Association Between Standing and Lying Behavior and Udder Health in Free-stall Housed, Lactating Dairy Cows

By

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ABSTRACT

RELATIONSHIP BETWEEN STANDING AND LYING BEHAVIOR AND UDDER HEALTH IN FREE-STALL HOUSED LACTATING DAIRY COWS

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This thesis investigates the relationship between cow lying behavior and udder health in free-stall housed dairy cows milked 3x/d. Two longitudinal studies were undertaken; in the first, associations with risk of elevated somatic cell count (eSCC) were determined and, in the second, associations between management practices, post-milking standing duration (PMSD), and risk of intramammary infection (IMI) were determined. A PMSD of >90 min was associated with reduced odds of eSCC. A PMSD of 90 to 120 min was associated with reduced odds of CNS IMI, as was provision of feed around time of milking. Providing ample feed bunk space, having lower free-stall stocking densities, and providing feed around the time of milking promoted PMSD. Overall, these results suggest that management practices which promote PMSD may help to improve udder health in free-stall cows milked 3x/d.

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Chapter 1: Introduction

Adequate daily lying durations are necessary to ensure optimal health and welfare in dairy cattle. Lying behavior has been identified as an element which can be used to measure a cow's welfare status and is also often referred to when assessing cow comfort (O'Driscoll et al., 2009; Tolkamp et al., 2010). When cows are unable to achieve a sufficient amount of lying time, the negative health impacts can include increased cortisol concentrations and levels of ACTH, decreased levels of growth hormone, and suboptimal hoof health leading to conditions such as lameness or sole haemorrhages (Singh et al., 1993; Munksgaard and Simonsen, 1996; Munksgaard et al., 1999).

Udder health is also impacted by lying behavior and consequently influences cow welfare. Mastitis is the most economically significant disease impacting the dairy industry, with losses related to culling, decreased production, decreased fecundity, and treatment costs (Seegers et al., 2003). It is also a complex disease, with various causal pathogens, and given its complexity, complete eradication is, at present, not feasible (Smith, 1983). Therefore, it is essential to understand the causal factors, including management practices and behavioral patterns, which influence its incidence. This understanding is important not only from an economic standpoint, but also from a cow welfare perspective (Barkema et al., 1999b). Therefore, this review will focus on lying behavior, udder health, and the relationship between lying behavior and udder health.

1.1 Standing and Lying Behavior

The modern free-stall housing system represents an intensive housing and management condition for dairy cows. The behavioral repertoires of dairy cows are arguably influenced under these conditions as they are impacted by the timing of various management practices, such as provision of feed, milking practices, and herd health practices. It is thus, important to understand what may be deemed the natural lying behavior patterns of cows housed in free-stall barns so as to determine how various management practices may cause variations in this behavior.

Several recent studies have quantified lying and standing behavior in free-stall housed cows. Mattachini et al. (2011) observed daily lying and standing behavior of cows housed in a 2x/d day parlor milking free-stall barn, noting that peak periods of lying behavior occurred during the early morning and nighttime hours and that standing and lying behavior was strongly influenced by management practices in the 1-2 h following milking times. Daily lying behavior for 43 British Columbia free-stall herds milking either 2 or 3x/d reported a herd average daily lying duration of 11 h/d, split into 9 bouts/d of approximately 88 min durations (Ito et al., 2009). While between-herd variation was low, throughout the study, there was significant variation between individual cow lying patterns. In a follow-up study of North American free-stall herds, a herd average daily lying duration of 11 h/d was once again observed, but with individual daily lying durations ranging from 3 to 21 h/d (von Keyserlingk et al., 2012). Variability in individual cow lying durations may be an indication of cow health, and this subject will be addressed later in this review. Daily lying durations of approximately 11 h/d in free-stall cows has been observed in several other studies of free-stall herds, both parlor milking (2-3x/d) and AMS (robotically milked herds) (12 h/d, Gomez and Cook, 2010; 11 h/d, Bewley et al., 2010: 11 h/d, Deming et al., 2013). Gomez and Cook (2010) observed a slightly longer daily lying duration than the other studies. However, this finding may be a result of only utilizing data collected on days when ambient temperature was below 19°C. There is some variability between studies in terms of frequency of lying bouts per day and the length of these lying bouts, with a range of 9 to 13 bouts/d and bout length ranging from 72 to 88 min (9 bouts of 88 min, Ito et al., 2009; 13 bouts of 72 min, Gomez and Cook, 2010; 11 bouts, Bewley et al., 2010; 9 bouts of 78 min, Deming et al., 2013). Thus, typical lying behavior for cows housed under free-stall conditions could be described as having a daily lying duration of 11 h/d split into 11 bouts of 80 min.

Given that free-stall housed cows devote almost half of their daily time budget to lying down, lying activity should be considered a high priority behavior. A motivation study concluded that dairy heifers have an inelastic demand for 12-13 h/d of lying time (Jensen et al., 2005). While the Jensen et al. (2005) study indicated a slightly higher lying duration than that observed in free-stall herds with cows of various ages, other studies have also demonstrated the importance of lying activity. Cooper et al. (2007) used 60 cows undergoing lying deprivation durations of 0, 2, or 4 h to determine the impacts of this deprivation on the subsequent 40 h of behavioral observations under normal circumstances. These authors observed that feeding activity increased as lying deprivation increased, but then dropped off in favor of lying activities following the end of the deprivation period. This pattern was more notable following 4 h of lying deprivation vs 2 h. Cows were also observed to have recovered 40% of their lost lying time by 40 h post-deprivation. This recovery time contradicts the findings of a 3 h deprivation study which observed cows recovering 50% of lost lying time in only 10 h following the deprivation period (Metz, 1985). However, while the Metz (1985) study indicated that the deprivation period was for 3 h, the deprivation period followed morning milking and feeding in which cows had already been prevented from lying for 2.5 h prior to the deprivation period. Thus, it may be more accurate to interpret the results of the Metz study as how 5.5 h of lying deprivation impacts behavior.

While Metz (1985) may not accurately depict the response to a 3 h lying deprivation, the study did indicate that lying behavior has a higher priority than feeding behavior. When cows were deprived of feeding opportunities only, they would reduce lying activity in the time period following the end of feed deprivation. However, when both feeding and lying opportunities were simultaneously deprived, cows would engage in lying activity ahead of feeding activity once deprivation had ceased (Metz, 1985). This relationship has important implications when considering how management practices may impact subsequent behavior, and the effects this may have on cow health and production.

Given the intensive management of dairy cows, it is essential to ensure that they are provided with comfortable and adequate space in which to lie down when not engaged in milking activities. Lying durations have been observed to be longer when cows are housed in tie-stalls, along with more frequent lying bouts (Krohn and Munksgaard, 1993). Along with increased bout frequency, tie-stall cows were observed to spend more time engaged in movements associated with lying down than their free-stall housed counterparts; possibly in relation to the confines placed on them by being tethered in their stall (Krohn and Munksgaard, 1993). While tie-stalls have an inherent degree of confinement, stall level effects, which impact lying behavior, are also observed in free-stall housing systems.

Stocking density (the number of cows per available stalls) has implications from both a welfare and economic perspective. Overstocking practices have been employed as a minimizing measure of housing cost that maximizes cow numbers thereby resulting in consistent stall occupancy. However, at certain levels this balance between economics and animal welfare can be disrupted. An analysis of the impacts of stocking densities from 100 to 142% indicated that 113% stocking density was the threshold after which negative impacts on lying behavior are observed (Hill et al., 2009). The authors observed a linear relationship with lying behavior decreasing as stocking density increased; daily lying duration was reduced from 12 h/d at 100% stocking density to 11 h/d at 142% stocking density. Fregonesi et al. (2007a) undertook a dose response study looking at the effect of stocking density on 2x/d milked cows stocked from 100 to 150% and observed a linear decline in total daily lying time as stocking density increased; 13 h/d at 100% down to 11 h/d at 150%. Not surprisingly, increased competition for stalls was observed as stocking density increased.

Aside from stocking density, other stall level impacts include stall size and bedding surface and condition. Longer lying times have been observed in wider stalls in both non-lactating and lactating cows (Tucker et al., 2004). While lying bouts were longer in wider stalls, there was no difference in the frequency of lying bouts and cows failed to show a preference for larger stalls. Tucker et al. (2004) speculated that given their evolutionary history of being plains dwellers cows place less emphasis on the spatial area when choosing to lie down but that the available stall area would impact the duration of a lying bout. While cows may not consider spatial area when selecting a place to lie down it is conceivable that they do assess the quality of the lying surface and that surface attributes may impact lying duration. An analysis of the impacts of various bedding depths on cow lying behavior noted that daily lying duration was decreased by 1 h/d when bedding was 6 cm below the cubicle curve as compared to bedding at curb height, indicating that lying duration is decreased with loss of bedding depth (Drissler et al., 2005). In fact, the authors concluded that for every 1 cm decrease in bedding depth, lying duration decreased by 11 min/d. As bedding depth declines over time by cow activity, the importance of a management program ensuring adequate bedding maintenance becomes paramount. Greater daily lying duration as bedding depth is increased was also observed on tie-stall housed cows indicating that the quality of the lying surface is important to promote lying behavior regardless of housing style (Tucker et al., 2009).

As with bedding depth, the quality of the bedding also influences lying activity. Researchers have observed a reduction in daily lying duration when cows were forced to lie solely on wet bedding (9 h/d) vs dry bedding (14 h/d) (Fregonesi et al., 2007b). Cows housed on the wet bedding also spent more time perching with two feet in the stall indicating a potential aversion to even standing on reduced quality bedding. When cows were provided a choice they showed a distinct predilection for dry bedding with daily lying times of 13 h/d in the dry bedded stalls vs 1 h/d in the stalls with wet bedding.

Understanding stall usage and design is essential to ensure adequate daily lying durations but other management activities can also influence the time available to a cow for lying. Specifically, milking activities can have a large impact on the daily time budgets of a lactating dairy cow. When assessing the time spent milking for free-stall herds milking 2 or 3x/d, the mean daily duration of milking was 3 h/d (Gomez and Cook, 2010). However, within cow variability showed a range of milking duration from 0.5 to 6 h/d (Gomez and Cook, 2010). Not surprisingly, daily lying durations decline as milking duration increases. Cook and Nordlund (2009) noted that while parlor design and group sizes generally target a milking time of 45-60 min per group, individual cow's daily time away from her pen can be variable, ranging from 1.5 h/d to over 3 h/d in 3x/d milked cows. Others have, however, failed to show a relationship between milking durations and lying time, irrespective of milking frequencies (see O'Driscoll et al., 2011; Österman and Redbo, 2001). To my knowledge no work has attempted to disentangle the effects of management factors (including milking frequency) on lying behavior.

Cow-level factors may also impact lying behavior. In comparing 2x/d and 3x/d milking cows, increased standing time was observed in the 4 h prior to the morning milking in the 2x/d milking cows; 128 vs 65 min for 2x/d vs 3x/d milked cows (Österman and Redbo, 2001). These authors conjectured that the reduced lying time in the 2x/d milked cows was likely in response to discomfort associated with the fuller udder than the more frequently milked 3x/d milked cows. However, this speculation should be viewed with caution given that the 3x/d milked cows had equal milking intervals of 8 h while the 2x/d cows had an interval of 9 h between morning and afternoon milking and 15 h between afternoon and morning. Thus in this particular case the increased udder fill is likely a consequence of an unbalanced milking interval rather than milking frequency. Therefore, remarks on the impact of milking frequency may be less relevant under these circumstances.

Despite the fact that the design of the Österman and Redbo (2001) study may be slightly skewing response to udder fill, connections between production and lying behavior have been extensively reported in the literature. In a study of 77 free-stall housed cows, increased lying duration was observed in later lactation cows (Bewley et al., 2010). Though the variable was not retained in their final multivariable model, the authors also observed a significant relationship between milk production and lying time when considered at the univariate level (increased production was associated with decreased lying duration). The relationship of increased lying duration with increased DIM has also been demonstrated by Chaplin and Munksgaard (2001) and Vasseur et al. (2012). Further, Vasseur et al. (2012) noted a response at the lying bout level, with decreased bout frequency, but increased bout duration, driving the longer daily lying durations in cows of greater DIM. As well, these latter authors observed decreased daily lying duration in higher producing cows. This relationship was originally demonstrated by Fregonesi and Leaver (2001) with high yield cows having daily lying durations of 12 h/d compared to 14 h/d for low yield cows.

Lying behavior can also be affected by the foot and udder health of the cow. Recent studies have consistently found a positive association between lameness and lying duration (Gomez and Cook, 2010; Ito et al., 2010; Blackie et al., 2011). But the manner in which these longer lying durations are occurring, in terms of lying bouts and bout duration, have been variable across studies likely due to the cause and affect nature of lameness. Both Ito et al. (2010) and Blackie et al. (2011) observed longer bout durations in lame cows. Although Blackie et al. (2011) noted no differences in bout frequency between non-lame and lame cows, Gomez and Cook (2010) observed a reduction in the number of lying bouts but no difference in bout duration between these two health categories. Plausible explanations for the difference in lying behavior observed between these studies is likely a result of various management and housing conditions. For instance, Gomez and Cook (2010) noted variation in the behavior of lame cows housed on sand bedding compared to those herds utilizing mattresses.

Udder health can also have an impact upon lying behavior. Specifically, changes in lying behavior can potentially be used as a crude detection method for cows with an intramammary infection (**IMI**). While sickness behavior is generally associated with lethargy and a sedentary state, recent research has indicated that cows with mastitis may deviate from the classic sickness behavior. Cows with experimentally induced endotoxin mastitis lay down less following onset of mastitis compared to their baseline behavior; moreover, when lying, the mastitic cows spend less time lying on the side of their experimentally induced quarters (Siivonen et al., 2011). These researchers also injected a saline solution into the control quarters as a sham treatment, so this aversion to lying on the infected quarter should not be associated with treatment affects and could be considered solely a response to pain or discomfort when lying on that side. Medrano-Galarza et al. (2012) also observed a reduction in lying time in mastitic cows compared to non-mastitic cows when looking at naturally occurring instances of clinical mastitis. However, they did not note any differences in lying laterality between mastitic and non-mastitic quarters, an observation further observed by Cyples et al. (2012) in cows with experimentally induced mastitis. Anecdotally, Medrano-Galarza et al (2012) did observe more laterality in the cows with mastitis which may be a response to discomfort. Collectively these studies highlight the importance of lying behavior in dairy cows and how behavioral patterns can be used as indicators of health issues. With respect to udder health, understanding the impact of lying behavior is of particular importance not only in detection of a problem, but also potentially as a risk factor for infection.

1.2 MASTITIS

Intramammary infections classified as mastitis are normally categorized into either clinical or subclinical mastitis. Clinical mastitis refers to an inflammatory response within the udder that is accompanied by a suite of visible signs such as visibly abnormal milk, swollen udder, discoloration of the udder, as well, the potential for systemic involvement. Whereas subclinical mastitis is frequently asymptomatic with regards to visible inflammation or systemic impacts but is characterized by an increase in somatic cell count (**SCC**) in the infected quarter (Merck Veterinary Manual, 2013).

Mastitis, both clinical and subclinical, is a ubiquitous disease throughout the dairy industry. A comprehensive study of clinical mastitis rates across Canada observed an incidence rate of 23 cases/100 cow-years at the national level with an elevated clinical mastitis rate of 32 cases/100 cow-years in Ontario (Olde Riekerink et al., 2008), while a recent study noted a 26% prevalence of subclinical mastitis among 1^{st} lactation Dutch dairy heifers (Santman-Berends et al., 2012). In a cohort of 91 Canadian dairy herds, *Staphylococcus aureus* was the predominate causal pathogen of clinical mastitis while *Coagulase-negative staphylococcus* (**CNS**) species were the most commonly isolated pathogens in lactating cows (Reyher et al., 2011).

Cost by case of clinical mastitis has been estimated to be between $95 and $211 (USD) depending on the causal pathogen (Cha et al., 2011). The contributions to economic losses associated with mastitis are pathogen specific, with treatment costs being the dominate factor in cases of gram-positive infections and milk loss being the dominate cost associated with cases caused by gram-negative infections (Cha et al., 2011). At the cow-level, mastitis can result in premature culling, reduced production, and decreased fecundity (Seegers et al., 2003; Hagnestam-Nielsen et al., 2009; Hertl et al., 2010). At the herd-level, mastitis cases can result in increased bulk tank SCC, which can lead to penalties or reduced value of milk depending on the resident country's regulations (Geary et al., 2012).

There appears to be a species-specific relationship between causal pathogens and housing type with higher incidence rates of *Staphylococcus aureus*, *Streptococcus uberis*, CNS, and other *Streptococcus* infections observed in tie-stalls while free-stall herds record more cases of *Klebsiella* spp. and *Escherichia coli* mastitis (Olde Riekerink et al., 2008). Furthermore, robotic milking herds have been observed to have a higher incidence of subclinical mastitis among 1st lactation heifers (Santman-Berends et al., 2012). A link between production and mastitis has also been observed in previous studies with a high milk yield being a risk factor for clinical mastitis (Gröhn et al., 2004; Hagnestam et al., 2007; Sato et al., 2008). An immediate suppression of production is observed after onset of clinical mastitis, but this suppressed milk yield is often carried throughout the duration of the lactation and impact on production varies based on causal pathogen. *Staph. aureus*, *E. coli*, *Arcano pyogenes*, *Klebsiella* spp., and *Strep.* spp. have been identified as the primary causal pathogens associated with production losses (Coulon et al., 2002; Gröhn et al., 2004).

There is also an established link between bulk tank SCC, mastitis pathogens, and management. Barkema et al. (1998) noted that *E. coli*, *Klebsiella* spp., and *Pseudomonas* spp. are the three most commonly isolated pathogens in low bulk tank SCC herds while *Staph. aureus, Strep. dysgalactiae*, and *Strep. agalactiae* were most prevalent in high bulk tank SCC herds. Farms with lower bulk tank SCC have also been described as having more hygienic conditions. However in instances where stall hygiene was poor or when rubber mats were used in stalls an increased incidence of clinical mastitis has been reported (Schukken et al., 1990; Barkema et al., 1999b).

1.3 IMI AND SCC

Intramammary infection cannot be categorized as clinical or subclinical mastitis without the relevant information on SCC and milk quality. However, as SCC and IMI are indicators for mastitis, understanding what influences fluctuations in SCC and incidence of IMI is vital to creating mastitis prevention strategies.

