was greatest during early lactation ($P < 0.05$), but did not vary after peak lactation. From Capuco et al, 2001.

Cell turnover rate as calculated by Capuco et al. (2001) included 0.3% proliferation rate on a 24-h period as assessed by BrdU and a 0.56% apoptotic rate calculated by TUNEL which was extrapolated to a 24-h period, resulting on a constant total net loss of epithelial cells per day throughout the entire lactation.

Sorensen et al. (2006) studied mammary cell dynamics by performing mammary biopsies on 10 cows at 7 timepoints during different stages of lactation as well as the dry period. The researchers analyzed cell proliferation using Ki67 and cell apoptosis using TUNEL. Cell proliferation was highest during the late dry period (11%) compared with the early dry period (6%) and cell proliferation was lowest during lactation, ranging from 0.4% to 0.9%. These data are in agreement with the 0.3% proliferation rate reported by Capuco et al. (2001).

Sorensen et al. (2006) also found that cell apoptosis was higher during the early dry period (0.37%) than during the late dry period (0.17%). These values represented a 3-h period -- when extrapolated to a 24-h period, the cell apoptotic rates were 2.96% and 1.36% for early and late dry periods, respectively. During lactation, Sorensen et al. (2006) determined that cell apoptotic rate to be highest at 14 d postpartum (0.76% over 3-h, or 6% extrapolated to a 24-h period). During the rest of lactation, cell apoptotic rate varied between 0.13% and 0.08% (1% to 0.6% over a 24-h period) and were not significantly different from each other (Sorensen et al., 2006).

Pregnancy status also affects milk yield during lactation. Lee et al. (1997) showed that as days open increased, 305-d milk yield

increased. Bormann et al. (2002) reported lower milk yields on pregnant cows after only 90 d of gestation compared to non-pregnant cows. The effect of pregnancy on milk yield was greater on the last third of gestation. In New Zealand, a study using twins analyzed differences in milk yield caused by concurrent pregnancy during lactation. Using one of the twins as a nonpregant control and the other pregnant, the researchers found no difference in milk yield between twins until 126 d of gestation (~30 wk of lactation). The cows on this experiment were managed on an intensive grazing system and both groups of cows had peak milk yields of 18.4 kg/d (Roche, 2003). Milk yield of these cows is lower than averages in other studies; these could be the source of difference in results.

The shape of the lactation curve is different for primiparous cows than for multiparous cows. Primiparous animals have a lower milk peak yield and their lactations are more persistent (Bormann et al., 2002). Miller et al. (2006) conducted an experiment where gene expression, DNA content, and fatty acid synthesis were analyzed from mammary biopsies taken during three stages of lactation on multiparous and primiparous animals. The researchers found an interaction between stage of lactation and parity for the expression of genes related to milk production, finding lower expression on the mammary gland of primiparous cows. The DNA content of the mammary gland on first lactation animals was also lower, suggesting a lower density of secretory cells that may explain lower milk yields. The conclusions of this experiment included the suggestion that the

mammary gland of multiparous cows is more metabolically active than the mammary gland of primiparous cows. (Miller et al., 2006).

Endocrine control of mammary cell function

The endocrine system plays an important role during mammogenesis (development of the mammary gland), lactogenesis (initiation of milk synthesis), and galactopoiesis (continuation of milk secretion). Different hormones control each of these processes. Some of the hormones involved in the control of these stages include estrogen, progesterone, prolactin, placental lactogen, oxytocin, and growth hormone (Svennersten and Olsson, 2005; Akers, 2006).

The mammary gland grows in proportion to the rest of the body with the exception of two periods. The first period of allometric growth is before puberty and is regulated primarily by estrogen produced by the ovaries. The ovaries have been shown to produce estrogen waves in calves as early as two weeks of age and continuing until puberty (Evans et al., 1994b). Duration of the estrogen waves is approximately 8 d. The concentration of follicle stimulating hormone (FSH) during these waves increases as puberty approaches. Concentrations of FSH during these prepubescent waves can reach that of FSH concentrations in mature cows during normal ovulatory cycles (Evans et al., 1994a). The specific pathway through which estrogen influences the mammary parenchyma to grow is not well defined but the fat pad in the mammary gland may play an important role in local regulation of this process (Meyer, 2005).

The second period of allometric growth occurs after conception. The mammary gland grows at a rate of 10% per month during the first gestation and does not differ from body growth on total gland weight until the last third of gestation. However secretory epithelium, blood vessels, connective tissue, and ducts grow at a rate of 31% to 36% per month during the entire lactation when assessed on a DFFT (Dry Fat Free Tissue, mammary gland weight after removing fat and water) and total nitrogen basis. These changes are not reflected in total weight of the mammary gland because adipose tissue is being replaced by the developing epithelium. The reproductive hormones are responsible for the accelerated growth during this period (Swanson and Poffenbarger, 1979; Meyer et al., 2006a). Research has also suggested that the placenta plays a role in the development and growth of the mammary gland. Birth weight has been positively correlated to placenta size (Freemark et al., 1992). Byatt et al. (1997) showed that milk yield was positively correlated to birth weight and placental mass. The researchers suggested that the sire effect on milk yield may come through the placenta. The placenta is a fetal organ; therefore, its size and metabolic activity depend on the genotype of the fetus.

The development of the mammary gland during pregnancy is mainly regulated by ovarian steroids; however, lactogenic hormones are required to achieve full lobuloalveolar growth and if prolactin secretion is restricted, lobuloalveolar growth is constrained (Hart and Morant, 1980). Results from experiments in which prolactin production was reduced during late gestation in goats, sheep, and cows indicated that mammogenesis was not affected by treatment,

suggesting that a placental hormone could substitute for prolactin (Byatt et al., 1994).

In order to determine whether placental lactogen (PL) was able to substitute for prolactin, non-pregnant heifers were induced to lactate, prolactin production was reduced with bromocriptine and heifers were assigned to one of four treatments: control, two doses of PL, and prolactin. Mammary development of heifers administered PL and prolactin was greater than controls, but was not significantly different between these two treatments (Byatt et al., 1994).

Subsequently, Byatt et al. (1997) conducted another experiment where heifers were induced to lactate and prolactin secretion was suppressed by bromocriptine on all heifers. The treatments consisted of either 40 mg/d of PL or water for 18 d. Milk yield was 20% higher for heifers treated with PL but this difference was not statistically significant (Byatt et al., 1997).

In an experiment designed to test the milk yield response in dairy cows to exogenous PL, Byatt et al. (1992) compared four doses of PL with a negative control and a positive control (20 mg/d rbST). Milk yield was increased by three of the four doses of PL in a dose-dependent manner; however, the response was lower than that achieved with rbST. Dry matter intake (DMI) was not affected by rbST treatment but was increased by two of the PL treatments in a dose-dependent manner. Figure 1.3 shows that the increase in DMI by cows administered PL explained most of the increases in milk yield, allowing them to maintain positive energy balance whereas this did not happen with rbST (Byatt et al., 1992).This study confirms the possible actions

of PL as an agonist of bST receptors with less potency. However, the finding that PL but not rbST increased DMI suggests the possibility of other specific effects of PL. Administration of PL did not alter insulin sensitivity and did not appear to have an effect on fat reserves.

A similar experiment (Kann et al., 1999) was performed in non-pregnant ewes in which the mammogenic and lactogenic properties of PL were compared to growth hormone (GH). All ewes were induced into lactation and for the last 9 d prior to the start of milking, ewes were treated with saline (control), ovine GH (oGH) or oPL. Plasma levels of GH were significantly higher for ewes on oGH treatment but were not different for ewes administered oPL or control. Prolactin levels were not different among groups. Circulating PL was only detected in the oPL treatment group and IGF-I levels were elevated by oGH treatment beginning one day after the initiation of treatment. Ewes administered oPL had increased IGF-I concentrations only after 6 d of treatment (Kann et al., 1999).

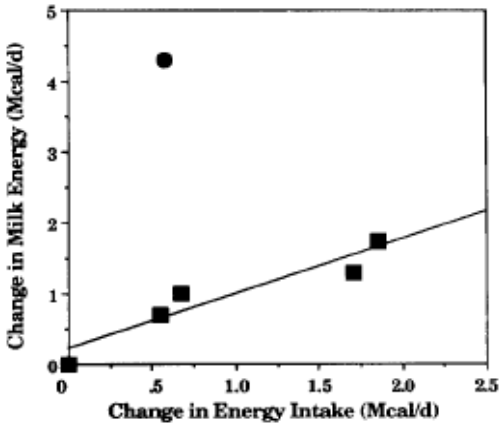

Figure 1.3. Change in milk energy versus the change in energy intake stimulated by recombinant bPL (■) and bST (●). The correlation for bPL and control groups is R2 = 0.892. From Byatt et al. (1992).

The effect of oGH and oPL on the mammary glands of the ewes was monitored during lactation. The ewes treated with oGH and oPL produced more milk compared to the ewes in the control group from week one through week six. The treated ewes produced twice the amount of milk compared to the control ewes, suggesting a hormonal effect during mammogenesis as well as during lactogenesis (Kann et al., 1999). Milk production of ewes assigned to each group is shown in Figure 1.4.

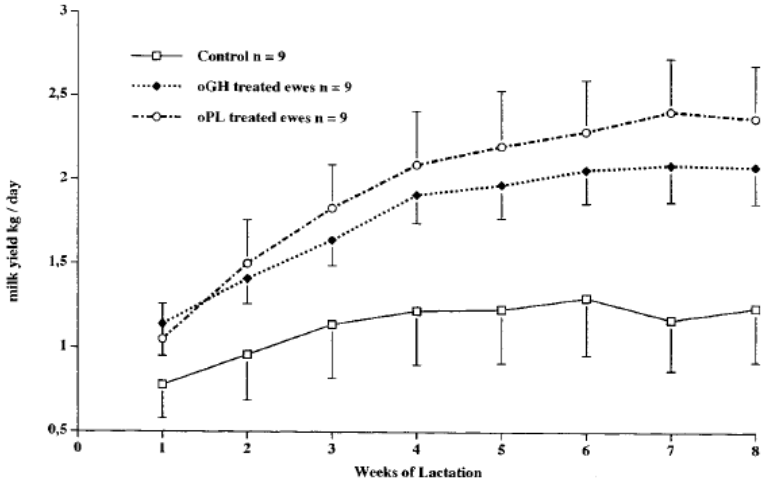

Figure 1.4. Mean daily production (+ s.e.m.) of milk during 8 wk for steroid-primed ewes induce to lactate after a previous 10-d treatment with either oGH or oPL. The last injection of oGH or oPL was on the last day of wk 0 (d 20) and ewes were not submitted to any pre-milking stimulus before d 21. From Kann et al., 1999.

The ejection of milk from the alveolar region of the mammary gland into the mammary cisterns depends on the pituitary hormone, oxytocin. The role of oxytocin in milk let down was first described in 1941 by Ely and Petersson (1941). Pre-milking stimulus is needed to elicit the release of oxytocin. Oxytocin is released from the pituitary

gland after physical stimulation of the teat; the release of oxytocin is signaled by the nervous system. Pre-milking techniques have been tested and applied for many years and have been linked with total milk yield and milk flow (Nostrand, 1989; Bruckmaier, 2005).

Every lactation ceases with the involution of the mammary gland, which is characterized by cell apoptosis. In dairy cattle where lactation overlaps with pregnancy, the dry period at the end of lactation is essential to maximize productivity during the next lactation (Annen et al., 2004). Hormones control every aspect of mammary development and function, including involution. Accorsi et al. (2002) conducted an experiment to evaluate the effects of prolactin (PRL), GH, and IGF-I on mammary involution. Using mammary gland explants from cows, they demonstrated that the lack of PRL, GH or IGF-I increased cell apoptosis. The effects of PRL and GH through IGF-I on mammary involution have been also described in rats, mice, and goats (Accorsi et al., 2002).

Factors affecting mammary cell dynamics and milk secretion

Pre-pubertal growth

The mammary gland in heifers starts its development shortly after birth. Prior to puberty, the mammary gland goes through a stage of allometric growth. The pre-pubertal growth of the mammary gland consists only of the elongation and branching of ducts into the surrounding fat pad (Meyer et al., 2006a).

The development of the mammary gland in prepubertal heifers has been studied under different levels of nutrition; many studies have found a decrease in parenchyma tissue at puberty on heifers raised on a higher rate of gain (Sejrsen et al., 1982; Sejrsen et al., 1983; Capuco et al., 1995). In a study to determine the effects of levels of nutrition on mammary development at different body weights, calves were fed to gain either 650 or 950 g/d and calves were slaughter every 50 kg of weight from 100 until 350 kg (Meyer et al., 2006a). Mammary parenchyma DNA on a weight basis was lower for heifers raised to gain 950 g/d. However, when age was used as a covariate the difference between groups disappear; this experiment indicates that mammary parenchyma tissue grows according to age and not to plane of nutrition.

Some studies have found a negative correlation between prepubertal body weight gain and first lactation milk yield (Gardner et al., 1977; Van Amburgh et al., 1998; Meyer et al., 2006a). Gardner et al (1977) described a negative correlation between age and number of estrous cycles before breeding with first lactation milk yield within groups. Heifers fed a high energy diet during growth calved at 19.7 months compared with heifers raised on a traditional hay diet that calved at 26.9 months. Milk yields per month of age were comparable at the end of the first lactation between the two groups. Within each group, heifers that calved earlier produced less milk than heifers on the same treatment that calved one month later (Gardner et al., 1977).

Milk yield of heifers raised to achieve 3 different rates of gain (0.6, 0.8 and 1 kg/d), was significantly lower when evaluated as 305 d

and 4% fat-corrected milk for heifers on the higher rate of gain (Van Amburgh et al., 1998). However in this study prepubertal body weight (BW) gain only explained 2% of residual milk from test day model. Factors such as postcalving BW explained more of the variation in first lactation milk yield. Findings from this study are in agreement with other studies that found no significant effect of prepubertal growth rate on first lactation milk yield (Capuco et al., 1995; Silva et al., 2002). Silva et al. (2002) found BCS at breeding to be a significant covariant for milk production in heifers raised on different planes of nutrition. Furthermore, Meyer et al. (2006b) demonstrated that prepubertal BW gain had no effects on epithelial cell proliferation or rate of accretion; neither of these has an effect on the dynamics of allometric and isometric growth of the mammary gland.

Prepartum milking

Milking cows prior to calving is known as prepartum milking and is another technique that has been researched to increase milk production in dairy cattle. In an experiment designed to compare mammary histology between udder halves when one-half of the udder was milked beginning 10 d prepartum, Akers et al. (1977) found 15.6% more epithelium cells on those halves milked pre-partum; the mature epithelium tissue was increased by 24% and the immature epithelium was decreased by 8.9% compared to that from mammary glands from cows not milked until after calving. Despite this increase in mammary epithelial cells, other studies have found no effect on milk yield

between cows milked prepartum and cows milked beginning after parturition (Greene et al., 1988; Grummer et al., 2000).

Effects of exogenous hormones: bST, Prolactin, Oxytocin

There are different management techniques that can help maximize milk production by maintaining a higher number of productive cells during lactation. Some of these management techniques include the use of exogenous hormones, dry period length variation, photoperiod management, and milking frequency variation among others (Van Amburgh et al., 1997; Dahl et al., 2000; Capuco et al., 2001; Capuco et al., 2003; Hale et al., 2003; Annen et al., 2007).

The first biotechnology product available for producers to maximize milk production and profitability was rbST. The experimental use of rbST started in 1982; prior to that time extracts from pituitary glands of slaughter animals were used. Administraton of rbST coordinates a series of metabolic changes that allows for increased partitioning of nutrients for milk production (Bauman, 1992). The response of each individual cow to rbST is mainly correlated to the management level the cow is under (Bauman, 1992).

The commercial use of rbST in the United States began in 1994 following approval by the Food and Drug Administration. The response in milk yield, milk protein, milk fat and somatic cell count (SCC) on commercial farms was evaluated in 1998. Bauman et al. (1999) selected a total of 340 herds in the Northeast; farms were selected depending on the use or non use of rbST from February 1994 to March 1998. The evaluation of rbST response was made using Dairy

Herd Improvement (DHI) records and a linear model that accounts for biological variations called the Test Day Model (TDM). The TDM generates residuals that account for within-herd variation in monthly test day milk yield resulting from herd test day, age, DIM, month fresh, and stage of pregnancy (Van Amburgh et al., 1997; Bauman et al., 1999).

Using records for 8 yr (4 yr pre-approval and 4 yr post-approval), the overall response of rbST on 305-d lactation was estimated to be 894 kg of milk, 27 kg of milk fat, and 31 kg of milk protein (Bauman et al., 1999). Figure 1.6 expresses the residuals for milk yield of both groups of farms during the 8-yr study period.

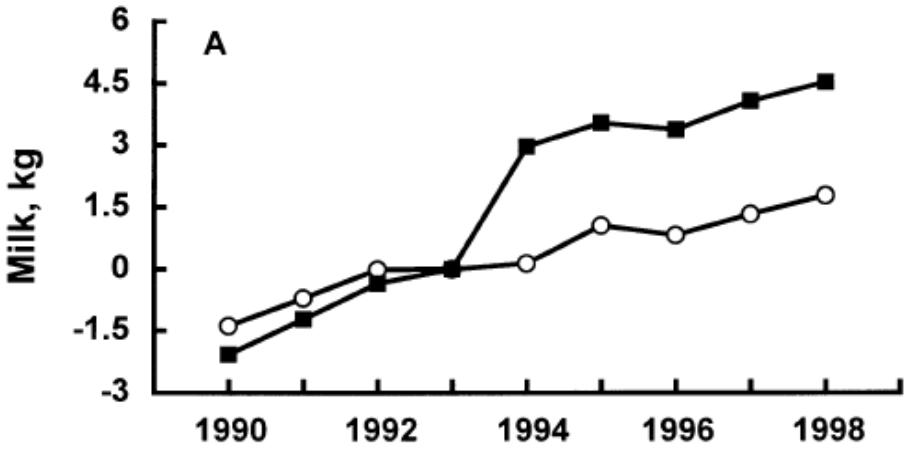

Figure 1.6. Comparison of herd management for daily yields of milk for control (circles) and rbST (squares) herds. Values represent averages for all cows milking on test day and SE was 0.1 kg. For ease of comparison, yearly test-day averages were expressed relative to 1993, the year prior to rbST approval. From Bauman et al., 1999

The study also analyzed the effect of rbST on the individual lactations of multiparous and primiparous cows. In both cases the

response to rbST was low during the post calving period and increased after peak yield until it reached a plateau. In both primiparous and multiparous cows, rbST when administered according to label specifications increases the persistency of lactation as shown in Figure 1.7 (Bauman et al., 1999).

