Impact of Dietary Transition at Dry Off on the Behavior and Physiology of Dairy Cows

by
Kaitlyn Marie Dancy

A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Master of Science
in
Animal Biosciences

Guelph, Ontario, Canada

© Kaitlyn Marie Dancy, April, 2018

ABSTRACT

IMPACT OF DIETARY TRANSITION AT DRY OFF ON THE BEHAVIOR AND PHYSIOLOGY OF DAIRY COWS

Kaitlyn Marie Dancy **Advisor:**

University of Guelph, 2018 **Dr. Trevor DeVries**

This thesis, firstly, investigated the impact of dietary transition at dry off on the behavior and physiology of Holstein dairy cows, and secondly, evaluated the efficacy of utilizing reductions in dietary nutrient density and milking frequency to mediate the dry off process and promote cow welfare. From the start of a 5-d dry off, wherein cows were milked intermittently, until 3 wk dry, cows received 1 of 2 dry cow total mixed rations which differed in nutrient density. Cows fed the dry cow diet of lower nutrient density had lower milk production at the time of dry off compared with cows fed the diet of higher nutrient density, likely as a result of dry matter intake being limited in cows fed the former, due to greater fill of the rumen. Cows fed the lower nutrient density diet fed at a slower rate, spent more time feeding, and sorted to a greater extent for the smaller, more nutrient dense components of the diet compared with cows fed the higher nutrient density diet. Regardless of sorting activity, cows fed the lower nutrient density diet spent more time ruminating per unit of dry matter consumed and had higher reticulorumen pH. Dietary transition and dry off similarly affected the energy balance and inflammatory response of cows. The results of this study indicate that the lower nutrient density diet more effectively reduced milk production before dry off, while not negatively impacting cow behavior and physiology.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Trevor DeVries, for all that he's done as a supervisor and mentor these past few years. I will always be grateful that you were willing to take me on as a summer student before I'd ever been in a pen with cows to realize just how tall they were/how short I am. Thank you for giving me the opportunity to pursue this degree and for all the support and patience you've shown me over its course. Even when things didn't go according to plan, you were always calmly reassuring and had a simple solution ready. I genuinely appreciate all the time you've put in answering my questions and providing me with feedback; regardless of the time of day, day of the week, or your travel schedule, your email reply times are record-breaking. Your dedication to your work and students is astounding and we're very lucky to have an advisor as accessible and approachable as you.

Secondly, I would like to thank my advisory committee member, Dr. Eduardo Ribeiro, for all his input and suggestions. I really appreciate your time and positive attitude that helped me to appreciate the value of my work.

I would also like to thank the staff of the Elora Dairy Research and Innovation Centre for making this project possible. Beyond keeping my cows well-looked after, thank you for answering my questions, helping me when it looked like I needed it, and for being patient with me about those feed samples. I am especially grateful to Laura Wright for all her advice and assistance, technical and otherwise, that helped make this project run smoother than I could have imagined.

Next, words cannot express how grateful I am to the wonderful lab-family that is CowCrew. Meagan King, thank you for taking me under your wing as a wee undergrad; you've been an amazing friend and mentor and you never cease to make me laugh. Rachael Coon, Robin Crossley and Emily Kaufman, I really appreciate all you taught me in barn and in the lab; without you three I really don't know where I would have been. I also owe a huge thank you to all those who put in their time to help me out at the barn, with data, or with tackling the seemingly never-ending mound of feed samples: Felipe Pádua, Sarah McPherson, Isabela Salmazo, Michelle Thompson, Kaitlin Sparkman, Vanessa Mutlak and Evan Richardson. To the more recent additions to CowCrew, Sarah Parsons, Sydney Moore and Casey Havekes, thank you for being absolute delights to be around; I'm looking forward to working with you more this summer!

To my family and friends, thank you so much for all the love and support you've shown me. I am eternally grateful to my parents, Sue and Steve, for being my biggest cheerleaders and for making me into who I am today. You've shared in my excitement, reassured me when I felt overwhelmed, and worked to make Guelph feel not so far away. I am also very thankful to my other parents, Gil and Laurie, for making me feel like their own. To my sister, Krystal, thank you for matching my sense of humour perfectly and keeping me laughing with all the videos sent back and forth. To my grandparents, Ruth, John and Mary, thank you for spoiling me with goodies and attention. I'd also like to thank Kayo Takeshima and Sarah Sheppard for being awesome friends, listeners and sources of motivation. Finally, thank you to Lucy Ngo, for being my best friend and partner in crime for six years now. Through good times and tougher ones, you've always been there to talk, encourage me and make me laugh; thanks for being my rock.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADF – acid detergent fiber

APP – acute phase protein

BCS – body condition score

BHB – beta-hydroxybutyrate

BW – body weight

CP – crude protein

DCT – dry cow therapy

DIM – days in milk

DM – dry matter

DMI – dry matter intake

FCM – fat-corrected milk

IMI – intramammary infection

MUN – milk urea nitrogen

NDF – neutral detergent fiber

NEB – negative energy balance

NEFA – non-esterified fatty acid

NE_L – net-energy, lactation

peNDF – physically-effective neutral detergent fiber

PSPS – Penn State Particle Separator

SAA – serum amyloid A

SCC – somatic cell count

TMR – total mixed ration

VFA – volatile fatty acid

Chapter specific terminology

Chapter 2:

HND – higher nutrient density

LND – lower nutrient density

rum/DMI – rumination time per kg of DMI

CHAPTER 1. INTRODUCTION

In the modern dairy industry, to maintain financial viability, producers often target a lactation cycle comprised of both a 305-d lactating period and a short, dry (non-lactating) period. The dry period is widely regarded as a rest period for the cow, and provides an opportunity for mammary epithelial cell turnover, thereby promoting greater milk production in the next lactation (Capuco et al., 1997). A long-held standard for dry period length has been 6 to 8 wk, and indeed, dry periods shorter than 4 wk have been shown to negatively affect milk production in the following lactation (Sørensen and Enevoldsen, 1991; Bachman and Schairer, 2003). Additionally, management, especially of nutrition, during the dry period is a key determinant of cow health and fertility in the next lactation (Bradley and Green, 2004; Beever, 2006; Dann et al., 2006).

Due to improvements in nutrition, management and genetics, overall milk production and lactation persistency have greatly increased in recent decades. In the 1980s, it was uncommon for the daily milk production of a Holstein cow to surpass 30 kg/d at peak lactation (Schutz et al., 1990). Presently, Canadian multiparous Holsteins milked 2x/d typically produce well in excess of 40 kg/d at peak lactation and it is not uncommon for cows to be producing 25 kg/d at 305 DIM, when the dry period is generally targeted to commence (Dingwell et al., 2001; Stefanon et al., 2002; López et al., 2015). Thus, the transition from lactating to dry rarely occurs naturally on commercial dairies, and the dry period is instead initiated by the producer via cessation of milking (dry off). In fact, it has been estimated that the modern Holstein would require 700 DIM, on average, to attain a level of milk production at which she would have been dried off in 1975 (Cole et al., 2011).

At dry off, cows experience regrouping and dietary transition, as well as various other stressors (e.g. increased udder pressure) that may be amplified by high milk production at the time of dry off. The dry off procedure most commonly recommended (NMC, 2006; Blowey and Edmondson, 2010) and practiced is abrupt cessation of milking, accompanied by dry cow therapy (DCT), which includes intramammary antibiotics, and often by internal and external teat sealers/sealants (Dingwell et al., 2001). However, the practice of abrupt dry off may negatively impact the health and welfare of cows, especially those with high production at dry off (Dingwell et al., 2001; Stefanon et al., 2002). Cows with greater production at dry off are at an increased risk of intramammary infections (IMI) post-calving (Dingwell et al., 2004; Rajala-Schultz et al., 2005) and experience higher udder pressure and greater stress levels at dry off (Bertulat et al., 2013). As an alternative to abrupt cessation of milking, producers may mediate dry off by reducing milk production through implementation of a gradual or intermittent milking schedule, and/or through a reduction in nutrient availability around the time of dry off (Bushe and Oliver; 1987; Skidmore et al., 1997). Despite the efficacy of these strategies in reducing milk production, researchers have indicated that dry off strategies that restrict feed quantity or quality (e.g. feeding only straw or hay) may cause cows to experience hunger (Valizaheh et al., 2008; Tucker et al., 2009) and a state of negative energy balance (NEB; Odensten et al., 2005; Odensten et al., 2007), potentially depressing immune function (Ster et al., 2012).

This literature review will firstly discuss the relationship between dry off and intramammary health, including the incidence of IMI. Welfare concerns with dry off practices and alternatives will then be discussed. Finally, this review will outline changes in feeding behavior that may occur as a result of dietary changes at dry off.

1.1 Dry off and Intramammary Health

A large body of literature exists exploring the ways in which dry off affects the intramammary health of cows. Firstly, substantial research has been conducted exploring the cellular processes that occur in the mammary tissue during dry off. The process by which milk production by the mammary glands declines is called involution, of which there are three types: gradual involution occurs as a cow's lactation progresses past its peak (Wilde et al., 1999), senile involution occurs as the cow ages, and acute involution occurs when milking is ceased at the end of a lactation. Abrupt cessation of milking creates an accumulation of intramammary pressure caused by the lack of milk removal (Oliver and Sordillo, 1989). Although apoptosis of mammary epithelial cells occurs in conjunction with each type of involution, the greatest extent of apoptosis occurs with acute involution. Despite the fact that apoptosis does not involve an inflammatory response (Wilde et al., 1997), inflammation can be observed at dry off, likely due to high intramammary pressure and resulting tissue damage, especially in cows with high production at dry off (Silanikove et al., 2013).

A major health concern surrounding the dry period is IMI, which can potentially develop into clinical mastitis. Not only do IMI have the potential to exert large financial pressure on the producer (Halasa et al., 2007), they can also compromise the welfare of the dairy cow. A time of particular risk of contracting IMI is during mammary involution. However, once a mammary gland has completed the process of involution, contracting an IMI-causing pathogen is unlikely (Bradley and Green, 2004). Increasing levels of lactoferrin, immunoglobulins, and leukocytes as involution progresses help to discourage bacterial growth and improve resistance against infection (Bushe and Oliver, 1987; Sordillo et al., 1997). Cows with higher milk production at dry off have been shown to have a greater likelihood of contracting IMI at parturition (Rajala-Schultz et al., 2005). Silanikove et al. (2013) observed that 5 cows with high milk production at dry-off had a less effective antibacterial response to an abrupt dry off than 5 cows with lower production at dry off, influencing susceptibility to IMI. Because cows in that study had similar genetic potential for milk production, the authors suggested that the reason for this difference was that cows with lower milk yields at dry off had metabolic and immunological adaptations that helped to mediate involution and reduce the risk of IMI. Cows with greater milk production at dry off may also be at greater risk of IMI because higher milk production increases milk leakage and slows formation of the keratin plug in the teat canal (Dingwell et al., 2004; Bertulat et al., 2013), potentially leaving the mammary gland susceptible to environmental pathogens. By drying cows off gradually with a short period of intermittent milkings and feeding a lower energy diet, milk leakage was reduced compared with drying cows off abruptly (Zobel et al., 2013). Tucker et al. (2009) observed that 28% fewer cows had milk leakage 2 d after dry off when fed restrictively compared to when fed double the amount. However, they found that milking less frequently the week before dry off did not reduce milk leakage at dry off. Other factors, such as the physical characteristics of the teat, may also affect the likelihood of milk leakage at dry off (Klaas et al., 2005).