Somatic cell count is primarily an indicator of the amount of leukocytes present in milk and thus is often used as a proxy of any immune responses within the mammary gland and as such can signify presence of infection (Bradley and Green, 2005). A long accepted threshold in Canada to predict presence of infection is a change in SCC from below 200,000 cells/mL to a SCC >200,000 cells/mL (Dohoo and Leslie, 1991). However, while this threshold provides a high specificity, it is a relatively insensitive method to detect instances of IMI or subclinical mastitis (Dohoo and Leslie, 1991). Contributing factors to difficulties in utilizing SCC as a parameter for IMI detection include the fact that there is a natural variation in SCC within cows as SCC is increased with greater parity, decreased milk production (likely due to a lack of dilution effect) and in earlier lactation (Schepers et al., 1997). As well, while major pathogens such as *Staph. aureus* often elicit a pronounced change in SCC, the response to minor pathogens such as CNS can be suppressed with SCC often remaining as low as 50,000 cells/mL in animals with an IMI caused by a minor pathogen (Bradley and Green, 2005). Similarly, composite SCC data has limited use as a predictor for IMI and for longitudinal monitoring of udder health (Dufour and Dohoo, 2013). Using a SCC threshold of 200,000 cells/mL to indicate infection, Dufour and Dohoo (2013) did note that composite SCC has some predictive value for IMI caused by major pathogens, but found quarter-level SCC to be a more useful measure if not using bacteriology. That being noted, the use of composite SCC as a means to identify at risk cows may still have merit as a cost-effective, crude measurement.

The term IMI specifically refers to the presence of an infectious organism within the udder in detectable levels (Berry and Meaney, 2006). Coupling SCC and IMI data together can indicate subclinical and clinical mastitis, but on its own, the detection of IMI is still a valuable means of assessing udder health. CNS has repeatedly been reported as the most prevalent pathogen isolated in milk samples globally (Ferguson et al., 2007; Sampimon et al., 2009; Dufour et al., 2012). A recent study in Canada identified its prevalence as 43%, with an incidence rate of 0.3 new IMI/quarter-month (Dufour et al., 2012). A Dutch study found CNS IMI was higher in heifers while multiparous cows had higher prevalence of major pathogens *Strep. uberis*, *Strep. dysgalactiae*, and *Staph. aureus* (Sampimon et al., 2009).

The effect of season on the incidence of IMI and whether changes in SCC can be used as an indicator is a frequently discussed topic within the dairy industry. Although Green et al. (2006) found that herds in the UK did experience higher SCC in the summer as compared to the winter months, these authors failed to show a correlation between higher SCC and IMI. These researchers surmised that the observed increased SCC in the summer months was not the result of new infections, but rather an indicator of chronic infection and lower cure rates within the summer months. A seasonal variation in pathogen prevalence has also been described. Prevalence of *Staph. aureus* was reported to be higher in the winter months while the highest CNS prevalence has been observed in the spring and summer months (Ferguson et al., 2007). This is likely related to the fact that many CNS species have an environmental reservoir and the spring and summer temperatures and moisture levels are likely more conducive for proliferation of environmental pathogens (Ferguson et al., 2007; Piessens et al., 2011). Along with seasonal variation, management has an important impact on both SCC and IMI.

The relationship between bedding style and pathogen prevalence has been described in various studies. Ferguson et al. (2007) noted a higher prevalence of *Staph. aureus* on herds not utilizing bedding as compared with herds using organic or sand bedding; while prevalence was similar, the herds using organic bedding did have a slightly higher prevalence than the herds using sand bedding. In contrast, CNS prevalence was found to be lowest on herds with no bedding and highest on herds with organic bedding: likely the result of a poor growth environment for CNS in herds that lacked bedding. Similarly, sand bedding was associated with lower odds of CNS IMI than wood bedding and sand bedding was also associated with a higher elimination rate of CNS IMI or less persistence of infection (Dufour et al., 2012).

Associations between management and SCC and IMI also include the effect of post-milking teat dip and overall barn hygiene. When evaluating the efficacy of a post-milking teat dip for prevention of CNS IMI, Quirk et al. (2012) observed decreased CNS IMI when an iodine-based post-milking teat dip was applied, however, efficacy did vary by CNS species. Relatedly, low bulk milk SCC herds were found to be more diligent in their hygiene practices and as a consequence had cleaner cows and stalls which would decrease the likelihood of infection (Barkema et al., 1998).

There is a well-established link between hygiene and SCC. Cow cleanliness and, more specifically, udder and hind limb hygiene scores have been repeatedly related to SCC. Specifically, increased SCC are observed in cows classified as dirty or as having dirty udders and hind limbs (Reneau et al., 2005; Sant'Anna and Paranhos da Costa, 2011; Schreiner and Ruegg, 2003). Interestingly, Reneau et al. (2005) calculated that a one-unit change in their composite udder-hind limb score was associated with a corresponding 40,000 to 50,000 cells/mL change in SCC, indicating the importance of hygiene as a risk factor for IMI. Aside from the link between cleanliness and SCC, prevalence of mastitis causing pathogens has also been linked to udder cleanliness. An increased prevalence of IMI causing environmental pathogens was noted with increasing udder scores (indicating dirtier udders) and cows assessed as having a dirty or very dirty udder were 1.5x more likely to have major pathogens present in their milk (Schreiner and Ruegg, 2003). Presence of CNS, of which many species have an environmental reservoir, has been previously identified as a factor that may increase the risk of *Staph.*

aureus IMI (Reyher et al., 2012), further emphasizing the need to assess management practices which may promote or prevent IMI.

Production, IMI, and SCC are all related and impacted by management. Herds identified as having low bulk tank SCC have greater milk production rates than higher SCC herds (Barkema et al., 1998). At the cow-level this relationship is also maintained with lower SCC cows having higher production than higher SCC cows (Jones et al., 1984); although the dilution effect with higher producers may be magnifying and contributing to this relationship (Jones et al., 1984; Ferguson et al., 2007). In fact, lower SCC herds also report lower prevalence of mastitis and *Staph. aureus*; this again is likely tied in with better management and more hygienic conditions (Barkema et al., 1998; Ferguson et al., 2007). High production, low SCC, and low prevalence of *Staph. aureus* is not an inconsequential relationship as this pathogen (and other IMI pathogens) and increases in SCC have been linked with decreased production (Ferguson et al., 2007), with the effects greatest for multiparous as compared with primiparous cows (Jones et al., 1984). This latter affect may be a consequence of higher baseline production in the multiparous cows (Jones et al., 1984). While maintaining hygienic conditions within the parlor and barn is essential to decrease risk of IMI, and can be beneficial in terms of milk production and quality, milking interval may also have an important impact on udder health.

Very short, <6 h, and very long, >12 h, milking intervals have been associated with increased SCC; the lowest SCC has been shown with a milking interval between 6 and 10 h (Fernando and Spahr, 1983; Hamann and Halm, 2004; Nielsen et al., 2005; Mollenhorst et al., 2011). In an AMS herd study, higher SCC was associated with longer milking intervals, more variable intervals, lower production, later lactation, and greater parity (Mollenhorst et al., 2011). In contrast, Allen et al. (1986) noted that 3x/d milked cows had higher CMT scores (in cows of parity ≤3), and one could surmise poorer udder health as a consequence, as compared to 2x/d milked cows. However, 3x/d milked cows would have a milking interval of 8 h that has recently been linked to improved udder health as measured by SCC. Two hypotheses abound on the relationship between 3x/d milking and udder health. The first being that increased exposure to the milking machine would increase transmission of infections, as well, more frequent milking may increase susceptibility to bacteria through increased teat canal penetrability (Logan et al., 1978). The second hypothesis indicates a beneficial effect of 3x/d milking, as the teat canal is being flushed more regularly and pathogens have a shorter incubation period between milkings (Jarrett, 1977). The differences in udder health observed under 3x/d milking may be attributed to changes in management; whereby teat canal penetrability are altered in such a way that the risk of opportunistic infections is decreased.

Keeping cows standing for a period of time after milking is a long accepted practice to promote udder health. By keeping the cow standing the teat canals will have had time to close prior to the udder contacting the stall substrate (Tyler et al., 1997; Johansson et al., 1999); thus, decreasing the likelihood of bacterial penetration into the teat canal and thereby decreasing the risk of IMI (Tyler et al., 1997; Johansson et al., 1999). The only empirical evidence for this relationship is from a study of tie-stall herds and a study of an AMS herd. In tie-stall herds, a post-milking standing duration (**PMSD**) of 40 to 60 min was associated with a 1.4x reduced odds for acquiring an environmental IMI while odds for environmental IMI increased as PMSD increased past 60 min

(DeVries et al., 2010). In an AMS herd, only CNS IMI was found to be associated with PMSD, whereas a PMSD of >2.5 h increased the odds for CNS IMI (DeVries et al., 2011a). The findings in both of these studies are in alignment with the current knowledge of teat morphology following milking, as teat canals are wide open immediately after milking and a second period of increased teat canal diameter has been identified 2 h after milking (McDonald, 1975; Schultze and Bright, 1983; Neijenhuis et al., 2001). Furthermore, teat-end width and teat-canal length have been noted as taking >8 h to recover following milking and, thus, teat susceptibility to bacterial penetration may be variable under different milking frequencies (Neijenhuis et al., 2001). Given this, PMSD may be utilized as a preventative measure to improve udder health through altering management practices to encourage specific PMSD.

1.4 Post-milking Standing Behavior

Previous research has reported varying PMSD under different housing and management conditions. Mean PMSD in a study of tie-stall herds was 79 min (DeVries et al., 2010) and recent research on free-stall, AMS herds has reported a comparable PMSD (75 min: Deming et al., 2013; 77 min: DeVries et al., 2012; 78 min: DeVries et al., 2011a). However, earlier studies of free-stall housed cows parlor milked 2x/d reported much shorter PMSD (55 min: DeVries and von Keyserlingk, 2005; 62 min: DeVries et al., 2005; 39 min: Fregonesi et al., 2007a; 35 min: Tyler et al., 1997). The variation in PMSD indicates variable behavior among the different housing systems and likely a variable management impact as well.

A long accepted practice to promote PMSD has been to deliver fresh feed to cows following milking. Tyler et al. (1997) observed a 20 min increase in PMSD when free-stall cows were provided with access to fresh feed following milking. DeVries and von Keyserlingk (2005) also observed a similar increase for PMSD when cows were provided with fresh feed. However, in another study it was observed that when delivering fresh feed 2x/d vs 1 x/d cows stood longer following the morning milking when feed was delivered 1x/d; it is of note that the second feed delivery did not coincide with the second milking (DeVries et al., 2005). Thus, while provision of fresh feed encourages PMSD, there may be an optimal time in which to deliver this feed to influence cows' PMSD.

Recent research in tie-stall herds and an AMS herd have provided evidence as to how timing of feed delivery impacts PMSD. In tie-stall herds, the longest PMSD was observed when fresh feed delivery occurred 30 min before to 60 min after milking (DeVries et al., 2010). In an AMS herd, feed manipulation (fresh feed delivery or feed push-ups) 60 to 120 min after milking produced the longest PMSD while feed manipulation >240 min after milking resulted in the shortest PMSD (DeVries et al., 2011a). It is of note that a similar study of 13 AMS herds produced no correlation between PMSD and feeding management (Deming et al., 2013). The variations observed in these studies indicate further research is needed in this area to determine the impacts of housing and management on PMSD.

While recent research has focused on tie-stall and AMS herds, Fregonesi et al. (2007a) observed an effect of stocking density on PMSD in free-stall housed, 2x/d parlor milked cows. PMSD was decreased by approximately 15 min when stocking density increased from 100 to 150% (Fregonesi et al., 2007a). Given the relationship between

PMSD and risk of IMI, and the multitude of factors which may be influencing PMSD, research into understanding this relationship across housing and management systems will potentially yield vital information into prevention strategies leading to improved udder health and dairy cow welfare.

1.5 Objectives and Hypotheses

The objective of this thesis was to determine the relationship between lying behavior and udder health in lactating dairy cows by analyzing herd- and cow-level factors. Two longitudinal studies were completed on free-stall herds milking 3x/d. In the first study (Chapter 2), our objectives were to quantify the standing and lying behavior of free-stall housed cows milked 3x/d, to determine what factors were influencing lying behavior, and to determine how this impacted udder health using elevated SCC (**eSCC**). It was hypothesized that PMSD would be shortest when cows were not offered fresh feed near the time of milking and that shorter PMSD would be associated with an increased risk of a new eSCC. In the second study (Chapter 3), our objectives were to determine which factors were associated with PMSD in free-stall housed cows milked 3x/d and to determine the relationship between PMSD and IMI. It was hypothesized that longer PMSD would be observed when cows received fresh feed or freshly pushed-up feed around the time of milking and that shorter PMSD would be associated with an increased risk of IMI.

Chapter 2: Associations of herd- and cow-level factors, cow lying behavior, and risk of elevated somatic cell count in free-stall housed lactating dairy cows

2.1 Introduction

Mastitis is the most economically significant disease impacting the global dairy industry (Seegers et al., 2003). Given the complexity of the disease, complete elimination of intramammary infection is not feasible at this time (Smith, 1983). Thus, understanding farm management practices that reduce the incidence of mastitis is essential, from both an economic and animal welfare standpoint (Barkema et al., 1999a).

A long-accepted management practice assumed to reduce the risk of mastitis is to promote longer standing times after milking. The presence of feed, particularly fresh feed, has been shown to encourage longer post-milking standing times (Tyler et al., 1997, DeVries and von Keyserlingk, 2005 and DeVries et al., 2010). Keeping cows on their feet for a certain period of time after milking will increase the likelihood that the teat canals have closed prior to the udder contacting the stall surface when the cow lies down, thus decreasing the odds of bacterial penetration of the teat (Tyler et al., 1997 and Johansson et al., 1999). A study of tie-stall housed cows observed that cows that laid down, on average between 40 and 60 min after milking, tended to have a decreased risk of acquiring a new environmental intramammary infection (IMI) compared to those cows that laid down within 40 min after milking (DeVries et al., 2010).

In the study by DeVries et al. (2010), the mean post-milking standing duration of the studied tie-stall housed cows was 79 min. This is much longer than that previously reported for free-stall housed cows (milked 2×/d), ranging from 33 to 62 min (Tyler et al., 1997, DeVries and von Keyserlingk, 2005, DeVries et al., 2005 and Fregonesi et al., 2007). These results provide some evidence that in free-stall housing systems, a large percentage of cows lie down relatively quickly (within 40 min) after returning from milking. One possible explanation for this may be the additional time required for these cows to access feed in addition to the time spent standing before and during milking. In a study of 16 free-stall farms, it was found that increased time spent milking was associated with a decrease in time spent feeding and lying (Gomez and Cook, 2010). Further, lying behavior has been shown to increase during the night and early morning hours, unless disrupted by management practices (Mattachini et al., 2011). Decreased post-milking standing durations observed in free-stall housed, parlor milked, cows may thus be a consequence of these animals attempting to regain lost lying times. One management practice that could further exacerbate this behavior is milking frequency. In herds with 3×/d milking, cows are being milked on average every 8 h; a practice that arguably disrupts their diurnal behavior patterns. Therefore, it is expected that the tendency for free-stall housed cows to lie down soon after milking may be further exacerbated when cows are milked more frequently.

There were three objectives for this study: (1) to quantify the standing and lying behavior, with particular emphasis on post-milking standing time, of dairy cows milked 3×/d, (2) to determine the cow- and herd-level factors associated with lying behavior, and (3) to relate these findings to the risk of experiencing an elevation in somatic cell count (SCC). It was hypothesized that post-milking standing times would be shorter when fresh feed was not offered near the time of milking, and that those cows which lie soonest after milking would have a higher risk of acquiring a new elevated SCC (eSCC).

2.2 MATERIALS AND METHODS

2.2.1 Farm selection

Five commercial dairy farms in Eastern Ontario, Canada were recruited for participation in this study. Herds were selected as a convenience sample. Selection was based on the criteria that they had free-stall housing, milked cows in a parlor, milked 3×/d, participated in a dairy herd improvement (DHI) program, were predominantly Holstein-Friesian (>90%), and had >120 lactating cows in the herd. Overall, these farms had a mean herd size of 337 (range: 144–566) lactating cows and a mean adjusted 305 d milk yield of 10,097 kg (range: 9541–10,699 kg). The mean annual bulk milk SCC was 247,000 cells/mL (range: 141,000–316,000 cells/mL). The study was conducted on these herds between September 2011 and February 2012. The farms were on a rolling start date corresponding to their DHI test dates.

2.2.2 Animal selection

Forty focal cows per herd were randomly selected from all herds. Eligible cows were <200 days in milk (DIM), ensuring that they would be available for 2 further DHI tests prior to dry-off, and had a SCC <100,000 cells/mL at the most recent DHI test. Lactation number and DIM were recorded from farm records for each focal cow and validated using DHI data.

2.2.3 Milk sampling and analysis

Cow-level SCC testing was conducted using composite milk samples taken approximately every 35 days, as part of the DHI program, and on-line reports of results were made available a few days after testing. Farms were enrolled in the study immediately following a regularly scheduled DHI test date, with each subsequent period beginning after the next DHI test date (which occurred on average at 5-wk intervals; average interval was 37 ± 13 d (mean ± SD)). A total of 4 observation periods were targeted on each farm. Milk sampling was conducted by the DHI customer service representative assigned to the farm and milk samples shipped overnight to the CanWest DHI Laboratory (Guelph, ON, Canada), and were analyzed for SCC using the Fossomatic method (Fossomatic 5000 series, Foss Electric, Hillerød, Denmark).

Somatic cell counts (eSCC) were used as a proxy for subclinical mastitis (Dufour et al., 2011 when >200,000 cells/mL). A new case was identified as one where the previous test was <100,000 cells/mL and the most recent test was >200,000 cells/mL (Dohoo and Leslie, 1991, Schepers et al., 1997 and Schukken et al., 2003). Thus, over the 4, 5-wk periods, the maximum number of new eSCC any individual cow could develop was 2. Incidence rate of eSCC was calculated as: number of new eSCC/# of cows at risk/d at risk × 365 d/year (Dohoo et al., 2009). To determine required number of cows and observation periods, a power analysis was conducted a priori using PASS software (PASS, Kaysville, UT, USA). For the calculation, beta was set to 0.20 (80% power), and alpha was set to 0.10. Estimates for incidence rates of infection, as well as for variability in observation of standing and lying behavior, were taken from previous findings (DeVries et al., 2010 and DeVries et al., 2011a).