The shift in the shape of the lactation curve with the use of bST allows for other management practices that can help increase the profitability of dairy farms. By having more persistent lactations, calving intervals (CI) can be increased, allowing for more productive days in the lives of the cows. Increasing CI also reduces the number of days a cow spends in the periparturient period, the period where most metabolic diseases occur. A study designed to evaluate the economic advantage of increasing CI with the use of bST showed an increase in milk yield of 4,468 kg when milk production was standardized to 4.35 years (an average productive life) and CI was increased from 13.2 months to 16.5 months (Van Amburgh et al., 1997).

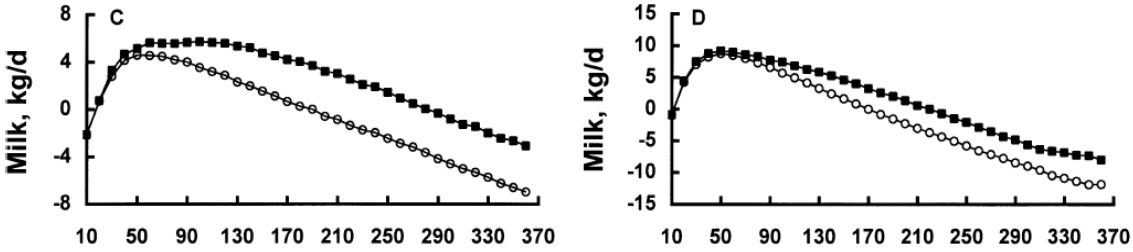

Figure 1.7. Comparison of lactation curves of milk yield for control and bST herds during the pre-approval period (January 1990 to February 1994) open circles; and the post-approval period (July 1994 to March 1998) dark squares. Panel C is for primiparous cows and panel D for multiparous cows. From Bauman et al., 1999

The effects of exogenous oxytocin as a management tool to increase milk production was examined in the early 1990s. Nostrand et al. (1991) conducted an experiment using 84 cows (30% primiparous and 70% multiparous cows), and followed their production for at least 150 DIM. Cows were either administered 1 ml of exogenous oxytocin immediately before machine attachment at every milking throughout the entire lactation or assigned to a control group. Treated cows produced 849 kg more milk for the first 305 d of lactation than the control group, which represented an 11.6% increase in milk production. Milk fat and protein content was not affected by treatment (Nostrand et al., 1991). Most of the differences in milk yield for cows administered oxytocin occurred after peak milk yield, as milk yield was similar between treatments during early lactation The positive effects on total milk yield of exogenous oxytocin may be due to a slower degree of involution in the mammary gland due to more complete milking, reducing possible negative feedback signals (Nostrand et al., 1991).

Photoperiod management

Dairy cows are not seasonal breeders; however, a number of studies have suggested that photoperiod can affect milk yield (reviewed by Dahl et al., 2000). Although the specific endocrine factor or factors regulating milk yield responses to increased photoperiod has not been identified, circulating IGF-I levels increase when cows are exposed to 16 h of light (Dahl et al., 2000). The increase in IGF-I has been detected in both heifers and cows and it is accompanied by an average

increase in milk yield of 2.5 kg/d. The IGF-I response appears to occur independently of GH or PRL (Dahl et al., 2000).

The effects of long photoperiods during lactation are not the only researched effects of photoperiod on milk yield. Short photoperiods, described as 8 h of light and 16 h of darkness during the dry period, have been shown to enhance milk production during the subsequent lactation. Different mechanisms have been proposed to explain the metabolic processes responsible for this response. Auchtung et al. (2005) related the response of short photoperiods on dry cows to increased prolactin sensitivity. Short photoperiods reduced the levels of circulating prolactin, but the expression of prolactin receptors and milk yield was enhanced; milk yield was increased by an average of 5 kg/d between wk 4 and 8 of lactation. In this study, cows assigned to the short photoperiod treatment increased their DMI during the dry period, but lactational DMI was not affected by treatment.

In order to compare mammary development during the dry period as affected by photoperiod, Wall et al. (2005) measured cell proliferation and apoptosis as well as the expression of IGF-I, IGF-II and IGF binding protein-5 in cows exposed to 8 vs. 16 h of light during the dry period. Mammary cell proliferation for cows exposed to the short day photoperiod increased from d -40 to -20, and was greater than cows exposed to long-day lighting on d -20. Apoptosis rate was lower for cows exposed to a short day treatment. Circulating concentrations of IGF-I and IGF binding protein-5 did not differ between treatments. However, cows assigned to the short day treatment had increased expression of IGF-II. The authors concluded

that short day photoperiods may enhance mammary development through the action of IGF-II.

Dry period length

Current management systems for dairy cows result in considerable overlap of pregnancy and lactation. Most cows are in their last trimester of gestation when milk stasis occurs. The importance of a period during which dairy animals are not being milked (dry period) before parturition has been widely accepted (Capuco and Akers, 1999).

The length of the dry period has traditionally been 60 d; many researchers have studied the effects of shorter or longer dry periods. Most studies have found a reduction in milk production in subsequent lactations when cows received dry periods shorter than 40 to 60 d (Sorensen and Enevoldsen, 1991; Gulay et al., 2003; Rastani et al., 2005; Kuhn et al., 2006; Annen et al., 2007).

Milk productions of subsequent lactations were evaluated by Sorensen and Enevoldsen (1991) on dry periods of 4, 7 and 10 wk. Using cows on 8 different commercial farms, the researchers enrolled 434 cows. The days dry for each treatment averaged 29.6, 49.9, and 70.6 d. The cows that were dry for 4 wk before calving produced 2.8 kg/d less 4% fat-corrected milk than cows dried off at 7 wk precalving; differences in milk production between cows provided 7- or 10-wk dry periods were not significant. They found no interaction between lactation number and dry period length on milk production.

In another experiment designed to compare dry periods of 30 and 60 d, Gulay et al. (2003) did not find significant differences in milk production during the first 21 wk of the subsequent lactation. They determined that cows with shortened dry periods had higher DMI postpartum expressed as percent of BW and maintained higher BCS than cows with longer dry periods.

The use of different management techniques that increase milk production throughout lactation may influence the response of cows to different dry period lengths. Annen et al (2004) studied the use of rbST in combination with different dry period lengths in cows on 3 dairy farms. Cows were assigned to one of 4 treatments. A 60-d dry period with label use of rbST (60d), a 30-d dry period with label use of rbST (30d), a no dry period treatment with label use of rbST (CM) and a continuous milking (no dry period) with continuous use of rbST (CMbST). Milk yield prepartum for all animals was higher for CM and CMbST groups then those assigned to the 30-d or 60-d dry period length treatments. Heifers yielded more milk during the last 8 wk than multiparous cows. Heifers assigned to the 60-d dry period treatment yielded more milk during wk 2 through 17 of the subsequent lactation than cows assigned to all the other treatments. Postpartum milk yield did not differ between cows assigned to the 30-d dry period, CM and CMbST treatments. However, combined milk production from the last 8 wk of previous lactation and the first 17 wk postpartum did not differ between any of the groups for heifers. Dry period length did not affect subsequent milk yield of multiparous cows in this study (Annen et al., 2004).

Considerations on the length of the dry period may also have implications for nutritional management of dry cows. Rastani et al. (2005) designed an experiment to compare a traditional dry period of 56 d during which cows were fed a low energy diet for the first 28 d and a moderate energy diet for the last 28 d (treatment T), a shortened dry period of 28 d in which cows were fed a high energy diet for the entire time (treatment S) and a no dry period (treatment N) treatment in which cows were fed the lactating diet throughout. They found differences in DMI during the prepartum period for all treatments, with DMI of cows assigned to treatment N being higher than S and S higher than T. Postpartum DMI was not affected by treatment. Cows assigned to treatment S produced 422 kg more milk prepartum than cows assigned to treatment T, and cows assigned to N produced another 328 kg more than those assigned to treatment S prepartum. Milk yield during the postpartum period was higher for cows assigned to treatment T compared to those assigned to S by 4.5 kg/d and was higher for those assigned to treatment S than for N by 4 kg/d. during the first 10 wk of lactation. Parity by treatment interactions was not significant.

The effects of bST on milk production of cows without a dry period were tested by Annen et al. (2007). The researchers used first lactation animals approaching their second calving. Treatments were the use of bST during late lactation and into early lactation or no bST; one udder half in each cow was randomly assigned to treatment of continuous milking (CM) or 60 d dry. Cows supplemented with bST produced 1.7 kg/d more milk during the late lactation period than

non-supplemented cows. The udder half that had a 60-d dry period produced more milk during the subsequent lactating period compared to the CM half (10.6 vs. 22.2 kg/d), independent of bST treatment.

Most of the studies regarding dry period length focused on milk production of the subsequent lactation; a different approach to determine the proper length of the dry period was the approach used by Kuhn et al. (2006). The researchers used information from DHI to determine lifetime milk production as well as milk production in adjacent lactations according to the number of days dry (DD). Based on the fact that fewer DD imply more days on milk at the end of the previous lactation, total lifetime yield should be a better parameter to determine the optimal dry period.

The minimum DD to maximize milk yield on the subsequent lactation was dependent on parity; first and second lactation animals required a minimum of 40 to 45 DD, while after second and third lactation 55 to 65 DD were required to maximize milk yield on the subsequent lactation. However, lifetime milk yield was maximized by 40 to 50 DD for first lactation animals and for 30 to 40 DD for cows at the end of second and later lactations. The researchers found that 30 to 40 DD can be used in second and later gestations but the gain in lifetime milk yield is minimal. This research showed that dry periods of less than 30 d or longer than 70 d were costly to lifetime milk production; dry periods of more than 80 d were more costly than dry periods of less than 30 d (Kuhn et al., 2006).

Milking frequency

Milk production is regulated by both systematic and local factors. Increased milking frequency (IMF) has been shown to increase milk production in all dairy animals (Henderson and Peaker, 1984; Wilde et al., 1987; Erdman and Varner, 1995; Wilde et al., 1995). The removal of milk from the udder acts as a local mechanism to signal the production of milk. The regulation of milk secretion after the removal of milk from the udder is an acute response that happens within hours of milking and responds to the frequency of milk removal as well at the completeness of milking. One of the signaling mechanisms that has been proposed is the presence of a protein in milk that may act as a feedback inhibitor of milk production (Henderson and Peaker, 1984; Wilde et al., 1987; Wilde et al., 1995).

Henderson and Peaker (1984) conducted an experiment to study the effects of a feedback inhibitor in milk and physical distention of the udder. The experiment used goats and consisted of three treatments: those milked twice daily, those milked thrice daily or those milked thrice daily but after one of the milkings the harvested volume of milk was replaced with the same amount of a sucrose solution. Goats milked three times daily and three times daily with the same volume replaced by sucrose solution produced 10% more milk than goats milked twice daily. The researchers concluded that physical distention was not a factor affecting milk production, but the removal of a feedback inhibitor was responsible for the increase in milk production on goats milked thrice daily and thrice daily with sucrose replacement.

The effects of milk whey proteins on milk secretion were tested using rabbit mammary glands explants. Milk secretion from rabbit mammary explants was inhibited by a whey protein of M_r 7600 (Wilde et al., 1995) and a protein between 10000 and 30000 Da (Wilde et al., 1987). The protein fraction reduced milk secretion in rabbits when injected through the teat canal; other proteins did not have the inhibiting effect. Milk secretion was restored when the protein was removed from the udder and the overall effect was dose-dependent (Wilde et al., 1987; Wilde et al., 1995).

Whole lactation

Many authors have reported increased milk yields with increased milking frequencies. Milk yield responses in literature vary from 10% to 30% when cows are milked three times per day instead of two times per day (Amos et al., 1985; Barnes et al., 1990; Klei et al., 1997; Smith et al., 2002; Salama et al., 2003).

Erdman and Varner (1995) analyzed information from 19 different studies and concluded that the differences in percentage increase of milk yield found by other researchers when cows went from two times per day milking to three times per day milking was explained by the production level of the cows. The authors pointed out that, regardless of the production level, cows milked three times per day compared with twice daily produced 3.5 kg/d more milk with a 95% confidence interval of $\pm$ 0.4 kg/d and 92 g/d of fat with a 95% confidence interval of $\pm$ 25. Increasing milking frequency from two to three times per day decreased milk fat and protein percentages;

however, the increase in milk yield more than compensates for an increase fat-corrected and/or energy-corrected milk yield (Barnes et al., 1990; Klei et al., 1997; Smith et al., 2002).

Barnes et al. (1990) studied the effects of frequent milking on two different sets of cattle; the researchers used daughters of selected commercial sires and daughters from random non-commercial sires and milked them either two times per day or three times per day during two lactations. Daughters from selected sires produced 25 to 30% more milk than daughters of non-commercial sires. Increased milking frequency increased milk yield in both groups; however, the daughters of non-commercial sires had a higher percent production response. All animals had a higher percentage milk yield response to frequent milking during their first lactation.

Using information from DHI records, Smith et al. (2002) analyzed the production differences among farms that milked two times per day with farms that milked three times per day. Comparisons were made for three years of milk production; 7% of the farms milked their cows three times per day and those farms had a 15% higher milk yield than farms milking two times per day. Frequent milking farms had lower milk fat percent; however, fat-corrected milk yield was still 13.5% higher for frequent milking farms. The researchers found increased days open, calving intervals, and services per pregnancy for farms milking three times a day. These results are opposite to other studies that reported no effect on reproduction parameters when milking frequency was increased (Amos et al., 1985; Barnes et al., 1990). The effects of frequent milking on udder health

have been measured in various studies and it has been consistently shown that frequent milking decreases SCC and has no other effects on udder health (Waterman et al., 1983; Klei et al., 1997; Smith et al., 2002).

Hillerton et al. (1990) studied the effects of frequent milking on the mammary gland by increasing milking frequency from two to four times a day on half of the udder. The researchers found an increased milk yield of 10.4% on the glands milked more frequently. Samples of tissue were collected twice through mammary biopsies and the researchers found a trend for increased activities of acetyl CoA carboxylase (13.8%), fatty acid synthetase (11.1%), galactosyl transferase (17.1%), and glucose-6-phosphate deydrogenase (31.8%) in treated quarters and increased synthesis of DNA. Histological sections revealed more and larger epithelial cells in frequently milked quarters.

Early lactation

Milking cows more frequently clearly has positive effects on milk yield (Amos et al., 1985; Hillerton et al., 1990; Erdman and Varner, 1995; Klei et al., 1997; Smith et al., 2002; Salama et al., 2003). However there is an associated cost with milking cows more frequently, especially on those farms where labor or milking parlor capacity is limited.

More recently, researchers have reported positive carryover effects on milk yield of cows milked more frequently (4x or 6x) during the early lactation period followed by a return to normal milking routines (2x or 3x; Bar-Peled et al., 1995; Hale et al., 2003; Dahl et al.,

2004; Wall et al., 2006; Wall and McFadden, 2007). Bar Peled et al. (1995) compared 3x (control) with 6x milking for 6 wk (M6) or 3x plus allowing the calves to suckle 3 times a day (S) for 6 wk. They found that cows assigned to M6 and S groups produced 7.3 kg/d and 14.7 kg/d more milk than the 3X controls respectively. Carryover effects were present in cows assigned to the M6 treatment through wk 18 of lactation. Frequently milked cows produced 13.6% more milk than control cows. Milk composition was not significantly different among treatments; therefore, fat and protein yields of cows on M6 group were higher than control cows (Bar-Peled et al., 1995).

In a subsequent study, Dahl et al. (2004) tested a shorter treatment period by frequently milking cows during the first 21 d postpartum. Cows were milked either 3x (control) or 6x for 21 d and 3x thereafter (FM) Milk production was monitored at monthly test days; FM cows produced more milk than control cows during the first 6 test days, the effects of frequent milking were significant for milk yield and milk yield as adjusted for mature equivalent and component production (Dahl et al., 2004).

The window of opportunity to initiate frequent milking was evaluated in one study. Hale et al. (2003) studied the effect of milking cows 4x starting at d 1 after parturition (FM1) or starting at d 4 after parturition (FM4) and continuing until d 21 compared with 2x controls. Cows milked 4x were milked at the beginning and the end of each milking in a herd milked 2x; the interval between these milkings was 3 h. Both groups of frequently milked cows produced about 3 kg/d more milk compared to 2x cows from wk 1 through wk 44.

Tissue from mammary biopsies conducted at d 7 and d 14 were used to evaluate cell proliferation and cell apoptosis on 4 cows per treatment. Rates of mammary cell proliferation on d 7 as assessed by [^{3}H]-thymidine incorporation were greater for cows assigned to FM1 than control and FM4; on d 14 rates of mammary cell proliferation tended to be higher for cows assigned to FM1. The percentage of epithelial and stromal cells expressing Ki-67 antigen as an index of cellular proliferation did not differ between treatments. However, cows assigned to FM1 had labeling indices numerically higher on d 7, as FM4 had on d 14 (Hale et al., 2003). A higher rate of apoptotic epithelial cells assessed using TUNEL was found on d 7 on FM4 cows and was significantly different than the apoptotic rate of control cows on d 7 and of FM1 cows on d 14. There was a tendency for lower apoptotic rate on FM1 cows on d 14 (Hale et al., 2003).