Although reducing milk production before dry off was once a common practice for dairy producers to assist cow resistance to IMI, DCT has made this practice relatively rare. The efficacy of DCT, including antibiotics, internal teat sealants and external teat sealants, in preventing IMI has been the subject of a great deal of research interest (Halasa et al., 2009). Dry cow therapy is administered to cows at dry off following the final milking of their lactation. Intramammary administration of antibiotics at dry off helps to prevent and treat infection by gram-positive bacteria during the dry period (Smith et al., 1966; Bradley and Green, 2004), although there is evidence that they become less effective as the dry period progresses (Pinedo et al., 2012). Additionally, these antibiotics provide limited protection against environmental, gram-negative bacteria. There are also growing concerns for blanket-use of antibiotics due to antimicrobial resistance (Bos et al., 2013), encouraging a reduction in antibiotic use through selective DCT. Although selective-use of antibiotics has been shown to reduce the risk of new IMI compared with no use of antibiotics (Halasa et al., 2009), the efficacy of selective-use may depend on the efficiency and accuracy of criteria used to identify cows with IMI at dry off (e.g. Torres et al., 2008; Kiesner et al., 2016). Milk somatic cell count (SCC) is commonly used to detect possible cases of IMI (Schukken et al., 2003). However, in a study wherein cows had SCC below thresholds of IMI at dry off, Scherpenzeel et al. (2014) still observed a higher incidence rate of clinical mastitis in udder quarters that were untreated compared with those that were treated with antibiotics. Due to the limitations of antibiotics and the selective-use thereof, the producer may opt to administer internal teat sealants and external teat sealants at dry off to help prevent bacterial infection by creating a physical barrier in the teat canal (Rabiee and Lean, 2013) or over the teat-end (Lim et al., 2007). A keratin plug should form naturally in the teat canal of the cow following involution, however, this does not always occur or otherwise is frequently delayed, increasing vulnerability to environmental IMI (Dingwell et al., 2003). Indeed, internal teat sealants containing bismuth subnitrate have been shown to have similar (Bhutto et al., 2011) or higher (Huxley et al., 2002) efficacy compared with antibiotic treatments in the prevention of new IMI and cases of clinical mastitis postpartum. Additionally, Sanford et al. (2006) observed fewer new IMI when an antibiotic was paired with an internal teat sealant than when used alone. In contrast, the efficacy of external teat sealants is dependent on adherence to the teat, which can be relatively short-lived (e.g. 3 d; Lim et al., 2007) when compared with internal teat sealants that can remain in the teat canal for the duration of the dry period.

1.2 Welfare Concerns with Abrupt Dry off and Alternatives

The conventional lactation cycle of cows reared in the commercial dairy industry bears little resemblance to that seen under natural conditions. In a natural setting, the cow produces milk to meet the nutritional requirements of her suckling calf. As the calf ages, it becomes less dependent on milk as a nutrient source, transitioning to solid feed over a period of several months (Krohn, 2001), similar to what is still observed in the beef industry and feral cattle populations (Vitale et al., 1986). Thus, the natural lactation cycle of the cow is tailored to the nutrient demands of the calf, and milk production gradually declines as the calf consumes less milk (Wilde et al., 1997). By contrast, the calves of dairy cows in a commercial setting are removed from their dams almost immediately after birth and milk is harvested until milking is, most commonly, abruptly ceased. Current production levels of commercial dairy cows have far exceeded a natural level of milk production needed for calf nutrition, which brings into question the adequacy of the practice of an abrupt dry off from a welfare perspective, as it differs so starkly from what is natural for a dairy cow. One strategy to help decrease milk production and mitigate welfare concerns at dry off is to reduce milking frequency prior to cessation of milking (e.g. Valizaheh et al., 2008; Tucker et al., 2009; Zobel et al., 2013). The following section will discuss aspects of abrupt dry off that may negatively affect the welfare of cows and how gradual dry off may work to attenuate those effects. Another strategy to reduce milk production prior to dry off is to decrease the quality or quantity of feed offered around dry off (e.g Odensten et al., 2005; 2007; Valizaheh et al., 2008). Finally, this section will discuss alternatives to the conventional lactation cycle targeted by dairy producers, including shortening or omitting the dry period and extending the lactating period.

There is research to suggest that dairy cows experience discomfort or pain in response to dry off, especially when an abrupt approach is taken, since cessation of milking causes pressure accumulation in the udder (Peaker, 1980). When milk production at dry off is greater, this pressure may be higher and result in a greater degree of discomfort (Silanikove et al., 2013). Silanikove et al. (2013) observed that cows with high milk production at dry off (>25 kg/d) vocalized more and had udders that were more engorged than cows with lower milk production at dry off (<14 kg/d) following an abrupt dry off. As these cows were fed the same diet, it was suggested the increased rate of vocalizations were indicative of pain due to higher intramammary pressure. However, increased vocalizations in cattle have also been associated with hunger (Watts and Stookey, 2000) and, to the author's knowledge, the amount fed following dry off is not described by Silanikove et al. (2013); thus hunger can not be eliminated as a possible source for the observed rates of vocalizations. Nevertheless, research from Leitner et al. (2007) supports that an abrupt dry off may be painful for cows. When abruptly dried off, cows producing 17-35 L/d, treated with casein hydrolysate, had longer lying bouts and lower udder pressure than untreated cows. Casein hydrolysate increases the speed at which mammary involution occurs, thus decreasing intramammary pressure. Therefore, it was suggested that treatment with casein hydrolysate improved cow comfort following dry off, promoting longer lying bouts. Lying behavior has also been used as an indicator of cow comfort in studies where milking frequency of lactating cows was reduced or a milking event was skipped (Tucker et al., 2007; O'Driscoll et al., 2011). In a case study where abrupt dry off was practiced on a commercial herd, Chapinal et al. (2014) observed that, for primiparous cows, milk production before dry off was negatively associated with lying time on the day of dry off. Furthermore, in that study both primiparous and multiparous cows had a higher frequency of shorter lying bouts following dry off, potentially as a result of discomfort.

Cows may also experience frustration in response to an abrupt dry off. When frustrated, an animal may exhibit abnormal behaviors (Dawkins, 1988), which may be used to assess their affective state. Some recent work with dairy cows has examined time spent standing at the gate to the milking parlor as an indication of motivation to be milked. Zobel et al. (2013) reported that cows dried off abruptly were much more likely to stand at the gate following dry off than before. These authors hypothesized that this behavior was indicative of frustration due to a lack of milking to relieve intramammary pressure. The strength of the cows' motivation to be milked after abrupt dry off was not tested, however, and should be explored in future research. In contrast, cows in that study that were dried off gradually over a 5-d period stood at the gate for a similar amount of time as prior to dry off. Tucker et al. (2007) work supports that a gradual transition may be sufficient to reduce frustration caused by a reduced frequency of milking; they found that cows milked 2x/d at mid-lactation did not increase time spent standing at the gate when transitioned to 1x/d milking.

There is work to suggest that the discomfort occurring during the dry off process may also cause cows to experience stress. By measuring fecal glucocorticoid metabolites, which have been shown to accurately depict chronic stress (Morrow et al., 2002), and udder pressure of cows that were abruptly dried off, Bertulat et al. (2013) provided evidence that an abrupt dry off was stressful for cows, particularly those with high production at dry off. These authors observed that udder pressure peaked 2 d following an abrupt dry off; most notably in cows producing high amounts of milk at dry off (>20 kg/d). Correspondingly, fecal cortisol metabolites of cows with high production at dry off increased 129% from baseline in fecal samples taken 3 d after dry off compared with 40% in cows with low production at dry off (<15 kg/d). Since an 18-h time lag exists between the systemic cortisol level and appearance of those metabolites in the feces, this implies that stress was highest 2 d after dry off, at which time udder pressure was also highest. Thus, the udder pressure caused by an abrupt dry off seems sufficient to cause elevated stress levels in dairy cows.

In an effort to reduce milk production, producers may begin to feed cows at a lower plane of nutrition several days before dry off. This change in feeding management may include feeding low quality forages, with or without supplementation, and/or feeding a lesser amount of total DM. While a cow transitions to such a new diet, it may experience hunger due to an inability to reach satiety. For example, Tucker et al. (2009) reported that cows offered a lesser quantity of fresh pasture and pasture silage (8 kg/d DM) before dry off vocalized more often than cows offered a greater quantity (16 kg/d DM). Vocalizations in cattle have been associated with periods of distress, such as social isolation, pain and hunger (Watts and Stookey, 2000). Since treatments were otherwise identical, it is likely that the higher frequency of vocalizations observed in the cows offered 8 kg/d DM were a result of feed restriction, and thus indicative of hunger. Similar results have been observed in dairy cows offered hay of differing quality in unlimited quantities prior to dry off. Valizaheh et al. (2008) observed that cows receiving ad libitum oat hay consumed less and vocalized more than cows receiving ad libitum grass hay. It was proposed that the higher protein content and lower acid detergent fiber (ADF) content of the grass hay were likely more effective in allowing cows to achieve satiety, as protein has been shown to promote satiety in humans (Johnstone, 2013) and the lower ADF content would improve digestibility.

Dry off feeding strategies may also result in metabolic stress. Odensten et al. (2007) noted higher blood cortisol levels in cows fed only ad libitum straw during a 5-d dry off, compared with cows offered an additional 4 kg DM/d of silage. Those authors proposed that the higher cortisol levels observed under these circumstances were the result of an energy deficit, as Agenas et al. (2003) had previously demonstrated that elevated cortisol levels may occur in response to feed restricting cattle. In a study where cows were fed either straw or straw with 4 kg/d of DM silage during a 5-d dry off, increased plasma NEFA concentration was observed in both groups, especially in cows fed only straw (Odensten et al., 2005). Similarly, increased NEFA was reported when cows were fed only dry hay in the 5 d (Ollier et al., 2014) or 7 d (Bernier-Dodier et al., 2011) prior to dry off. Although feeding these forages ad libitum was effective in reducing milk production, the extent that NEFA was increased in these studies may be indicative of NEB similar to that seen in early lactation (Loiselle et al., 2009). As elevated NEFA concentration has been shown to impair some immune functions (Ster et al., 2012), the benefit of having lower milk production at dry off from an intramammary health perspective may be counteracted by immunosuppression. Furthermore, an accumulation of triglycerides in the liver may accompany an increase in NEFA (Kobayashi et al., 2002), which may in turn be associated with higher levels of the acute phase protein (APP) serum amyloid A (SAA) (Katoh, 2002). Indeed, Odensten et al. (2007) observed an increase in plasma SAA concentration following the start of dry off, although there was no difference in SAA between cows fed straw and those fed straw and silage, despite the fact that cows fed only straw had higher NEFA during the same time period (Odensten et al., 2005). Serum amyloid A and haptoglobin, another important APP in cattle, have been shown to increase in instances of inflammation, trauma, or other external or internal stressors, and may signify clinical or sub-clinical illness (Alsemgeest et al., 1994; Murata et al., 2004). The elevated SAA observed by Odensten et al. (2007) may, therefore, have been indicative of an inflammatory response from involution of the udder.