2.2.4 Standing and lying behavior

Lying behaviors were recorded using data loggers (HOBO Pendant G Data Logger, Onset Computer Corporation, Pocasset, MA, USA) that record leg orientation in 1-min intervals (Ledgerwood et al., 2010). Data loggers were placed on the hind limb of focal cows with veterinary bandaging tape (Vetrap Bandaging Tape, 3 M, London, ON, Canada) for the first 5 d of each period following DHI testing. Ito et al. (2009) demonstrated that recording lying behavior for 3 d produced an accurate estimate of herd-level lying behavior using the same technology as utilized in this study. Thus, a 5 d recording period was chosen to improve the accuracy of the observations. Recordings made in the first 5 d following the DHI test were assumed to reflect a cow's normal (typical) behavior and cows were identified as infection free when SCC <100,000 (Medrano-Galarza et al., 2012). A cow's typical lying behavior (when not infected) was assumed to be related to her risk of acquiring a new infection based on the SCC obtained at the next DHI test. Feed delivery and feed push-up were recorded by the herd manager on the same days as lying behavior. The interval between feed delivery and milking time was calculated for each cow.

Standing and lying times (min/d), lying bout duration (min/bout), and lying bout frequency (#/d) were calculated, as per description by Ledgerwood et al. (2010), using the recorded data. Pre-milking standing duration (min) was defined as the interval between the last observation of lying prior to milking and the termination of milking. Post-milking standing duration (min) was defined as the interval between the termination of milking and the initial observation of lying following milking.

2.2.5 Cow-based measures

Individual milking times and milk production were obtained from parlor records obtained at each farm. Focal cows were scored for cleanliness in the milk parlor using a 4-point scale (1 = very clean to 4 = very dirty) hygiene scoring system (www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf; Cook and Reinemann, 2007) on the first day of each 5 d recording period. The udder, lower legs, and upper legs-flank were evaluated separately. Locomotion was scored as cows exited the parlor on the first day of the 5 d recording period using a 5-point scale (1 = normal to 5 = severely lame; Flower and Weary, 2006). For statistical analysis, lameness was dichotomized into lame (gait score = ≥3) and not lame (gait score = <3). Both scoring procedures were completed by the same two trained observers on all farms for all periods and their scores were averaged for each cow. For both hygiene and locomotion scoring, training occurred by providing the two observers the scoring scales, along with visual examples (photographs for hygiene and video for lameness) for each score to study and practice with. The observers practiced scoring individual cows (n = 40) before the start of the study; inter-observer reliability of this practice scoring was tested and correlated to acceptable standards (r > 0.75).

2.2.6 Herd, housing, and management data

A questionnaire, adapted from Dufour et al., 2010 and Dufour et al., 2011, was administered on each farm before the commencement of the study. Farm owners or herd managers were questioned regarding housing and management practices, including milking practices, stall dimensions, and bedding (type, quantity, frequency of cleaning). Although all farms fed a TMR, bedding type and frequency of delivery, feeding regimen (both frequency of feed delivery and feed push-ups), and cleaning systems varied by farm. Feeding frequency was either 1x/d (n = 3) or 2x/d (n = 2). Feed push-ups ranged in frequency from 0 to 5x/d. Mean free-stall stocking density (# cows/stall) for all 5 farms was 104% (range: 95–114%). Linear bunk space per cow averaged 0.66 m/cow (range: 0.36–1.22 m). The median parlor capacity was 24 (range: 12–36) cows. While daily milking times were variable by farms, each farm did maintain an 8 h interval between milking start times. Only one farm used sand bedding while the other 4 used mattresses or waterbeds partially covered with either straw or composted manure solids. The sand bedded farm used deep bedding (>2 cm) while two of the mattress based farms provided bedding (<2 cm), and the other farms used >2 cm of bedding. Four of the farms docked tails and 3 clipped or flamed udders. Two farms had scheduled gait scoring; one farm recorded gait scores 1x/year while the second recorded 2x/year, a third farm just observed cows for lameness at milking time, and the other two farms had no specific gait scoring practices. All farms cleaned stalls 3x/day, corresponding to milking times, while alleyways were cleaned either by automatic scrapers (3 farms) or tractors (2 farms), with cleaning schedules ranging from 2x/week to 24x/d. Cows entered a holding pen prior to milking and farms milked by group according to production and/or parity. Focal animals were randomly selected to ensure representation across all of the farms' milking groups. All farms engaged in pre- and post-milking cleaning procedures employing teat dips or foams pre- and post-milking; 2 farms utilized foam in their pre-milking cleaning procedure while all farms used a teat dip post-milking. Milking practices on all farms ensured that all teats were cleaned and dried before applying the milking machines that had automatic take offs. Two of the farms encouraged post-milking standing by pushing-up feed at the time cows returned from milking while the other 3 farms had no specific practices to encourage post-milking standing. Any variables that were unique to a specific herd were not retained as an explanatory variable in the statistical analyses since its measure of association would be perfectly confounded by other characteristics specific to that herd.

2.2.7 Statistical analysis

Prior to analyses, all data were screened for normality using the UNIVARIATE procedure of SAS (SAS Institute Inc, 2009). Cow SCCs at the study beginning were slightly right skewed and, thus, transformed by the natural logarithm. All cow-level data were summarized across each of the 5-d observation periods for each cow. The associations between dependent behavioral variables (lying time, lying bout frequency and duration, post-milking and pre-milking standing time) and possible cow-level and herd-level explanatory (independent) fixed-effect variables were analyzed with multivariable linear mixed models using the MIXED procedure of SAS (SAS Institute Inc, 2009), treating period as a repeated measure. The models included the random effects of herd and cow within herd. Cow within herd was included in the models as the subject of the repeated statement. Initially, unconditional associations between independent variables and dependent variables were examined. Only independent variables with $P \leq 0.10$ in this initial screening were included in multivariable linear regression models. The CORR procedure of SAS was used to check for correlations between the retained explanatory variables. If two variables were highly correlated ($r > 0.8$), the one with the lower P value in the unconditional associations was retained. For the multivariable models, effects were considered significant at $P \leq 0.10$. Manual backward elimination of non-significant effects was used to construct the final multivariable models. Period was not retained in any of the final models, thus no estimates for this effect are further presented. From the resultant models, plausible 2-way interactions were examined and retained if $P \leq 0.10$. Only those results retained in the final multivariable models are further presented. The covariance structure was first-order autoregressive or compound symmetry depending upon best fit according to Schwarz's Bayesian information criterion.

The association of the various cow-level and farm-level explanatory variables on the occurrence or non-occurrence of an eSCC was assessed using a random intercept mixed logistic model using the GLIMMIX procedure (distribution = binomial and link = logit) of SAS (SAS Institute Inc, 2009). As new eSCC were assessed at the cow level, both herd and cow within herd were considered random. Unconditional associations were estimated in the described model to screen all potential explanatory variables. For variables measured on a continuous scale, linearity of the relationship between the variable and the occurrence of an eSCC was assessed by categorizing the continuous variable and visual inspection of plots of the odds ratio against mean values of the categories. Variables with $P \leq 0.20$ were retained for model building. The CORR procedure of SAS was used to check for correlations between the kept explanatory variables. If two variables were highly correlated ($r > 0.8$), the one with the lower P value in the unconditional associations was retained. These kept variables were then included in a multivariable analysis using the above mentioned model. Effects in the multivariable logistic model were considered significant at $P \leq 0.10$. Manual backward elimination of non-significant effects was used to construct the final multivariable model. From the resultant model, plausible 2-way interactions were examined and retained if $P \leq 0.10$. Only those results retained in the final multivariable logistic model are further presented.

One of the farms was removed from the study after period 1 due to switching from parlor milking to an automated milking system; when this farm was enrolled in the study they had indicated they would not be making the switch between the 2 milking systems until a later date however unforeseen circumstances pushed this date forward. Two farms completed only 3 periods due to one farm switching from 3x/d to 2x/d milking due to a staff shortage and the final DHI test for the second farm failed to include SCC data. One cow on one farm was sold before the end of the first period, thus 199 cows completed the first period. Throughout the remainder of the study various cows were removed from the study as they were either culled or dried off. A complete data set (for 4 periods) was obtained for 67 cows from 2 of the 5 farms, however, all cow data were used in the analysis regardless of whether a cow had a complete data set.

2.3 Results

Parity, DIM, initial SCC, and milk production of the study cows were similar across study herds (Table 2.1). Due to low selection availability on one farm (i.e. the farm lacked 40 cows with baseline SCC <100,000 cells/mL), one of the 40 enrolled cows had a baseline SCC of 125,000 cells/mL. Low selection availability on another farm required selection of a cow at 203 DIM. Across the study, a total of 669 observations of lameness and hygiene scores were made. The average, SD, and range values for these measures, as well as for standing and lying behavior, across all cows, are found in Table 2.2.

2.3.1 Factors associated with lying behavior

Lameness was associated with both lying time and lying bout duration (Table 2.3). Lame cows were more likely to have longer daily lying times and lying bout durations than non-lame cows. Daily milk yield was negatively associated with lying time, with cows of higher milk production having shorter daily lying times; those cows above the study average daily milk yield (37 kg/d) had an average daily lying time of 11 h/d compared to 12 h/d for those cows below the average daily milk yield. Days in milk were associated with lying time, lying bout frequency, and lying bout duration. Cows with higher DIM were more likely to have longer lying times, fewer lying bouts, and longer lying bouts than cows earlier in their lactation. Parity was associated with lying bout frequency; cows of greater parity had fewer lying bouts per day.

2.3.2 Factors associated with pre- and post-milking standing time

Feed manipulation (feed delivery or push-up) occurred on average 62 min before milking (range: 867 min before milking to 432 min after milking). The relationship between duration of post-milking standing time and interval between milking time and feed manipulation time was non-linear ($P = 0.3$; Figure 2.1). Delay between milking time and feed manipulation time was a predictor of post-milking standing time (Table 2.4). The longest post-milking standing times were observed when feed was manipulated in the hour before milking or in the immediate time period after milking. Parity was associated with both pre- and post-milking standing time; older cows spent more time standing both before and after milking than younger cows. Days in milk were associated with pre-milking standing time; cows spent less time standing before milking when they were further along in lactation.

2.3.3 Factors associated with eSCC

A total of 591 pairs of milk samples (i.e. consecutive DHI samples) were collected throughout the study (Table 2.5). Eighty-seven pairs of milk samples were excluded due to high initial SCC (i.e. SCC on first sample within a pair was >100,000 cells/mL) resulting in 504 pairs of samples enrolled for the analysis of eSCC. From these paired samples, 48 cases of new eSCC were detected. Thus, the mean herd eSCC incidence rate was 0.91 eSCC/cow-year (SD: 0.49 eSCC/cow-year).

The incidence of eSCC across the range of average post-milking standing duration is presented in Figure 2.2. Incidence of eSCC peaked in those cows that stood for approximately 80 min post-milking with the incidence of eSCC tending to decrease after this cut point. Non-linearity of the relationship between average post-milking standing time and risk of a new eSCC was clear ($P = 0.8$; Figure 2.3). This allowed for more meaningful categories to be chosen for average post-milking standing times based on variable distribution and on existing knowledge concerning teat canal closure following milking (McDonald, 1975 and Schultze and Bright, 1983). Two categories of average post-milking standing time were defined: 0–90 min and >90 min based on the distribution of post-milking standing durations (where the median post-milking standing time was

92 min) and the observed relationship between post-milking standing time and incidence of eSCC (Figure 2.2 and Figure 2.3).

Unconditional estimates of association between independent explanatory factors and odds of having an incident eSCC are described in Table 2.6. Post-milking standing time >90 min, encouraging post-milking standing time through feed push up, and greater stall width were all associated with decreased odds of experiencing eSCC. Other factors, including greater parity, DIM, SCC at the study start, lameness, having poorer udder hygiene and use of foam teat disinfection, were associated with increased odds of acquiring a new eSCC. The final logistic regression model is presented in Table 2.7. Three variables were retained in the final model: post-milking standing time, parity, and baseline SCC. The model showed decreased odds for acquiring a new eSCC when post-milking standing time averaged >90 min. In addition, increased parity and a greater baseline SCC were associated with increased odds of a new eSCC.

2.4 DISCUSSION

The existence of an association between post-milking standing time and udder health, in terms of SCC and infection rates, has long been hypothesized. A study of tie-stall housed cows by DeVries et al. (2010) provided the first direct empirical evidence of an association between post-milking standing time and incidence of IMI. Evidence for this relationship in free-stall housed cows milked with an AMS was also recently reported (DeVries et al., 2011a). To our knowledge, this is the first study to report the association between post-milking standing duration and udder health, of free-stall housed cows that are parlor milked 3x/d. Mean post-milking standing time across all 5 herds was 103 min (range: 1–798 min), which is much longer than previously reported for free-stall housing systems milking 2x/d in a parlor, range 33–62 min, (Tyler et al., 1997, DeVries and von Keyserlingk, 2005 and DeVries et al., 2005) or milking 2.3x/d in an AMS (78 min: DeVries et al., 2011a). In addition, the present duration of post-milking standing time was also much longer than the 79 min reported in the tie-stall study by DeVries et al. (2010). The average duration of pre-milking standing of 87 min was comparable to results from free-stall housed cows milked by an AMS (94 min: DeVries et al., 2011a). Given the duration of average pre-milking standing time, the duration of the post-milking standing times observed in the present study were unexpected, as one could hypothesize that cows would lie down sooner post-milking given their increased pre-milking standing time demands when milked 3x/d. While we did not collect data on time spent in the holding pen prior to being milked, one possible explanation for the long pre-milking standing times observed in these cows could be that parlor milked, free-stall housed cows often have a forced standing period in the holding pen of 30 min to >1 h prior to being milked (Cook and Nordlund, 2009). A more recent study of North American free-stall farms which were milking either 2 or 3x/d found that the average amount of time cows were spending away from their pens for milking was 249 min, indicating that these cows are likely spending long periods of time in the holding pen waiting to be milked (von Keyserlingk et al., 2012). Despite the pre-milking standing time of the study cows, indicating that they are likely spending long periods of time in the holding pen waiting to be milked, their post-milking standing times did not appear to be reduced. Also, across the study farms, higher producing animals (milk production >37 kg/d) stood on average for 12 min longer post-milking than the lower producing focal cows. Therefore, a possible explanation for the duration of post-milking standing time would be that these cows may have greater metabolic demands as a consequence of the higher milk production normally observed with increased frequency of milking and thus increased feeding motivation (O'Driscoll et al., 2010).

It is well established that the presence of fresh feed promotes longer post-milking standing time in cows (Tyler et al., 1997, DeVries and von Keyserlingk, 2005 and DeVries et al., 2010). Given the variation in both the frequency of feed delivery (1 to 2x/d) of fresh feed and feed push-up (0 to 5x/d) across farms it was not surprising that feed manipulation (both feed delivery and push-up) was associated with post-milking standing time. On average feed manipulation occurred 62 min before milking with post-milking standing times reduced when feed manipulation occurred >60 min before milking. The longest post-milking standing times were observed when cows were fed in the hour before milking or in the short period of time after the return from milking (DeVries and von Keyserlingk, 2005, DeVries et al., 2010 and DeVries et al., 2011a).

The average daily lying time of 11 h/d was comparable to the average lying time reported for 16 free-stall herds milked either 2x/d or 3x/d (12 h/d; Gomez and Cook, 2010) and was comparable to the lying time reported in a study on high-producing Holsteins from North American free-stall herds (von Keyserlingk et al., 2012). However, the average lying times for the 5 herds observed in the present study were slightly higher than that reported by Calderon and Cook (2011: 3x/d milked herd; 10–11 h/d) and lower than the 13 h/d reported by Krawczel et al. (2012). The average number of lying bouts (9 bouts/d) and average bout duration (85 min/bout) were also similar to other studies observing 2x/d milking herds (Krohn and Munksgaard, 1993 and Ito et al., 2009).

However, the reduced lying bouts observed in our study compared to previous work (13 bouts/d; Gomez and Cook, 2010; 13 bouts/d; Krawczel et al., 2012) is difficult to explain. Previous work has speculated that cows increase their bout frequency in relation to increased milking frequency but we failed to show this effect. Rather it appears that cows with long post-milking standing times compensate by increasing the bout duration of their lying and standing events, thus decreasing the total number of daily lying and standing bouts. The reduction in lying bout frequency may also be the result of cows prioritizing their feeding times to coincide with milking times, this would also explain the long post-milking standing times of the cows in this study. Daily lying times of cows from farms which provided >0.66 m/cow at the bunk averaged 11.3 h/d compared to 11 h/d on farms which provided <0.66 m/cow. This would indicate that despite an increased number of milkings per day, 3x/d milked cows are driven to maintain long daily lying durations and that adequate space at the feed bunk may enable them to economize feeding time, thus leading to fewer but longer lying bouts per day. Our finding that DIM was associated with all facets of lying behavior confirms the observational results previously reported by Bewley et al. (2010), who found that DIM was a predictor of lying duration with lying duration increasing in later lactation. Longer lying durations in later lactation may be a result of lessened metabolic demands as lactation progresses; therefore, cows may be spending less time feeding and spending more time engaged in resting behaviors (Bewley et al., 2010). Increased lying times observed in later lactation cows may also be a result of decreased udder fill. Österman and Redbo (2001) observed that morning pre-milking standing time was reduced in cows milked 2x/d as opposed to cows milked 3x/d (15 vs 8 h time between milking). These latter researchers hypothesized that this decrease in lying behavior before the morning milking in cows milked 2x/d was a result of discomfort caused by increased udder fill and pressure. When comparing production, DIM, and daily lying times across this study, cows producing between 30 and 40 kg/d tended to be higher in DIM compared to cows producing between 40 and 50 kg/d and those producing in excess of 50 kg/d (127 d vs 98 d and 80 d, respectively) and as production increased with decreasing DIM, average daily lying time also decreased from 11 h/d to 10 h/d. Thus, it is plausible that greater milk production in early lactation and consequently greater udder fill, may also result in reduced lying times compared to later lactation cows.