Different mechanisms have been proposed to explain the carryover effect that frequent milking during early lactation has on milk yield. Wall et al. (2006) tested the effects of prolactin and frequent milking by assigning cows to either 2x milking (control), 2x milking plus prolactin for 3 wk (2x PRL), or 4x milking for 3 wk (FM). Milk yield for cows assigned to 2x PRL and FM was higher than control for the first 6 wk of lactation; these findings suggest a similar effect of exogenous prolactin and frequent milking on milk yield and carry over effect. Mammary cell proliferation measured by [^{3}H]thymidine incorporation was not significantly different between treatments but tended to be higher for FM cows. Differences in apoptosis rates for

stromal and epithelial cells were also not significant, but apoptosis tended to be decreased on 2x PRL and FM cows (Wall et al., 2006).

Wall and McFadden (2007) also conducted a cross-udder experiment in which one udder half was milked 4x during the first 21 d of lactation and the other half 2x the entire lactation. Quarters milked 4x produced 3.5 Kg/d more milk during the first 21 d than quarters milked 2x; front and rear quarters were equally responsive to treatment. Half udders milked 4x had a drastic decrease in milk yield once treatment ended but still yielded a significant difference of 1.8 kg/d more milk than udder halves milk 2x during the remaining of the lactation. This experiment clearly showed that the mechanisms controlling the increase of milk yield and the carry over effect are locally regulated within the mammary gland as both half udders would have bee subjected to the same systemic effects. Milk component percentages between udder halves were not significantly different (Wall and McFadden, 2007).

Although many researchers have shown the positive effects of frequent milking during early lactation, some other studies have failed to replicate these effects. A study conducted on a commercial herd using 300 animals compared milk yield of cows milked 6x during the first 7, 14 or 21 DIM to cows milked 3x during the entire lactation. Milk yield for cows milked 3x was higher during the first 9 wk of lactation than cows milked 6x for 7 or 21 d. After 9 wk all groups had similar milk yields. Fat and protein percentages did not differ among treatments. In that study, the cows assigned to 6x spent on average 6.5 h/d away from their pen; therefore, the author hypothesized that

time away from the pen for 6x cows may have contributed to the lack of response to increased milking frequency (VanBaale et al., 2005).

Another study conducted on a commercial farm compared 4x milking during the first 21 DIM with 2x milking during the entire lactation; 105 cows were assigned to one of these treatments and milk yield and composition was measured at monthly test days (Fernandez et al., 2004). Cows milked 4x during the first 21 DIM yielded significantly more milk during the first two test days but differences in milk yield were not significant thereafter. Frequently milked cows tended to produce only 1.6 kg/d more milk than controls across the first 9 monthly test days (Fernandez, 2004). Milk yields of different IMF studies are summarized in Table 1.1.

Focus of the research reported herein

Controlled studies have demonstrated the potential for increased milk yield throughout lactation following increased milking frequency during early lactation. Results from a limited number of studies conducted on commercial farms suggest mixed results to this practice. Studies to be included as part of this thesis will evaluate the effects of increased milking frequency across multiple commercial dairy farms and also will evaluate whether increased milking frequency affects aspects of energy metabolism and mammary cellular dynamics.

Table 1.1 Milk, fat and protein yield responses to increased milking frequency reported in the literature for studies of increased milking frequency (IMF) during early lactation.

Author	Milking scheme[1]	Length of IMF[2]		Milk yield response[3]	Fat yield response[3]	Prot. yield response[3]
			DIM	kg/d	kg/d	kg/d
Bar-Peled et al., 1995	6x-3x	42 d	1-42 d	7.31	0.19	0.2
			42-126 d	5.1	0.15	0.15
Hale et al., 2003	4x-2x	21 d	1-21 d	8.8	0.02	0.23
			21-70 d	4.6	0.09	0.07
			21-308 d	2.6	0.03	0.06
Fernandez, 2004	4x-2x	21 d	~ 15 d	3	nr[6]	nr
			~ 45 d	3.5	nr	nr
			1-270 d	1.6[5]	0	0.01
Dahl et al., 2004	6x-3x	21 d	1-21 d	8.4	nr	nr
			305 d	3.6	nr	nr
VanBaale et al., 2005	6x-3x	7 d	1-63 d	-1.7[5]	-0.12	-0.03[5]
			63-308 d	-0.8[5]	-0.03[5]	0.04[5]
		14 d	1-63 d	0.2[5]	-0.04[5]	0
			63-308 d	-0.2[5]	0.01[5]	-0.01[5]
		21 d	1-63 d	-2.3[5]	-0.15	-0.1[5]
			63-308 d	*-0.6*[5]	*-0.04*[5]	*-0.02*[5]
Wall and McFadden, 2007	4x-2x	21 d	1-21 d	3.5[4]	nr	nr
	Unilateral FM		21-305 d	1.8[4]	nr	nr

[1]Milking scheme represents the number of milking the treated group was exposed to during the length of IMF – the number of milkings the control group was exposed to during the entire experiment as well as the treated group after the length of IMF and for the reminder of the period

[2]Represents the length of time the increased milking frequency was applied.

[3]Milk yield response, fat yield response and protein yield response represent the average value of treated cows minus the average value for the control cows.

[4]Represents the response in half udders

[5]Indicates no significant difference.

[6]Not reported.

CHAPTER II

The effects of increased milking frequency during early lactation on milk yield and milk composition on commercial dairy farms

Abstract

Early lactation increased milking frequency (IMF) has been a research focus during the past several years because of the potential for carryover responses following the return to normal herd milking frequency. The objective of this experiment was to determine the consistency of response of cows in commercial dairy farms to early lactation IMF. Data from Holstein cows (n=385) entering either first or second and greater lactation on four commercial farms were used in this study; each of the farms had a control group in which cows were milked 2x for the entire lactation and an IMF group in which cows were milked 4x during the first 21 d postpartum followed by 2x for the remainder of lactation. Cows in the IMF group were milked at the beginning and again at the end of the normal milking routine. This resulted in minimum milking intervals for the 4x cows of 3.5, 4, 5, 5.5, and 6 h for each farm. Results from the analysis of data from the three farms from which 7 monthly test day milk yields and composition was available suggested that early lactation IMF increased milk yield by 2.1 kg/d during the first 7 months of lactation ($P < 0.01$). Interactions of treatment with lactation group were not significant. Although percentages of milk fat ($P = 0.02$) and true protein ($P = 0.05$) decreased by early lactation IMF, overall milk fat yield ($P = 0.04$) and milk true protein yield ($P < 0.01$) were increased by early lactation IMF.

Early lactation IMF did not affect udder health as assessed by linear score in this experiment and did not appear to increase body fat mobilization as assessed by BCS; however, there was a tendency for serum NEFA to increase (P = 0.08) and serum BHBA was increased (P = 0.03) by IMF but the number of cows diagnosed with subclinical ketosis (BHBA > 14 mg/dL) did not differ among treatments (P = 0.87). Early lactation IMF has the potential to increase milk yield on commercial dairy farms. However the magnitude of the response is different among farms and appears to be influenced by management practices specific to farms.

Introduction

The effects of frequent milking on performance of dairy cows have been widely studied. For many years researchers focused on the effect of increased milking frequency (IMF) during the whole lactation, consistently finding an increase in the yields of milk and components when cows were milked more frequently (Amos et al., 1985; Barnes et al., 1990; Klei et al., 1997; Smith et al., 2002). Erdman and Varner (1995) compiled data from 19 studies and concluded that cows milked 3x yielded an additional 3.5 kg/d of milk, 92 g/d of fat, and 82 g/d of protein than cows milked 2x.

Recent studies have demonstrated the potential for IMF during early lactation only to affect lactational milk yield. Researchers have observed carryover effects on yields of milk and milk components in cows milked 4x or 6x during the first 21 or 42 d postpartum followed by a return to a 2x or 3x milking routine (Bar-Peled et al., 1995; Hale

et al., 2003; Dahl et al., 2004). Other studies have demonstrated either minimal carryover effects (Fernandez et al., 2004) or negative effects (VanBaale et al., 2005) of IMF during early lactation on overall performance.

Most of the experiments conducted to date included either small numbers of cows per treatment or were conducted on only one dairy farm. Therefore, the objective of the present experiment was to evaluate the effect of increased milking frequency of cows in early lactation on performance on several commercial dairy farms in order to evaluate the consistency of responses under commercial dairy farm conditions.

Materials and Methods

Herd enrollment, treatments, and housing

Holstein cows (n=421) across four commercial dairy farms in central and northern New York were assigned randomly at calving within each of the farms to either a 4x or 2x milking frequency treatment for the first 21 d postpartum. After d 21 postpartum, all cows were milked 2x for the remainder of the experiment. Milking and health management was conducted according to each of the herds' protocols as well as culling decisions were made by the farm owners. The numbers of primiparous and multiparous cows assigned to the treatments on each farm as well as the number of animals removed from the experiment on each treatment are summarized in Table 2.1.

On farm 1, cows (n=107) were housed in separate pens in a freestall barn and milked 2x or 4x for the first 21 d postpartum. After d 21, all cows were housed in the same pens sorted by lactation group and milked 2x for the remainder of lactation. Cows were milked at 0315 and 1515 h; cows assigned to the 4x treatment were milked again at 0715 and 1915 h. Four cows did not finish the experiment and their data were not used.

On farm 2, cows (n=107) were housed in a freestall barn and the fresh cow pen was divided in two. All cows were milked at 0800 and 1800 h; the cows milked 4x were milked again at 0245 and 1330 h. In addition to the treatment in which cows were milked 4x for 21 d, an additional group of cows (n=50) continued the 4x treatment until d 60. Management practices at this farm included milking fresh cows postcalving 4x during the first 60 d postpartum; the producers wanted to evaluate their current management practice with the treatments described in this experiment. After completion of the 4x milking treatment at either 21 or 60 d postpartum, cows were moved to a 2x milking group for the remainder of lactation. Two cows did not finish the experiment and one cow was removed from the additional 4x for 60 d group.

On farm 3, cows (n=100) were housed in a tiestall barn and milked either 2x or 4x for the first 21 d postpartum. After d 21, all cows were moved to a freestall barn and milked 2x for the remainder of lactation. All cows were milked at 0300 and 1500 h; cows assigned to the 4x treatment were milked again at 0630 and 1830 h. Eleven cows did not finish the experiment.

On farm 4, cows (n=107) were housed in a divided pen in a freestall barn after calving and milked 2x or 4x for the first 21 d postpartum. After d 21, all cows were housed together in a pen and milked 2x for a minimum of 5 months of lactation before the entire herd was changed to a 3x milking scheme. Cows were milked at 0600 and 1800 h; cows assigned to the 4x treatment were milked again at 1200 and 2400 h. Six cows did not finish the experiment.

Table 2.1. Summary of number (n) of primiparous (1st lact) and multiparous (2+ lact) cows by farm for cows milked 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period. The number in parenthesis represents the number of cows removed from the experiment.

Farm	1st lact cows, n	2+ lact cows, n	1st lact cows control, n	2+ lact cows control, n	1st lact cows IMF, n	2+ lact cows IMF, n	Interval 4x[1],
1	35 (1)	68 (3)	19 (1)	31 (1)	16	37 (2)	4
2	44	61 (2)	22	30 (1)	22	31 (1)	5
3	32 (2)	57 (9)	16 (1)	26 (5)	16 (1)	31 (4)	3.5
4	36	65 (6)	16	39 (2)	20	30 (4)	5.5
Total	**147 (3)**	**251 (20)**	**73 (2)**	**126 (9)**	**74 (1)**	**129 (11)**	

[1]Interval 4x is the number of hours between the first and second milkings, also equal to the number of hours between the third and fourth milkings. The interval between the first and third milkings of the 2x treatment was 12 h for all farms. The interval between milkings for 2x cows was 12 h.

Sampling and laboratory analysis

Milk yield was measured at monthly test days by Dairy Herd Improvement technicians and milk samples collected on the same day were analyzed for content of fat, true protein, somatic cell count, and milk urea N using midinfrared spectroscopy according to AOAC (2001) methods (DairyOne Cooperative, Ithaca, NY).

Body condition score (BCS) was assessed by one person using a 1 (thin) to 5 (fat) scale (Wildman et al., 1982). The BCS of each cow

was assessed once during the month prior to calving, once during the first month postcalving, and one time thereafter on each farm.

A single blood sample was collected from each cow during the first 21 d post calving; blood was collected through venipuncture of the coccygeal vein or artery into evacuated test tubes (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ). Samples were placed on ice for transport to the laboratory where they were centrifuged (1380 x g for 15 min). Serum was transferred to polycarbonate test tubes and frozen at -20°C until subsequent analysis for nonesterified fatty acids (NEFA) and B-hydroxybutyrate (BHBA). Serum NEFA concentrations were analyzed by enzymatic analysis (NEFA-C; WAKO Pure Chemical Industries, Osaka, Japan) using modifications described by McCutcheon and Bauman (1986). Serum BHBA concentrations were determined by enzymatic analysis (BHBA dehydrogenase; kit #310, Sigma Chemical).

Statistical analysis

Farms 1, 2, and 3 all had milk yield and milk composition information for at least 7 monthly test days for cows enrolled in the study. However, the farm owner of Farm 4 began whole-herd 3x milking 6 mo after the experiment commenced. Therefore, data for milk yield and composition from the first 7 monthly test days of lactation and BCS from farms 1, 2, and 3 were subjected to analysis of variance for a completely randomized design with repeated measures using the MIXED procedures of SAS (2001). The denominator degrees of freedom were adjusted using the method of Kenward Rogers, farm

was included as a random variable, and each model was tested using 4 different covariance structures (autoregressive order one, autoregressive order one with heterogeneous variance, compound symmetry, and compound symmetry with heterogeneous variance). The model and covariance structure with the lowest Akaike's Information Criterion was selected; in almost all cases this was the model using the autoregressive order one with heterogenous variance covariance structure. Terms in the model included treatment, lactation group (primiparous vs. multiparous), month of lactation, and all 2-way, and 3-way interactions. In the analysis of BCS all 4 farms were included and precalving BCS was used as a covariate.

Data for concentrations of BHBA and NEFA in serum were analyzed using the MIXED procedures of SAS (2001). Terms in the model included treatment, lactation group, and the 2-way interaction. Farm was included in the model as a random variable. These data also were evaluated for the frequency of cows categorized as subclinically ketotic (BHBA > 14 mg/dl; Nielem et al. 1994; Walsh et al. 2007) and having high NEFA concentrations (NEFA > 800 μeq/L) using the LOGISTIC procedure of SAS (2001). Terms in the model included treatment, lactation and the 2-way interaction thereof.

Data were also analyzed by farm, where information from 7 monthly test days was used in the analyses of Farms 1 and 3; Farm 2 had an additional treatment in which cows were milked 4x for 60 d and then returned to 2x milking; therefore, the independent analysis of data from Farm 2 included both treatments and data from 10 monthly test days. The analysis of Farm 4 only included data from 5

test days due to the management decision to milk all cows 3x before the study had concluded. The statistical analysis by farm was conducted for a completely randomized design with repeated measures using the MIXED procedures of SAS (2001). Terms in the model included treatment, lactation group, month of lactation, and all 2-way and 3-way interactions.

Significance was declared at $P = 0.05$ and trends were declared at $0.05 < P < 0.10$. Least squares means are presented throughout.

Results

Overall results for milk yield and milk components for the first 7 monthly test days for multiparous and primiparous cows milked 4x for the first 21 DIM and 2x thereafter (IMF) and for control cows milked 2x throughout the entire period on farms 1, 2, and 3 are presented in Table 2.2. With the exception of MUN values, none of the production-related variables had significant interactions of treatment by lactation group, or treatment by lactation group by month. Therefore, results for milk yield and composition are reported as overall results across the three farms.

Milk yield was increased by 2.2 kg/d during the first 7 months for cows subjected to early lactation IMF compared to controls (33.9 vs. 31.8 kg/d; $P < 0.01$). The interaction of treatment by month was not significant ($P = 0.27$; Figure 2.1), suggesting that a carryover effect of early lactation IMF existed and was persistent across the period evaluated.

Milk fat percent and milk true protein percent decreased with IMF treatment (3.73% fat and 3.03% true protein for control vs. 3.62% fat and 2.97% true protein for IMF; P = 0.02 and P = 0.05 for milk fat and true protein percentages, respectively; Table 2.2). Early lactation IMF increased milk fat yield by 0.04 kg/d (1.21 vs. 1.17 kg/d; P = 0.04) and the interaction of treatment and month for milk fat yield was not significant as illustrated in Figure 2.2. Overall yield of true protein in milk was increased by early lactation IMF (1.0 vs. 0.95 kg/d P < 0.01); a treatment by month interaction also existed for true protein yield such that differences were larger at the first two monthly test days and smaller throughout the rest of the study period (Figure 2.3).

Table 2.2. Least squares means and standard errors for milk yield and milk components for multiparous (2+ lact) and primiparous (1st lact) during the first 7 test days of lactation for cow from three farms milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the reminder of the period.