Alternatively, there has been work to explore strategies that deviate from the traditional lactation cycle, including shortening or omitting the dry period or extending the length of lactation. In omitting the dry period, cows may be subjected to fewer management changes, such as regrouping and dietary changes, and avoid the welfare concerns associated with dry off altogether. Shortening the dry period may promote cow welfare by providing an opportunity for milk production to further decrease before drying off, particularly in the case of multiparous cows. Indeed, multiparous cows with a 35-d dry period were shown to produce ~7 kg less milk on the day of dry off compared with those with a 56-d dry period (Pezeshki et al., 2007). As discussed, lower milk production at the time of dry off is favorable for the welfare of the cow from various standpoints. Furthermore, shortening or omitting the dry period may improve metabolic status early in the next lactation, at least in primiparous cows (Pezeshki et al., 2007). It must be noted, however, that as previously mentioned, lower milk production in the next lactation may occur if the dry period is shortened or omitted completely (Sørensen and Enevoldsen, 1991; Rastani et al., 2005; Kok et al., 2017b). However, in reducing milk production in the next lactation, energy balance postpartum can be improved, as evidenced by lower plasma NEFA and higher glucose in cows managed without a dry period compared with those with a short or traditional dry period (Rastani et al., 2005). Meanwhile, DMI may be similar or greater for cows with no dry period compared with those with a short or traditional dry period (Rastani et al., 2005; van Knegsel et al., 2014; Kok et al., 2017a). Kok et al. (2017a) also reported a low positive correlation between energy balance and lying time, with cows managed without a dry period having greater lying time in early lactation compared with those with a dry period, perhaps related to relative comfort level. With potential improvements to the incidence of postpartum diseases, like ketosis (Schlamberger et al., 2010), and fertility (Watters et al., 2009), as well as additional milk yield at the end of the previous lactation compensating for potential losses in the next lactation (Bachman and Schairer, 2003), shortening the dry period may be economically viable, at least for Canadian Holstein herds (Santschi et al., 2011).

Another alternative strategy with potential welfare benefits is delaying re-breeding and, thereby, extending lactation. Because the early postpartum period is a period of high health risk for the dairy cow (Erb et al., 1984), extending a cow's lactation would mean that she is exposed to this stressful time less often over her lifetime. An apparent problem with extending the lactating period is, again, potential milk yield losses. Although further work is warranted, Sorensen et al. (2007) work suggested that management, in particular increasing milking frequency from 2x/d to 3x/d, can be used to increase lactation persistency and thus make extending lactations from 12 months to up to 18 months economically competitive. Extending lactations may be especially worthwhile in situations where reproductive efficiency is further reduced by heat stress (Mellado et al., 2016). Furthermore, extending lactations also translates to less frequent dry off and potentially lower milk production at the time of dry off.

1.3 Dietary Factors Influencing Feeding Behavior at Dry Off

In transitioning from the lactating diet to that provided at dry off, cows may experience several changes in feeding behavior due to differences in the characteristics of the feed. Firstly, whether the dry off feeding strategy includes decreased ration quality or quantity, the ration provided should reduce DMI to facilitate a reduction in milk production. Valizaheh et al. (2008) observed that cows transitioned from a lactating TMR to either ad libitum grass hay or oat hay at the start of a 6-d dry off period consumed ~5 kg and ~11 kg less DM/d, respectively. In that study, the detected difference in DMI was in response to a difference in in vitro NDF digestibility, wherein the grass hay was more digestible than the oat hay. Lower NDF digestibility has been associated with reduced DMI and FCM (Oba and Allen, 1999). If forage

NDF is of higher digestibility, it will have a faster rate of passage through the rumen, thereby decreasing rumen physical fill, and promoting voluntary DMI (Allen and Oba, 1996). Diets with a higher NDF content are often of greater bulk density, increasing rumen fill and decreasing voluntary DMI (Mertens, 1997; Allen, 1996). Further, a greater proportion of longer particles in the diet will limit DMI by increasing rumen retention time (Allen and Mertens, 1988). Although Valizaheh et al. (2008) observed similar feeding times between cows fed the two types of hay (grass or oat), since cows fed the grass hay had higher DMI, they consumed this hay at a faster rate compared with cows fed the oat hay. In another study, when cows fed a lactating TMR were transitioned to ad libitum oat straw and ~10 kg/d of grass hay at dry off, cows demonstrated an increase in feeding time from ~5 to ~6 h/d (Zobel et al., 2013). This is in agreement with the findings of Huzzey et al. (2005), who observed a decrease in time spent at the feed bunk post-calving when cows were switched to a higher energy (higher proportion of concentrate) lactating diet compared with the pre-calving lower energy dry diet. Higher feeding rates have been associated with higher concentrate content in the diet (Friggens et al., 1998), as time spent feeding has been linearly associated with an increased proportion of roughage, or forages, in the diet (Jiang et al., 2017). Huzzey et al. (2005) also observed an increase in meal frequency from pre-calving to post-calving. It was suggested that this increase may have been in response to higher competition following regrouping, as increased feed visits have been observed with higher competition (Olofsson, 1999), or to greater energy requirements due to lactation. It is possible, however, that the differences in diet characteristics could have also contributed to the difference in meal frequency.

Optimal nutrition strategies for the dry period have been extensively studied due to their importance in promoting cow health and welfare at calving and into lactation. Whether feeding distinct diets between the "far-off" and "close-up" (e.g. last 3 wk dry) dry periods or feeding a single diet throughout the entire dry period, producers most often feed cows a TMR. It has been recommended that the dry cow TMR include a high proportion of wheat straw, or other low nutritive roughage, and contain similar components to the lactating diet to reduce the impact of dietary change at dry off (Beever, 2006). Although a TMR is meant to be a homogeneous mixture of feed components that promotes cow health and discourages preferential selection of certain components (Coppock et al., 1981), cows maintain the ability to selectively consume (sort) the feed, often in favor of the small, palatable concentrate components and against the longer, forage components (Leonardi and Armentano, 2003; Greter and DeVries, 2010). While lactating and receiving the lactating diet, this pattern of sorting activity may put cows at an increased risk of subacute ruminal acidosis, a disorder wherein cows experience a decrease in ruminal pH due to over-consumption of concentrates (Plaizier et al., 2009). Due to a greater inclusion of forages and a lesser inclusion of concentrates in the dry cow diet, this disorder is less of a concern during the dry period. However, sorting in favor of the smaller components could put certain cows at risk of exceeding nutrient requirements, and could increase intake variability depending on the degree of competition occurring at the feed bunk (Crossley et al., 2017). Cows that overfeed and exceed nutrient requirements during the far-off dry period are more at risk of developing metabolic diseases, like ketosis, postpartum (Dann et al., 2006; Mann et al., 2015). Thus, finding ways to minimize the risk of sorting of dry diets is important for maintaining nutrient intake consistency and long-term health and production.

The extent that cows engage in feed sorting may be dependent on a variety of factors related to the diet provided. Various studies have investigated the effects of manipulating the forage components of the diet on feed sorting. Leonardi and Armentano (2003) observed that cows sorted against long particles to a greater extent when the amount and particle size of alfalfa hay was increased in the diet. However, sorting behavior was not affected by the quality of hay included, in terms of relative NDF-content. In a study comparing 2 diets of differing forage-concentrate ratios, DeVries et al. (2007) observed that lactating cows fed a lower forage diet (50.7% forage) had greater sorting activity than cows fed a higher forage diet (62.3% forage), sorting more against the long particles and more in favor of the small particles. This result opposed their hypothesis that cows fed the higher forage diet would have greater motivation to sort in favor of the small, concentrate components of the diet since they were less abundant. It is possible that because the higher forage diet was of higher moisture content than the lower forage diet it may have been more difficult to sort, as sorting has been shown to be reduced by adding water to diets of low moisture content (Leonardi et al. 2005). DeVries et al. (2007) also suggested, however, that a greater degree of sorting for the small particles may have occurred simply because the lower forage diet contained a higher proportion of concentrate, making them more easily sorted for. In a study where additional straw was added to the mixed diets of dairy heifers, Greter et al. (2008) observed that heifers receiving a diet that was 20% straw sorted for the medium and fine particles, in addition to sorting against long particles and in favor of short particles. Further, Suarez-Mena et al. (2013) investigated the effects of various lengths of straw particle size on the sorting behavior in two experiments using late-lactation and dry cows. Those authors observed a linear increase in sorting against the long particles as hay or straw particle size increased. These studies are a small sample of a large number of studies that have been conducted to investigate dietary factors influencing feed sorting (see review by Miller-Cushon and DeVries, 2017). To our knowledge, no studies have been conducted to investigate how transition to the dry cow diet at dry off may influence sorting behavior. However, based on previous research, transitioning to a diet that is of greater forage content, and lower in moisture content (Miller-Cushon and DeVries, 2017), is likely to facilitate sorting, in addition to potentially encouraging sorting for the higher energy components of the diet, due to continued nutrient requirements (for maintenance and gestation) after dry off and the relatively lower overall palatability of the dry diet.

1. Objectives and Hypotheses

The objectives of this thesis were to investigate the impact of the magnitude change in dietary nutrient density during and after gradual dry off on the behavior and physiology of dairy cows. A secondary objective was to evaluate the efficacy of reducing dietary nutrient density and milking frequency to decrease milk production prior to dry off. It was hypothesized that cows experiencing a greater reduction in dietary nutrient density at dry off would exhibit differences in feeding behavior, including decreased DMI, increased feeding time and decreased feeding rate, compared with cows experiencing a lesser reduction in dietary nutrient density. It was also hypothesized that feeding a diet of lower nutrient density would encourage a greater degree of sorting against the long particles and in favor of the short particles, compared with feeding a diet of higher nutrient density. Despite these predictions for sorting activity, it was still hypothesized that cows fed the lower nutrient density diet would spend more time ruminating and have higher reticulorumen pH than cows fed the higher nutrient density diet. It was also predicted that a greater magnitude of change in ration nutrient density would result in greater physiological differences between treatments, especially immediately following dietary transition. Finally, it was hypothesized that milk production would be reduced to a greater extent in cows fed a diet of lower nutrient density compared with those fed a diet of higher nutrient density, and would better promote comfort at dry off, as indicated by increased lying duration.