Parity was negatively associated with lying bout frequency, with cows of greater parity having fewer lying bout/d. This may be driven in part by the higher milk yield normally observed in multiparous cows (DeVries et al., 2011b); first lactation cows in the current study were producing on average 31 kg/d compared to 39 kg/d for the multiparous cows. Again the higher metabolic demands associated with elevated milk production in the older cows may have resulted in increased feeding times post milking (and thus longer post milking standing times).

In the current study, on average, 6% and 13% of primiparous and multiparous cows were considered to be clinically lame (gait score ≥3), respectively. It is well recognized that higher producing cows are at greater risk for lameness (Archer et al., 2010) and that lame cows have fewer lying bouts/d (Gomez and Cook, 2010). In concordance with previous work, lameness was associated with lying behavior in our study; lame cows had greater lying times (11 h/d for non-lame cows vs 12 h/d for lame cows) and lying bout duration (83 min/bout for non-lame cows vs 97 min/bout for lame cows). Calderon and Cook (2011) also observed an increase in lying times in lame cows that were parlor milked 3x/d in a free-stall barn. Lame animals likely have an increased daily lying duration and longer bout durations in an effort to mitigate pain they may be experiencing when standing (Chapinal et al., 2009).

The study farms selected were representative of commercial Ontario dairy herds in terms of milk quality. The average bulk milk SCC of 247 ± 106 (×1000) cells/mL was similar to that reported for Ontario (205,000 cell/mL: Olde Riekerink et al., 2008). The 0.91 eSCC/cow-year incidence rate observed in this study was lower than recent studies reporting IMI incidence rate for the same geographic region (1.45 new IMI/quarter-year: DeVries et al., 2010; 1.95 new IMI/quarter-year: DeVries et al., 2011a). This observed difference may be the result of differing sensitivities between the two methods; SCC vs bacteriological sampling. SCC is an effective screening tool to identify potential infections, but fails to explicitly confirm IMI status. Moreover, older cows naturally have higher SCC levels and there is a dilution effect when assessing cow-level SCC as the inflammatory process is at the quarter-level (Bradley and Green, 2005). While SCC may be a less sensitive method of identifying IMI, it is a more practical and cost-effective method (Pyörälä and Pyörälä, 1997), and it is routinely measured on commercial farms. In subsequent studies, bacteriological culture of aseptically collected milk samples should be used to identify IMI and causal pathogens and validate eSCC measures.

Parity, baseline SCC, and post-milking standing time were the only variables retained in the final model as factors associated with eSCC. Higher parity cows are more likely to have pendulous udders and thus poorer udder hygiene (Reneau et al., 2005). Milk production and mastitis have also been linked with parity, with mastitic cows having higher pre-mastitis milk yields compared to non-mastitic cows (Hagnestam et al., 2007). With increased milk yield comes increased metabolic demands and, thus, increased time spent standing to feed. As multiparous cows tend to be higher producers, they likely spend more time standing at the feed bunk and are thus at greater risk of becoming dirty (DeVries et al., 2011a and Nielsen et al., 2011). In fact, those animals in third lactation or higher had a numerically poorer udder cleanliness score (2.5 vs 2.4) compared to cows in their first or second lactation. Further, primiparous cows stood on average 1 h less than multiparous cows. Thus, higher parity cows may be at increased odds of eSCC because of reduced cleanliness, as a result of increased standing time and lower udder carriage, which increases the risk of exposure of the teat ends to manure from the alley floor.

Parity has also been linked with baseline SCC, with multiparous cows naturally having a higher SCC (Olde Riekerink et al., 2007). Even though there was no interaction between parity and baseline SCC on the risk of eSCC, the higher baseline SCC was, in addition to parity, associated with higher risk of eSCC. For the purposes of this study, a SCC level of >200,000 cells/mL was considered an eSCC, and used as a proxy for potential infection, while an SCC <100,000 cells/mL was considered normal (Dohoo and Leslie, 1991 and Bradley and Green, 2005). While cows needed to have a SCC <100,000 cells/mL to be considered at risk for acquiring a new eSCC, those cows which had an SCC closer to 100,000 cells/mL were at increased odds of acquiring a new eSCC. A potential limitation of our study is the presence of false negatives; cows that were categorized as not infected according to our eSCC definition but in fact did have an IMI. Bradley and Green (2005) noted that while major pathogens cause SCC to increase above

200,000 cells/mL, minor pathogens have a lesser impact on SCC. In fact, SCC may be as low as 50,000 cells/mL even though a cow is infected. In future studies, bacteriological culture is recommended to help ensure that those animals selected as uninfected based on SCC were actually true culture-negative.

Cows with post-milking standing times averaging >90 min were at decreased odds for acquiring a new eSCC. Previous research (McDonald, 1975 and Schultze and Bright, 1983) would suggest that there are 2 periods of time whereby teat canal diameter may be expanded and, thus, more susceptible to bacterial penetration following milking: immediately after removal of the milking machine and 2 to 4 h after milking. Given that in the current study few cows chose to lie down quickly after milking (only 6% of cows studied laid down within 40 min after milking), it is not that surprising that we did not detect any change in odds of incidence of eSCC in those cows. The decreased odds of eSCC with post-milking standing times >90 min in the current study contrasts to that found by DeVries et al., 2010 and DeVries et al., 2011a, who noted increased odds of IMI when cows spent >1.5 and 2.5 h, respectively, standing after milking. These latter authors speculated that the threshold for the second period of susceptibility might be at the lower end of the 2–4 h period after milking. In the current study, just over 23% of cows spent between 90 and 120 min standing after milking, 25% of cows spent between 2 and 4 h standing after milking, while only 3% stood for over 4 h after milking. These findings would suggest that the start of the second period of susceptibility might under certain conditions be closer to the 4 h mark after milking. Further research is required to fully understand these factors that may affect teat canal diameter at various time points post-milking, and the resultant influence this has on the risk of acquiring infections during those time periods.

2.5 Conclusions

Free-stall housed cows that are milked 3×/d that were in later lactation, produced less milk, or were lame had the longest daily lying times. Lying bout frequency was greater in older cows or cows in earlier lactation, while lying bout duration increased with DIM and in those cows identified as being lame. The results of the current study suggest that free-stall housed cows milked 3×/d that remain standing for greater than 90 min after milking are at decreased odds for acquiring a new eSCC compared to those cows that lay down within 90 min after milking. Provision of feed in the hour before milking, or in the time period immediately after milking, led to the longest duration of post-milking standing. Management practices that promote post-milking standing duration, such as providing new feed or pushing-up feed around milking time may help to improve udder health.

2.6 Acknowledgements

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Table 2.1 Descriptive summary of the cows sampled within each study herd at the beginning of the study period.

Herd	Cows sampled/herd	# of periods	Mean parity	Mean DIM	Mean beginning SCC (x 1000 cells/mL)	Mean 305 d milk production (kg)
1	40	3[1]	2.2	115	51	9,845
2	39[2]	3[3]	2.1	73	41	9,820
3	40	4	2.2	68	36	10,777
4	40	1[4]	2.0	79	34	10,250
5	40	4	2.5	66	33	11,845
Total	199	-	2.2	74	39	10,521

[1]This herd was switched to 2x/d milking before the completion of period 4.

[2]One of the focal animals was sold before the completion of period 1.

[3]The final DHI test for this herd failed to include SCC data for the end of period 4.

[4]Herd switched to robotic milking before the completion of periods 2.

Table 2.2 Descriptive summary of data from focal cows (n=199) across all 5 study herds[1].

Variable	Mean	SD	Minimum	Maximum
Lying time (h/d)	11.2	2.1	1.2	17.2
Lying bouts (#/d)	8.6	2.9	1.2	26.4
Lying bout duration (min/bout)	84.6	27.2	11.7	221.1
Post-milking standing time (min)	103	96	1	798
Pre-milking standing time (min)	88	87	1	867
Lameness score[2]	1.5	0.8	1	4
Hygiene score[3]				
Udder	2.4	0.7	1	4
Upper leg/flank	2.6	0.6	1	4
Lower legs	4	0.2	3	4

[1]Data is averaged across the recording periods for each of the 40 focal cows from each of the 5 herds (with the exception of 39 cows at one herd) that were observed for 5 d observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

[2]Lameness: no = gait score <3; yes = gait score ≥ 3). Gait score was assessed on a 5-point scale (1= normal to 5= severely lame) (Flower and Weary, 2006; Ito et al., 2010).

[3]Hygiene score was assessed on a 4-point scale (1=very clean to 4=very dirty) (www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf; Cook and Reinemann, 2007).

Table 2.3 Final general linear model for factors associated with lying behavior[1] of free-stall housed cows.

	Lying time (h/d)			Lying bout frequency (bouts/d)			Lying bout duration (min/bout)		
Variable	B[2]	SE	*P*-value	B	SE	*P*-value	B	SE	*P*-value
Intercept	11.7	0.62	<0.001	10.26	0.74	<0.001	64.43	6.95	<0.001
DIM[3]	0.009	0.002	<0.001	-0.008	0.002	<0.001	0.15	0.017	<0.001
Parity			-			0.09			-
1	-	-		Ref[4]	-		-	-	
2	-	-		-0.52	0.38		-	-	
≥ 3	-	-		-0.82	0.38		-	-	
Milk yield, kg/d	-0.046	0.013	<0.001	-	-	-	-	-	-
Lameness[5]			0.001			-			0.005
No	Ref	-		-	-		Ref	-	
Yes	0.75	0.023		-	-		7.51	2.63	

[1]Data is averaged across the recording periods for each of the 40 focal cows from each of the 5 herds (with the exception of 39 cows at one herd) that were observed for 5 d observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

[2]β = estimated regression coefficient.

[3]DIM = days in milk at the beginning of each observation period.

[4]Ref = Reference category.

[5]Lameness: no = gait score <3; yes = gait score ≥ 3). Gait score was assessed on a 5-point scale (1= normal to 5= severely lame)

(Flower and Weary, 2006; Ito et al., 2010).

Table 2.4 Final general linear model for factors associated with pre- and post-milking standing time of free-stall housed cows[1].

	Pre-milking standing time (min)			Post-milking standing time (min)		
Variable	B[2]	SE	*P*-value	β	SE	*P*-value
Intercept	101.6	6.4	<0.001	96.1	15.5	0.001
Milking-feeding interval[3]						0.10
< - 60 min	-	-	-	-11.1	5.4	
-60 to 0 min	-	-		-0.5	5.5	
> 0 min	-	-		Ref[4]	-	
DIM[5]	-0.18	0.04	<0.001	-	-	-
Parity			0.07			0.06
1	Ref	-		Ref	-	
2	9.3	5.7		11.6	6.4	
≥ 3	12.3	5.7		14.7	6.3	

[1]Data is averaged across the recording periods for each of the 40 focal cows from each of the 5 herds (with the exception of 39 cows at one herd) that were observed for 5 d observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

[2]β = estimated regression coefficient.

[3]Minimum difference in time between when cows were milked and when fresh feed was provided.

[4]Ref = Reference category.

[5]DIM = days in milk at the beginning of each observation period.

Table 2.5 Number of pairs of milk samples taken per farm and number of newly elevated somatic cell count (eSCC) across the study period[a].

Herd	Total pairs of samples	Total pairs at risk[b]	Number of new eSCC[c]	eSCC incidence rate (eSCC/cow-year)
1	118	108	12	1.13
2	117	91	15	1.58
3	160	133	7	0.51
4	40	39	2	0.37
5	156	133	12	0.96
Total	591	504	48	0.91

[a]Data is averaged across the recording periods for each of the 40 focal cows from each of the 5 herds (with the exception of 39 cows at one herd) that were observed for 5 d observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

[b]Pairs were considered at risk when SCC <100,000 cells/mL at the beginning of each observation period.

[c]New eSCC was defined as SCC >200,000 cells/mL at the end of each period when SCC <100,000 cells/mL at the beginning of each period.

Table 2.6 Unconditional estimates of association between explanatory factors and odds of having an incident elevated somatic cell count[1].

Variable	Percentage or Mean (±SD)[2]	Odds ratio (95% CI)[3]	*P*-value
Parity			0.007
1	26.0	Ref[4]	
2	40.7	5.31 (1.86, 15.19)	
≥ 3	33.3	2.99 (1.02, 8.75)	
DIM[5]	113.5 (50.9)	1.31 (0.95, 1.81)	0.09
LnSCC[6]	3.44 (0.63)	2.13 (1.51, 3.02)	<0.001
Post-milking standing time			0.035
0-90 min	49.2	Ref	
> 90 min	50.8	0.47 (0.23, 0.95)	
Encourage post-milking standing[7]			0.03
No	64.1	Ref	
Yes	35.9	0.40 (0.18, 0.92)	
Lameness[8]			0.09
No	88.9	Ref	
Yes	11.1	1.27 (0.97, 1.67)	
Udder hygiene	2.49 (0.69)	1.24 (0.90, 1.71)	0.19
Teat dip method			0.20
Dip cup	53.5	Ref	
Foam	46.5	1.81 (0.72, 4.60)	
Stall width (cm)	121.7 (6.0)	0.70 (0.46, 1.07)	0.10

[1]Data is averaged across the recording periods for each of the 40 focal cows from each of the 5 herds (with the exception of 39 cows at one herd) that were observed for 5 d observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

[2]Proportion of observations for categorical variables or mean and standard deviation for continuous variables.

[3]Odds ratio and 95% confidence interval for 1 SD increase for continuous variables presented.

[4]Ref = Reference category.

[5]DIM = days in milk at the beginning of each observation period.

[6]LnSCC = natural log of individual cow SCC (×1,000 cells/mL) at the beginning of the study period.

[7]Encourage post-milking standing : no = feed was not intentionally pushed up in the bunk at the return from milking; yes = feed was intentionally pushed up in the bunk at the return from milking.

[8]Lameness: no = gait score <3; yes = gait score ≥ 3). Gait score was assessed on a 5-point scale (1= normal to 5= severely lame).

Table 2.7 Final logistic regression model for factors associated with the incidence of elevated somatic cell count[1].

Variables	Coefficient	SE	Odds ratio (95% CI)	*P*-value
Intercept	-7.73	1.19	-	-
Random effects				-
Herd-level variance	0.18	0.22	-	
Cow-level variance	0.09	0.49	-	
Post-milking standing time				0.008
0-90 min	Ref[2]	-	-	
> 90 min	-1.04	0.39	0.35 (0.16, 0.76)	
Parity				0.002
1	Ref	-	-	
2	1.76	0.54	5.83 (2.03, 16.73)	
≥ 3	0.95	0.55	2.59 (0.88, 7.60)	
LnSCC[3]	1.30	0.29	2.26 (1.58, 3.24)[4]	<0.001

[1]Data is averaged across the recording periods for each of the 40 focal cows from each of the 5 herds (with the exception of 39 cows at one herd) that were observed for 5 d observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

[2]Ref = Reference category.

[3]LnSCC = natural log of individual cow SCC (×1,000 cells/mL) at the beginning of the study period.

[4]Odds ratio and 95% confidence interval for one standard deviation increase in initial LnSCC.

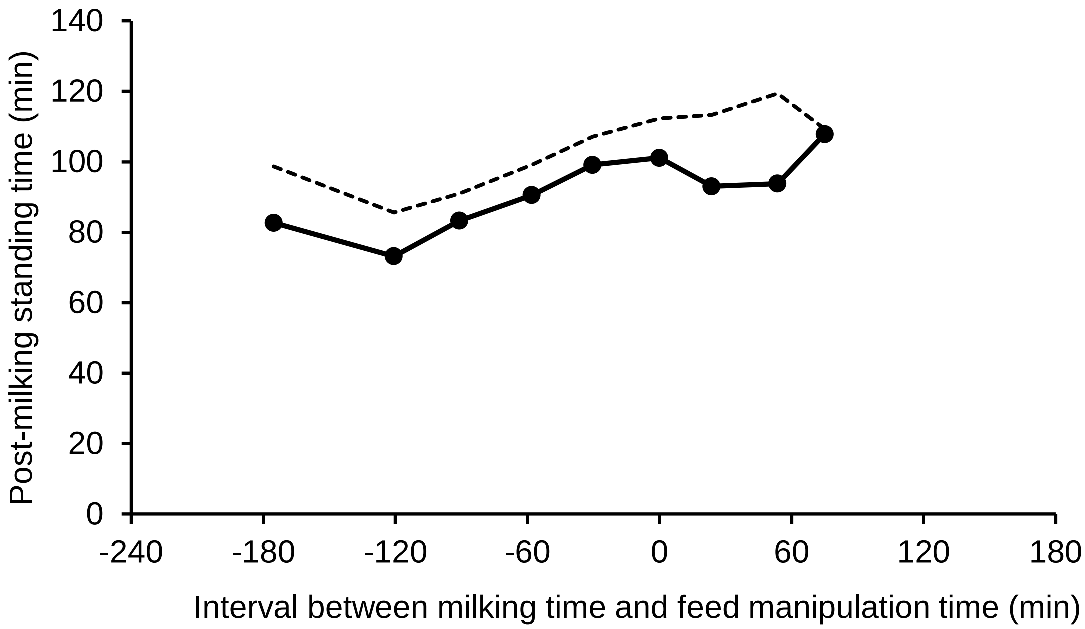

Figure 2.1 Moving average (with width of 60 min and increments of 30 min) of median (full line) and mean (dashed line) post-milking standing time across time interval between milking and feed manipulation (feed delivery and feed push-up). Data is averaged by period for each of the 40 focal cows from each of 5 herds (with the exception of 39 cows at one herd) that were observed for 5 d observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