	Control		IMF			P		
	1st lact	2+ lact	1st lact	2+ lact	SEM	Trt	Lact	Trt*Lact
Milk, kg/d	27.7	35.9	29.9	37.9	0.7	<0.01	<0.01	0.93
Fat, %	3.68	3.76	3.60	3.64	0.07	0.02	0.21	0.78
Fat, kg/d	1.01	1.33	1.07	1.36	0.03	0.04	<.001	0.64
True protein, %	3.04	3.02	3.02	2.93	0.03	0.05	0.06	0.24
True protein, kg/d	0.83	1.07	0.90	1.10	0.02	<0.01	<0.01	0.46
3.5% FCM[1], kg/d	28.4	37.0	30.3	38.4	0.7	<0.01	<0.01	0.76
ECM[2], kg/d	27.9	36.2	29.8	37.5	0.7	<0.01	<0.01	0.68
MUN[3], mg/dL	13.45	12.78	12.88	12.76	0.29	0.64	0.02	0.16
LS	2.07	2.55	2.08	2.45	0.16	0.73	<0.01	0.78

[1]Formula for 3.5% fat-corrected milk [(0.4324* kg milk)+(16.216* kg Fat)]

[2]Value corrected for 3.5% fat and 3.2% true protein using formula from NRC (2001) [(0.3246* kg milk)+(12.86* kg Fat)+(7.04* kg True protein)]

[3]Values for milk urea nitrogen (MUN) come from two farms; the third farm did not test for MUN

Consistent with the increased yields of milk and true protein and trend toward increased yield of milk fat for cows subjected to early lactation IMF, overall yields of 3.5% fat-corrected milk and energy-corrected milk were increased ($P < 0.01$) by early lactation IMF compared to 2x controls (Table 2.2). In IMF cows, 3.5% fat-corrected milk yield was increased by 1.6 kg/d (34.3 vs. 32.7 kg/d) and energy-corrected milk yield was increased by 1.5 kg/d (33.6 vs. 32.1 kg/d). No treatment by month interaction existed for yields of either fat-corrected milk or for energy-corrected milk ($P > 0.10$). Energy-corrected milk yields by month for cows assigned to the two treatments are presented in Figure 2.4.

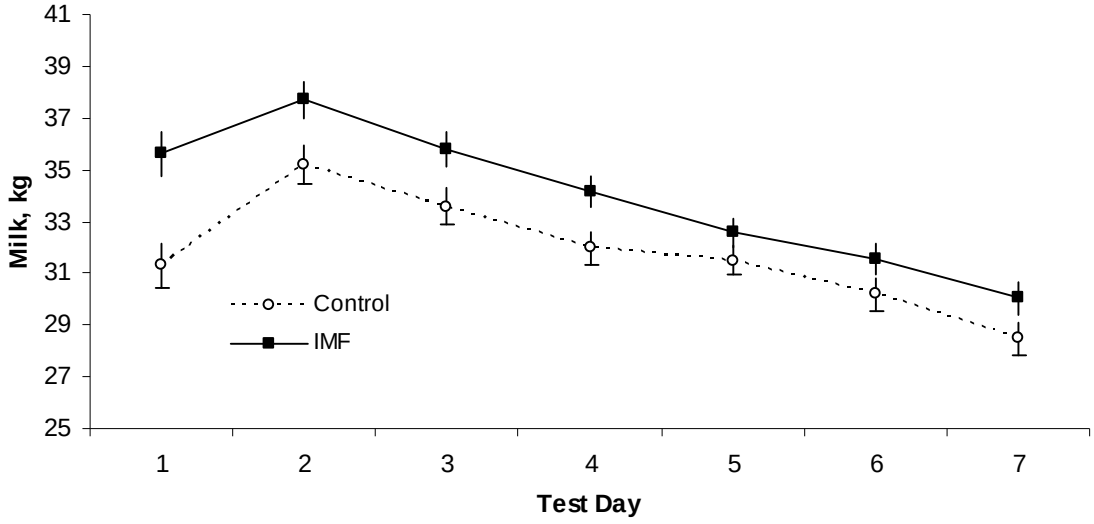

Figure 2.1. Least squares means and standard errors for milk yield during the first 7 test days of lactation for cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period. The P value for the effect of treatment was < 0.01 and the interaction of treatment and month was 0.27.

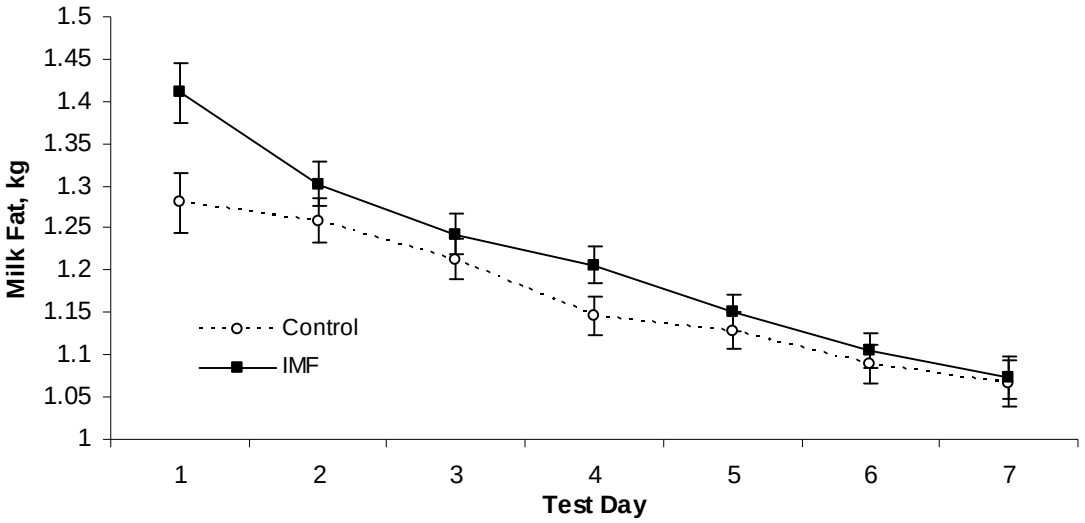

Figure 2.2. Least squares means and standard errors for milk fat yield during the first 7 test days of lactation for cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period. The P value for the effect of treatment was 0.04 and the interaction of treatment and month was 0.30.

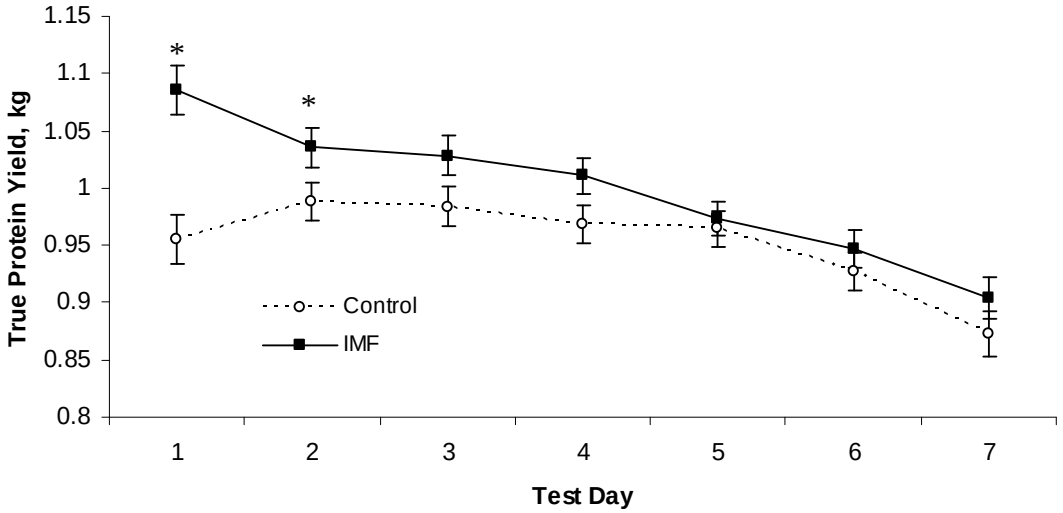

Figure 2.3. Least squares means and standard errors for true protein yield during the first 7 test days of lactation for cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period. The P value for the effect of treatment was < 0.01 and the interaction of treatment and month was 0.02. Significant differences at individual timepoints are denoted by an asterisk.

Only farms 1 and 3 tested for MUN. Of those farms, no significant differences were detected among treatments (P = 0.64). Somatic cell linear score was higher (P < 0.01) in multiparous compared with primiparous cows; however, there were no differences between treatments (Table 2.2).

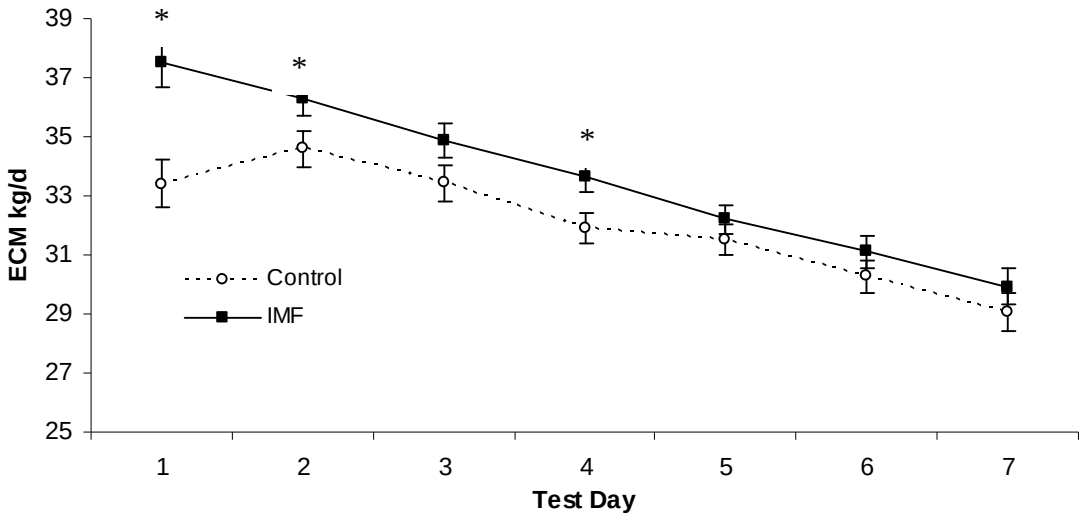

Figure 2.4. Least squares means and standard errors for yield of energy-corrected milk (ECM; corrected to 3.5% fat and 3.2% true protein) during the first 7 test days of lactation for cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period. The P value for the effect of treatment was < 0.01 and the interaction of treatment and month was 0.11.

Precalving BCS was used as a covariate for analysis of postpartum BCS. Differences between treatments during early or mid lactation and the interactions of treatment with lactation group were not significant (Table 2.3).

Blood samples taken within the first 21 d postcalving were analyzed for BHBA and NEFA concentrations. Cows subjected to early

lactation IMF had increased circulating concentrations of BHBA (12.2 vs. 10.4 mg/dl, P = 0.03); however, the proportion of cows with circulating BHBA greater than 14 mg/dL was not affected by treatment (Table 2.3). Concentrations of circulating NEFA tended to increase with IMF (485 µEq/L for control vs. 427 µEq/L for IMF; P = 0.08). However, the proportion of cows with NEFA greater than 800 µEq/L (14/145 for control vs. 18/160 for IMF; P = 0.56) was not affected by treatment (Table 2.3)

Table 2.3. Least squares means and standard errors for body condition score (BCS) and blood variables for multiparous (2+ lact) and primiparous (1st lact) cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period for multiparous (2+ lact) and primiparous (1st lact) cows

	Control		IMF			P		
	1st lact	2+ lact	1st lact	2+ lact	SEM	Trt	Lact	Trt*Lact
BCS								
Early Lact.	3.46	3.12	3.4	3.19	0.07	0.94	<0.01	0.45
Mid Lact.	3.27	2.92	3.13	2.96	0.12	0.45	<0.01	0.20
NEFA, µEq/L	350	504	423	548	43	0.07	<0.01	0.65
BHBA, mg/dL	10.0	10.8	10.9	13.5	1.1	0.03	0.03	0.25
NEFA[1]	2/59	12/86	3/52	15/108		0.56	0.22	0.58
BHBA[1]	5/58	15/94	7/57	27/107		0.87	0.66	0.80

[1] Threshold for BHBA were values > 14 mg/dL and threshold for NEFA were values > 800 µEq/L

Data analysis by farm allowed the determination of differences in the magnitude of the response to IMF treatment among farms (Table 2.4). In Farm 1, IMF increased milk production during the first 7 monthly test days by 3.2 kg/d or 10% above that of control cows (32.3 kg/d for control vs. 35.4 kg/d for IMF; P = 0.03 (Table 2.4)). Fat percent, fat yield, and true protein percent were not different among

Table 2.4. Least squares means and standard errors for milk yield for multiparous (2+lact) and primiparous (1st lact) cows during the first 7 test days of lactation for cows milked either 2X (control) for the first 21 d or 4X (IFM) for the first 21 d postpartum followed by 2X for the remainder of the period.

Farm	Control		IMF		SEM	Increase yield	%	Increase yield	%	Control	IMF	SEM	Increase yield	%	P
	1st lct	2+ lct	1st lct	2+ lct		1st lct		2+ lct					Overall		
1	29.1	35.7	30.9	40.1	1.7	1.8	6%	4.4	12%	32.4	35.5	1.0	3.1	10%	0.02
2	28.9	39.4	31.3	39.9	1.0	2.4	8%	0.5	1%	34.1	35.6	0.7	1.5	4%	0.24
3	25.2	32.5	27.6	33.7	1.0	2.4	10%	1.2	4%	28.9	30.7	0.6	1.8	6%	0.05
4*	32.2	38	32.8	41.3	1.8	0.6	2%	3.4	9%	35.1	37.1	1.1	2	6%	0.18
1, 2 and 3	27.7	35.9	29.9	37.9	0.7	2.2	8%	2.1	6%	31.8	33.9	0.5	2.1	7%	<0.01

*Only 5 monthly test days were use for this farm.

treatments (P > 0.10). However, true protein yield increased with IMF by 0.1 kg/d (P = 0.05). Energy-corrected milk yield tended to be increased for IMF by 2.3 kg/d (31.8 kg/d for control vs. 34.1 kg/d for IMF; P = 0.08). Linear score and MUN did not differ among treatments (P > 0.5).

Farm 2 implemented an additional treatment in which cows were milked 4x for 60 DIM (IMF2) and milk yield was monitored for 10 months. Early lactation IMF increased (P = 0.04) milk yield compared to the 2x controls; overall milk yield for IMF2 was increased compared to controls (35.0 vs. 32.5 kg/d; P = 0.01) and milk yield of cows subjected to IMF for 21 d (IMF1) was intermediate (33.6 kg/d) to the other two treatments (Figure 2.5; Table 2.5). Interactions of treatment with lactation group or month were not significant for milk yield. Percentages and yield of milk fat and true protein were not significant (P > 0.10; Table 2.4). Linear score also was not affected by treatment.

Increasing milking frequency in Farm 3 increased milk yield during the first 7 monthly test days by 1.8 kg/d or 6% above that of control cows (28.9 kg/d for control vs. 30.7 kg/d for IMF; P = 0.05 (Table 2.4)). Fat percent and fat yield did not differ among treatments (P > 0.1). True protein content was significantly decreased by 0.1% (P = 0.04). However, true protein yield was not significantly different among treatments (P = 0.3). Energy-corrected milk yield tended to increase by 1.4 kg/d (29.4 kg/d for control vs. 30.8 kg/d for IMF; P = 0.08). Linear scores and MUN did not differ among treatments (P > 0.1).

Farm 4 interrupted the study by switching all cows to 3x milking 6 months after beginning the experiment, so the data from this farm

was analyzed independently using only the first 5 monthly test days of lactational data per cow. Differences in overall milk yield resulting from early lactation IMF were not significant (37.1 vs. 35.1 kg/d; P = 0.18). The interaction of treatment by month was significant for this farm (P < 0.01) in that cows subjected to early lactation IMF had increased milk yield during the first month of lactation (Table 2.4). With the exception of MUN, which was increased in IMF treated cows (13.6 mg/dL for control vs. 14.6 mg/dL for IMF; P = 0.02), percentages and yields of all other milk components were not significantly different between treatments.

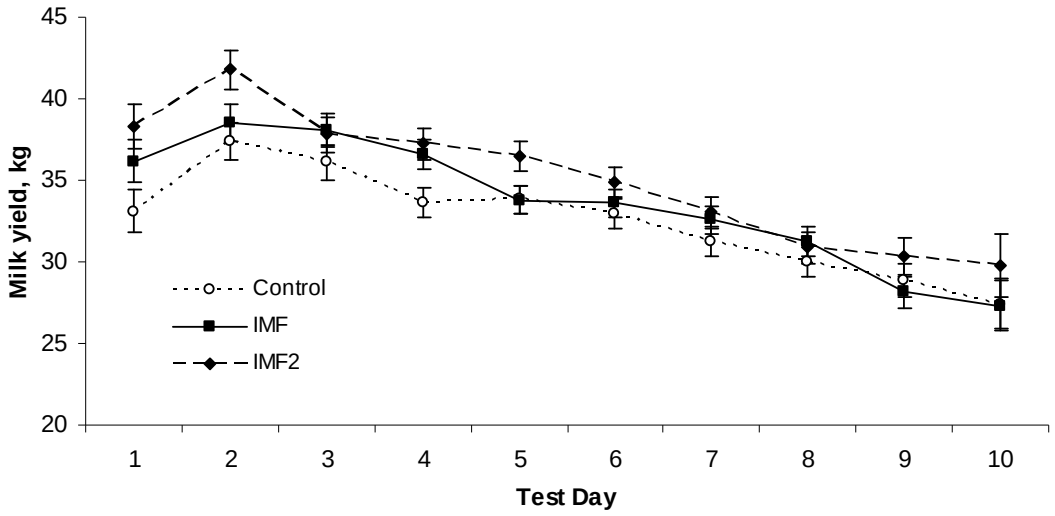

Figure 2.5. Least squares means and standard errors for milk yield of farm 2 during the first 10 test days of lactation for cows milked either 2X (control) for the first 21 d, 4X (IMF) for the first 21 d postpartum or 4X (IMF2) for 60 d postpartum followed by 2X for the remainder of the period. The P value for the overall effect of treatment was 0.04 and the interaction of treatment and month was 0.27. The P value for the overall effect of the control compared with IMF 21 D was 0.24, for control compared with IMF 60 D was 0.01, and for IMF 21 D compared with IMF 60 D was 0.16.