CHAPTER 2. Impact of dietary transition at dry off on the behavior and physiology of dairy cows

2.1 INTRODUCTION

Dairy cows undergo substantial physiological changes during late gestation. Apart from increased nutrient demands for fetal growth, a key event involved in these changes is dry off, wherein the cow transitions from a lactating to a non-lactating state. The non-lactating, or dry, period is widely recognized for its role in promoting successful transition into the subsequent lactation. Additionally, the dry period is important for optimizing milk production in the next lactation, with a dry period length of 40 to 60 d being commonly practiced (as reviewed by Bachman and Schairer, 2003). Due to high milk production, and the target of 12-mo calving intervals, dry off rarely occurs naturally in modern dairy herds. Instead, the dry period is often initiated by abrupt cessation of milking and administration of dry cow therapy (e.g. antibiotic, teat sealer/sealant; Blowey and Edmondson, 2010). The practice of abrupt dry off may have consequences for cow health and welfare, particularly for cows with high production at dry off (e.g. 25 to 30 kg/d; Dingwell et al., 2001; Stefanon et al., 2002). Researchers have demonstrated that cows with greater production at dry off are at an increased risk of IMI post-calving (Dingwell et al., 2004; Rajala-Schultz et al., 2005) and experience high udder pressure and greater stress levels at dry off (Bertulat et al., 2013).

As an alternative to an abrupt dry off, producers may mediate cessation of milking by gradually reducing milking frequency or by reducing nutrient availability around the time of dry off (Bushe and Oliver; 1987; Skidmore et al., 1997). However, dry off strategies that restrict feed quantity or quality may also cause cows to experience reduced welfare, at least in the short-term. For example, feeding hay of lower digestibility at dry off may cause cows to experience distress due to hunger, as indicated by increased vocalizations (Valizaheh et al., 2008). Increased rates of vocalization have similarly been observed in cows that were restrictively fed before dry off (Tucker et al., 2009). Increased plasma NEFA and cortisol have been observed in cows fed only straw at dry off, likely signaling an insufficient supply of nutrients (Odensten et al., 2005; Odensten et al., 2007). From these studies, it has been recommended that cows be provided low-quality feed *ad libitum*, rather than be fed restrictively. However, even when fed *ad libitum*, some forages may not be digestible enough to allow cows to achieve satiety (Valizaheh et al., 2008), and may require supplementation to prevent cows from experiencing negative energy balance (NEB; Odensten et al., 2005) and, potentially, depression of immune function (Ster et al., 2012).

As an alternative to feeding solely a low-quality forage at dry off, producers may opt to transition cows to the dry cow diet that they will be fed after dry off. Potential benefits to this strategy are that it limits the number of dietary changes cows must undergo, thereby reducing stress and promoting rumen health, and provides cows with a higher plane of nutrition, which addresses the physiological and welfare concerns mentioned above. However, the ability of cows to manipulate a mixed ration allows for preferential consumption (sorting) of certain components, and, thus, may result in cows consuming a ration that is nutritionally different than intended (Leonardi and Armentano, 2003; DeVries et al., 2007).

To our knowledge, the behavioral and physiological impact of transition at dry off to diets of lower nutrient densities has not been thoroughly elucidated. Therefore, the objective of the present study was to investigate the impact of the magnitude change in dietary nutrient density during and after gradual dry off on behavior and physiology of dairy cows. A secondary objective was to evaluate the efficacy of reducing dietary nutrient density and milking frequency to decrease milk production prior to dry off.

2.2 MATERIALS AND METHODS

2.2.1 Animals, Housing and Experimental Design

This study included 26 primiparous and 22 multiparous Holstein cows (parity = 2.5 ± 0.7; mean ± SD) housed at the University of Guelph, Livestock Research and Innovation Centre - Dairy Facility (Elora, Ontario, Canada). Cows were enrolled in late lactation in 8 groups of 6 cows, beginning in June 2016, with the last group being enrolled in December 2016. Groups of 6 were composed of cows with similar expected calving dates, and time of enrollment (d -16 relative to the start of dry off) was determined to allow for an average dry period length of 60 d (actual = 62.6 ± 8.0 d). At the time of enrollment, cows were on average 297.7 ± 49.6 DIM, had an average BW of 741.9 ± 57.9 kg, an average BCS of 3.3 ± 0.3, and produced, on average, 26.0 ± 5.6 kg/d of milk during the final 16 d before the start of dry off. Inclusion criteria required cows be producing at least 10 kg/d of milk at enrollment. The use of cows and all experimental procedures were in compliance with the guidelines of the Canadian Council on Animal Care (2009) and were approved by the University of Guelph Animal Care Committee (Animal Use Protocol #3245).

At least 3 d before enrollment, each group of 6 cows was moved from the main herd to a separate lactating free-stall pen and trained to access individual automated feed bins (Insentec, B. V., Marknesse, the Netherlands). This lactating pen had 15 automated feed bins, 30 lying stalls, and 2 water troughs, which offered free access to water. The number of cows in the lactating pen never exceeded 15 cows to ensure feed bin stocking density remained lower than 100%. The base of the lying stalls was mattresses (Pasture Mat; ProMat, Woodstock, ON, Canada) that were bedded with chopped straw. The 3 d prior to enrollment served as an adjustment period during which cows became accustomed to their new social group and to using the feed bins. Cows were then followed for a period of 16 d, during which time baseline data were collected. During the baseline period, cows were milked 2x/d, beginning at 0500 and 1700 h, in a rotary parlor (DeLaval, Peterborough, ON, Canada) and were fed a lactating cow TMR (Table 2.1) 1x/d between 1330 and 1430 h. The lactating cow feed bins were cleaned out each day at approximately 1300 h, prior to the next feed delivery. Lactating cows were fed *ad libitum*, targeting approximately 10% refusals (actual = 11.7 ± 9.5 % on a DM basis).

At the end of the 16-d baseline period, each group of cows was moved to a free-stall, dry cow pen that contained 12 automated feed bins, 24 lying stalls (mattress base with chopped straw bedding) and 2 water troughs. Similar to when cows were in the lactating pen, the number of cows in the dry pen never exceeded the number of feed bins. In this pen, cows were again assigned to an individual feed bin. Once moved to the dry pen, all cows were dried off over a 5-d period following an intermittent milking schedule. During dry off, all cows were milked only 1x/d on d 1, 2, 3, and 5 at 0500 h in the parlor, and not milked at all on d 4. Additionally, while a group of cows was being dried off, the dry pen was divided in half by gates to facilitate sorting of cows undergoing dry off from dry cows at milking. After the final milking on d 5, all udder quarters of all cows were treated with a tube of dry cow antibiotic (Dry-Clox, Boehringer Ingelheim Vetmedica Inc., Duluth, Georgia, USA), followed by a tube of teat sealant (Orbeseal; Pfizer Inc., Kirkland, Quebec, Canada). Prior to infusion, all teats were disinfected with alcohol.

At the start of gradual dry off (d 1), cows within each group were randomly assigned to 1 of 2 dry cow treatment diets (Table 2.1, 2.2), balanced for parity and milk production between treatments. The treatments were: 1) higher nutrient density (**HND**; n=24) or 2) lower nutrient density (**LND**; n=24) dry cow diet. The treatment diets contained the same feed ingredients, but differed in the proportion of each feed ingredient. From the start of dry off (d 1), cows were fed 1x/d between 1030 and 1130 h. The dry cow feed bins were cleaned out each day at approximately 1000 h, before the next daily feed delivery. After dry off (d 5), cows continued to receive their treatment diet for an additional 21 d, for a total of 26 d on their assigned treatment diet. Both treatment diets were fed *ad libitum*, again targeting approximately 10% refusals (actual =11.8 ± 9.3% on a DM basis). Access to water was also *ad libitum* while cows were in the dry cow pen, with no water restriction occurring during dry off. After d 26, cows were fed the HND diet until calving, which was the standard dry cow ration for the herd.

2.2.2 Milk Yield and Components

From enrollment (d-16) until the end of dry-off (d 5), milk yield was recorded automatically at every milking by DelPro software (DeLaval, Peterborough, ON, Canada). Milk samples were collected from each cow at both milkings on d -11, -10, -4, -3, 3 and 5. These samples were sent to the DHI testing laboratory (CanWest DHI, Guelph, ON, Canada) for component analysis (fat, protein, MUN, and SCC) using a Fourier Transform Infrared full spectrum analyzer (Milkoscan FT+ and Milkoscan 6000; Foss, Hillerød, Denmark). One value per cow on each sampling day was obtained by calculating the average across milkings.

2.2.3 Feed Sampling and Analysis

Throughout the duration of the study, 2 duplicate samples of each of the 3 diets (lactating and 2 dry cow treatment diets) were collected 3 d/wk at feed delivery time. One of the duplicate samples was used to determine DM content and chemical composition, while the other was used to determine particle size distribution. At the beginning of each month, samples of each of the TMR components (for all diets) were taken for DM and composition analysis. In addition to samples taken of offered (fresh) feed, refusal (orts) samples were also collected regularly, for particle size determination, from the automated feed bins of enrolled cows, with a total of 17 refusal samples for each cow. Sampling frequency of refusals was approximately every 3 d, but occurred more frequently around the time of dry off and dietary transition (d -14, -11, -8, -5, -2, -1, 1, 3, 5, 6, 7, 10, 13, 16, 19, 22, and 25). Upon collection, all samples were frozen at -20°C until further analysis. Before analysis, all samples were given a period of at least 24 h to thaw in a refrigerator.

Each fresh feed sample taken for nutrient determination was oven-dried at 55°C for 48 h to determine DM. To assess sorting behavior, the other fresh feed sample and all orts samples were separated by particle size using a 4-screen Penn State Particle Separator (PSPS; Maulfair et al., 2011; Maulfair and Heinrichs, 2013). The PSPS separated samples into four particle size fractions: long (>19mm), medium (8-19mm), short (4-8mm) and fine (<4mm). After being separated into fractions, PSPS samples were also oven-dried at 55°C for 48 h.

Sorting behavior of each particle size was quantified by comparing the particle size distribution of the offered feed to that of the refused feed of individual cows. Specifically, sorting of each PSPS fraction was calculated as the actual intake of a fraction divided by the predicted intake of the fraction, expressed as a percentage (Leonardi and Armentano, 2003). For each fraction, the actual intake was calculated as the amount refused of the fraction subtracted from the amount offered (on a DM basis), while the predicted intake was calculated as the cow's DMI of the complete ration multiplied by the % of the fraction in the offered ration (on a DM basis). A sorting value of 100% indicated that no sorting of the fraction occurred: actual intake equaled predicted intake. A sorting value <100% indicated that sorting occurred against the fraction: actual intake was less than predicted intake. Finally, a sorting value >100% indicated that sorting occurred in favor of the fraction: actual intake was greater than predicted intake. The difference of the sorting value from 100% indicated the degree to which sorting occurred.