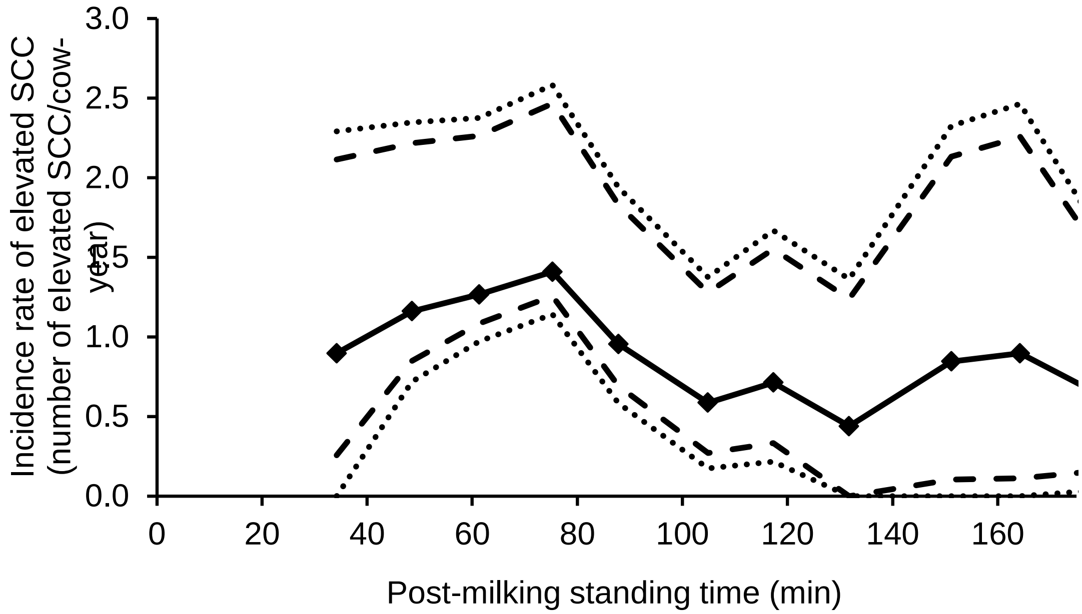

Figure 2.2 Moving average (with width of 30 min and increments of 15 min) of incidence rate of elevated somatic cell count across average post-milking standing time. The elevated SCC incidence rate (full line) with 90% (dashed lines), and 95% (dotted line) confidence limits. Data is averaged by period for each of the 40 focal cows from each of 5 herds (with the exception of 39 cows at one herd) that were observed for 5 d observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

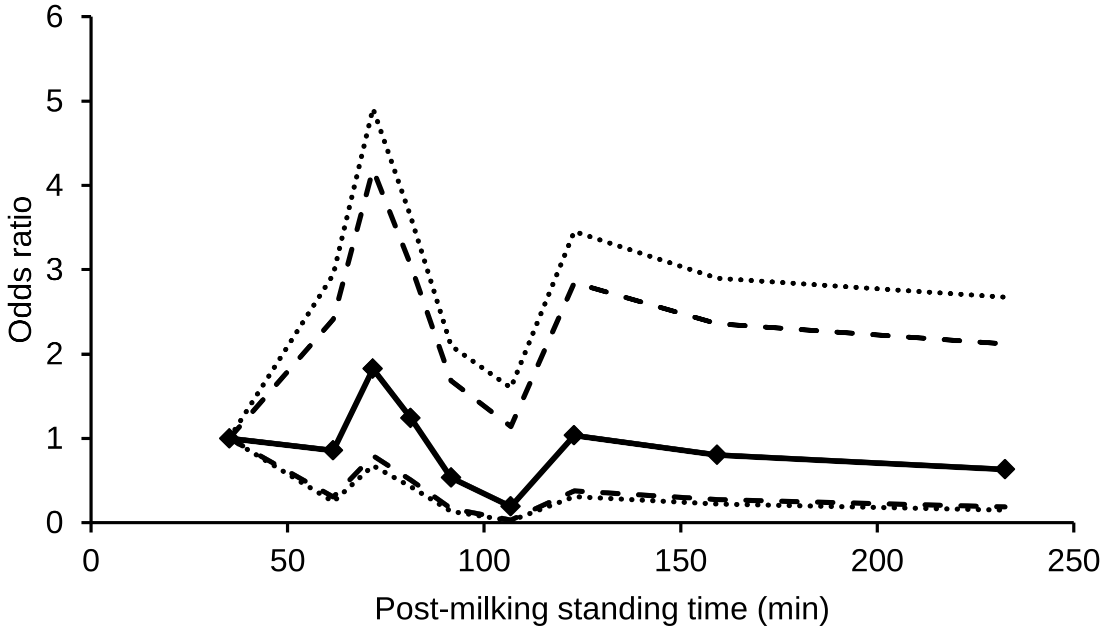

Figure 2.3 Adjusted estimates of the odds of acquiring a newly elevated somatic cell count across deciles of average post-milking standing time (solid line) with 90% (dashed lines), and 95% (dotted lines) confidence limits. Data is averaged by period for each of the 40 focal cows from each of 5 herds (with the exception of 39 cows at one herd) that were observed for 5 d observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

CHAPTER 3: RELATIONSHIP BETWEEN POST-MILKING STANDING DURATION AND RISK OF INTRAMAMMARY INFECTION IN FREE-STALL HOUSED DAIRY COWS MILKED THREE TIMES PER DAY

3.1 INTRODUCTION

Encouraging cows to remain on their feet after milking has been a long accepted practice surmised to decrease the incidence of intramammary infection (IMI) in lactating dairy cows. This practice is thought to increase the likelihood that the teat canals will have closed prior to the udder contacting the stall substrate when the cow lies down, thus decreasing the odds of bacterial penetration of the teat, and by association resulting in a decreased risk of IMI (Tyler et al., 1997; Johansson et al., 1999). Recent research on the effects of farm management on lying behavior and the incidence of IMI provides some evidence of a relationship between incidence of IMI and post-milking standing duration (PMSD) (DeVries et al., 2010; DeVries et al., 2011a).

In a recent longitudinal study a decreased odds of environmental IMI in tie-stall housed cows was observed with PMSD of 40 to 60 min as compared to those animals lying down within 40 min of milking, while the incidence of new environmental IMI increased when PMSD were greater than 60 min (DeVries et al., 2010). A similar type of study but undertaken with free-stall, robotic-milked cows noted that the only IMI pathogen associated with PMSD were *coagulase-negative staphylococci* (CNS) (DeVries et al., 2011a). In that study, the incidence of new CNS IMI was similar in cows with PMSD up to 2.5 h, after which the risk tended to increase (DeVries et al., 2011a). Interestingly, few of the robotic-milked cows laid down soon after milking, which may have contributed to a lack of an increased risk observed in the time period immediately following milking when risk has traditionally thought to be greatest.

Given the tremendous variation in management of free-stall housed dairy cows observed on commercial farms (von Keyserlingk et al., 2012) it follows that IMI risk and PMSD may be affected by differences in management that in turn affect lying behavior. DeVries et al. (2010) reported a mean PMSD of 79 min for tie-stall housed cows. This value was comparable to subsequent studies observing cows under free-stall housing conditions milked by AMS (78 min, DeVries et al., 2011a; 77 min, DeVries et al., 2012). However, a shorter PMSD was reported by Tyler et al. (1997) when observing the effects of feed provision following milking on free-stall housed cows milked 2x/d in parlor; 48 min for cows with access to feed vs 21 min for cows without access to feed. This reduced latency to lie down post-milking in 2x/d milked free-stall cows as a response to fresh feed access was also found by DeVries and von Keyserlingk (2005); cows provided with fresh feed had an average PMSD of 66 min vs 45 min for cows without access to fresh feed. Also under free-stall housing and 2x/d milking, Fregonesi et al. (2007) observed an average PMSD of 39 min under 100% stocking conditions.

It is plausible that the reduced latency to lie observed in free-stall 2x/d parlor milking conditions is a result of the forced period of standing inherent to parlor milking practices. A study of 16 free-stall herds, milking either 2 or 3x/d, revealed that mean daily milking time was 3 h/d with a range of 0.5 to 6 h/d on the 2x/d milking farms and 1 to 6 h/d on the 3x/d milking farms (Gomez and Cook, 2010). In a study of North American free-stall herds, mean time away from pen was reported as almost 4 h/d in Western herds and almost 5 h/d in Eastern herds indicating that regardless of milking frequency, parlor milked cows experience long periods of time outside of their pens (von Keyserlingk et al., 2012). While long group milking times have been reported, there is also individual variability in milking time and Cook and Nordlund (2009) noted that while parlor design and group sizes generally target a milking time of 45-60 min per group, individual cow's daily milking times may be quite variable ranging from 1.5 h/d to over 3 h/d in 3x/d milked cows. Thus, reduced PMSD may be anticipated in cows milked 3x/d given the increased potential for standing demands associated with this management practice, and the expectation that cows will choose to lie down more quickly following milking if not provided with an incentive to remain standing, such as fresh feed.

The objectives of this study were, for free-stall cows milked 3x/d, to determine: 1) which factors are associated with PMSD, and 2) whether there is an association of PMSD with incidence of IMI. It was hypothesized that cows that had shorter PMSD would be at an increased risk to experience an IMI, and that PMSD would be longer when feed delivery and feed push up occurred close to the time of milking.

3.2 Materials and Methods

3.2.1 Farm Selection

Four commercial dairy farms in Eastern Ontario, Canada were recruited for participation in this longitudinal study. Herds were selected as a convenience sample for their proximity to the University of Guelph, Kemptville Campus (Kemptville, Ontario, Canada). Selection criteria was as follows; free-stall housing, parlor-milking, milked 3x/d, participated in a dairy herd improvement (DHI) program, were predominantly Holstein-Friesian (>90%), had > 120 lactating cows in the herd. Given that there were too few farms using sand bedding in the study area we also did not enroll farms which utilized sand bedding. Farms had a mean herd size of 326 (range: 133 to 566 cows), mean adjusted 305 d milk yield of 11,670 kg (range: 10,801 to 13,012 kg), and a mean annual bulk milk SCC of 249,000 cells/mL (range: 161,000 to 330,000 cells/mL). The study was conducted between April 2012 and August 2012. Initiation of the study on each farm was based on a rolling start date corresponding to each farm's DHI test dates. The study was comprised of three, 28-d periods beginning on the initial on-farm milk sample date.

3.2.2 Animal Selection

Forty cows per herd were randomly selected as focal animals from the group of cows on each farm that were <200 DIM and SCC <100,000 cells/mL at the most recent DHI test. A DIM of <200 d was targeted to ensure that focal cows would remain in the milking herd for at least 2 periods of the study. A SCC of <100,000 cells/mL was targeted as a pre-screening method for IMI and these cows were assumed to be infection free (Dohoo and Leslie, 1991; Schepers et al., 1997; Schukken et al., 2003); bacteriological culture of quarter milk samples later confirmed the IMI status of all focal cows at the start of the study. Lactation number and DIM were recorded from farm records for each focal cow and validated using DHI data.

3.2.3 Milk Sampling and Analysis

The study began following a regularly scheduled DHI milk test to allow for selection of focal animals based on SCC. All focal cows enrolled in the study were subjected to an initial quarter aseptically milk sampling regime (Hogan et al., 1999) undertaken by two trained personnel. This initial sample served as a baseline sample to determine whether a cow was truly culture negative and to allow for determination of new instances of infection in subsequent periods of the study.

Milk samples were then collected on a 28-d cycle, not necessarily concurrent with a DHI test date, with 4 sets of milk samples taken from each focal cow on each farm. All milk sampling was done at the second daily milking on each farm. Cows entered the parlor and were prepped by the farm milking personnel according to their normal milking procedures (pre-dip, forestripping, etc.). Immediately following, researchers disinfected teat ends with cotton swabs soaked in 70% alcohol and allowed teats to dry. A 30-mL milk sample was then drawn into a labeled sterile container for each quarter. The milking unit was then placed on the cow and normal milking procedures proceeded. All milk samples were stored at -20°C prior to being shipped for bacteriological analysis.

Samples were shipped overnight to the University of Calgary, Faculty of Veterinary Medicine (Calgary, Alberta, Canada) for milk bacteriological culture. The bacteriological culturing protocol and pathogen species identification followed the National Mastitis Council (NMC) guidelines (NMC, 2004; Harmon et al., 1990). Briefly, blood agar plates were segregated into four sections with each zone representing one quarter. An inoculum of 10 µL of milk was then spread over each respective section using a sterile 10 µL inoculating loop. All plates were then incubated at 37°C and examined for bacterial growth at 24 and 48 h. Following 48 h of incubation colonies were enumerated and speciated. Sub-culturing of colonies was performed where necessary to ensure sufficient sample for further tests to determine pathogen type.

Samples that were culture positive for 3 or more pathogens, except for those that included *Staphylococcus aureus* or *Streptococcus agalactiae*, were classified as contaminated and no longer considered for further analysis (Sampimon et al., 2009; DeVries et al., 2011a). A milk sample was considered culture positive when ≥100 cfu/mL of a major pathogen (i.e. *Staph. aureus, Strep. agalactiae*, *Strep. uberis*, *Strep. dysagalactiae*, *E. Coli*, *Enterobacter spp.*, *Klebsiella spp.*) or ≥500 cfu/mL of a minor pathogen (i.e. *CNS*, *Corynebacteria spp.*, *A.*

pyogenes, Staph. Hyicus, Enterococcus spp., Streptococcus spp., Nocardia) was cultured (Sampimon et al., 2009). *Mycoplasma spp*. were not cultured for this study.

IMI were identified at the quarter level (Harmon et al., 1990). The occurrence of a new IMI was considered to have occurred when a culture-positive sample of the specific pathogen was preceded by a culture-negative sample collected during the previous milk sampling. Animals were only considered to be at risk for a new IMI if they had experienced a culture-negative for that specific pathogen at the previous testing. Non-occurrence of a new IMI was defined as two consecutive culture-negative samples for the specific pathogen. To be considered at risk for a new IMI during the second, third, or fourth milk sampling, a quarter would have to have been culture-negative during the first, second, or third milk sampling, respectively. Based on these definitions, a cow could only experience a maximum of two new IMI for each pathogen throughout the study duration.

3.2.4 Standing and Lying Behavior

Standing and lying behaviors were recorded using data loggers (HOBO Pendant G Data Logger, Onset Computer Corporation, Pocasset, MA, USA). These devices allowed standing and lying behavior to be electronically recorded by measuring leg orientation in 1-min intervals (Ledgerwood et al., 2010). Data loggers were affixed to the rear metatarsal area of the focal cows with veterinary bandaging tape (Vetrap Bandaging Tape, 3M, London, ON, Canada) for the first 5 d of each period following the first 3 milk samplings. Ito et al. (2009) and Vasseur et al. (2012) demonstrated that recording lying behavior for 3 and 4 d, respectively, produced an accurate result of herd-level lying behavior using the same technology utilized in this study. By using 5 d of recording we sought to improve the accuracy of our results and increase the likelihood that we were recording a cow's normal (typical) behavior. Lying behavior was recorded at the start of each period to determine lying times when they were culture-negative for bacteriology; this behavior was then related to future risk for acquiring an IMI (Medrano-Galarza et al., 2012). The lying behavior of culture-positive cows was not considered in the risk analysis. Standing and lying duration (min/d), lying bout frequency (#/d), and lying bout duration (min/bout) were calculated using the recorded data. Pre-milking standing duration (min) was defined as the interval between initiation of milking and the last observation of lying prior to milking. PMSD (min) was defined as the interval between the initiation of milking and the initial observation of lying following milking.

3.2.5 Cow Measures

Individual milking times and milk production for each focal cow were obtained from parlor records. Focal cows were hygiene scored (Cook and Reinemann, 2007) and locomotion scored (Flower and Weary, 2006) by two trained observers for all three periods at the conclusion of each 5 d recording period. Given that the focal cows were randomly distributed throughout the herd, the herd manager was requested to record feed delivery and feed push-up times for each milking group in which there were focal cows. The interval between all feed manipulation events, including fresh feed delivery, feed push up and milking time was calculated for each focal cow by determining the time (in minutes) between each focal cow's individual milking time and the feed manipulation event that took place closest to the time of that milking.

3.2.6 Herd, Housing, and Management Data

Prior to the commencement of the study, a questionnaire, adapted from Dufour et al. (2010) and Dufour et al. (2011), was distributed and completed by the owner or herd manager. In addition to the written survey, the individuals were questioned by a single interviewer on the general housing and management present on each farm, including among other things, milking practices, stall dimensions, bedding (type, quantity, frequency of cleaning), and management style. Although all farms fed a TMR to their lactating cows, bedding, stall design, feeding regimen (including feed delivery and feed push-ups), and cleaning systems varied by farm. For instance, although 2 farms delivered feed 2x/d, 1 farm had a morning and evening feed delivery while the other farm topped-up their first feed delivery 1 h after the first feeding. The other two farms delivered feed 1x/d. Feed push-up frequency ranged from 4x/d (n=1) to 5x/d (n=3). Mean free-stall stocking density (#cows/stalls) and linear bunk space per cow was 103 ± 10 % (range: 93 to 116%) and 0.53 ± 0.12 m/cow (range: 0.35 to 0.67 m/cow), respectively, for the 4 farms. For stall base, one farm used rubber mats, two used mattresses, and the fourth farm had a combination of rubber mats and waterbeds. Two of the farms bedded with composted manure solids, one used compost (Orgaworld Canada Ltd, Ottawa, Ontario), one used straw and shavings, and the fourth farm used sawdust and shavings coupled with the addition of lime 1x/wk. One farm stated that their aim was to bed to a depth of >2 cm, while two of the farms stated that their bedding depth was <2 cm, and the fourth farm had no targeted bedding depth. All farms scraped manure and dirty bedding from the stalls 3x/d. Farms also differed in stall cleanliness techniques with 2 farms scraping the entire stall, one only the back third, and the remaining farm only removing the portion of the stall that was dirty. Addition of new bedding occurred either 1x/wk (n=2) or 2x/wk (n=2). The average stall dimensions across all farms were

175 ± 8 cm x 112 ± 4 cm (range stall length: 163 to 183 cm; range width: 107 to 117 cm). All farms utilized automatic alley scrappers to clean passageways (one farm also had slatted floors on one passage) with cleaning frequency ranging from 3x/d to 24x/d. Hoof trimming frequency ranged from 1 to 4x/yr with 3 of the farms using a certified hoof trimmer. Two of the farms specified that they conducted locomotion scoring to detect lameness. In terms of milking procedures, parlor capacity ranged from double ten to double fourteen parallel parlors. Daily milking times were variable across all farms, however all farms maintained an 8-h interval between milkings. Average number of cows in the holding area was 92 ± 37 (range: 50 to 130). Cows entered a holding pen prior to milking on all farms, and farms grouped lactating cows based on either production and/or parity. All farms used a pre-milking and post-milking teat treatment. For pre-milking teat cleaning procedures, the farms used either a foam (n=2), spray (n=1), or dip (n=1). The two farms that used a pre-milking foam treatment also fore-stripped before attaching the milking units. Post-milking teat disinfection procedures used either a foam (n=1), spray (n=1), or dip (n=2). The two farms that used a pre-milking foam treatment utilized a post-milking dip treatment, while the farm that utilized a dip as a pre-milking teat treatment used a foam post-milking. One farm stated that they cleaned >80% of the teat surface prior to milking while the other farms stated they cleaned 100% of the teat. All of the milking units had automatic take-off and none of the farms cleaned the units between cows; some farms rinsed the units with a spray of water following a bucket cow (a fresh or treated cow depending on the particular farms practices) or between milking groups after the equipment had been soiled. All farms flamed the cows’ udders and three of the farms tail docked their cows. Two of the farms stated that they did not specifically encourage cows to remain standing after milking while the other two farms stated that they pushed-up feed to encourage cows to remain on their feet for a

time after milking. Any variables that were unique to a specific herd were not retained as an explanatory variable in the statistical analyses since its measure of association would be confounded by other characteristics specific to that herd.