Analysis of BCS by farm revealed a difference in pre-calving BCS in farm 3 (3.59 for control cows vs. 4.01 for IMF cows; P = 0.04 (Table 2.6)). No other differences were found among treatments.

Table 2.5. Least squares means and standard errors for milk yield and milk components for multiparous (2+lact) and primiparous (1st lact) cows in farm 2 during the first 10 test days of lactation for cows milked either 2X (control) for the first 21 d, 4X (IFM) for the first 21 d postpartum, or 4X (IMF2) for 60 d postpartum followed by 2X for the remainder of the period.

	Control[1]		IMF		IMF2			P		
	1st lact	2+ lact	1st lact	2+ lact	1st lact	2+ lact	SEM	Trt	Lact	Trt*Lact
Milk, kg/d	27.7	37.2	30.1	37.1	31.5	38.6	1.2	0.04	<.001	0.38
Fat, %	3.88	3.84	3.83	3.79	3.77	3.66	0.09	0.26	0.36	0.91
Fat, kg/d	1.08	1.41	1.13	1.39	1.17	1.38	0.04	0.58	<.001	0.22
True protein, %	3.11	3.05	3.07	3.04	2.98	3.03	0.04	0.11	0.68	0.33
True protein, kg/d	0.86	1.13	0.92	1.12	0.93	1.15	0.03	0.18	<.001	0.42
LS	2.38	2.50	2.15	2.56	1.88	2.85	0.33	0.94	0.03	0.31

[1]Milk yield of control cows was significantly different than IMF2 (P=0.01); Control cows were not significantly different than IMF (P=0.24); IMF cows were not significantly different than IMF2 (P=0.16)

Differences in BCS during the first 21 d post-calving were only detected among lactation groups for farms 2 and 3 (P < 0.05). There were no differences among treatments in any farm (P > 0.1) (Table 2.6).

Plasma BHBA concentrations, when analyzed by farm, were not different between treatments (Table 2.6). The only differences for BHBA concentrations were detected between lactation groups on farm 1 (10.7 for primiparous cows vs. 15.0 for multiparous cows; P = 0.03). Farm 2 had a tendency (P = 0.06) for higher BHBA concentrations in multiparous compared to primiparous cows. This was the only farm

with a significant interaction for treatment by lactation group (P = 0.04) in that primiparous cows milked 2x had higher BHBA than multiparous cows milked 2x and multiparous cows milked 4x had higher BHBA than primiparous cows milked 4x (Table 2.6).

Table 2.6. Least squares means and standard errors for BCS, BHBA concentrations and NEFA concentrations for multiparous (2+lact) and primiparous (1st lact) cows for cows milked either 2X (control) for the first 21 d or 4X (IFM) for the first 21 d postpartum followed by 2X for the remainder of the period.

Farm	Control		IMF		Control	IMF	SEM	P	
	1st lct	2+ lct	1st lct	2+ lct				Trtmt	Lact
Pre-calving BCS									
1	4.1	4.1	4.0	4.0	4.1	4.0	0.1	0.48	0.84
2	3.9	3.7	3.9	3.6	3.8	3.8	0.1	0.89	0.02
3	3.3	3.9	4.1	4.0	3.6	4.0	0.1	0.04	0.29
4	4.4	3.7	3.9	3.7	4.1	3.8	0.2	0.21	0.05
Early Lactation BCS[A]									
1	3.7	3.5	3.7	3.5	3.6	3.6	0.1	0.88	0.14
2	3.4	2.9	3.1	3.1	3.1	3.1	0.1	0.98	0.04
3	3.2	2.5	3.2	2.8	2.9	3.0	0.1	0.66	0.01
4	3.3	3.8	3.5	3.4	3.5	3.4	0.1	0.67	0.30
Mid Lactation BCS[A]									
1	3.0	2.6	2.6	2.8	2.8	2.7	0.2	0.65	0.63
2	3.6	3.2	3.6	3.3	3.4	3.4	0.1	0.54	<0.01
3	3.2	2.7	2.9	2.7	2.9	2.8	0.1	0.48	0.06
4	3.6	3.3	3.6	3.0	3.5	3.3	0.1	0.41	0.02
BHBA mg/dL									
1	9.8	13.1	11.5	16.8	11.5	14.1	1.4	0.17	0.03
2	10.2	9.9	8.7	14.3	10.1	11.5	1.0	0.32	0.06
3	10.1	10.1	13.5	11.2	10.1	12.3	1.0	0.10	0.39
4	10.8	10.2	11.4	10.4	10.5	10.9	0.6	0.56	0.30
NEFA Eq/L									
1	337.2	552.2	403.1	608.8	444.7	506.0	198.0	<0.01	<0.01
2	328.8	494.0	423.6	503.4	411.4	463.5	33.7	0.26	0.01
3	262.8	629.9	252.9	547.0	446.3	399.9	64.6	0.60	<0.01
4	510.2	295.2	626.5	492.7	402.7	559.6	51.2	0.03	0.02

[A] Pre-calving BCS was use as a covariant for this analysis.

Plasma NEFA concentrations were significantly different among treatments in farms 1 and 4 with higher NEFA concentrations in the IMF group ($P < 0.03$); but no differences were found for farms 2 and 3 ($P > 0.3$) (Table 2.6). Differences in NEFA concentrations among lactation groups were detected on all farms ($P < 0.02$). For farms 1, 2 and 3, primiparous cows exhibited lower blood NEFA concentrations while primiparous cows on farm 4 displayed higher NEFA concentrations than multiparous cows.

Discussion

Cows subjected to early lactation IMF in this study had increased milk yield during the treatment period and sustained this production after they returned to a normal (2x) milking routine, exhibiting a carryover effect from the treatment through the first 7 monthly test days. This translated into an average increase of 2.2 kg/d or 7% for the 210 d measured. In comparison, Bar-Peled et al. (1995), used a milking scheme of 6x during the first 42 d postpartum and 3x thereafter and measured a milk response of 5.8 kg/d during the first 126 d. This difference in response may be due to the length of time following treatment that milk yields were measured and the duration and frequency of milking both during and after the treatment period.

Hale et al. (2003) determined that 4x milking for the first 21 d postcalving followed by 2x milking increased milk yield by 5.9 kg/d during the first 70 d postpartum compared with 2x controls. This milk yield increase was comparable with that of Bar-Peled et al. (1995). Furthermore, Hale et al. (2003) reported increased milk yield of 3 kg/d

during the 308-d lactational period for cows milked 4x/2x, which is comparable with the 2.1 kg/d response we measured through 210 days. Dahl et al. (2004) milked cows 6x for the first 21 d postpartum followed by 3x for the rest of lactation and reported increased milk yield of 3.6 kg/d during the 305-d lactational period, which is greater than the response found in the present study; however this is consistent with the carry over effect generated from IMF. Wall and McFadden (2007) measured the effects of 4x milking for the first 21 d postpartum using a half-udder model in which the contralateral udder half was milked 2x throughout lactation. They determined that 305-d lactational milk yield was increased by 1.8 kg/d in the udder half subjected to early lactation IMF, which would be extrapolated to a 3.6 kg/d increase in milk yield for the whole udder.

In contrast to the results reported above, Fernandez (2004) determined that 4x milking for the first 21 d postpartum increased milk yield during early lactation; however, carryover responses were minimal. This response resulted in a trend toward increased milk yield of 1.6 kg/d during the first 9 monthly test days. The difference in results with the current study may have been due in part to the number of cows and the statistical power gained in the present study through the increased number of cows in the treatment.

A lack of response to early lactation IMF was reported by VanBaale et al. (2005), who conducted an experiment with a similar number of cows to the current experiment (300 animals). They milked the cows 6x for 7, 14, or 21 d postpartum followed by 3x milking and reported that IMF did not increase milk yield either during or following

the 6x period. The experiment was conducted in a commercial farm and cows assigned to the 6x treatment spent nearly 6 h (double that of 3x-milked cows) out of their pen for milking, which may have contributed to the lack of response to 6x milking in their study.

Bar-Peled et al. (1995) reported that early lactation IMF tended to decrease overall milk fat and true protein percentages; however, increased milk yield by cows assigned to the IMF treatment more than compensated for the decreased component percentages and resulted in increased total yields of milk fat and true protein in their experiment. In the current study, overall milk fat yield tended to be increased and milk true protein yield was increased by 0.05 kg/d. Hale et al. (2003), reported no difference for 3.5% fat-corrected milk yield during the first 10 wk; in the current study IMF cows increased 1.7 kg/d of 3.5% fat corrected milk.

Results from the additional treatment in farm 2 suggested that increasing the period of IMF from 21 to 60 d may slightly increase the overall response to IMF. The only other study conducted in which different lengths of time for IMF were analyzed had no effects from treatment and may have been compromised by factors unrelated to the milking frequency treatment as described above (VanBaale et al., 2005).

Milk yield responses on Farm 4 were similar to a previous study conducted by our group in which cows receiving the IMF treatment increased production during the first 2 months but had minimal carryover effect (Fernandez, 2004). In the case of Farm 4, IMF treatment significantly increased production only for the first monthly

test day. Differences in the magnitude of the response to IMF among farms suggest an interaction between the effects of IMF and other management practices. Some of the management practices that may have an effect in the response to IMF include overcrowding of the fresh pen, grouping cattle by lactation group, and genetic selection as reported by Barnes et al. (1990). Linear score for somatic cell count did not differ between treatments. These findings are different that those from Smith et al. (2002) as well as those from Klei et al. (1997) who reported a decrease in LS and SCC when cows were milked 3x compared with cows milked 2x.

In this study, early lactation IMF did not affect postpartum BCS but tended to increase serum NEFA concentrations; however, the proportion of cows having elevated NEFA in the sample collected during the treatment period did not differ among treatments. These results suggest that early lactation IMF did not result in a higher degree of negative energy balance or body fat mobilization. Early lactation IMF did increase mean concentrations of serum BHBA, but did not affect the proportion of cows categorized as subclinically ketotic. Reasons for this increase in serum BHBA independent of serum NEFA are uncertain as the primary potential for IMF to increase BHBA would seem to be through increased body fat mobilization.

Conclusions and implications

Early lactation IMF for the first 21 d postpartum resulted in consistent overall increases in yields of milk and milk components on commercial dairy farms varying in both overall management schemes

and with minimum milking intervals ranging from 3.5 to 6 h. Increasing the duration of IMF to 60 d on one farm resulted in slightly increased responses to IMF. In contrast to previous reports, early lactation IMF did not affect udder health as assessed by changes in linear score. Early lactation IMF did not appear to affect BCS during early lactation and did not increase body fat mobilization as assessed using serum NEFA concentrations during the treatment period; however, serum BHBA concentrations were increased by early lactation IMF. In conclusion, early lactation IMF has the potential to increase yields of milk and milk components and should be evaluated in the context of economic decision making on commercial dairy farms.

CHAPTER III

The effects of increased milking frequency on metabolism and mammary cell proliferation in Holstein dairy cows

Abstract

Results presented in Chapter II of this thesis and by other researchers have suggested that early lactation increased milking frequency (IMF) results in increased milk yield not only during the period of IMF but also after cows have returned to a decreased milking frequency. The mechanisms underpinning this increase in milk yield and the overall effects of IMF on metabolism have not been well-characterized. The objective of this study was to determine the effects of IMF on metabolism and mammary epithelial cell dynamics in dairy cows. Thirty primiparous and 30 multiparous Holstein cows were randomly assigned at calving to one of 2 treatments. The control group was milked 2x for 119 d while the IMF group was milked 4x from d 2 postcalving until d 21 and 2x from d 22 until d 119. Milk yield was not significantly different ($P > 0.1$) between treatments throughout the 119 d monitored; however, the interaction of treatment by week was significant ($P < 0.01$) in that IMF cows yielded 4.8 kg/d more milk than control cows during wk 2 and 3 and comparable amounts of milk during the remainder of the study period. Analysis of data in which cows receiving mammary biopsies were excluded suggested that the mammary biopsy procedure contributed to the lack of overall responses of milk yield. Plasma NEFA concentrations were elevated in multiparous cows subjected to IMF during the period of IMF; NEFA

concentrations did not appear to be affected by treatment in primiparous cows. Plasma BHBA concentrations were not affected by treatment ($P > 0.1$). Mammary tissue was collected by biopsy in a subset of cows (n=8 cows per lactation group and treatment) at calving, 21 d postpartum, and 75 d postpartum and used for immunohistochemical localization of Ki-67. A treatment by day interaction existed ($P = 0.03$) for the percentage of labeled epithelial cells such that the IMF treated cows had a lower percentage of labeled epithelial cells on d 21 postpartum but a higher percentage of labeled epithelial cells at 75 d postpartum. Further analysis is needed for determination of apoptosis rates to determine difference in cell turnover between treatments as well as cell activity to pinpoint possible mechanisms for the milk yield responses to early lactation IMF.

Introduction

Increased milking frequency (IMF) of dairy cows during early lactation has been demonstrated to increase milk yield not only during the treatment period but throughout lactation (Bar-Peled et al., 1995; Hale et al., 2003; Dahl et al., 2004; Wall et al., 2006; Wall and McFadden, 2007; Chapter II, this thesis). In addition, some studies have reported decreased somatic cell counts in cows subjected to early lactation IMF (Smith et al., 2002; Dahl et al., 2004), suggesting that early lactation IMF may improve udder health. Other authors have reported no effects of early lactation IMF on SCC or udder health (Bar-Peled et al., 1995; Hale et al., 2003; Wall and McFadden, 2007).

Few studies have focused on the effects of IMF on energy metabolism. However, effects of early lactation IMF on indicators of energy metabolism such as plasma concentrations of BHBA and NEFA as well as BCS and BW have been reported. Andersen et al. (2004) compared plasma concentrations of BHBA and NEFA of cows milked 2x versus cows milked 3x during the first 8 wk postpartum. Cows milked 3x had a 19% higher plasma BHBA concentrations than cows milked 2x and plasma concentrations for NEFA did not differ between milking frequencies. Another study comparing cows milked 2x versus cows milked 4x during the first 21 d and 2x thereafter (IMF) observed a tendency for higher BHBA levels in IMF cows, but no differences in the proportion of cows categorized as subclinically ketotic or concentrations of NEFA (Fernandez, 2004). Hale et al. (2003) reported that early lactation IMF (4x) did not result in differences in BCS compared to 2x controls. However, Bar-Peled et al. (1995) reported decreased BCS of cows milked 6x compared to 3x controls.

In contrast to the multiple studies focused on performance or udder health of cows subjected to early lactation IMF, few studies have focused on the underlying potential cellular mechanisms for carryover responses to IMF. The production of milk in the udder is a consequence of the number of secretory cells present in the udder and the activity level of each cell (Capuco et al., 2001). If there is an increase or a reduction in milk synthesis, it is due to an increase or a reduction in cell number or cell activity.

Norgaard et al. (2005) compared mammary cell proliferation and apoptosis when cows were fed high or low energy diets in conjunction

with 2x or 3x milking for 8 wk followed by 2x thereafter. In this study researchers found increased milk yields in cows fed the high energy diet but only a tendency for increased production in cows milked 3x during the first 8 wk; there was no difference in milk yield between milking intensity groups after wk 8. Norgaard el al. (2005) did not find differences among groups for DNA concentrations in mammary gland tissue. However, cows consuming the high energy diet had 8.6% more proliferating cells at wk 8 than cows fed the low energy diet. Apoptotic rates did not differ among treatments. Frequency of milking did not result in differences in cell proliferation or apoptosis at wk 8 or 16.

Milking frequency has been shown to increase milk yield in the frequently milked glands without affecting the yield of the opposing gland when the treatment is given in only half the udder. This response suggests a local regulation that is responsive to frequent milking (Hillerton et al. 1990, Wall and McFadden, 2007).

The objectives of this study were to analyze the effects on energy metabolism and mammary cellular proliferation of cows milked 4x during the first 21 d and 2x thereafter compared to cows milked 2x during the entire period. These effects were evaluated by measuring changes in plasma metabolites, BCS and BW, and mammary cell proliferation through incorporation of Ki-67 antigen.

Materials and Methods

Animals, treatments and sampling

The Cornell University Institutional Animal Care and Use Committee approved all procedures involving animal use prior to the commencement of the experiment. The study started in January 2007 and ended in November 2007. Holstein cows (n=60) were assigned to either a 2x or 4x milking treatment one day after parturition. Half of the cows were multiparous (n=30) and the other half were primiparous cows (n=30). A total of 15 multiparous cows and 15 primiparous cows were assigned to the 4x milking group and 15 multiparous cows and 15 primiparous cows were used as 2x controls. All cows were milked at 900 h and 2030 h and the cows on the IMF treatment were milked again at 1600 h and 0400 h. After d 21 postpartum, all cows were milked 2x until 119 d of lactation. Cows were housed in individual tiestalls throughout the experiment.

Milk weights were collected electronically at every milking and milk samples were collected once weekly from all milkings and composited into a single weekly sample. The composite sample was analyzed for content of milk fat, true protein, somatic cell count, lactose and milk urea N using midinfrared spectroscopy according to AOAC (2001) methods (DairyOne Cooperative, Ithaca, NY).

All cows were fed the same diet (Table 3.1) for ad libitum intake once daily at 0900 h. Amounts of feed offered and refused were recorded on a daily basis. A weekly sample of the TMR was dried at 55°C until static weight and weekly DM content of the TMR were used

Table 3.1. Ingredient and chemical composition (DM basis) of postpartum diets.