After being dried, all DM samples (feed components and fresh TMR samples), as well as fresh PSPS fraction samples of the 3 diets, were ground through a 1-mm sieve (Model 4 Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ, USA). Ground samples were then pooled by sample type and date, and shipped to Cumberland Valley Analytical Services Inc. (Waynesboro, PA, USA) for analysis of DM (135°C; AOAC, 2000: method 930.15), ash (535°C; AOAC, 2000: method 942.05), ADF (AOAC, 2000: method 973.18), NDF with heat-stable α-amylase and sodium sulfite (Van Soest et al., 1991), and CP (N x 6.25; AOAC 2000: method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI).

2.2.4 Feeding Behavior

Automated feed bins were used to individually measure each cow's feeding behavior and dry matter intake (DMI), as validated by Chapinal et al. (2007). At each visit to the bin, duration of the visit (feeding time) and feed consumed (start weight−end weight; as-fed intake) were recorded. To determine DMI, the as-fed intake at each bin visit was multiplied by the weekly average DM percent of the corresponding diet, as determined by DM analysis of fresh feed samples. Feeding rate at each bin visit was calculated as DMI (kg/d) divided by time spent feeding (min/d). These data were then summarized to determine total DMI (kg/d) and feeding time (min/d), as well as the average feeding rate (kg/min) for each trial day for each cow. To analyze meal patterning, individual bin visits were combined into meals using individual meal criteria for each cow, as described by DeVries et al. (2003). Separate meal criteria (the minimum time between meals) were determined for each cow, for both the lactating and dry period, using a software package (MIX 3.1.3; MacDonald and Green, 1988) to fit normal distributions to the frequency of log_{10} transformed time intervals between bin visits. If an interval of time between 2 bin visits exceeded the determined meal criteria then this indicated a different meal. The number of different meals was classified as meal frequency (no./d). Total meal time was the time between the start of the first bin visit until the end of the last bin visit within the meal criterion. Meal length (min/meal) was calculated as the total meal time divided by the meal frequency. Finally, meal size (kg/d) was calculated as DMI divided by the meal frequency.

2.2.5 Rumination

As validated by Schirmann et al. (2009), an electronic monitoring system (HR-TAG-LD, SCR Engineers Ltd., Netanya, Israel) was used to monitor rumination activity. A rumination data logger attached to a nylon collar was fitted to each cow before enrollment and was used to measure rumination activity 24h/d for the 6-wk period. Rumination data were automatically uploaded to receiver at 2-h intervals and downloaded at least 1x/wk. Data were then summarized by cow and trial day.

2.2.6 Lying Behavior

Lying and standing behavior were recorded with electronic data loggers (HOBO Pendant G Data Logger, Onset Computer Corporation, Bourne, MA, USA), which took measurements of leg orientation at 1-min intervals, as validated by Ledgerwood et al. (2010). Data loggers were wrapped in veterinary bandage (VetRap; 3M products, St. Paul, MN) and attached to the medial side of the lower hind leg, also using veterinary bandage. Cows were fitted with their first data logger before enrollment. Data loggers were removed 1x/wk to download data and to prevent prolonged exposure to rubbing on a particular leg. Prior to removing one data logger, a new data logger was fitted to the other hind leg of the cow, so as to keep data continuous. Data collected was downloaded using Onset HOBOware Software (Onset Computer Corporation, Bourne, MA, USA) and exported to Microsoft Excel (Microsoft, Redmond, WA, USA). Excel macros were used to process and summarize lying data as total lying time, frequency of lying bouts, and average bout length (total lying time/frequency of bouts).

2.2.7 Reticulorumen pH

To measure rumen health, each cow was administered a wireless telemetric bolus (eBolus, eCow Ltd., Devon, UK), as validated by Falk et al. (2016), before enrollment using a balling gun. Each bolus took measurements of reticulorumen pH at 15-min intervals, 24h/d. Data were downloaded from the boluses at least 1x/wk using a handset and antenna. Downloaded files were combined into a continuous record for each cow and data were summarized as mean, maximum, and minimum pH, as well as range in pH, by day and cow.

2.2.8 Blood Sampling and Analyses

A total of 8 blood samples were taken from the coccygeal vein of each cow into 10-ml red top vacutainer tubes on d -14, -7, 1, 5, 7, 13, 19, and 25. All blood samples were taken at the same time each day (between 1000 h and 1100 h while cows were in the dry pen and 1200 h and 1300 h while cows were in the lactating pen). Blood samples were left to sit at room temperature for a period of 1 h following collection to allow coagulation and to facilitate fibrinogen breakdown. After 1 h, samples were centrifuged at 3000 rpm for 15 min to separate cells from serum. For each blood sample, 1.5 ml of serum were placed into 3 ml tubes to be frozen at -20°C until the time of analysis. Serum samples were sent to the Animal Health Laboratory, University of Guelph, where they were analyzed for non-esterified fatty acids (NEFA), glucose and haptoglobin concentrations using a photometric test. Additionally, an electronic hand-held meter (FreeStyle Precision Neo, Abbott Diabetes Care, Saint Laurent, QC, Canada) as validated by Kanz et al. (2015), was used to measure beta-hydroxybutyrate (BHB) concentration (mmol/L) immediately following blood collection by placing one drop of blood on a blood ketone test strip.

2.2.9 Body Weight and Body Condition Score

Body weight and BCS were recorded periodically for each group of cows. Body weight was measured by bringing cows to a scale (1-20W scale, Ohaus, Dundas, ON, Canada) at enrollment (d -16 and -15), during dry off (d 3 and 5) and at the end of each study period (d 25 and 26). Cows were weighed twice at each time point so an average could be calculated to better account for daily variation. During dry off, BW was not measured on consecutive days to avoid bringing cows to the scale on days when they were not being milked, since the scale was near the milking parlor. Body condition score was conducted on a five-point scale as described by Wildman et al. (1982), at 0.25 increments, at enrollment (d -16), at dry off (d 5) and at the end of the study period (d 26). All BCS assessments were performed by a single assessor.

2.2.10 Statistical Analyses

All statistical analyses were conducted using SAS 9.4 software (SAS Institute Inc., 2013). Significance was declared if $P \leq 0.05$ and tendencies reported if $0.05 < P \leq 0.10$. Before analyses, the UNIVARIATE procedure was used to assess the normality of all measures. The assumptions of normality were met for most variables, however, the natural logarithm was used to normalize SCC, NEFA, and haptoglobin data sets. Dietary treatment was applied at the level of the cow; thus cow was the experimental unit. Due to the technical failure of 2 rumination data loggers, all rumination activity analyses were conducted using a sample size of 46 cows (HND, n=23; LND, n=23). The data for one cow was removed from all analyses involving feeding behavior, lying behavior, and blood parameters as its data were consistently an outlier due to chronic lameness and illness (HND, n=24; LND, n=23). In instances where malfunctioning of automated feed bins, data loggers, or telemetric boluses occurred, only days with complete data were included in the analyses.

To investigate the impact of dry off and dietary treatment on milk yield and composition, DMI, feeding behavior, feed sorting, rumination, lying behavior, reticulorumen pH, energy balance, and haptoglobin, data were summarized by day and cow and analyzed in mixed-effect, linear regression models using the MIXED procedure of SAS, treating day after the start of dry off as a repeated measure. In each model, treatment, day and the treatment by day interaction were included as fixed effects. To account for pre-existing variability, the average values of each measure during the baseline period were included as covariates in all models. As allocation to treatments was balanced for milk production and parity, these variables were tested in the models, but not included, as they did not change the estimates or significance for fixed effects. The random effect was group, referring to the 8 groups of 6 cows included in the study. The subject of the repeated measures statement was cow within group. For each model, several covariance structures were considered, and that with the best fit, based on Schwarz's Bayesian information criterion, was utilized for each model. Covariance structures used were: compound symmetry, heterogeneous compound symmetry, autoregressive (1), and heterogeneous autoregressive (1). Treatment by day interactions were considered at $P < 0.05$ and investigated using the PDIFF procedure in the LSMEANS statement. Additionally, a difference from the baseline period was assessed for each variable, for each treatment. This was performed on a within cow-basis by subtracting the baseline average of each measure from the value of that measure for each day during the treatment period. These models were created identically to those described above, except the dependent variable in each case was the difference in that variable from their baseline average (and the baseline average was not included as a covariate). Finally, to determine if sorting occurred within treatments and during the baseline period, the summarized sorting values for each particle size were tested for a difference from 100 using t-tests.

To determine if there was an effect of dietary treatment on BW and BCS, models were created, again using the MIXED procedure, wherein BW and BCS at the end of the study were the dependent variables, treatment was the fixed effect, and BW or BCS at enrollment were included as a covariate. Group was also included as a random effect. The change in BW and BCS was calculated as the difference between enrollment and end values, and was modelled similarly, with treatment as a fixed effect.

2.3 RESULTS

Across the 5-d dry off period, cows experienced a reduction in milk production of approximately 10 kg/d compared with the baseline period (Table 2.3). Furthermore, milk yield during the 5 d of dry off differed between treatments, with LND cows producing approximately 1 kg/d less milk than HND cows. Compared with the baseline period, milk samples taken during dry off had increased milk fat %, protein % and log SCC, and had decreased 4% FCM, fat yield and protein yield. However, MUN was similar to baseline for both treatments. There were no differences in milk composition between treatments during the dry off period.

Following introduction to the treatment diets, DMI was lower for all cows during the treatment period compared with the baseline period (Table 2.4). A treatment by day interaction for DMI was detected, wherein cows fed the LND diet had consistently lower DMI than cows fed the HND diet, however, the extent of the difference between treatments varied across days (Figure 2.1a). Cows spent more time feeding in the treatment period compared with the baseline period, with cows fed the LND diet spending more time feeding compared with cows fed the HND diet (Figure 2.1b). Correspondingly, cows consumed their feed at a slower rate during the treatment period compared with the baseline period, with LND cows feeding at a slower rate than HND cows (Figure 2.1c). During the treatment period, cows had a lower meal frequency compared with the baseline period (Table 2.4). A treatment by day interaction indicated similar meal frequency between treatments, with the exception of d 4, 13, 17, 22, 23 and 24 where cows fed the LND diet had lower meal frequency than cows fed the HND diet. Meal length was higher during the treatment period than during the baseline period, and a treatment by day interaction was detected wherein HND cows tended to have longer meals than LND cows on d 3, but LND cows had longer meals than HND cows on d 5, 7, 13, 17, 20, 22, 23 and 24 and tended to have longer meals on d 12. Compared with baseline, HND cows had a similar average meal size, while LND cows had a smaller average meal size. Meal size during the treatment period demonstrated a treatment by day interaction wherein LND cows had smaller average meal size than HND cows on d 1, 3, 10, 14 and 16, and tended to have smaller meal size on d 2, 12, and 15. No treatment differences were detected for the interval between meals, within meal interval, non-feeding time within meals, or total meal time.