3.2.7 Statistical Analysis

Prior to analyses, all data were screened for normality using the UNIVARIATE procedure of SAS (SAS Institute, 2011). Cow baseline SCC and PMSD were slightly right skewed and, thus, transformed by the natural logarithm. All cow-level data were summarized across each of the 5-d observation periods for each cow. The associations between PMSD (for each milking of the day), and possible cow-level and herd-level explanatory (independent) fixed-effect variables were analyzed with multivariable linear mixed models using the MIXED procedure of SAS, treating period as a repeated measure. The models included the random effects of herd and cow within herd. Cow within herd was included in the models as the subject of the repeated statement. Initially, unconditional associations between independent variables and dependent variables were conducted. Only independent variables with $P \leq 0.25$ in this initial screening were included in multivariable linear regression models (Dohoo et al., 2009). The CORR procedure of SAS was used to check for correlations between the kept explanatory variables. If two variables were highly correlated ($r > 0.6$), the one with the lower P-value in the unconditional associations was kept. Manual backward elimination of non-significant ($P > 0.10$) variables was used and from the resultant models, plausible 2-way interactions were examined and retained if $P \leq 0.10$. Only those results retained in the final multivariable models are presented. The covariance structure was compound symmetry by best fit according to Schwarz's Bayesian information criterion.

The association of the various cow-level and farm-level explanatory variables on the occurrence or non-occurrence of an IMI was assessed using a random intercept mixed logistic model using the GLIMMIX procedure (distribution = binomial and link = logit) of SAS. As new IMI were assessed at the quarter level, with herd, cow within herd, and quarter within cow within herd considered as random. Unconditional associations were estimated in the described model to screen all potential explanatory variables. For variables measured on a continuous scale, linearity of the relationship between the variable and the occurrence of an IMI was assessed by categorizing the continuous variable and visual inspection of plots of the odds ratio against mean values of the categories. Variables with $P \leq 0.25$ were submitted to the multivariate model. The CORR procedure of SAS was used to check for correlations between explanatory variables. If two variables were highly correlated ($r > 0.6$), the one with the lower P-value in the unconditional associations was kept. These kept variables were then included in the previously described multivariable analysis. Manual backward elimination of non-significant ($P > 0.10$) effects was used and from the resultant models, plausible 2-way interactions were examined and retained if $P \leq 0.10$. Only those results retained in the final multivariable logistic model are presented.

3.3 RESULTS

A total of 10 cows were eliminated in the study due to drying-off (1), culling (4), illness (1), or death (4). A descriptive summary of focal cow parameters, including parity, DIM, baseline SCC, and 305 d milk production, is presented in Table 3.1. Table 3.2 contains a descriptive summary for data on all 160 focal cows collected across the study periods.

3.3.1 Factors Influencing Post-milking Standing Duration

Average PMSD across all 3 periods of the study was 96 min (median = 80 min). The average before milking milk-feed manipulation delay (difference in time between individual milking time and closest to fresh feed delivery or feed push-up) was 20 ± 63 min, while the average after milking-fresh feed delay (difference in time between individual milking time and closest fresh feed delivery) was 100 ± 357 min. The relationship between PMSD and feed manipulation was non-linear with PMSD peaking around 60 min before milking with a second incidence of increased PMSD between 30 to 60 min following milking (Fig. 3.1). The relationship between PMSD and fresh feed delivery was also non-linear with PMSD decreasing 120 to 300 min before milking and a second lesser decrease of PMSD between 360 and 600 min following milking (Fig. 3.2). Given the non-linear relationship between these variables meaningful categories were chosen based on data distribution, with similar numbers of observations per category (DeVries et al., 2010; DeVries et al., 2011a). Average milk-feed manipulation delay was categorized as follows: >60 min before milking, 60 to 30 min before milking, 30 min before to 60 min after milking, and >60 min after milking. Average milk-fresh feed delivery delay was categorized as: >300 min before milking, 300 to 60 min before milking, 60 min before to 90 min after milking, 90 to 180 min after milking, 180 to 540 min after milking, and >540 min after milking.

The multivariable model for PMSD indicated a significant interaction between daily milking time (1st, 2nd, or 3rd milking of the day) and the milk-fresh feed delay, therefore separate analyses were done for PMSD for each daily milking (Table 3.3).

Mean PMSD for the first daily milking was 86 ± 54 min. An increase in PMSD following the first daily milking tended to be associated with increased frequency of feed push-ups and was associated with increased bunk space per cow (m/cow) (Table 3.3). Each additional feed push-up per day was associated with a 6 min increase in PMSD, while every 0.1 m/cow increase in bunk space was associated with a 19 min increase in PMSD. Increased stocking density (%) was associated with a decrease in PMSD (Table 3.3); every 10% increase in stocking density was associated with a 2 min decrease in PMSD. Manipulation of feed between 60 to 30 min before milking and between 30 min before and 60 min after milking were associated with an 8 and 5 min, respectively, reduction in PMSD (Table 3.3). Delivery of fresh feed between 180 to 540 min after milking was associated with a 23 min decrease in PMSD (Table 3.3).

Mean PMSD following the second daily milking was 99 ± 71 min. Increased PMSD following the second daily milking was associated with increased feed push-ups (Table 3.3); each additional feed push-up was associated with a 7 min increase in PMSD. Delivery of fresh feed between 90 to 180 min after milking was associated with a 20 min increase in PMSD after the second daily milking (Table 3.3).

Mean PMSD following the third daily milking was 94 ± 62 min. Increased PMSD following the third daily milking was associated with increased feed push-ups (Table 3.3); each additional feed push-up was associated with a 10 min increase in PMSD. Decreased PMSD was associated with DIM and milk order (Table 3.3). Later lactation cows had a reduced PMSD, with PMSD decreasing by 4 min for every additional 50 DIM. Cows coming later in the milking order also had a decreased PMSD, with PMSD 11 min longer when comparing a cow milked 25th out of 100 cows to one milked 75th.

3.3.2 Association of Post-milking Standing Duration and Incidence of IMI

A total of 2,460 milk samples were collected over the course of the study. Twenty-eight (1.1%) of the samples were identified as being contaminated. Overall, across the three, 28-d observation periods, 1,846 quarters were considered at risk for a new IMI. A total of 456 new IMI were detected throughout the study for an incidence rate of 3.22 IMI/quarter-year. CNS and *Corynebacterium* spp. were the most commonly observed pathogens causing 45% and 31%, respectively, of the total culture-positive results. Other pathogens responsible for new IMI were: 46 *Streptococcus* spp., 40 *Enterococcus* spp., 10 *Staph. hyicus*, 8 *T. pyogenes*, 3 *Staph. aureus*, 2 *Strep. dysagalactiae*, 1 *E. coli*, 1 *Enterobacter* spp., 1 *Nocardia* spp., 1 *Strep. agalactiae*, and 1 *Strep. uberis*. A total of 202 new CNS IMI were detected for an incidence rate of 1.62 CNS IMI/quarter-year and a total of 140 new *Corynebacterium* spp. IMI were detected for an incidence rate of 1.10 *Corynebacterium* spp. IMI/quarter-year. A complete description of the total IMI, CNS IMI, and *Corynebacterium* spp. IMI by herd can be found in Table 3.4. As CNS and *Corynebacterium* spp. were the pathogens responsible for the most new IMI, the analyses were focused on these two specific pathogens.

Separate analyses of CNS and *Corynebacterium* spp. IMI revealed that only CNS IMI risk was associated with PMSD. Therefore, only the results of the CNS IMI analysis are presented below. Incidence of CNS IMI steadily increased until approximately 50 min PMSD, this was followed by a steady decline until approximately 100 min post-milking before the incidence of CNS IMI increased again (Fig. 3.3). A second decrease in incidence was observed when PMSD was greater than 180 min. Given the non-linearity of the relationship between PMSD and risk of a new CNS IMI (Fig. 3.4), meaningful categories of PMSD were chosen based on data distribution and existing knowledge on teat morphology following milking

(McDonald, 1975; Schultze and Bright, 1983). PMSD was categorized as follows: <60 min, 60 to 75 min, 75 to 90 min, 90 to 120 min, and >120 min. Table 3.5 shows the unconditional associations for CNS IMI of the independent variables from the univariable analyses prior to running the multivariable analysis. Increased risk of CNS IMI was associated with increased fresh feed delivery frequency, DIM, and delivery of fresh feed between 180 to 540 min after milking. Decreased risk of CNS IMI was associated with increased frequency of feed push-ups, increased pre-milking standing time, a PMSD of 90 to 120 min, and delivery of fresh feed between 60 min before to 90 min after milking and >540 min after milking. Three of these variables were retained in the multivariable model (Table 3.6). Decreased risk of CNS IMI was associated with a PMSD of 90 to 120 min, fresh feed being delivered between 60 min before to 90 min after milking and >540 min after milking, and increased frequency of feed push-ups.

3.4 DISCUSSION

The study median PMSD, 80 min, is much longer than that previously reported in free-stall herds parlor milking 2x/d (35 min: Tyler et al., 1997; 39 min: Fregonesi et al., 2007; 55 min: DeVries and von Keyserlingk, 2005; 62 min: DeVries et al., 2005). Post-milking standing duration was more comparable to that reported under tie-stall and free-stall AMS conditions (79 min: DeVries et al., 2010; 78 min, DeVries et al., 2011a; 77 min, DeVries et al., 2012; 75 min: Deming et al., 2013). One would anticipate a shorter PMSD under 3x/d milking conditions given that cows would be assumed to have longer periods of forced standing. However, given that the cows in this study only had 9 lying bouts/d it is possible that they remained standing to feed for a longer period of time following milking and in turn increase the length of their lying bouts when they do lie down, it may also be a reflection of time away from the pen for milking.

DeVries et al. (2011a) and Deming et al. (2013) reported a similar number of daily lying bouts and subsequently longer bout durations when observing free-stall housed cows, milked in a robotic system > 2x/d.

High free-stall stocking densities also have the potential to decrease PMSD, as cows may elect to lie down quickly following milking to secure a lying place rather than access the feed bunk and face increased competition for stalls later on. Fregonesi et al. (2007) observed a 13 min decrease in PMSD when cows were stocked at 150% compared to those stocked at 100%. Despite a minimal amount of over-crowding in the current study (average 103%), a negative relationship was observed between PMSD and increased stocking density. This indicates that keeping free-stall stocking densities lower is advisable to discourage cows from lying down quickly following milking.

Another factor influencing PMSD is competition at the feed bunk. The mean linear feed bunk space of 0.53 m/cow for this study is below the industry recommendation (0.61 m/cow: Grant and Albright, 2001). Previous research has established that providing more bunk space per cow improves feed access and decreases competition (DeVries et al., 2004; DeVries and von Keyserlingk, 2006; Huzzey et al., 2006). Thus, it is unsurprising that increasing feed bunk space was associated with longer PMSD, as cows would be more likely to access the feed bunk following milking if competition is reduced. It is interesting that the relationship between stocking density and feed bunk space was only found for the first daily milking. To our knowledge, this is the first study on PMSD to separately analyze the factors influencing this behavior at each individual milking. However, given that 2 of the farms only had one fresh feed delivery, and one farm that delivered fresh feed 2x/d had both deliveries around the first daily milking we caution the extrapolation of this finding and encourage work specifically asking this question.

Increasing frequency of feed push-ups was associated with longer PMSD for all 3 daily milkings. While the relationship between provision of fresh feed and PMSD is well-established (Tyler et al., 1997; DeVries and von Keyserlingk, 2005), the impact of feed push-ups on encouraging cows to remain standing is less well understood. DeVries et al. (2011a) noted that feed-manipulation (fresh feed delivery and feed push-ups) impacted PMSD in AMS cows fed 1x/d with multiple feed push-ups. Given that cows in this study were milked on average 2.4x/d it is conceivable that the cows in the present study, under 3x/d milking conditions, are similarly stimulated to visit the feed bunk by feed push-ups following milking

A relationship between the milk-feed manipulation delay and PMSD was only found for the first daily milking. The finding that feed manipulation between 1 h before milking until 1 h after milking decreased PMSD as compared to feed manipulation occurring greater than 60 min before milking is contradictory to the findings of DeVries et al. (2011a), where, in an AMS herd, feed manipulation between 60 min before to 120 min after milking resulted in the longest PMSD. However, the fact that in the DeVries et al. (2011a) study the AMS-milked cows did not enter a holding pen before milking could explain these differences. Average pre-milking standing duration in this study was 106 min; although the cows were not in the holding pen for the entire time, the holding pen durations were approximately 60 min in duration. Thus, if feed was manipulated >60 min prior to milking, the cows were likely being stimulated to feed just prior to being removed from the pen for milking and this preference continued when they returned from milking. Delivery of fresh feed has been identified as having a greater impact on stimulating feeding behavior than returning from milking, therefore the coupling of feeding and milking may have resulted in a greater effect (DeVries and von Keyserlingk, 2005).

It is unsurprising that looking at the impact of fresh feed-delivery in relation to time of milking on PMSD following the first milking, that again, delivery of fresh feed well before milking time (between 1-5 h before milking) produced longer PMSD than delivery of fresh feed 2-9 h after milking. This differs from the impact of fresh-feed delivery on PMSD following the second milking, where increased PMSD were observed when feed was delivered 90 to 180 min after milking. However, the majority of focal cows did not receive a second fresh feed delivery, therefore this finding is indicative of the fact that the provision of a second fresh feed delivery likely has positive impacts on PMSD. While delivering fresh feed 90 to 180 min after milking resulted in increased PMSD, it should be noted that the average cow only stood for 99 min following the second milking. Unfortunately, it is beyond the scope of this study to speculate given that holding pen times may have affected this relationship. Thus, future studies should seek to further understand the impact of holding pen time on PMSD in 3x/d milked cows.

The factors affecting PMSD at the third daily milking were distinctly different given the failure to identify any associations with factors related to feeding, with the exception of feed push-up frequency. The relationship between PMSD for the third milking and DIM is likely related to production and metabolic requirements, which are expected to be exacerbated under 3x/d milking conditions. Milking 3x/d leads to increased milk yield and increased milk yield has been associated with decreased daily lying durations and increased feeding activity following milking (Allen et al., 1986; Norring et al., 2012). Later lactation animals may spend less time feeding and more time lying due to lower metabolic requirements and the reduction in PMSD observed in this study in relation to DIM may be an indicator of less motivation to stay standing to feed following milking, particularly after the third milking of the day (Bewley et al., 2010).

As with DIM, the fact that milking order was only associated with PMSD following the third milking was an intriguing finding that warrants further research. To date there has been no specific link between milking order and impact on PMSD, although DeVries et al. (2011a) did report a positive correlation between pre-milking standing duration and PMSD in AMS cows. Given that cows with a later milking order would likely be experiencing increased pre-milking standing demands it seems intuitive to expect that they may choose to lie down more quickly following milking. It is also likely that cows further down in the milking order may have less space at the feed bunk available to them upon return from milking, thus they may choose to lie down rather than compete for access to feed. A further explanation may be that sorting by the cows milked earlier in the order has led to a less high quality feed being available to the cows at the end of milking thus the feed is less enticing and as a result they may lie down sooner; at the herd-level, cows actively sort for highly palatable starch-rich short and fine particles (Sova et al., 2013). Therefore, a possible means for farmers to both mitigate competition at the feed bunk and sorting, as well as increase PMSD may be to have a higher feeding frequency (DeVries et al., 2004; Sova et al., 2013).

The geometric mean annual bulk milk SCC for the study herds of 240,000 cells/mL was comparable to the Ontario average of 205,000 cells/mL (Olde Riekerink et al., 2008). The herd IMI incidence rate of 3.22 new IMI/quarter-year is higher than that reported by both DeVries et al. (2010) and DeVries et al. (2011a), despite those studies reporting a similar distribution of causal pathogens with predominately CNS and *Corynebacterium* spp. being cultured. The prevalence of CNS IMI reported in this study is comparable to several other recent studies

(Sampimon et al., 2009; Reyher et al., 2011; Quirk et al., 2012). However, the incidence rate of 1.62 CNS IMI/quarter-year is much lower than that recently reported by Dufour et al. (2012) (3.48 CNS IMI/quarter-year) in a cohort of 90 Canadian dairy herds. This is unsurprising given that the current study used a threshold of ≥500 cfu/mL for CNS compared to ≥200 cfu/mL by Dufour et al. (2012). Thus, it is possible that some CNS-infected cows were missed by using a more critical threshold, but by being more critical, a higher sensitivity in identifying actual cases of CNS IMI was hoped for. This does not, however, account for the high overall IMI incidence rate observed in these cows. The high overall IMI incidence rate may also be a response to the frequency of CNS IMI observed in these herds, as a potential link between presence of CNS and increased incidence of a new major pathogen IMI has been described but not confirmed in a previous study (Reyher et al., 2012). The incidence rate of IMI seen in this study may also be due to the sampling period that took place during the spring/summer months, a time of year noted for increased risk of high SCC and IMI (Makovec and Ruegg, 2003; Olde Riekerink et al., 2007).