Ingredient	% DM
Corn silage	36.67
Haycrop silage	15.69
Shelled corn, finely ground	12.39
Distillers grains	8.66
Wheat middlings	7.05
Corn germ meal	6.03
Soybean meal, (47.5% CP)	4.21
Molasses	1.56
Calcium carbonate	0.65
Whole cottonseed	0.98
Blood (Flash-dried)	1.10
SucraPlex	1.76
Energy Booster 100™ [1]	0.62
Sodium bicarbonate	0.61
Mixer fat	0.45
Urea	0.37
Salt	0.32
1000 vit-min premix [2]	0.69
Magnesium oxide	0.07
Alimet[3]	0.05
Soybean hulls	0.00
Calcium sulfate	0.04
Sel-Plex 2000™ [4]	0.01
Chemical[5]	
NE_L, [6] Mcal/kg	1.68 (0.01)
CP	17.8 (0.4)
Acid detergent insoluble CP	0.87 (0.22)
ADF	19.2 (1.1)
NDF	31.4 (1.6)
Ca	0.99 (0.07)
P	0.44 (0.02)

[1]Prilled saturated free fatty acids, MS Specialty Nutrition, Dundee, IL.
[2]Contained 36% Ca, 0.009% P, 0.949% Mg, 0.839% S, 1,274 ppm Cu, 6,040 ppm Mn, 165 ppm Co, 128 ppm I, 7,371 ppm Zn, 1,204 IU/kg of vitamin A, 225 IU/g of vitamin D, and 2,305 IU/kg of vitamin E.
[3]2-hydroxy-4-(methylthio)-butanoic acid (Novus International, St. Louis, MO)
[4]Selenium yeast, Alltech Inc., Nicholasville, KY.
[5]Means and standard error of 10 composite samples of TMR.
[6]Calculated by Dairy One Cooperative (Ithaca, NY) using NRC (2001) equations in calculations of DMI.

weekly samples were ground through a 2-mm screen in a Wiley mill and used to prepare 4-wk composite samples. Composite TMR samples were analyzed using wet chemistry techniques (Dairy One Cooperative Inc., Ithaca, NY) for DM (method 930.15; AOAC, 2000), CP (method 990.03; AOAC, 2000), ADF and NDF (Van Soest et al., 1991), acid detergent insoluble CP (Licitra et al., 1996), and macro- and microminerals (Sirois et al., 1994).

Body weights were measured for each cow on one day per week after the 0900 h milking. Body condition scores were assessed by three individuals on the same day using a 1 to 5 scale (Wildman et al., 1982); BCS values from the 3 individuals were averaged prior to statistical analysis.

Plasma and tissue sampling and analysis

Blood samples were collected via venipuncture of the coccygeal vein or artery into evacuated test tubes containing sodium heparin (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ) within the first 24 h after parturition, three times per week for the first 21 d postpartum, and twice weekly thereafter until 56 d postpartum. All blood samples were taken at 800 h before feeding. Blood samples were centrifuged (2,800 x *g*, 15 min, 4^0C), plasma was aliquotted into microcentrifuge tubes, and stored at -20°C until analysis.

Plasma NEFA concentrations were analyzed by enzymatic analysis (NEFA-C; WAKO Pure Chemical Industries, Osaka, Japan) using modifications described by McCutcheon and Bauman (1986).

Plasma BHBA concentrations were determined by enzymatic analysis (BHBA dehydrogenase; kit #310, Sigma Chemical).

Mammary biopsies were performed on eight multiparous cows and eight primiparous cows in each treatment within 24 h postpartum, at 21 d postpartum, and 75 d postpartum. Biopsies (two sites per sampled quarter) were performed using a Magnum biopsy gun (Magnum Instrument MG1522, Bard Peripherial Vascular, Tempe, AZ). The quarter was shaved and washed with a diluted Betadine solution prior to biopsy. The site of the incision was scrubbed twice with spiral movements starting at the incision site with surgical scrub [Betadine Surgical Scrub (7.5% povidone-iodine); Purdue Frederick, Stamford, CT] and then rinsed with ethyl alcohol (70%). Cows were administered 0.5 cc of the sedative xylazine hydrochloride (Rompun 2%, Bayer Inc., Sarnia, Ontario, Canada) via venipuncture of the coccygeal vessels. Twenty cc of a local anesthetic (Lidocaine-HCl, 2%; Butler Animal Health, Dublin, OH) was administered subcutaneous above the incision. A perpendicular incision (~ 2.5 cm) was made on the outside of the quarter using a scalpel blade (size 22), a guide was inserted into the quarter (C12168, Bard TruGuide Coaxial Biopsy Needle, 11g x 13 cm. Bard Peripherial Vascular, Tempe, AZ), on a slight downward angle and to the side. The biopsy needle was inserted into the guide (MN1216, Bard Magnum Disposable Needle, 12g x 16cm. Bard Peripherial Vascular, Tempe, AZ), and three samples were taken. This process was repeated after repositioning the guide about 5 cm lateral to the first sampling site. One sample was placed into a foil pack, frozen in liquid N_2 and stored at -80° C; another sample was

fixed, frozen in liquid N_2 and stored at -80° C; the last sample was immersed in a solution of 10% formaldehyde for 24 h at 4° C and then transferred to a solution of 93% ethanol to be stored at 4° C. Samples were submitted to a commercial laboratory to be mounted in paraffin for histochemistry (American Histolabs, Gaithersburg, MD).

Immunohistochemistry and slides analysis

Immunohistochemical localization of Ki-67 cell proliferation antigen was performed using a procedure modified from that described by Capuco et al. (2001). Samples were deparaffinized and hydrated in a graded series of ethanol. Slides were heated in a microwave at high power (650 W) in 400 mL of citrate buffer (10mM) in a covered glass staining dish for 5 min. They remained in the dish for 5 min after which they were microwaved for another 5 min. Thereafter, the slides remained in the buffer solution for a 30 min cooling period. Samples were washed in double distilled water (3 x 2 min). Samples were then quenched in a 3% H_2O_2 solution for 10 min. Samples were washed in double distilled water (1 x 2 min) and then in PBS (3 x 2 min). Slides were incubated for 10 min with 100 uL of CAS block (5% non-immune goat serum in PBS). Slides were then incubated for 60 min with MIB-1 (monoclonal antibody (Prediluted MIB-1, Zymed Invitrogen, Carlsbad, CA). Samples were washed in PBS with 0.05% Tween-20 (3 x 2 min). Slides were then incubated for 30 min with the secondary antibody, broad spectrum poly HRP conjugate (Super picture kit, Zymed Invitrogen, Carlsbad, CA). Samples were washed in PBS with 0.05% Tween-20 (3 x 2 min). Slides were incubated with DAB chromogen

(Super picture kit, Zymed Invitrogen, Carlsbad, CA) for 4 min. Samples were washed in double distilled water in the dark (3 x 2 min); then samples were counterstained with hematoxylin for 1 min. Samples were washed in tap water (1 x 2 min) and set in PBS until color developed (~ 30 sec), rinsed in double distilled water (1 x 5), dehydrated and then mounted with Permaslip.

Slides were set under the microscope using a 10x magnification lens out of focus to randomly select between 6 to 10 sites to be photographed, once the site was selected a 40x magnification lens was used to photograph the slide. Each picture was analyzed for number of epithelial cells and number of ki-67 labeled cells using Cell Counter 2000 (Steve Ellis, Beltsville, USDA, MD).

Statistical analysis

All variables measured over time were subjected to analysis of variance for a completely randomized design with repeated measures using the MIXED procedures of SAS (2001). Terms in the model included treatment, lactation group, time (week or day), and all 2-way and 3-way interactions. For the analysis of BHBA and NEFA concentrations, results from plasma collected immediately postcalving were used as covariates. For the analysis of mammary biopsies percent label cells, cell counts of biopsy performed after calving was use as covariates. The denominator degrees of freedom were adjusted using the method of Kenward Rogers, and each model was tested using 4 different covariance structures (autoregressive order one, autoregressive order one with heterogeneous variance, compound

symmetry, and compound symmetry with heterogeneous variance). The model with the lowest Akaike's Information Criterion was selected; in almost all cases this was the model using the autoregressive order one with heterogenous variance covariance structure. In a separate analysis, statistical analyses for DMI and milk yield were repeated using a dataset in which all cows that had received mammary biopsies had been removed in order to assess the effects of treatment independent of the effects of mammary biopsy.

Difference in mammary epithelial cell-related measurements between individual biopsy days were analyzed as a completely randomized design using the MIXED procedure of SAS (2001). The model included the term of treatment.

Significance was declared at $P = 0.05$ and trends were declared at $0.05 < P < 0.10$. Least squares means are presented throughout.

Results

Overall results for milk yield and milk composition for multiparous and primiparous cows for the control group (milked 2x from calving until 119 d postpartum) and for the IMF group (milked 4x from d 1 to 21 and 2x from d 22 until d 119) are presented in Table 3.2. The interaction of treatment and lactation group was not significant for any of the production variables.

The overall milk yield of IMF cows was numerically increased by 1.4 kg/d during the first 17 wk (42.0 vs. 40.6 kg/d); however, this difference was not significant ($P = 0.25$). The interaction of treatment by week was significant for milk yield ($P < 0.01$) such that milk yield of

cows milked 4x was increased during the first 3 wk of lactation during the period of IMF, but similar to controls thereafter (Figure 3.1).

Table 3.2. Least squares means and standard errors for milk yield and milk components during the first 17 wk of lactation for multiparous (2+ lact) and primiparous (1st lact) cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period.

	Control		IMF			P		
Item	1st lact	2+ lact	1st lact	2+ lact	SEM	Trt	Lact	Trt*Lact
DMI, kg	18.2	23.5	18.2	23.5	0.4	0.97	<0.01	0.97
Milk, kg/d	35.1	46.1	37.8	46.2	1.3	0.25	<0.01	0.31
Fat, %	3.57	3.40	3.42	3.43	0.14	0.65	0.53	0.51
Fat, kg/d	1.21	1.51	1.26	1.56	0.06	0.41	<0.01	0.97
True protein, %	2.96	3.03	2.89	3.04	0.03	0.44	<0.01	0.22
True protein, kg/d	1.03	1.38	1.09	1.40	0.03	0.22	<0.01	0.66
Lactose, %	4.99	4.81	4.90	4.76	0.03	0.05	<0.01	0.50
Lactose, kg	1.76	2.22	1.86	2.22	0.06	0.41	<0.01	0.40
Total Solids, %	12.41	12.15	12.12	12.16	0.17	0.39	0.51	0.35
Total Solids, kg	4.31	5.54	4.55	5.62	0.12	0.20	<0.01	0.50
3.5% FCM[1], kg/d	34.8	44.5	36.8	45.5	1.4	0.27	<0.01	0.71
ECM[2], kg/d	34.2	44.1	36.1	45.1	1.3	0.25	<0.01	0.72
MUN, mg/dL	11.5	10.9	11.9	10.7	0.3	0.71	0.01	0.36
LS	1.9	2.0	2.0	2.0	0.1	0.62	0.64	0.63

[1]Formula for 3.5% energy corrected milk [(0.4324* kg milk)+(16.216* kg Fat)]
[2]Value corrected for 3.5% fat and 3.2% true protein using formula from NRC (2001) [(0.3246* kg milk)+(12.86* kg Fat)+(7.04* kg True protein)]

During the first 21 d postpartum, cows subjected to IMF produced 4.3 kg/d more than controls (38.1 vs. 33.9 kg/d; $P < 0.01$). Because of the potential that mammary biopsies conducted on d 21 and 75 postpartum on a subset of cows could have affected milk yield, statistical analysis was conducted using only data from cows that were not biopsied. Although overall effects of treatment were not significant ($P = 0.36$), there was an interaction of treatment and lactation group (P

< 0.01) such that early lactation IMF increased overall milk yield of primiparous cows (34.2 kg/d for control vs. 40.1 kg/d for IMF) but not multiparous cows (50.1 kg/d for control vs. 47.1 for IMF). Furthermore, there was a trend for an interaction of treatment, lactation group, and week (P = 0.07) such that milk yield was increased consistently throughout the experimental period in primiparous cows, but IMF increased milk yield during early lactation and appeared to result in decreased persistency in multiparous cows (Figure 3.2).

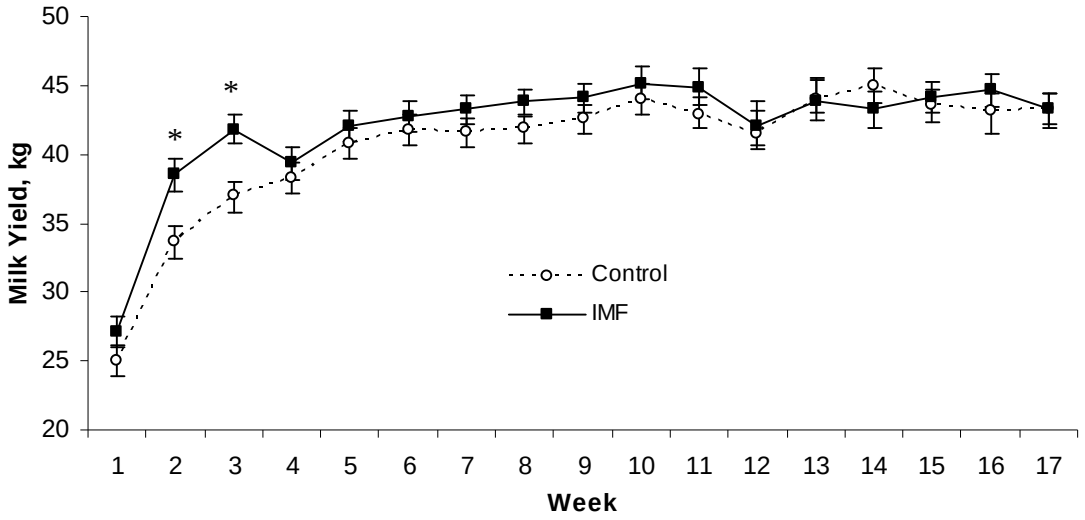

Figure 3.1. Least squares means and standard errors for milk yield during the first 17 wk of lactation for cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period. The P value for the effect of treatment was 0.25 and the interaction of treatment and week was < 0.01. Significant differences at individual timepoints are denoted by an asterisk.

With the exception of lactose percentage, none of the other milk components were different between treatments, expressed either as

percentages or yields (P > 0.10). The interaction of treatment by week, however, was significant for true protein yield, lactose yield, and total solids yield (P < 0.02) due to an increase of these component yields during the first 21 d in the IMF group after which these values decreased to converge with those of the control group. Linear score also had a significant interaction between treatment and week due to high values for the IMF group at wk 4 and 13; no difference was detected at any other timepoint. Overall yields of 3.5% fat-corrected milk and energy-corrected milk were not affected by treatment; however, both variables had treatment by week interactions (P < 0.01) such that yields were increased during early lactation by IMF and comparable to controls thereafter.

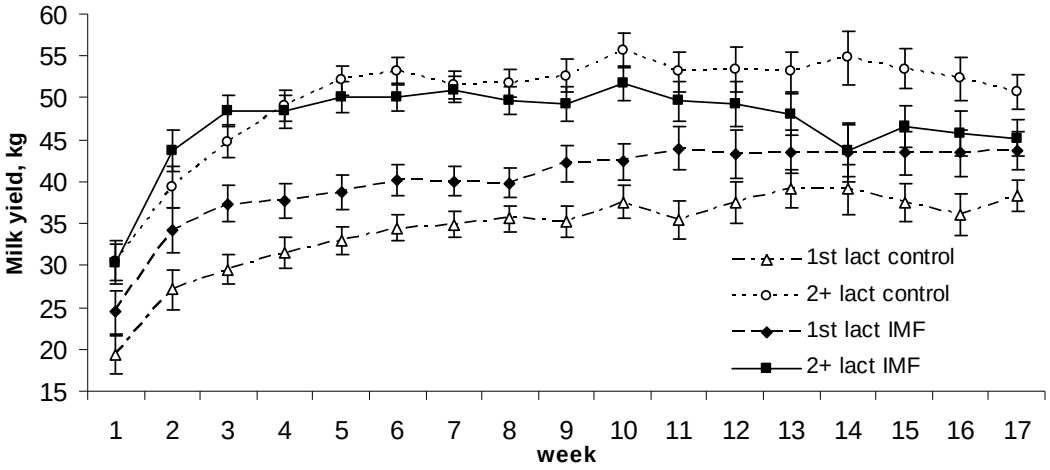

Figure 3.2. Least squares means and standard errors for milk yield during the first 17 wk of lactation for multiparous and primiparous cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period that not received mammary biopsies. The P value for the effect of treatment was 0.36, the interaction of treatment and week was 0.02 and the interaction between treatment and lactation group by week was 0.07

Overall DMI of cows was not affected by treatment (Table 3.2; Figure 3.3). When DMI was analyzed for only the first 21 DIM, cows in the IMF treatment tended to consume ~ 0.9 kg/d more DM than controls (16.1 kg/d for control vs. 17.0 kg/d for IMF P = 0.08).

Weekly measures of BCS and BW were compared between treatments; neither of these variables was affected by treatment (P > 0.2), and no significant interactions of other model terms with treatment were detected for either of these variables (Table 3.3).

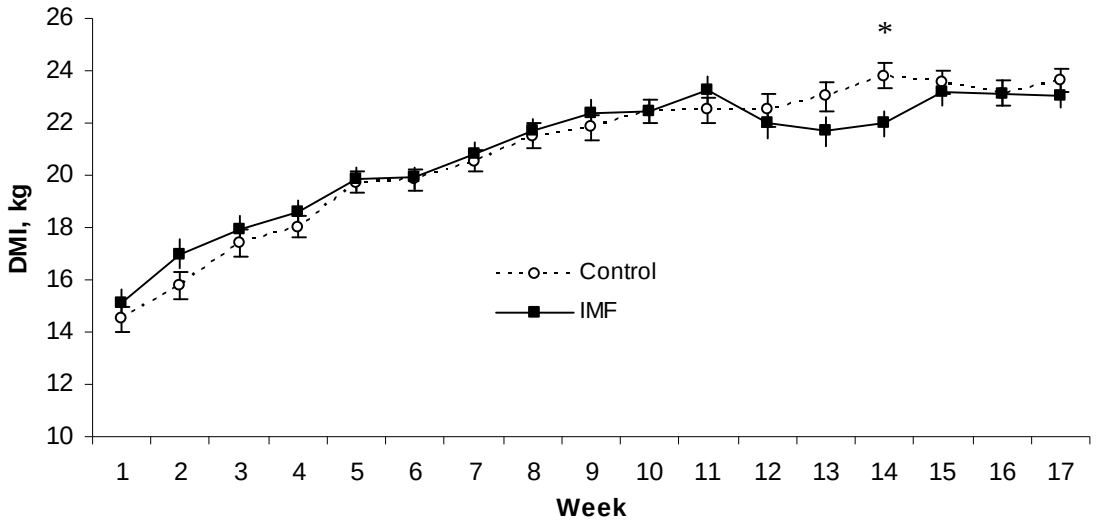

Figure 3.3. Least squares means and standard errors for dry matter intake (DMI) during the first 17 wk of lactation for cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period. The P value for the effect of treatment was 0.97 and the interaction of treatment and week was 0.14. Significant differences at individual timepoints are denoted by an asterisk.