During the baseline period, cows sorted against the longest particles (>19 mm) of the lactating diet, and continued to do so similarly between treatments after the dietary change (Table 2.5). Sorting values indicated that cows sorted neither for nor against the medium particles (8 to 19 mm) during the baseline period, however, during the treatment period sorting occurred slightly in favor of these particles. Sorting for the medium particle size was similar across days and between treatments. During the baseline period, cows tended to sort in favor of short particles (4 to 8 mm). Similarly, cows sorted in favor of the short particles during the treatment period, although this occurred to a greater extent for both treatments compared with baseline. A treatment by day interaction (Table 2.5) indicated that LND cows sorted more in favor of the short particles than HND cows on d 1 ($P = 0.03$) and tended to do so more on d 6, 13, 16, and 25 ($P < 0.10$). Finally, cows sorted in favor of the fine particles (< 4 mm) during the baseline period, and continued to do so similarly when fed the HND diet in the treatment period (Table 2.5). However, cows fed the LND diet sorted in favor of the fine particles to a greater extent than during the baseline period. A treatment by day interaction was detected for the treatment period (Table 2.5), wherein sorting of the fine particles was greater for LND cows than HND cows ($P < 0.06$) with the exception of d 13 and 25 ($P > 0.20$).

Compared with the baseline period, both rumination time and rumination time per kg of DMI (**rum/DMI**) were greater in the treatment period (Table 2.4). A treatment by day interaction indicated that rumination time was similar between treatments (Table 2.4), with the exception of d 6, 7 and 14 wherein LND cows spent more time ruminating than HND cows ($P < 0.05$) and d 4 wherein LND cows tended to spend more time ruminating than HND cows ($P < 0.07$). A treatment by day interaction was also detected for rum/DMI (Table 2.4), in which case rum/DMI was greater for LND cows than HND cows, except for d 5, 9, 13, 14, 20, 22, and 25 where rum/DMI was similar between treatments.

Compared with the baseline period, cows had higher daily mean and daily minimum reticulorumen pH in the treatment period (Table 2.6). Daily maximum pH was higher than baseline for LND cows, but was similar to baseline for HND cows. Mean, maximum, and minimum reticulorumen pH differed by treatment, with cows fed the LND diet having higher pH in each regard compared with those fed the HND diet. During the treatment period, cows had smaller within day range in pH compared with that observed during the baseline period. Daily range in reticulorumen pH also differed between treatments, with LND cows having a slightly lower daily range in pH compared with HND cows.

Although daily lying time was higher than baseline for HND cows, lying time did not differ between LND cows and HND cows during the treatment period (Table 2.4). Lying bout frequency was similar to that during the baseline period for both treatments, however, cows fed the LND diet had fewer bouts/d than cows fed the HND diet. Finally, cows fed the LND diet had longer bouts compared with their baseline and cows fed the HND diet tended to have longer bouts compared with their baseline. However, no difference in average bout length was observed between treatments.

Although no treatment differences were detected for glucose, NEFA or haptoglobin, these parameters varied by day relative to treatment implementation and dry-off, as well as from baseline (Table 2.7). Cows fed the HND diet had increased glucose from d 5 to 19 ($P < 0.05$), while cows fed the LND diet had increased glucose on d 5 and 7 ($P < 0.04$), and showed a tendency for elevated glucose on d 13 and 19 ($P < 0.08$; Figure 2.2a). Cows fed the HND diet had lower NEFA on d 7 and 25 compared with baseline ($P < 0.02$), while cows fed the LND diet tended to have higher NEFA on d 1 and lower NEFA on d 25 ($P < 0.08$), and had lower NEFA on d 7 ($P = 0.002$; Figure 2.2b). Cows fed the HND diet had increased haptoglobin on d 5, 7, 13 and 19 ($P < 0.04$), while cows fed the LND diet only demonstrated elevated haptoglobin on d 13 and 19 ($P < 0.001$; Figure 2.2d). By d 25, haptoglobin for both treatment groups was similar to that occurring during the baseline period. Finally, there was a tendency for cows fed the LND diet to have slightly lower BHB than cows fed the HND diet, and both treatments exhibited differences from baseline (Table 2.7; Figure 2.2c). Compared with baseline, cows fed the HND diet tended to have decreased BHB on d 7 ($P < 0.09$), and had decreased BHB from d 13 to 25 ($P < 0.02$). Similarly, cows fed the LND diet had decreased BHB from d 7 to 25 compared with the baseline period ($P < 0.02$).

At the end of the trial period (d 26), both treatments had similar BW (785.8 vs 794.3 for LND and HND cows, respectively; SE = 5.4 kg; $P = 0.14$) when accounting for BW at the time of enrollment. Although both treatment groups demonstrated increased BW from enrollment ($P < 0.001$), there was a tendency for cows fed the LND diet to gain less weight than cows fed the HND diet (+43.1 vs +53.1 kg; SE =5.1; $P = 0.08$). Similarly, no difference in BCS between treatments was detected at the end of the trial period (3.47 vs 3.50; SE = 0.05; $P = 0.66$). However, by d 25, both treatments exhibited an increase in BCS from the time of enrollment (+0.16 vs +0.15; SE = 0.05; $P < 0.006$).

2.4 DISCUSSION

The objectives of this study were to investigate the impact of dietary transition at dry off on the behavior and physiology of dairy cows, and the efficacy of using a greater magnitude change in ration nutrient density, coupled with a reduction in milking frequency, to decrease milk production prior to dry off. Following 5 d of intermittent milkings, average milk yield was reduced by ~10 kg/d from baseline for both magnitude changes in dietary nutrient density. The change in milking frequency from 2x/d to, at most, 1x/d was likely primarily responsible for the reduction in milk yield, as losses in milk yield when cows are switched to once-daily milking is well-documented (see review by Davis et al., 1997). The decline in milk yield observed in the current study was similar to that reported by Zobel et al. (2013), wherein cows producing 24 ± 5 kg/d at dry off experienced a decrease in milk yield of ~13 kg after a 5-d period of skipped milkings. During those 5 d, cows were fed oat straw *ad libitum* and ~10 kg/d of tall fescue hay, a lower plane of nutrition than the present study, which may have facilitated the greater decrease in milk yield. Restriction of feed has been demonstrated to reduce milk yield in cows in peak-lactation (Agenäs et al., 2003) as well as late-lactation before dry off (Tucker et al., 2009). Although both treatment diets in the current study were fed *ad libitum*, rather than restrictively, and provided, in comparison to similar studies a higher level of nutrition during dry off, treatment likely contributed to the decrease in milk production, as cows fed the LND diet experienced a greater reduction in milk yield than the HND cows during the dry off period. This may be attributed to both the lower nutrient density, and detected difference in DMI, wherein LND cows had consistently lower DMI compared with HND cows.

Additionally, the effect of intermittent milkings observed in the current study, at dry off, on milk fat %, protein % and SCC are consistent with changes observed in previous studies at dry off (Odensten et al. 2005; Odensten et al., 2007) and when lactating cows are switched to once-daily milking (Davis et al., 1999); a concentration effect due to decreased milk production was likely responsible for these increases.

Once introduced to their respective treatment diets, all cows experienced a reduction in DMI, in particular the LND cows. This was not surprising as both treatment diets had higher NDF content compared with the lactating diet due to greater inclusion of forages. With higher NDF, the bulk density of the feed is often increased, which promotes physical fill of the rumen and limits voluntary DMI (Mertens, 1997; Allen, 1996). The treatment diets also had a greater proportion of long particles than the lactating cow diet, which may have further limited intake due to increased rumen retention time (Allen and Mertens, 1988). Thus, greater NDF content and proportion of long particles in the LND diet would have limited DMI to a greater extent than the HND diet. Despite lower DMI, cows in the current study spent more time feeding after receiving their respective treatment diet, with LND cows spending more time feeding than HND cows. These results are consistent with work by Jiang et al. (2017), who reported a linear increase in feeding time with greater inclusion of roughage in the diets of mid-lactation cows. Other researchers have also observed increased feeding time when cows were switched from a lactating TMR to lower quality feeds prior to dry off (Valizaheh et al., 2008; Zobel et al., 2013). In the present study, lower DMI, but greater feeding time, corresponded with slower average feeding rates following dietary transition, especially for cows fed the LND diet. Further to the effect of higher roughage inclusion promoting longer feeding times, forage type in the diet has been shown to affect feeding rate, with straw and hay having been shown to encourage slower rates of intake compared with silage (Beauchemin et al., 2008). These changes in feeding behavior on a daily-basis were also reflected in meal patterning. Although cows had fewer meals per day following treatment diet implementation, total daily meal time increased, individual meals were of longer duration, with more non-feeding time within meals. These changes can be attributed to slower feeding rates and greater time spent chewing (Jiang et al., 2017). There was also more time between meals for both treatments, and meals were smaller on a DM-basis for LND cows, again likely a result of correspondingly increased gut fill from the greater bulk of both treatment diets, but especially the LND diet.

Unsurprisingly, cows in the current study sorted against the longest particles (which would be primarily straw) and at least tended to sort in favor of the short and fine particles (which would be primarily corn of silage and supplement) of their respective diets. These results are in agreement with previous studies that have shown cows prefer the smaller, more palatable concentrate components of a TMR to the larger, forage components (Leonardi and Armentano, 2003; Greter and DeVries, 2010). Furthermore, cows fed the LND diet sorted in favor of the short and fine particles to a greater extent than cows fed the HND diet. This contradicts work by DeVries et al. (2007) wherein lactating cows fed a lower forage diet (50.7% forage) had greater sorting activity than cows fed a higher forage diet (62.3% forage), especially in sorting against long particles. The treatment diets used in the current study were approximately 80% forage, which may have contributed to the observed difference. Further, the higher forage ration used by DeVries et al. (2007) contained almost 5% more long particles on a DM-basis compared with the LND diet used in the current study. This fact, coupled with the higher moisture content of the higher forage diet (41.4% DM) compared with the lower forage diet (47.6% DM; DeVries et al., 2007), may have resulted in a ration that was more difficult to sort (Leonardi et al., 2005).