Given that CNS species predominate in the environment (Piessens et al., 2011), it was not surprising that the final model for factors associated with CNS IMI included PMSD, milk-fresh feed delay, and frequency of feed push-ups. The tendency of PMSD to be associated with environmental (DeVries et al., 2010) or CNS (DeVries et al., 2011a) IMI has been previously established. DeVries et al. (2010) reported a reduced incidence of new IMI in tie-stall herds with a PMSD of 40 to 60 min and an increased incidence of new IMI as PMSD increased past 60 min. Comparably, in an AMS herd DeVries et al. (2011a) reported an increased incidence of new CNS IMI with a PMSD >150 min. In the present study, reduced incidence of new CNS IMI was associated with PMSD 90 to 120 min which, while somewhat contradictory to the findings of the aforementioned studies, gives further credence to the idea that different management practices have a varied influence on IMI incidence. Interestingly, DeVries et al. (2010; 2011a) noted an increased incidence of IMI with longer PMSD. However, the current study failed to show a relationship between longer PMSD and increased risk of IMI. The decreased risk of IMI when cows stood for 90 to 120 min after milking supports a recent finding by Watters et al. (2013) where a decreased risk of elevated SCC was noted when 3x/d milked cows stood >90 min after milking. Further research is needed to understand how teat morphology changes in relation to milked time under 3x/d milking conditions.

The decreased risk of CNS IMI when fresh feed was delivered 60 min before to 90 min after milking is likely related to PMSD and cows standing for 90 to 120 min following milking to access this feed. The second influence of fresh feed delivery >540 min following milking is more difficult to interpret. These instances where fresh feed delivery provided 9 h after milking take place on farms feeding once per day. In this case, feed delivery response may mask the feed manipulation response, which would impact the later milkings. The fact that increased frequency of feed push-ups was associated with decreased likelihood of CNS IMI provides some insight but more work is needed to disentangle these effects. The decreased risk of CNS IMI as frequency of feed push-ups is increased is likely also tied into PMSD. Increased frequency of feed push-ups may entice cows to the feed bunk following milking as the feed presented is somewhat novel following a feed push-up. Increased feed push-ups may also reduce competition at the feed bunk which could also promote PMSD and increase the likelihood that cows would remain standing for the 90 to 120 min following milking which was associated with reduced odds for CNS IMI.

3.5 CONCLUSIONS

Post-milking standing duration was longer in 3x/d milked free-stall housed cows provided with ample feed bunk space per cow, kept at a lower stall stocking density, milked earlier in the herd milking order, in earlier lactation, and provided with fresh feed in the time period after milking or feed push-up just before being removed for milking. Cows that stood for 90 to 120 min after milking had a decreased incidence of new CNS IMI. Risk for CNS IMI also decreased when fresh feed was provided around the time of milking and with an increased frequency of feed push-ups. In summary, management practices that promote post-milking standing duration, such as providing new feed or pushing-up feed around milking time, providing ample feed bunk space per cow, and keeping free-stall stocking densities low may help to improve udder health through reduced CNS IMI.

3.6 ACKNOWLEDGMENTS

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Table 3.1 Descriptive summary of the focal cows sampled within each study herd at the beginning of the study period.

Herd	Cows sampled/herd	Mean parity	Mean DIM	Geometric mean baseline SCC (× 1000 cells/mL)	Mean 305 d milk production (kg)
1	40	2.5	71	29	10,801
2	40	2.4	73	33	11,298
3	40	2.3	68	33	11,568
4	40	1.9	109	33	13,012
Total	160	2.3	81	32	11,670

Table 3.2 Descriptive summary of data from focal cows (n=160) across all 4 study herds[1].

Variable	Mean	SD	Minimum	Maximum
DIM[2]	80	43	15	209[3]
Parity	2.3	1.2	1	9
SCC (× 1000 cells/mL)[2]	32	21.3	10	98
Milk yield (kg/d)	41	8.5	20	66
Lying time (h/d)	10	2.2	2.2	17
Lying bouts (#/d)	9	3.1	2	27
Lying bout duration (min/bout)	73	22.2	22	169
Post-milking standing time (min)	96	52	20	371
Pre-milking standing time (min)	106	64	20	558
Lameness score[4]	1.6	0.8	1	5
Hygiene score[5]				
Udder	2.3	0.6	1	4
Upper leg/flank	2.4	0.6	1	4
Lower legs	3.9	0.3	2.5	4

[1]Data averaged across all 3 study periods for all of the 160 focal cows from each of the 4 herds.

[2]Baseline data collected at beginning of study.

[3]>200 DIM because of low selection availability.

[4]Gait score was assessed on a 5-point scale (1= normal to 5= severely lame) (Flower and Weary, 2006; Ito et al., 2009). Lameness was dichotomized for analysis into not lame = gait score <3; lame = gait score ≥ 3). Throughout the study 12.4% of focal cows were categorized as lame.

[5]Hygiene score was assessed on a 4-point scale (1=very clean to 4=very dirty) (www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf; Cook and Reinemann, 2007).

Table 3.3 Final general linear model for factors associated with post-milking standing duration of focal cows[1].

	Post-milking standing duration 1[2]			Post-milking standing duration 2			Post-milking standing duration 3		
Variable	β[3]	SE	*P*-value	β	SE	*P*-value	β	SE	*P*-value
Intercept	3.88	0.53	<0.01	3.74	0.18	<0.01	4.09	0.15	<0.01
Feed bunk space (m/cow)	1.59	0.34	<0.01	-	-	-	-	-	-
Stocking density (%)	-0.009	0.004	0.04	-	-	-	-	-	-
Milk-feed manipulation delay (min)[4]	-	-	0.04	-	-	-	-	-	-
<60 min before	Ref[5]	-	-	-	-	-	-	-	-
60 to 30 min before	-0.17	0.06	-	-	-	-	-	-	-
30 min before to 60 min after	-0.12	0.06	-	-	-	-	-	-	-
>60 min after	-0.05	0.07	-	-	-	-	-	-	-
Milk-fresh feed delay (min)[6]	-	-	0.02	-	-	0.02	-	-	-
<300 min before	-	-	-	Ref	-	-	-	-	-
300 to 60 min before	Ref	-	-	-0.03	0.1	-	-	-	-
60 min before to 90 min after	0.11	0.13	-	-	-	-	-	-	-
90 to 180 min after	0.04	0.15	-	0.38	0.1	-	-	-	-
180 to 540 min after	-0.65	0.28	-	0.11	0.09	-	-	-	-
>540 min after	-	-	-	-	-	-	-	-	-
Push-up frequency (#/d)[7]	0.08	0.04	0.06	0.11	0.04	<0.01	0.11	0.03	<0.01
Milk order[8]	-	-	-	-	-	-	-0.45	0.19	0.02
DIM[9]	-	-	-	-	-	-	-0.002	0.0008	0.02

[1]Data is averaged by period for each of the 40 focal cows from each of the 4 herds that were observed for 5 d for each period.

[2]Post-milking standing duration associated with each individual milking time (1 denotes first milking of the day etc.).

[3]β = estimated regression coefficient.

[4]Milk-feed manipulation delay = difference in time between individual milking time and closest feed manipulation (fresh feed delivery or feed push-up).

[5]Ref = Reference category.

[6]Milk-fresh feed delay = difference in time between individual milking time and closest fresh feed delivery.

[7]Push-up frequency = number of feed push-ups per day.

[8]Milk order = point at which the individual cow was milked within the overall order (tested as an index with cow's individual milking/overall number of cow's milked).

[9]DIM = days in milk.

Table 3.4 Number of quarter milk samples taken per farm and number of new IMI across the study period[1].

Herd	Total samples[2]	Total quarters at risk[3]	Number of new IMI[4]	IMI incidence rate (IMI/quarter-year)	Number of new CNS IMI[3]	CNS IMI incidence rate (IMI/ quarter-year)	Number of new *Coryne.* spp. IMI[3]	*Coryne.* spp. IMI incidence rate (IMI/ quarter-year)
1	610	467	124	3.46	42	1.32	54	1.78
2	612	450	174	5.04	77	2.65	39	1.32
3	611	457	64	1.83	14	0.42	28	0.85
4	627	472	94	2.60	69	2.29	19	0.56
Total	2460	1846	456	3.22	202	1.62	140	1.10

[1]Data is averaged across all 3 study periods for all of the 160 focal cows from each of the 4 herds.

[2]Total number of samples taken throughout the study across all 4 milk sampling collection dates.

[3]Quarters were considered at risk when the milk sample was analyzed as culture negative at the beginning of each observation period.

[4]New IMI was defined as a culture positive quarter milk sample for a specific pathogen at the end of each period when the quarter milk sample was culture negative for that specific pathogen at the beginning of each period.

Table 3.5 Unconditional estimates for factors associated with the incidence of CNS IMI[1].

Variables	Mean (± SD)	Odds ratio (95% CI)	*P*-value
Feeding frequency (#/d)[2]	1.5 (0.5)	1.92 (1.04, 3.55)[3]	0.04
Push-up frequency (#/d)[4]	4.5 (0.5)	0.52 (0.28, 0.96)[3]	0.04
Pre-milking standing (min)[5]	105.7 (61.36)	0.87 (0.72, 1.07)[3]	0.19
Milk-fresh feed delay (min)[6]			<0.01
<300 min before	-	-	-
300 to 60 min before	-81.1 (14.02)	Ref[7]	-
60 min before to 90 min after	39.1 (38.23)	0.51 (0.28, 0.94)	-
90 to 180 min after	129.5 (26.51)	0.69 (0.36, 1.33)	-
180 to 540 min after	206.9 (17.94)	2.75 (1.07, 7.06)	-
>540 min after	637.0 (13.48)	0.35 (0.14, 0.87)	-
Post-milking standing duration (min)			0.06
<60 min	47.6 (9.76)	Ref	-
60-75 min	67.5 (4.34)	0.74 (0.44, 1.24)	-
75-90 min	81.5 (4.54)	0.83 (0.48, 1.43)	-
90-120 min	103.5 (8.56)	0.39 (0.21, 0.73)	-
>120 min	178.7 (55.74)	0.73 (0.42, 1.28)	-
DIM[8]	106.7 (48.43)	1.25 (1.02, 1.54)[3]	0.03

[1]Data is averaged by period for each of the 40 focal cows from each of the 4 herds that were observed for 5 d for each period.

[2]Feeding frequency = the number of fresh feed deliveries per day.

[3]OR and 95% CI for 1 standard deviation in variable presented.

[4]Push-up frequency = number of feed push-ups per day.

[5]Pre-milking standing = the length of time (min) between the attachment of the milking unit and the previous recorded lying bout.

[6]Milk-fresh feed delay = difference in time between individual milking time and closest fresh feed delivery.

[7]Ref = Reference category.

[8]DIM = days in milk.

Table 3.6 Final logistic regression model for factors associated with the incidence of CNS IMI[1].

Variables	Coefficient	SE	Odds ratio (95% CI)	*P*-value
Intercept	4.19	3.11	-	0.31
Random effects				-
Herd-level variance	0.78	0.71	-	-
Cow-level variance	0.78	0.22	-	-
Post-milking standing duration (min)				0.08
<60 min	Ref[2]	-	-	-
60-75 min	-0.26	0.27	0.77 (0.45, 1.32)	-
75-90 min	-0.04	0.29	0.96 (0.54, 1.69)	-
90-120 min	-0.75	0.34	0.47 (0.24, 0.92)	-
>120 min	0.11	0.31	1.12 (0.60, 2.06)	-
Milk-fresh feed delay (min)[3]				<0.01
<300 min before	-	-	-	-
300 to 60 min before	Ref	-	-	-
60 min before to 90 min after	-0.66	0.31	0.52 (0.28, 0.95)	-
90 to 180 min after	-0.39	0.34	0.68 (0.35, 1.31)	-
180 to 540 min after	0.92	0.49	2.50 (0.96, 6.51)	-
>540 min after	-1.25	0.48	0.29 (0.11, 0.73)	-
Push-up frequency (#/d)[4]	-1.26	0.69	0.28 (0.07, 1.09)[5]	0.07

[1]Data is averaged by period for each of the 40 focal cows from each of the 4 herds that were observed for 5 d for each period.

[2]Ref = Reference category.

[3]Milk-fresh feed delay = difference in time between individual milking time and closest fresh feed delivery.

[4]Push-up frequency = number of feed push-ups per day.

[5]OR and 95% CI for 1 standard deviation in variable presented.

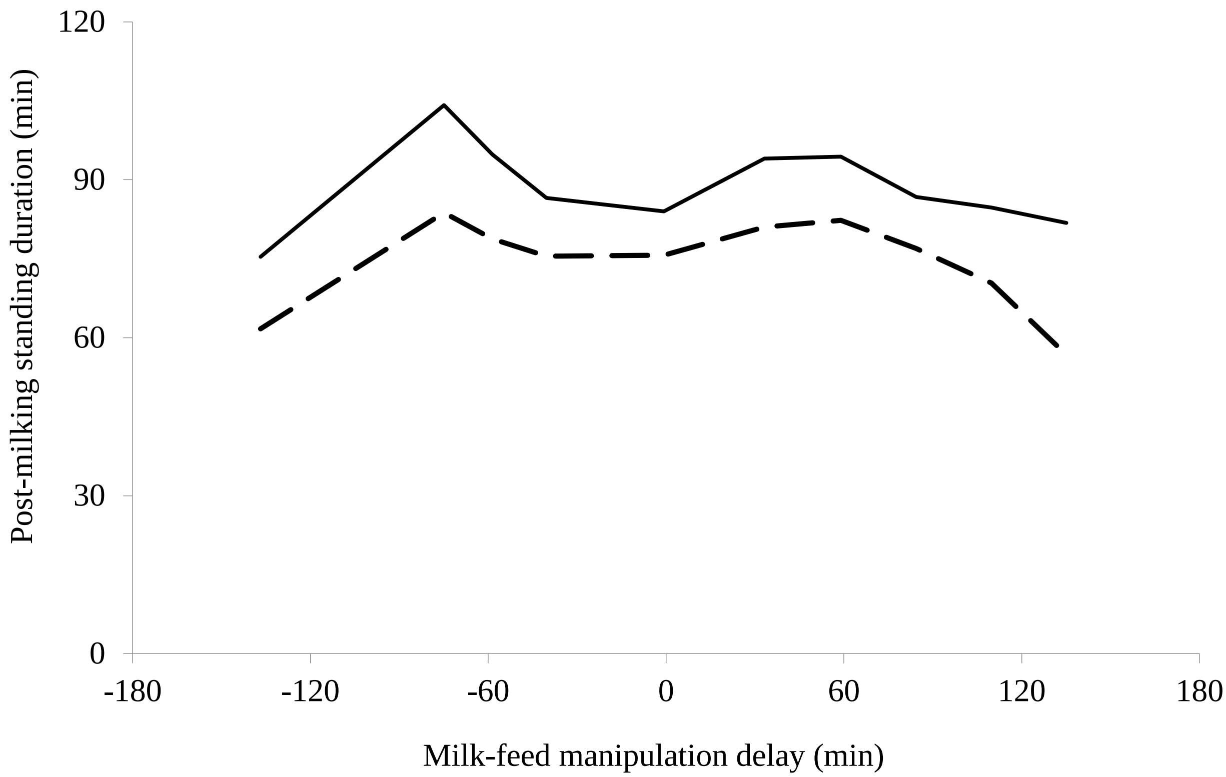

Figure 3.1 Moving average (with width of 60 min and increments of 30 min) of post-milking standing duration across milk-feed manipulation (fresh feed or feed push-up) delay (mean = solid line; median = dashed line).

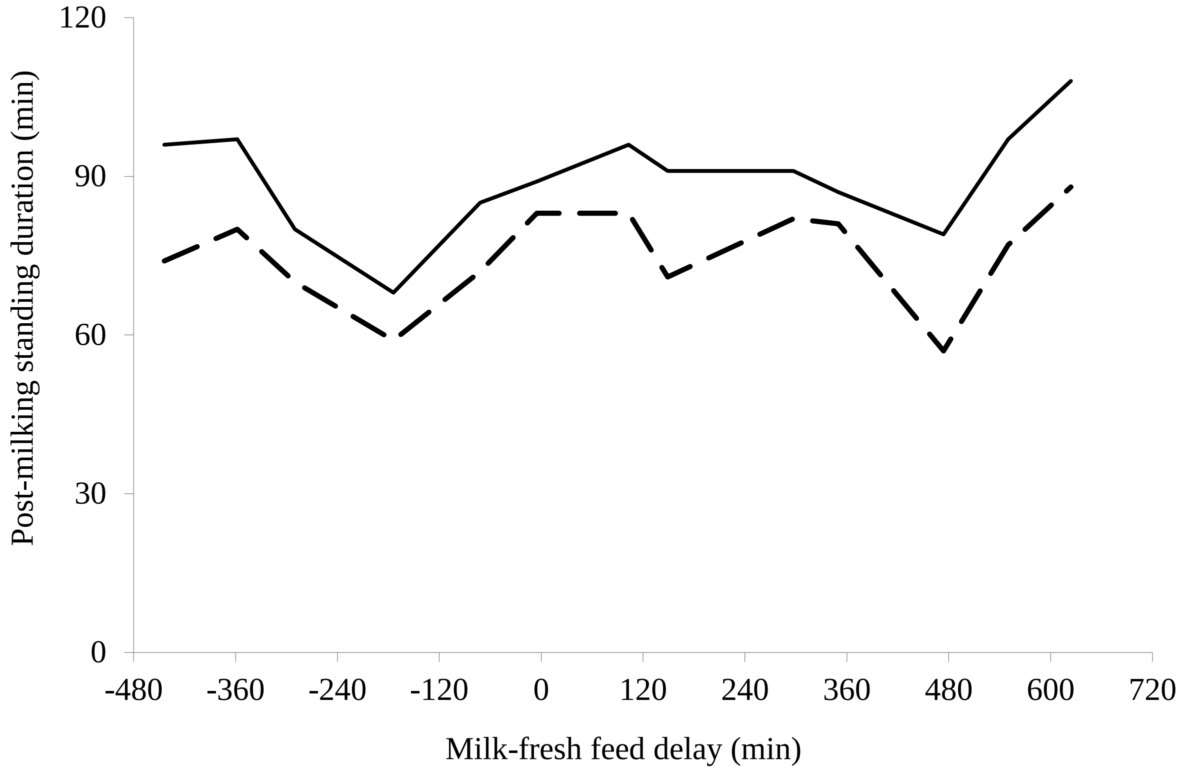

Figure 3.2 Moving average (with width of 120 min and increments of 90 min) of post-milking standing duration across milk-fresh feed delivery delay (mean = solid line; median = dashed line).