Energy balance was calculated using NRC (2001) equations for each cow. Difference in calculated energy balance between treatments

was not significant (P = 0.38; Table 3.3) and no significant interactions of treatment existed with other terms in the model (P > 0.10).

Table 3.3. Least square means and standard errors for body condition score (BCS), body weight (BW), energy balance, B-hydroxybutyrate (BHBA), and non-esterified fatty acid (NEFA) concentrations for multiparous (2+ lact) and primiparous (1st lact) cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period.

	Control		IMF			P		
Item	1st lact	2+ lact	1st lact	2+ lact	SEM	Trt	Lact	Trt*Lact
BCS	3.22	2.80	3.10	2.80	0.08	0.43	<0.01	0.48
BW	551	650	535	632	14	0.23	<0.01	0.98
Energy Balance[1]	-2.57	-1.47	-3.22	-1.67	0.50	0.38	<0.01	0.64
NEFA, µEq/L	563	468	583	679	49	0.02	0.99	0.05
BHBA, mg/dL	8.57	8.37	8.99	9.41	0.52	0.16	0.83	0.55

[1]Energy balance calculated using NRC (2001) equations

Concentrations of NEFA in blood plasma were also analyzed using concentrations at calving as a covariate. A three-way interaction of treatment, lactation group, and day existed (P < 0.05) for plasma NEFA such that multiparous cows subjected to IMF had increased plasma NEFA during the period of IMF, but were comparable to multiparous cows milked 2x thereafter (Figure 3.4). Concentrations of plasma NEFA in primiparous cows were comparable between treatments throughout the study period. This interaction was the primary contributor to the overall effect of treatment (P = 0.02) and the interaction of treatment and lactation group (P = 0.05) detected for plasma NEFA (Table 3.3). Despite these effects of treatment on plasma NEFA, plasma concentrations of BHBA were not affected (P = 0.16) by treatment (Table 3.3).

Effects of treatment and the interaction of treatment by day for the total number of mammary epithelial cells for biopsies performed at d 21 and d 75 postpartum were not significant. Significant differences were only observed for day (293 at 21 d vs. 273 at 75 d P = 0.03, although total number of cells is highly dependent upon the biopsy sample collected.

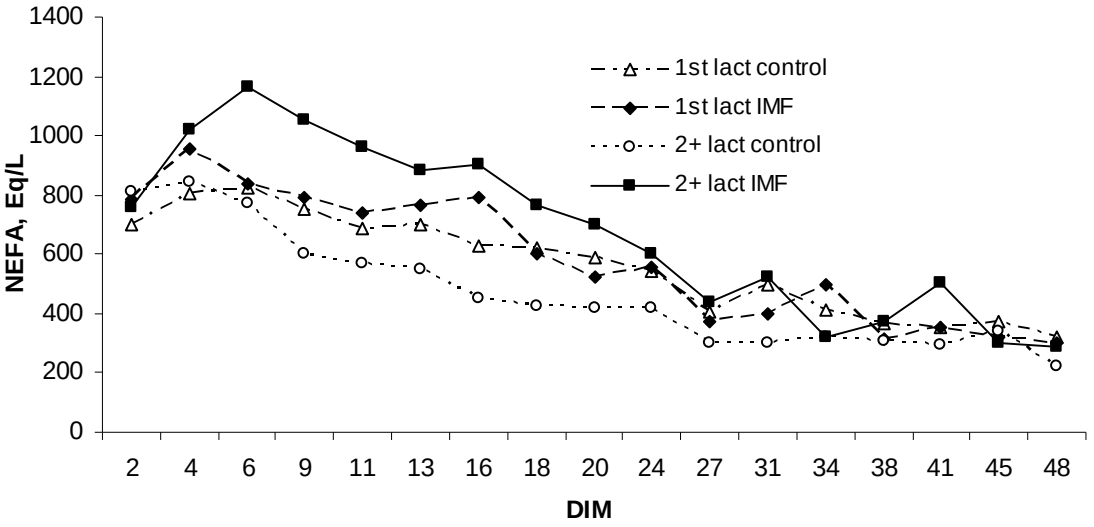

Figure 3.4. Least squares means and standard errors for plasma concentrations of nonesterified fatty acids (NEFA) during the first 48 d of lactation for multiparous (2+ lact) and primiparous (1st lact) cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period. The P value for the effect of treatment was 0.02 and the interaction of treatment, lactation group and day was 0.03.

The use of percent labeled epithelial cells accounts for differences in biopsy sites, therefore it is not affected by tissue collection. Effects of treatment, lactation group, day, or the interaction of treatment and lactation group for percent labeled epithelial cells were not significant. However, a treatment by day interaction (P =

0.03) existed such that IMF cows had 0.5% less labeled epithelial cells than control at d 21 (0.82% for control vs. 0.31% for IMF) but 0.7% more labeled epithelial cells at d 75 than control cows (0.65% for control v. 1.37% for IMF; Figure 3.5).

Differences in the percentages of labeled epithelial cells between the timepoints are presented in Table 3.4. Differences among treatments were not significant between d 0 and d 21 (P = 0.68); However, trends were detected for the effects of lactation group and the interaction of treatment and lactation group (P = 0.08). The differences between d 0 and d 75 were not different (P = 0.27), even though there was almost a three-fold difference in the calculated mean due to a high error (1.92 for control cows vs. 0.61 for IMF cows with a standard error of 0.83). The difference among treatments was mainly driven by multiparous cows, the interaction between treatment and lactation group presents a trend (P = 0.09) where first lactation animals had similar differences between treatments (0.11 for control primiparous cows vs. 0.85 for IMF primiparous cows).

The difference between d 21 and d 75 was significant for treatment (P = 0.04), control cows declined in percentage of label epithelial cells by 0.2 from d 21 to d 75 while IMF cows increased the percent label epithelial cells by 1.2 from d 21 to d 75. From d 21 to d 75 there was no difference for lactation group or treatment and lactation group interaction (P > 0.1); (Figure 3.6).

Table 3.4. Least squares means and standard errors for the difference in mammary epithelial cells labeled with Ki-67 for multiparous (2+ lact) and primiparous (1st lact) cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period.

	Control		IMF			P		
	1st lact	2+ lact	1st lact	2+ lact	SEM	Trt	Lact	Trt*Lact
Change between 0 and 21DIM[1]	0.13	-4.70	-1.78	-1.71	1.36	0.68	0.08	0.08
Change between 0 and 75 DIM[2]	-0.12	-3.73	-0.85	-0.36	1.31	0.27	0.2	0.09
Change between 21 and 75 DIM[3]	-0.55	0.13	0.55	1.79	0.65	0.04	0.14	0.66

[1]Values represent percent labeled epithelial cells at d 0 minus percent labeled epithelial cells at d 21

[2]Values represent percent labeled epithelial cells at d 0 minus percent labeled epithelial cells at d 75

[3]Values represent percent labeled epithelial cells at d 21 minus percent labeled epithelial cells at d 75

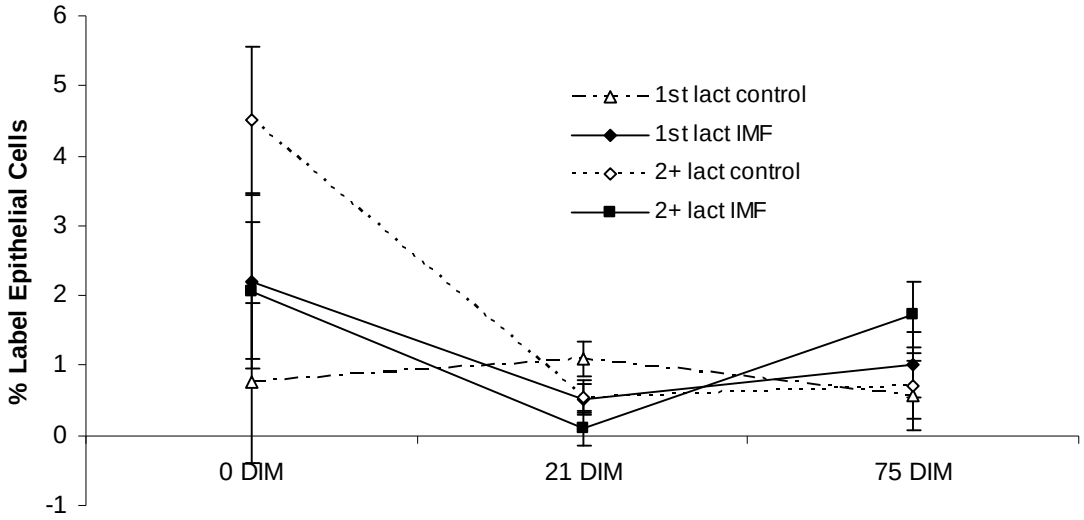

Figure 3.5. Least squares means and standard errors for percent labeled epithelial cells at day of biopsy for multiparous (2+ lact) and primiparous (1st lact) cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period. The P value for the effect of treatment was 0.67 and the interaction of treatment and day was 0.03.

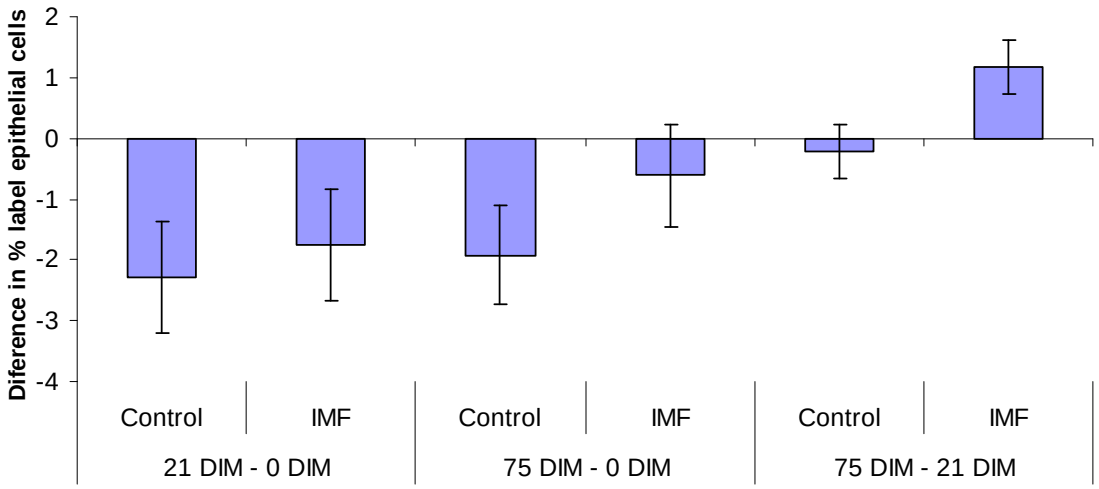

Figure 3.6. Mean difference and standard errors for percent labeled epithelial cells at each biopsy day for cows milked either 2X (control) for the first 21 d or 4X (IMF) for the first 21 d postpartum followed by 2X for the remainder of the period. The P value for the difference between 21 DIM and 0 DIM was 0.68; for the difference between 75 DIM and 0 DIM was 0.27 and for the difference between 75 DIM and 21 DIM was 0.04.

Discussion

The milk yield increase from IMF during the treatment period was similar to the milk yield increase found in the commercial farm experiment (Chapter II). In this experiment, milk yield was increased by 4.3 kg/d during the first 21 d for cows subjected to IMF; in the previous study IMF cows increased milk yield by 4.3 kg/d at the first test day. In a previous study in our group, Fernandez (2004) reported an increase of 3 kg/d during the first 2 test days for cows subjected to IMF. These responses are generally lower than those described by others. Bar-Peled et al. (1995) observed an increase in milk yield of 7.3 kg/d during the first 42 d for cows milked 6x compared to 3x controls, Hale et al. (2003) detected a milk yield response of 8.8 kg/d during 21 d of IMF for cows milked 4x compared to 2x controls, and Dahl et al.

(2004) observed 8.4 kg/d more milk by cows milked 6x during the first 21 d postpartum compared to 3x controls.

Overall milk yield responses to IMF were not significant when assessed across the first 117 d postpartum; this response was not expected and is not consistent with the results presented in Chapter II. Milk yield was increased by IMF during the treatment period by 4.3 kg/d but converged with milk production from the control group from wk 4 on. Results summarized from dataset in which the subset of cows receiving mammary biopsies was eliminated were different in that although multiparous cows continued to have minimal overall response to early lactation IMF, primiparous cows subjected to early lactation IMF consistently yielded more milk than controls throughout the study period. This suggests that the mammary biopsy procedure influenced milk yield responses in affected cows.

Despise an expected increase in DMI from IMF cows, overall differences between treatments were not significant. As expected, the DMI tended to increase during the 21-d period of IMF in which milk yield was increased by IMF. Consistent with the minimal response in overall milk yield, effects of treatment on BCS, BW, or calculated energy balance were not significant. Control cows and IMF cows did not differ from each other in BCS or BW throughout the 18 wk period. These results are in agreement with those in Hale et al. (2003), who did not find a difference in BCS between treatments.

Multiparous cows in the IMF group had higher blood concentrations of NEFA than control cows during the period of IMF. The interaction between treatment and lactation group for NEFA levels

and the trend for treatment by lactation group by week interaction for milk yield may suggest a detrimental effect of high NEFA concentrations on milk production; providing a possible explanation for the lack of carry over from multiparous cows on the IMF treatment.

In the experiment in Chapter II, two out of four farms had higher NEFA values for IMF cows, while the other two farms demonstrated no difference. Other studies have reported no effects of IMF on NEFA concentrations. Fernandez (2004) did not find a difference between treatments for NEFA concentrations. Andersen et al. (2004) reported that increasing milking frequency from 2x to 3x during early lactation did not affect plasma NEFA values, but increased plasma BHBA. The present study did not found differences between treatments for plasma concentrations of BHBA.

Results from mammary biopsies were inconclusive; the decrease in percent epithelial cells from 0 DIM to 21 DIM follows a similar pattern as that found by Hale et al. (2003) in one of their treatments. However differences between treatments were not significant in either study. The increase in percent labeled epithelial cells in the IMF group at d 75 is not comparable to previous research; since the cows subjected to IMF had a higher percent labeled cells, but did not differ in milk yield compared to controls at that timepoint. Norgaard et al. (2005) did not observe differences in milk production or cell proliferation for cows milked 3x for 8 wk versus cows milked 2x for 8 or 16 wk. However, they reported increased proliferation of mammary epithelial cells at wk 8 postpartum in cows feed a higher energy diet. Even though energy balance as calculated with NRC (2001) equations

was not different among treatments, high levels of plasma NEFA may help explain the decrease in mammary cell proliferation rates at d 21 for the IMF group in this study

As explained by Capuco et al. (2001), milk production is a function of both number and activity of epithelial cells in the mammary gland. The number of epithelial cells in the mammary gland is a factor of the rate of proliferation minus the rate of apoptosis or better described as the turnover rate. Capuco et al. (2001) described the increased milk yield at the beginning of the lactation as a function of the increased activity in epithelial cells and the decline in milk yield during mid and late lactation as a factor of the lost of epithelial cells. Even though activity of epithelial cells and apoptotic rate were not measured in this study, different behaviors were found in the proliferation rate of IMF cows and control cows throughout the first 75 d of lactation, which leads us to think there may be an effect of IMF on mammary cell dynamics. However, future research is needed to better understand these effects.

Conclusions and implications

Early lactation IMF resulted in increased milk yield during the period of IMF treatment, but minimal carryover effects on milk yield. Responses appeared to be greater in primiparous than multiparous cows. In general, IMF did not affect energy metabolism, although the increased plasma NEFA concentrations in multiparous cows may have affected the potential for overall response to IMF. Effects of IMF

appear to not be related to differences in mammary cell proliferation, and other potential mechanisms warrant investigation.

CHAPTER IV

Summary and economic analysis

Summary

When results of these experiments are considered collectively with other studies in the literature, IMF during early lactation generally increases milk yield not only during the period of IMF but also after treatment ceases. In most cases the increase in milk production does not result in apparently negative effects on energy balance. However, for reasons that remain uncertain, cows do not always have carryover responses to IMF. The lack of response may be associated with the need to increase fat mobilization to support production. Although the mammary gland response appears to be related to local mechanisms, it is still uncertain whether IMF increases overall milk yield through increased activity or number of mammary epithelial cells.

Milk fat and protein percentages are generally decreased by early lactation IMF; however, the increased milk yield more than compensates of the decreased percentages such that overall yields of milk components are increased.

The increase in milk yield by IMF was not clearly explained by mammary cell proliferation evaluated through Ki-67 incorporation. However, differences between IMF cows and control cows were found at d 75 where IMF cows had a higher percentage of proliferating epithelial cells than control cows. More research is needed to

determine the mechanisms through which IMF causes an increase in milk yield after treatment has ended.

Economic analysis

The final objective of any management tool implemented in a dairy farm is to increase profitability. A dairy producer has to consider the additional cost before making the decision to implement or not to implement a management technique. The additional milk production achieved with IMF is accompanied by an increase in cost of feed and extra labor.