Further, even the change from the lactating diet to the higher forage diets in the present study resulted in a greater extent of sorting for the short particles in both treatments and a greater extent of sorting for the fine particles for LND cows. In this case, the results indicate that the higher forage content of the LND diet facilitated sorting of the smaller, nutrient dense particles, or that cows were motivated to sort more to compensate for the lower nutrient density of the diet. Greter and DeVries (2010) made associations between sorting and feeding rate in lactating dairy cows. In that study, a greater extent of sorting against long particles was associated with a slower feeding rate. It is possible in the present study that the increased sorting activity of LND cows could have contributed to the observed lower feeding rate if more time was being invested into manipulating the diet. Regardless of the reason, the sorting behavior observed in this study indicates that, on average, cows on both treatments were consuming a diet of higher nutrient density than intended, which could potentially put cows at greater risk of health disorders, like ketosis, after calving (Mann et al., 2015). Even without considering the effects of feed sorting on nutrient intake, both treatment diets were of relatively high nutrient densities for far off dry diets (Dann et al., 2006). Consequently, cows gained both BW and BCS over the course of the 42-d study period, where ideally BCS would be maintained. At the observed rate of gain in BCS, cows would be at risk of exceeding the upper limit of the commonly recommended BCS range for calving (3.25-3.75).

Despite the preferential sorting for short and fine particles in the current study, reticulorumen pH was still increased compared with baseline, and was consistently higher in LND cows than HND cows, even though LND cows sorted for these particles to a greater extent. Sorting for the smaller components of the diet has been associated with increased production of VFA and decreased pH (Cook et al., 2004; DeVries et al., 2008). However, this effect may have been counteracted by the LND diet having greater NDF content in each of the particle size fractions compared with the HND diet, including the small and fine fractions, thereby stimulating more chewing activity and buffering of the rumen (Jiang et al., 2017).

Rumination time increased in both treatments following the dietary transition, which was to be expected due to the higher forage content of the treatment diets relative to the lactating diet. Increased forage inclusion increases physically effective fiber (peNDF), which works to stimulate rumination (Yang and Beauchemin, 2009). That said, rumination time was similar between treatments, except for select days where HND cows ruminated more, despite the LND diet having a higher fiber content. This was likely because the LND diet also limited voluntary DMI, and thus consumption of peNDF. When rumination was expressed as rum/DMI, however, the LND diet stimulated more rumination activity on the majority of days. Similarly, Jiang et al. (2017) also reported that increasing dietary roughage did not affect overall rumination time, but did increase rum/DMI.

Lying behavior has often been used as an indicator of cow comfort, whether that be for comparing different housing systems (Fregonesi and Leaver, 2001), for assessing the impact of illness (Medrano-Galarza et al., 2012), detecting illness (King et al., 2017), or most relevantly assessing the impact of different dry off strategies (e.g. Leitner et al., 2007; Tucker et al., 2009; Zobel et al., 2013). In relation to skipped milkings or dry off, decreased lying behavior is often associated with the discomfort of elevated udder pressure from milk stasis (O'Driscoll et al., 2011; Österman and Redbo, 2001). In the current study, however, daily lying time was similar to baseline for LND cows and was higher for HND cows relative to baseline. Because cows no longer needed to stand for milking 2x/d following dry off, it is expected that cows in both treatment groups would have more time to allocate to lying and feeding behavior. Cows in the HND group also spent less time feeding per day than LND cows, which could have allowed them to allocate more time to lying down compared with LND cows. Cows fed the HND diet had more lying bouts/d than cows fed the LND diet, and this may again be related to feeding behavior in that HND cows also had more meals/d on certain days, potentially interrupting lying bouts. In contrast, Zobel et al. (2013) observed reduced lying time and lying bouts following dry off, however, average lying time and the number of bouts of both abruptly and gradually dried off cows were of similar values to those observed after dry off in the current study. Those authors also suggested that the changes in lying behavior following dry off could at least partly be explained by increased feeding time. Thus, in the current study, the observed differences in lying behavior appear to be the result of management changes, rather than discomfort due to dry off and cessation of milking.

Following the final milking during the gradual dry off, serum glucose concentration was elevated from baseline in both treatment groups. These results are consistent with Odensten et al. (2005), wherein increased glucose concentration was observed during a 5-d dry off in cows fed *ad libitum* straw and 4 kg/d of DM silage. Cows fed only dry hay in the 5 d before dry off also demonstrated increased glucose following cessation of milking (Ollier et al., 2014). The detected increases in glucose may be the result of decreased mammary uptake of glucose for lactose synthesis (Shennan and Peaker, 2000). Although not measured in the present study, Odensten et al. (2005) observed decreased lactose concentration in milk during dry off. Those researchers saw glucose return to pre-dry off levels by d 4 of the dry off period, however, in the current study, glucose did not return to baseline levels until more than 19 d after the start of dry off. This difference may be associated with the relatively high plane of nutrition in the current study compared with that of Odensten et al. (2005). In the present study, NEFA increased briefly at the start of dry off on d 1. The reason for this increase was likely the magnitude change in ration nutrient density, wherein cows were at first reluctant to consume their respective treatment diets due to its lower palatability compared with the lactating diet. Since cows were still producing 26 kg/d, on average, at the time dry off began, the nutrient demands for milk and a reduction in DMI likely resulted in mobilization of body fat. That said, NEFA returned to levels similar or lower than baseline by the day of dry off. In comparison, Odensten et al. (2005) observed a marked increase in NEFA in cows fed straw and silage at dry off and especially in cows fed only straw, indicating a reduction in energy balance. From the start of dry off, BHB in the present study gradually decreased into the dry period. Odensten et al. (2005) also observed decreased BHB following the start of dry off, despite NEFA increasing, and other researchers have indicated that BHB may not be appropriate as an indicator of energy balance except during early lactation (Chilliard et al., 1995; Drackley et al., 1999).

Finally, haptoglobin is an acute phase protein (**APP**) that may be used to indicate disease in cattle (Alsemgeest et al., 1994) and is produced in response to acute stressors such as inflammation, infection or trauma. Following dry off on d 5, HND cows immediately exhibited increased haptoglobin until at least d 19, while LND cows only exhibited increased haptoglobin from d 13 to 19. In a study wherein haptoglobin was measured at dry off, close up, and following calving, Abuelo et al. (2016) reported haptoglobin concentrations at dry off similar to those observed in the current study during the baseline period and until d 5 of dry off. Unfortunately, the dry off protocol was not included in that study, as its objective was directed towards the development of claw lesions during lactation rather than investigating dry off practices. To our knowledge, the study of Odensten et al. (2007) is the only other than the present study to measure APP at multiple time points surrounding dry off, however, almost all haptoglobin concentrations in that study were below the range of detection. Those researchers did, however, test for the APP serum amyloid A (**SAA**) as well, and observed increased SAA during dry off in cows fed straw, and straw and silage. The serum haptoglobin concentrations observed in the current study, as well as the SAA concentrations observed by Odensten et al. (2007), may be comparable to those in cows with mild or moderate mastitis, respectively (Eckersall et al., 2001), and were likely elevated as a reaction to involution of the udder eliciting an inflammatory response.

CONCLUSIONS

In the current study, dietary transition at dry off resulted in both behavioral and physiological changes, especially when the magnitude change in dietary nutrient density was greater. In this study, cows fed the LND diet had lower DMI, spent more time feeding and fed at a slower rate than cows fed the HND diet. Feeding strategy also affected meal patterning and sorting behavior, with cows fed the LND diet sorting for short and fine particles to a greater extent than cows fed the HND diet. Based on the behavioral and physiological changes observed, feeding diets of lower nutrient density before dry off and milking intermittently did not compromise cow welfare at the time of dry off and facilitated a reduction in milk production before cessation of milking. However, there is concern that the plane of nutrition used in the present study was potentially too high for the far off dry period, especially with the observed feed sorting behavior likely resulting in greater intake of nutrients than intended. Future work should compare diets of lower nutrient density than the current study and follow cows into early lactation to observe effects on milk production and health outcomes.

ACKNOWLEDGEMENTS

Thank you to the staff of the University of Guelph, Livestock Research and Innovation Centre-Dairy Facility (Elora, ON, Canada), and especially to Laura Wright for all her assistance facilitating this research project. Special thanks to Michelle Thompson, Kaitlin Sparkman, Isabela Salmazo, Sarah McPherson, and Felipe Pádua for their assistance with data collection. This project was financially supported by a Natural Sciences and Engineering Research Council of Canada (NSERC; Ottawa, ON, Canada) Discovery Grant. Further, equipment for this project was supported through contributions from the Canadian Foundation for Innovation (CFI; Ottawa, ON, Canada) and the Ontario Research Fund (Toronto, ON, Canada).

Table 2.1 Ingredient and chemical composition (mean ± SD) of the lactating cow and treatment total mixed rations

Composition	Lactating Diet	Higher Nutrient-Density Dry Diet	Lower Nutrient-Density Dry Diet
Ingredient, % DM			
Corn Silage[1]	31	44	39
Wheat Straw[2]	4	21	29
Alfalfa Haylage[3]	32	14	12
High Moisture Corn[4]	19	-	-
Lactating Cow Supplement[5]	14	-	-
Dry Cow Supplement[6]	-	21	19
Chemical Composition[7]			
DM, %	48.2 ± 3.0	50.9 ± 2.8	53.1± 3.1
CP, % of DM	14.7 ± 0.8	12.9 ± 0.6	12.2 ± 0.8
ADF, % of DM	20.2 ± 0.5	28.0 ± 1.2	30.2 ± 1.1
NDF, % of DM	29.7 ± 1.3	41.9 ± 0.7	46.2 ± 0.8
TDN, % of DM	72.3 ± 1.0	67.5 ± 0.8	64.9 ± 0.6
NFC, % of DM	48.5 ± 1.8	38.7 ± 1.3	34.7 ± 0.8
Ca, % of DM	1.0 ± 0.1	0.8 ± 0.0	0.8 ± 0.0
P, % of DM	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
NE_L, Mcal/kg of DM	1.66 ± 0.02	1.55 ± 0.02	1.48 ± 0.02

[1]Corn silage had a DM of 39.5 ± 2.0% and chemical composition (DM basis) 7.0 ± 0.7% CP, 19.4 ± 0.5% ADF, 32.6 ± 0.5% NDF, and 54.0 ± 1.1% NFC.

[2]Straw had a DM of 90.8 ± 2.8% and chemical composition (DM basis) 4.4 ± 2.2% CP, 52.3 ± 0.5% ADF, and 76.3 ± 3.5% NDF.

[3]Alfalfa haylage had a DM of 38.0 ± 6.2% and chemical composition (DM basis) 19.2 ± 3.3% CP, 32.6 ± 1.1% ADF, and 39.0 ± 0.6% NDF.

[4]High moisture corn had a DM of 74.0 ± 2.6% and chemical composition (DM basis) 8.0 ± 1.3% CP, 2.8 ± 0.4% ADF, and 8.5 ± 0.8% NDF.