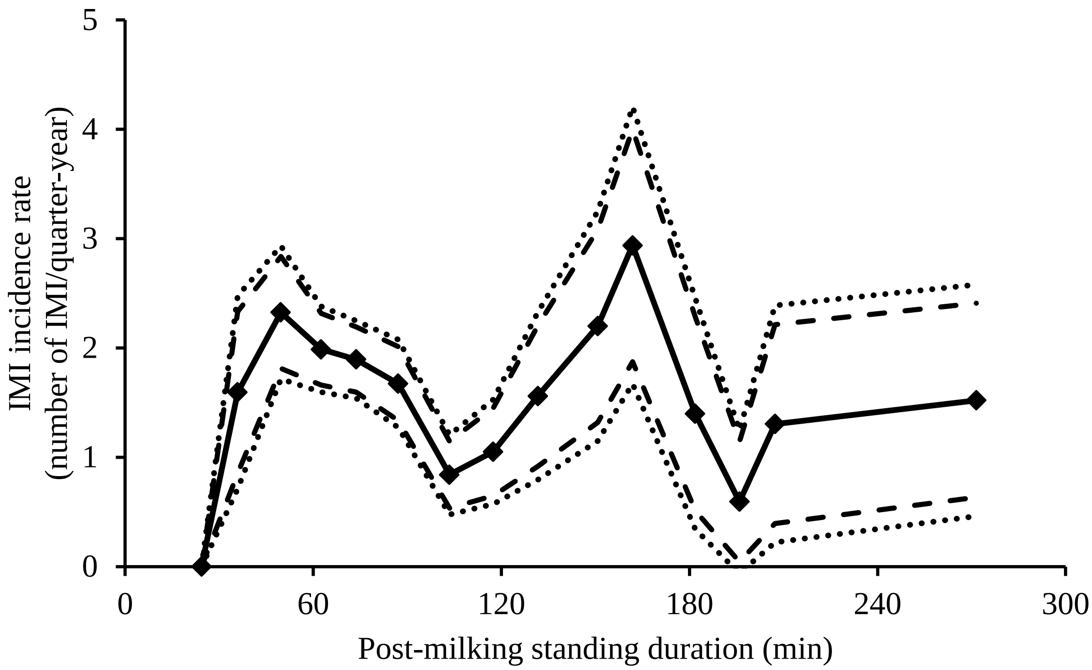

Figure 3.3 Moving average (with width of 30 min and increments of 15 min) of incidence rate of CNS IMI across average post-milking standing duration (solid line) with 90% (dashed lines), and 95% (dotted line) confidence limits. Data is averaged by period for each of the 40 focal cows from each of 4 herds.

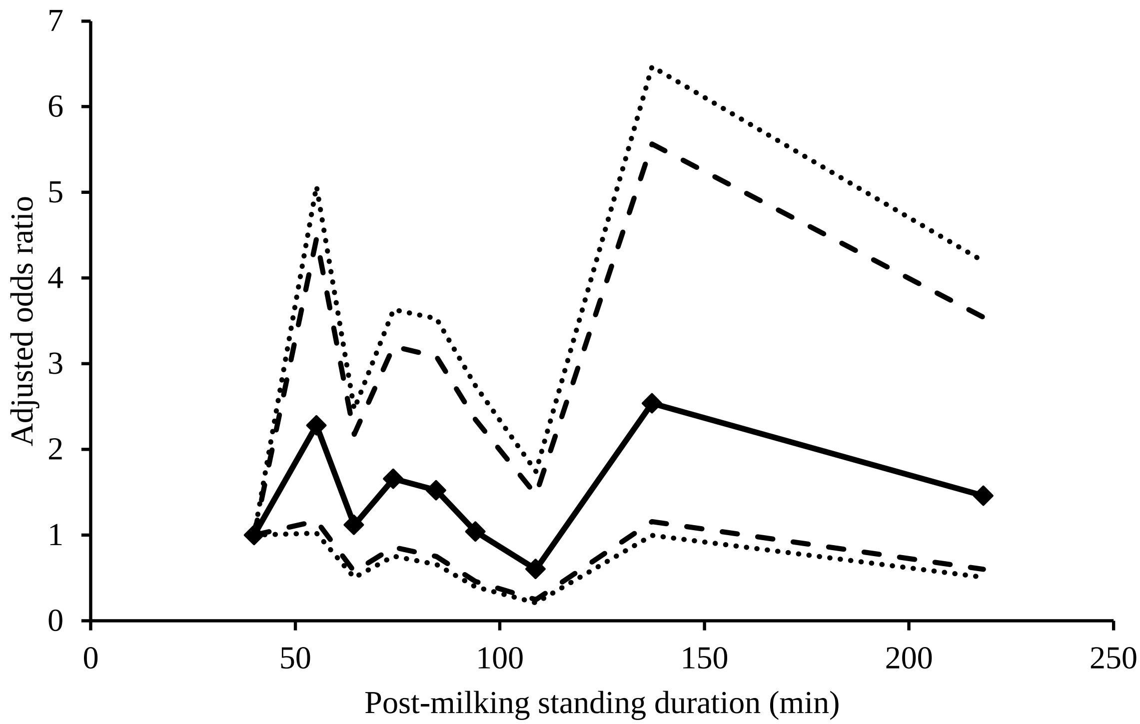

Figure 3.4 Adjusted estimates of the odds of acquiring a CNS IMI across deciles of average post-milking standing duration (solid line) with 90% (dashed lines), and 95% (dotted lines) confidence limits. Data is averaged by period for each of the 40 focal cows from each of 4 herds.

CHAPTER 4: GENERAL DISCUSSION

4.1 IMPORTANT FINDINGS

Recent evidence has suggested that PMSD can be altered by changes in management and thus could potentially be utilized as a prevention strategy to decrease risk and incidence of IMI in lactating dairy cows. However, the optimal PMSD may vary depending on housing and other management variables such as milking system and milking frequency. In Chapter 2 we sought to determine associations between herd- and cow-level factors, including lying behavior, and compromised udder health (as measured by eSCC) in free-stall housed cows milked 3x/d. We hypothesized that PMSD would be shortest when fresh feed was not offered around the time of milking and that those cows which have a shorter latency to lie following milking would be at an increased risk for an eSCC. Our results supported these hypotheses as the longest PMSD were observed when feed manipulation occurred in the hour before milking or the immediate time period after milking. Further, our research indicates that a reduced odds for eSCC was associated with a PMSD >90 min.

Cows of greater parity and those with a higher baseline SCC (closer to the cut-point of 100,000 cells/mL) were also at an increased risk to experience an eSCC. While parity cannot be altered through management, this result along with the observed variations in lying behavior as a result of parity, DIM, and milk production, indicate that management of cows to improve udder health is mostly influenced by cow-level factors. Greater parity animals are likely higher producers and as a result will have higher metabolic requirements and consequently, spend more time at the feed bunk to meet their increased metabolic demands (Bewley, 2010; DeVries et al., 2011a). Increased time spent standing at the feed bunk may increase the likelihood of reduced udder hygiene, especially in situations where poor management has led to reduced alley cleanliness, and coupled with the fact that older cows are more likely to have lower udder carriage, this may lead to a predisposition for IMI (Nielsen et al., 2011; Reneau et al., 2005). Thus, these results reinforce the needed for increased cleaning regimen of alleys and stalls, as well as efficient feeding management to reduce the risk of eSCC in all cows.

The increased risk for eSCC in cows with a higher baseline SCC may be indicative of the sensitivity of utilizing composite SCC as an indicator of infection. Without bacteriology, the infection status of these cows was unknown and despite the use of a SCC of 100,000 cells/mL to enroll culture negative cows, those cows with a baseline SCC closer to the cut-point may have had a subclinical infection with a minor pathogen. In turn, these latter cows may have been predisposed to an increase in SCC at the subsequent milk sample. Recently Dufour and Dohoo (2013) indicated that composite SCC has little power in detecting IMI with the exception of some major pathogens. While bacteriology is certainly a preferred method to explicitly determine IMI status, in terms of economics and practicality, SCC may be an effective broad-level tool to screen for potential at risk animals and is currently routinely measured on the majority of Canadian farms.

The results of Chapter 2 pointed towards some interesting associations between management, behavior, and udder health and suggested that certain management practices which impact cow behavior may have beneficial impacts on udder health. To elaborate on this, in Chapter 3, the association between PMSD and risk of IMI in free-stall housed cows milked 3x/d was investigated. It was hypothesized that those cows with shorter PMSD would be at an increased risk to experience IMI while provision of feed close to the time of milking would result in longer PMSD. In this study, an interaction between milking event (1^{st}, 2^{nd}, or 3^{rd} milking of the day) and PMSD was noted therefore, factors associated with PMSD were analyzed separately for each milking. This appears to be the first study to separately analyze PMSD by milking and given this, there may be unique attributes contributing to varied cow behavior in a 3x/d milking system. In support of the hypotheses a reduced odds for CNS IMI was observed with a PMSD of 90 to 120 min, while provision of feed around the time of milking both decreased risk of CNS IMI and promoted PMSD. However, the impact of feed provision on PMSD varied by milking event as did the length of PMSD.

The longest PMSD was following the second milking, while the shortest PMSD was observed after the first milking. It is interesting that the shortest PMSD was following the first milking as, on farms feeding 1x/d, this was the milking around which fresh feed was delivered. On average fresh feed delivery occurred 100 min after milking, while feed manipulation (delivery or push up) occurred 20 min before milking. The average feed manipulation during this study was much closer to milking times than the 62 min reported in the first study (Chapter 2); this is likely attributed to the farms in this study (Chapter 3) having more frequent feed push-ups. Feed manipulation within the hour before to the hour after the first daily milking resulted in a 5-8 min reduction in PMSD, which was much less than the results of Chapter 2 and those of a tie-stall study where feed manipulation or provision of fresh feed around the time of milking promoted PMSD (DeVries et al., 2010). However, PMSD was longer when feed was manipulated

>60 min before. Unfortunately, I was unable to disentangle the effects of other management factors such as holding times just before milking which may have affected the results of my study. That being said, anecdotally, milking time per group was around 60 min therefore, manipulation of feed just prior to milking may not only stimulate cows to stand, thus contributing to the increased pre-milking standing duration, but this stimulation may continue upon return from the parlor leading to an increased PMSD. This relationship was also seen with delivery of fresh feed around the time of the first milking, whereby delivery of feed >60 min before milking contributed to longer PMSD. This differs from the second daily milking where delivery of fresh feed 90 to 180 min after milking resulted in a 20 min increase of PMSD. Thus, the differences observed between Chapter 2 and Chapter 3 may be the result of analyzing by milking in Chapter 3 or it may be an indication of a factor that was not assessed in either study. Regardless, these results indicate that future investigation into behavioral differences and their causal factors at each milking is warranted, especially given the diurnal variation in behavior of cows.

That management factors impact PMSD is no longer up for debate. Variation in PMSD is influenced by feed bunk space and stocking density, particularly following the first milking (see Chapter 3). Our observation that increased bunk space per cow was associated with increased PMSD was likely a result of increased access to feed and reduced competition. Previous studies have noted that providing more bunk space per cow improves access to feed and reduces competition (DeVries et al., 2004; DeVries and von Keyserlingk, 2006; Huzzey et al., 2006). The fact that lower stocking density also increased PMSD following the first milking is also likely competition related. In cases of higher stocking densities a reduction in PMSD has previously been reported (Fregonesi et al., 2007a). When lying space is at a premium, such as in instances of overstocking, cows may elect to access lying space following milking rather than access the feed bunk, thereby mitigating the chances of facing increased competition for a stall later on. The fact that both of these factors were only observed at the first daily milking highlights the need for further exploration of this relationship.

In both Chapter 2 and 3, PMSD was much longer than that reported in similar studies for tie-stall cows and free-stall housed cows milked 2x/d or by AMS, although the median value was similar to that reported in tie-stall and AMS free-stall herds (DeVries et al., 2011a; DeVries et al., 2010; DeVries and von Keyserlingk, 2005; Tyler et al., 1997). Frequency of lying bouts were also reduced indicating that these cows were spending more time lying down during each lying event; with the bouts separated by longer periods of standing. These findings may be a unique attribute of 3x/d milking herds and may be the result of either an increased metabolic demand given the increased production or increased forced standing time due to increased milking frequency, or some other management impact. The results of both DeVries et al. (2010) and DeVries et al. (2011a) supported the evidence of a second period of teat canal opening between 2 to 4 h following milking, with their results indicating that this period of opening is likely closer to 2 h post-milking. However, the results of this study with reduced odds of eSCC when PMSD >90 min and reduced odds of CNS IMI when PMSD 90 to 120 min post-milking suggest that this association between PMSD and risk of infection may depend on milking frequency.

Overall, the findings of this thesis research suggest that PMSD is altered under variable management conditions, and provision of feed around the time of milking, ensuring adequate feed bunk space, and keeping stocking density low are management strategies which can be used to promote PMSD. Encouraging longer PMSD of 90 to 120 min may be an effective prevention strategy to reduce the risk of environmental infection, specifically CNS IMI. However, when comparing with previous studies, there is an indication that optimal PMSD and strategies to ensure adequate PMSD are variable under differing management and housing conditions; thus future research is needed to determine the precise cause-and-effect interactions.

4.2 Future Research

Longitudinal studies in commercial settings are useful to determine the impacts of real-world practices, but are limited in the amount of control over study variables. While intensive, controlled environment studies are effective in unequivocally pin-pointing causal variables' precise response to the area of interest, the applicability to a real-world setting is sometimes questionable. The studies presented in this thesis resulted in the collection of a large amount of data on the management practices and corresponding cow behavioral responses in commercial settings under 3x/d milking conditions. One limitation to the applicability of the data set is the limited number of farms used in the study, which were all located in a small geographic area. However, this data presents a baseline for future studies to assess the impact of PMSD on udder health across a broader spectrum of farms and highlights that more concentrated research is necessary to truly understand the impacts of management on PMSD and why such large variations in average PMSD across different milking frequencies are evident.

Our finding that 3x/d milked cows have long pre-milking standing duration and PMSD as well as a reduced number of lying bouts throughout the day raises many questions into the activities that these cows are engaged in during these long periods of standing. It was postulated in both Chapter 2 and 3 that holding pen time may be an important variable to assess in future studies involving 3x/d milked cows. Previous research has indicated that farm management may be more indicative of holding pen time than milking frequency, however, while it is likely that these cows are not under forced standing conditions during these extensive stretches of pre-milking standing, it would be interesting to determine what is truly initiating this prolonged standing behavior and what activities these cows are engaged in during this time. It would also be of interest to determine what proportion of PMSD is actually allocated to feeding activity. Also, given that this research was conducted during the spring and summer months, heat stress may be contributing to the extended PMSD following the second milking however, as this variable was not a study variable, this is only conjecture. The impact of season and ambient temperature on PMSD under different management conditions has yet to be analyzed and presents an area for consideration of future research. However, a study to answer these questions would be difficult to assess across commercial farms as it would most likely require extensive installation of video equipment.

The fact that a PMSD around the 2 h mark, where previous research has indicated a secondary incidence of teat canal opening, decreased risk for IMI in both Chapter 2 and 3 is an intriguing result that warrants further exploration. This may be an indication that previous observations of teat morphology following milking may not be applicable to a cow milked 3x/d. Updated research on how udder morphology is impacted by milking frequency may not only lead to an explanation of why longer PMSD is beneficial for 3x/d milked cows but may also provide insight for future mastitis prevention strategies.

Given the variations observed between PMSD, and how PMSD relates to risk of IMI, under different housing and milking systems future research should seek to understand the relationship between PMSD and IMI in free-stall housed cows milked 2x/d. To date, empirical evidence for PMSD and IMI does not exist for cows in this type of management situation. A controlled study seeking to understand the impact of milking frequency and milking system on PMSD and IMI in 2x/d milked free-stall cows would also be interesting and add to the present knowledge.

Given that IMI has a multitude of causal pathogens and that it can be contagious, environmentally acquired, and certain cow attributes may predispose an animal to infection, it is important that we work towards an understanding of how management impacts the risk of IMI and how certain behaviors may elevate a cow's risk for IMI. Overall, this project provided new insight as to how management and cow behavior impact udder health in free-stall housed cows milked 3x/d. It also highlighted the need for continued research into how management practices impact cow behavior. This line of research may be a viable and easily implemented strategy as a measure of mastitis prevention. Future research should seek to further determine optimal PMSD as a strategy to combat certain IMI in various management conditions and how milking frequency may impact udder morphology to improve our current understanding of teat canal susceptibility to bacterial penetration.

4.3 Implications

Ensuring optimal udder health is of concern to the global dairy industry from both an economic and animal welfare standpoint. When it comes to addressing issues of illness and disease, a primary prevention strategy is the optimal standard. Altering management systems to encourage cow behavior which decreases risk of reduced udder health, may be both an economical and feasible primary prevention strategy.

Our results indicate that PMSD is varied based on management practices and that PMSD of a specific duration may promote improved udder health in terms of reduced risk for eSCC and CNS IMI. In both Chapter 2 and 3, feeding management was observed as having an important impact on PMSD. These findings indicate that PMSD may be promoted through increased frequency of feed push-ups and by provision of fresh feed around the time of milking. The results of Chapter 3 also indicate that, aside from providing and pushing up feed to encourage cows to remain standing, factors that affect accessibility to feed and lying space also impact PMSD. In particular, increased feed bunk space per cow (m/cow) and lower free-stall stocking densities were related to increased PMSD.

Improved udder health in free-stall housed 3x/d milked cows may be achieved by encouraging PMSD. A PMSD of >90 min was related to a decreased risk for eSCC (Chapter 2) while a PMSD of 90 to 120 min was associated with a decreased risk for CNS IMI (Chapter 3). In relation to this, increasing frequency of feed push-ups and delivery of fresh feed around the time of milking was associated with a decreased risk for CNS IMI. Thus, altering feeding management to increase PMSD is an easily applied strategy to promote udder health.

Overall, the results of this thesis research suggest that PMSD may be integral to prevention strategies to prevent reduced udder health with specific applicability to CNS IMI. Specifically, management practices which promote PMSD of 90 to 120 min, such as increased provision of feed around milking time, establishment of adequate feed bunk space, and keeping free-stall stocking density low should be encouraged and may improve udder health in free-stall housed, 3x/d milked cows.

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