To better understand the benefits of IMF in dairy farms, this section compares the profitability of cows milked 4x for 21 d and 2x the remainder of the lactation (4x-2x) as well as cows milked 3x throughout the entire lactation (3x) to cows milked 2x throughout the entire lactation (2x).

Energy-corrected milk (3.5% fat and 3.2% true protein) yields were used to adjust milk yield based upon the differences in milk composition resulting from the different milking schemes. Milk response from Chapter II was used as the response to 4x-2x (1.52 kg/d of ECM) and the average response found by Erdman and Varner (1995) was used for the response of 3x over 2x (3.0 kg/d of ECM).

Milking parlor efficiencies vary among dairy farms in the number of workers per hour, number of cows milked per hour, electricity use per hour as well as a variety of other factors. The size of the dairy farm is another key difference that must be taken into consideration when performing an economic analysis.

The economic analysis presented here was performed for both small and large farms. Different assumptions were made accordingly. Assumptions that apply for both scenarios include the price of feed at $0.19 per kg, energy density at 1.68 Mcal/kg of feed (from wet chemistry analysis in Chapter III), a 10% reduction in the time a 3x cow takes to be milked each time it is milked (Thomas et al., 1995), a total cost per worker including all associated costs of $12.00 per h ($10.00 wage plus 20% social security and other taxes), a total electricity cost of running the milking parlor of $20.00 per h and the cost of milking supplies and chemicals of $0.15 per cow per milking. Additional feed consumed was estimated using NRC 2001 guidelines.

For the small farms, defined as having 200 milking cows, the assumption for total workers (equivalent as labor force in the milking parlor per h) was made at 2.5 workers. When the milking capacity is fixed at 50 cows per h and the only variable is the milk price per cwt, 3x milking is less profitable than 2x milking under milk prices of $15.70/cwt while 4x-2x milking remains more profitable than 2x at milk prices above $8.35/cwt. 4x-2x milking provides higher marginal profit than 3x milking at milk prices under $23.39/cwt (Figure 4.1).

Considering a fixed price of $18.00/cwt and varying milking capacity, 3x milking presents a marginal profit over 2x milking when milking more than 42 cows per h. In milking parlors with a capacity to milk 72 cows per h or less, 4x-2x is more profitable than 3x milking. Milking parlors that allow one to milk more than 72 cows per h with the same 2.5 worker equivalents per h are more profitable milking 3x at $18.00/cwt (Figure 4.2).

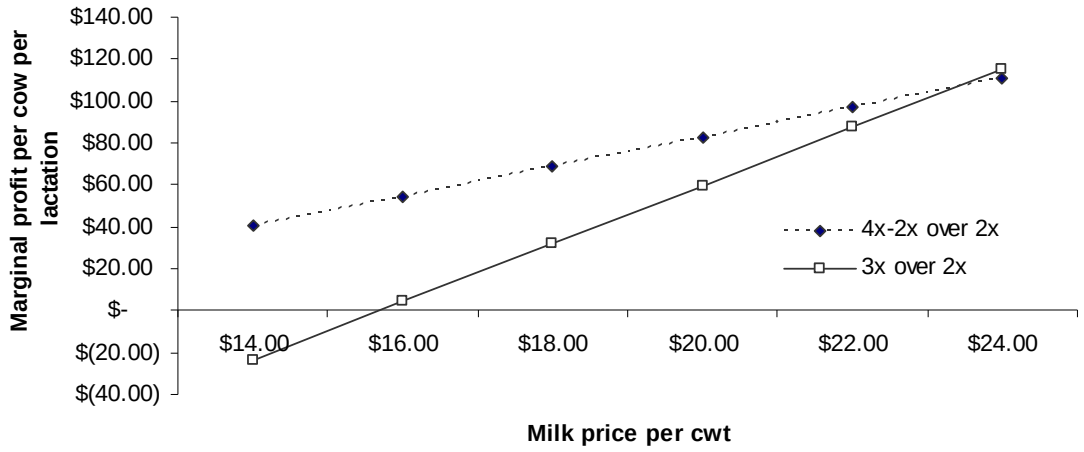

Figure 4.1. Additional income per cow per lactation for cows milked 4x during the first 21 d of lactation and 2x thereafter (4x-2x) or milked 3x during the entire lactation over cows milked 2x during the entire lactation at a farm with 200 cows in milk, a milking capacity of 50 cows per h and labor force of 2.5 worker equivalents at $12.00 per worker per h with variable milk prices.

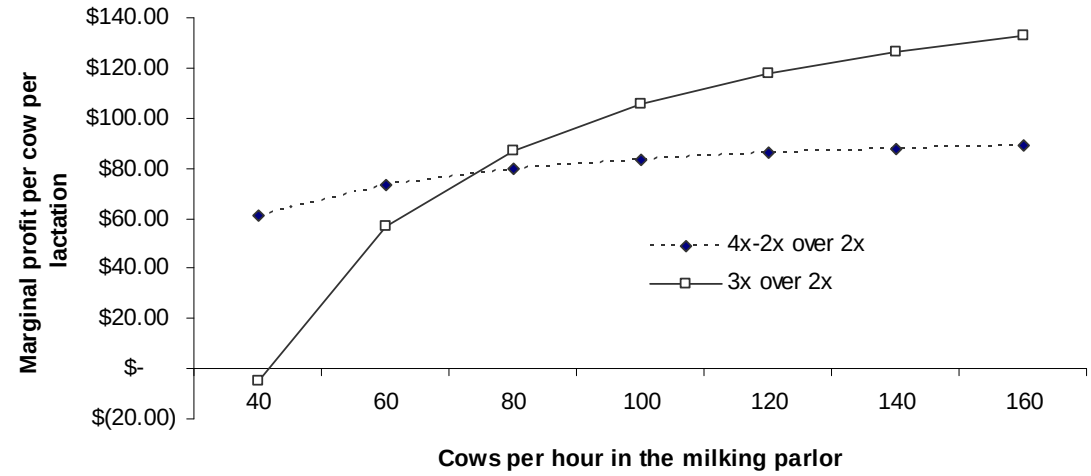

Figure 4.2. Additional income per cow per lactation for cows milked 4x during the first 21 d of lactation and 2x thereafter (4x-2x) or milked 3x during the entire lactation over cows milked 2x during the entire lactation at a farm with 200 cows in milk, a labor force of 2.5 worker equivalents at $12.00 per worker per h with a milk prices of $18.00/cwt.

At $18.00/cwt, a farm milking 50 cows per h with 2.5 worker equivalents will gain an additional profit per cow per lactation of $68.72 with a 4x-2x milking scheme and $31.99 with 3x milking over a 2x milking scheme (Figure 4.3).

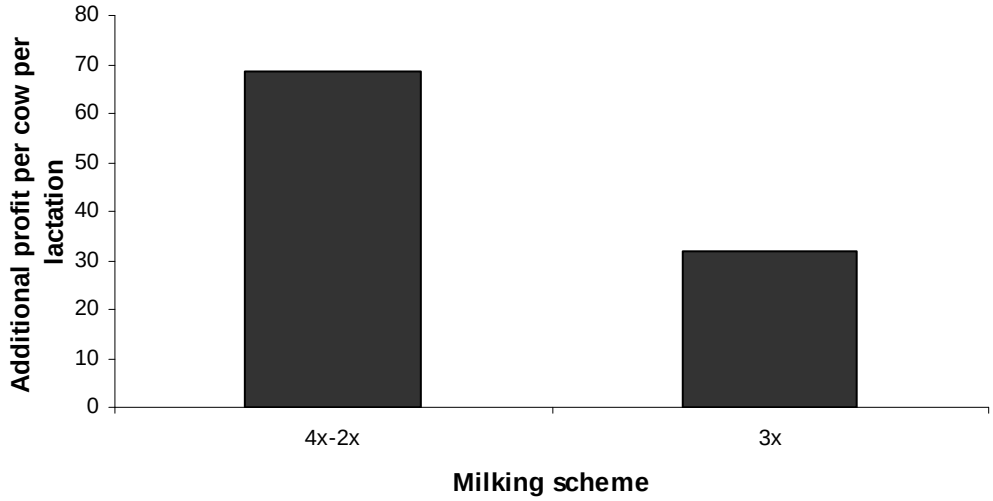

Figure 4.3. Additional income per cow per lactation for cows milked 4x during the first 21 d of lactation and 2x thereafter (4x-2x) or milked 3x during the entire lactation over cows milked 2x during the entire lactation at a farm with a milking capacity of 50 cows/h, a labor force of 2.5 worker equivalents at $12.00 per worker per h with a milk prices of $18.00/cwt.

When analyzing large farms, defined as having 1,000 milking cows, 5 worker equivalents were assumed per h. When using a fixed milking capacity of 200 cows per h and only varying milk price, 3x milking is more profitable than 2x when milk prices are above $9.35/cwt, while 4x-2x milking is more profitable than 2x at milk prices above $5.90/cwt. A 3x milking scheme on large farms is more profitable than 4x-2x milking at milk prices above $13.03/cwt (Figure 4.4).

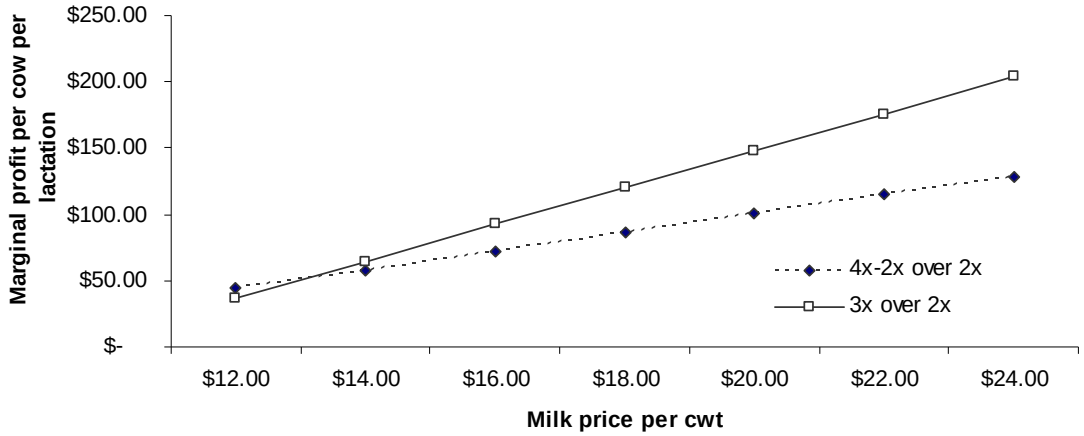

Figure 4.4. Additional income per cow per lactation for cows milked 4x during the first 21 d of lactation and 2x thereafter (4x-2x) or milked 3x during the entire lactation over cows milked 2x during the entire lactation at a farm with 1,000 cows in milk, a milking capacity of 200 cows per h and labor force of 5 worker equivalents at $12.00 per worker per h with variable milk prices.

When using a fixed milk price of $18.00/cwt, 3x milking is more profitable than both 2x and 4x-2x milking if the milking parlor has the capacity of milking 130 cows per h or more (Figure 4.5). This would be the minimum capacity for a parlor milking 1,000 cows three times a day (3x) because it requires 23 h per d in milking time.

At a milk price of $18.00/cwt, a farm milking 200 cows per h with 5 worker equivalents will have an additional profit per cow per lactation of $86.36 when milking 4x-2x compared to 2x milking and an additional $33.83 when milking 3x compared to 4x-2x milking (Figure 4.6).

The relationship between milk price and marginal profitability behaves differently for small herds than for large herds. Table 4.1 shows the relationship between milk price, milking capacity and number of worker equivalents with marginal profit per cow of 4x-2x vs

2x milking and 3x vs. 2x milking. In general terms, under the assumptions used for this evaluation, 4x-2x milking appears to be better suited for small herds while 3x milking appears to be a better option for large herds.

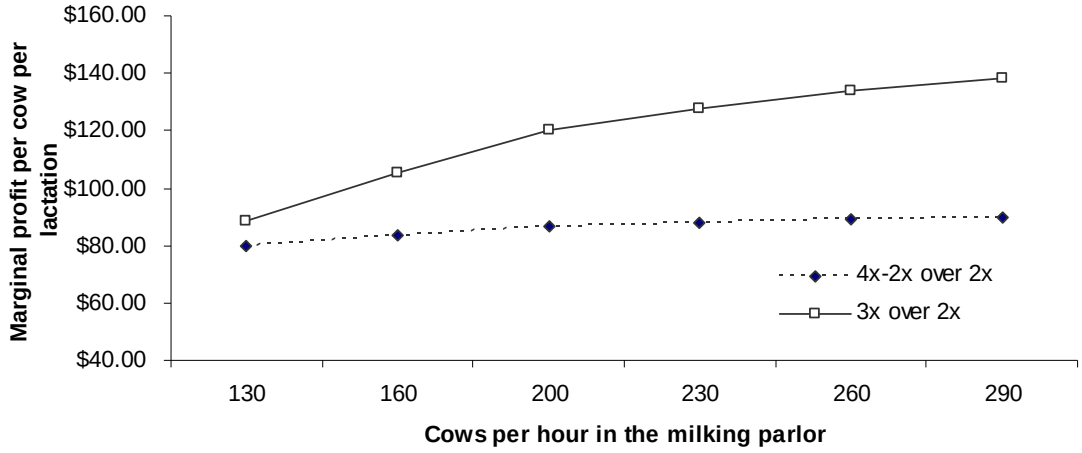

Figure 4.5. Additional income per cow per lactation for cows milked 4x during the first 21 d of lactation and 2x thereafter (4x-2x) or milked 3x during the entire lactation over cows milked 2x during the entire lactation at a farm with 1,000 cows in milk, a labor force of 5 worker equivalents at $12.00 per worker per h with a milk prices of $18.00/cwt.

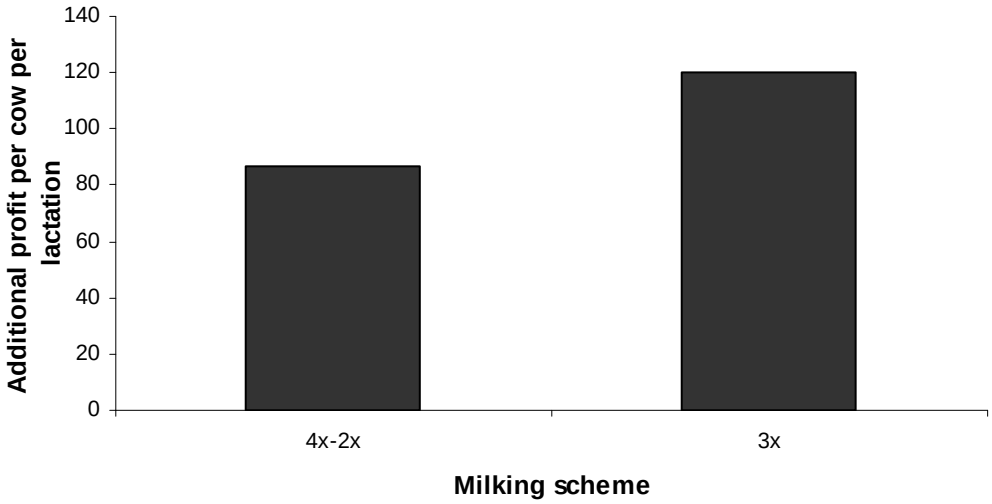

Figure 4.6. Additional income per cow per lactation for cows milked 4x during the first 21 d of lactation and 2x thereafter (4x-2x) or milked 3x during the entire lactation over cows milked 2x during the entire lactation at a farm with a milking capacity of 200 cows/h, a labor force of 5 worker equivalents at $12.00 per worker per h with a milk prices of $18.00/cwt.

Table 4.1. Additional income per cow per lactation for cows milked 4x during the first 21 d of lactation and 2x thereafter (4x-2x) or milked 3x during the entire lactation over cows milked 2x during the entire lactation under different conditions.

No. cows	Milking capacity cows/h	Milk Price/cwt	Worker equivalent/h	4x-2x	3x
100	40	16	1	$ 60.43	$ 33.60
100	40	19	1	$ 81.68	$ 75.28
100	40	22	1	$ 102.94	$ 116.95
100	40	16	2	$ 51.61	$ (10.50)
100	40	19	2	$ 72.86	$ 31.18
100	40	22	2	$ 94.12	$ 72.85
250	50	16	2	$ 58.08	$ 21.84
250	50	19	2	$ 79.33	$ 63.52
250	50	22	2	$ 100.59	$ 105.19
250	70	16	2.5	$ 62.95	$ 46.20
250	70	19	2.5	$ 84.20	$ 87.88
250	70	22	2.5	$ 105.46	$ 129.55
500	80	16	3	$ 63.37	$ 48.30
500	80	19	3	$ 84.62	$ 89.98
500	80	22	3	$ 105.88	$ 131.65
500	120	16	4	$ 67.29	$ 67.90
500	120	19	4	$ 88.54	$ 109.58
500	120	22	4	$ 109.80	$ 151.25
800	150	16	4	$ 70.62	$ 84.56
800	150	19	4	$ 91.88	$ 126.24
800	150	22	4	$ 113.13	$ 167.91
800	200	16	5	$ 72.19	$ 92.40
800	200	19	5	$ 93.44	$ 134.08
800	200	22	5	$ 114.70	$ 175.75
1000	250	16	5	$ 74.54	$ 104.16
1000	250	19	5	$ 95.80	$ 145.84
1000	250	22	5	$ 117.05	$ 187.51

If the assumptions change so that the number of cows becomes variable and the goal is to maximize milking parlor capacity by milking as many cows as possible per day, a 2x milking scheme becomes the most profitable option since it allows for a greater number of cows to be milked. The additional milk produced by either 4x-2x milking or 3x milking does not compensate for the additional income earned from

the increased number of cows being milked. Assumptions for this analysis included a total operating cost including interest per cow per day of $11.65 (Business Summary for NY dairy farms, 2006).

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