[5]Supplied by Floradale Feed Mill Ltd (Floradale, Ontario, Canada) including ingredients (as fed); 38.2% soybean meal, 24.1% Soy Plus, 12.3% canola, 4.1% fish meal (Herring), 3.9% limestone calcium carbonate, 3.5% sodium sesquicarbonate, 3.2% fine salt, 2.4% monocalcium phosphate, 1.7% magnesium oxide, 1.6% Diamond V Yeast XP, 1.4% biuret, 0.8% DCAD+ (potassium carbonate), 0.8% urea, 0.8% FFM Org Ruminant Micro P, 0.6% tallow, 0.4% sulfur 99.5%, 0.1% Metasmart, 0.04% Rumensin, and 0.02% Selplex 2000. Lactating cow supplement had a DM of 92.0 ± 0.9% and chemical composition (DM basis) 43.7 ± 1.3% CP, 9.0 ± 0.2% ADF, and 14.4 ± 0.5% NDF.

[6]Supplied by Floradale Feed Mill Ltd (Floradale, Ontario, Canada) including ingredients (as fed); 24.1% Soy Plus, 18.0% soy hulls (ground), 17% canola, 13.6% wheat shorts, 8.5% soybean meal, 2% Diamond V Yeast XP, 1.9% limestone calcium carbonate, 1.3% magnesium oxide, 1.0% vitamin E, 1.0% fine salt, 1.0% tallow, 0.5% FFM Org Ruminant Micro PRX, 0.07% Selplex 2000, 0.05% Rumensin, and 0.01% Rovimix Biotin 20,000. Dry cow supplement had a DM of 91.7 ± 1.4% and chemical composition (DM basis) 31.1 ± 0.1% CP, 18.4 ± 1.1% ADF, and 31.2 ± 1.1% NDF.

[7]Values were obtained from chemical analysis of TMR samples. NE_L was calculated based on NRC (2001) equations.

Table 2.2 Particle size distribution[1] (mean ± SD), and nutrient content (mean ± SD) by particle size of the fresh experimental diets

Item	Lactating Diet	Higher Nutrient-Density Dry Diet	Lower Nutrient-Density Dry Diet
% DM retained on screen			
Long	6.1 ± 2.4	11.1 ± 3.9	14.7 ± 5.5
Medium	44.0 ± 4.2	41.0 ± 4.4	38.1 ± 3.7
Short	16.3 ± 1.6	14.9 ± 1.5	15.3 ± 1.5
Fine	33.6 ± 4.1	33.0 ± 5.0	31.9 ± 4.7
ADF, % of screen DM[2]			
Long	-	35.8 ± 0.9	38.1 ± 0.9
Medium	-	29.9 ± 0.2	31.8 ± 0.2
Short	-	23.6 ± 1.6	26.3 ± 0.1
Fine	-	22.0 ± 0.7	24.6 ± 0.5
NDF, % of screen DM[2]			
Long	-	53.4 ± 1.5	56.6 ± 0.6
Medium	-	45.2 ± 0.6	49.2 ± 0.9
Short	-	36.5 ± 0.1	40.4 ± 1.6
Fine	-	33.5 ± 0.9	36.3 ± 0.6

[1]Particle size determined by processing feed samples with the Penn State Particle Separator, which has a 19 mm screen (long), 8 mm screen (medium), 4 mm screen (short) and a pan (fine).
[2]Values were obtained from chemical analysis of TMR samples separated by particle size with the Penn State Particle Separator.

Table 2.3 Effect of dietary treatments[1] and dry off[2] on the milk production and milk composition of Holstein dairy cows

	Baseline[3]		Treatment			*P*-Value				
	Mean	SE	HND	LND	SE	BvsH[4]	BvsL[5]	T[6]	Day	T × Day
Milk yield, kg/d	26.0	0.21	16.6	15.7	0.40	<0.001	<0.001	<0.05	<0.001	0.98
4% FCM, kg/d	27.3	0.43	21.1	20.2	0.52	<0.001	<0.001	0.21	<0.001	0.64
Fat, %	4.39	0.053	6.08	6.21	0.213	<0.001	<0.001	0.58	<0.001	0.79
Fat yield, kg/d	1.13	0.020	0.97	0.94	0.029	<0.001	<0.001	0.38	<0.001	0.60
Protein, %	3.60	0.022	4.09	4.21	0.065	<0.001	<0.001	0.11	<0.001	0.72
Protein yield, kg/d	0.92	0.013	0.65	0.63	0.018	<0.001	<0.001	0.47	<0.001	0.85
Natural log-transformed SCC[7]	4.72	0.076	5.67	5.85	0.092	<0.001	<0.001	0.17	<0.001	0.81
SCC[8], × 1,000 cells/mL	112	-	291	346	-	-	-	-	-	-
MUN, mg/dL	10.3	0.20	10.5	11.1	0.69	0.84	0.24	0.20	0.81	0.24

[1]HND = higher nutrient density diet (1.55 Mcal/kg of DM; n = 24); LND = lower nutrient density diet (1.48 Mcal/kg of DM; n = 23). Treatments diets were offered daily beginning at the start of dry off.
[2]Dry off occurred over a 5-d period, wherein cows were milked 1x/d on d 1, 2, 3 and 5.
[3]Baseline = 16-d period in late lactation immediately prior to the start of dry-off.
[4]BvsH = within-cow difference between baseline and HND treatment.
[5]BvsL = within-cow difference between baseline and LND treatment.
[6]T = treatment effect.
[7]Somatic cell counts (cells/mL) were log-transformed, given that they did not meet the assumption of normality.
[8]Back-tranformed SCC data.

Table 2.4 Effect of dietary treatments on the feeding behavior, rumination, and lying behavior of Holstein dairy cows

	Baseline[1]		Treatment[2]			*P*-Value				
	Mean	SE	HND	LND	SE	BvsH[3]	BvsL[4]	T[5]	Day	T × Day
DMI, kg/d	23.1	0.12	19.4	17.2	0.41	<0.001	<0.001	<0.001	<0.001	0.02
Feeding time, min/d	211.1	1.53	232.0	247.7	4.86	0.007	<0.001	0.004	<0.001	0.46
Feeding rate, kg DM/min	0.12	0.001	0.09	0.07	0.003	<0.001	<0.001	<0.001	<0.001	0.58
Meal criterion, min	22.6	0.94	32.0	29.2	2.26	<0.001	0.01	0.36	-	-
Meal frequency, no./d	7.7	0.06	6.7	6.4	0.18	<0.001	<0.001	0.13	<0.001	0.03
Interval between meals, min	155.6	1.36	180.2	187.3	5.94	<0.001	<0.001	0.20	<0.001	0.13
Within meal interval, min	49.7	1.07	57.3	53.5	3.92	<0.05	0.43	0.49	<0.001	0.76
Non-feeding time within meals, min/meal	6.7	0.15	9.1	9.0	0.75	0.003	0.005	0.91	0.12	0.42
Total meal time, min/d	260.8	1.93	289.4	302.1	6.09	<0.001	<0.001	0.14	<0.001	0.42
Meal length, min/meal	35.5	0.39	45.5	50.1	1.73	<0.001	<0.001	0.02	<0.001	<0.001
Meal size, kg DM/meal	3.2	0.03	3.1	2.9	0.10	0.43	0.004	0.02	<0.001	0.002
Rumination time, min/d	462.8	3.75	496.9	491.9	8.52	<0.001	<0.001	0.60	<0.001	0.009
Rumination/DMI, min/kg	20.6	0.20	26.0	29.3	0.58	<0.001	<0.001	<0.001	<0.001	0.03
Lying time, min/d	774.4	4.70	827.0	798.6	15.01	<0.001	0.20	0.18	<0.001	0.35
Lying bout frequency, no./d	8.9	8.7	9.1	8.7	0.22	0.30	0.18	0.01	0.013	0.82
Lying bout length, min/bout	91.9	98.4	97.5	98.4	3.46	0.09	0.04	0.85	<0.001	0.61

[1]Baseline = 16-d period in late lactation immediately prior to the start of dry-off.
[2]HND = higher nutrient density diet (1.55 Mcal/kg of DM); LND = lower nutrient density diet (1.48 Mcal/kg of DM). Treatment diets were fed over a 26-d period, beginning at the start of a 5-d dry-off and 21 d into the dry period.
[3]BvsH = within-cow difference between baseline and HND treatment.
[4]BvsL = within-cow difference between baseline and LND treatment.
[5]T = treatment effect.

Table 2.5 Effect of dietary treatments on the sorting (%)[1] behavior of groups of Holstein dairy cows

	Baseline[3]		Treatment[4]			*P*-Value				
Particle Size[2]	Mean	SE	HND	LND	SE	BvsH[5]	BvsL[6]	T[7]	Day	T × Day
Long	85.8*	1.30	85.6*	82.5*	1.78	0.74	0.46	0.11	<0.001	0.18
Medium	100.3	0.26	100.6*	100.5*	0.17	0.73	0.88	0.68	0.18	0.79
Short	100.7†	0.45	102.5*	103.1*	0.31	0.04	0.03	0.03	0.005	0.04
Fine	101.5*	0.47	102.9*	105.4*	0.54	0.47	0.03	<0.001	<0.001	0.04

[1]Sorting % = (actual DMI of a particle size/predicted DMI of a particle size) × 100. Sorting values of 100% indicate no sorting, <100% indicate sorting against the particle size, and >100% indicate sorting in favour of the particle size.
[2]Particle size determined by a Penn State Particle Separator, which has a 19 mm screen (long), 8 mm screen (medium), 4 mm screen (short) and a pan (fine).
[3]Baseline = 16-d period prior to the start of dry-off.
[4]HND = higher nutrient density diet (1.55 Mcal/kg of DM; n = 24); LND = lower nutrient density diet (1.48 Mcal/kg of DM; n = 23). Treatment diets were fed over a 26-d period, beginning at the start of a 5-d dry-off and 21 d into the dry period.
[5]BvsH = within-cow difference between baseline and HND treatment.
[6]BvsL = within-cow difference between baseline and LND treatment.
[7]T = treatment effect.
*†Difference in sorting values from 100% expressed as †$P < 0.10$, *$P < 0.05$.

Table 2.6 Effect of dietary treatments on the reticulorumen pH of Holstein dairy cows

	Baseline[1]		Treatment[2]			*P*-Value				
Reticulorumen pH	Mean	SE	HND	LND	SE	BvsH[3]	BvsL[4]	T[5]	Day	T × Day
Mean	6.38	0.009	6.55	6.62	0.017	<0.001	<0.001	0.001	<0.001	0.16
Maximum	6.80	0.009	6.84	6.90	0.025	0.12	<0.001	0.01	<0.001	0.69
Minimum	5.94	0.011	6.25	6.33	0.019	<0.001	<0.001	<0.001	<0.001	0.13
Range[6]	0.86	0.009	0.59	0.57	0.013	<0.001	<0.001	0.04	<0.001	0.31

[1]Baseline = 16-d period in late lactation immediately prior to the start of dry-off.
[2]HND = higher nutrient density diet (1.55 Mcal/kg of DM; n = 24); LND = lower nutrient density diet (1.48 Mcal/kg of DM; n = 24). Treatment diets were fed over a 26-d period, beginning at the start of a 5-d dry-off and 21 d into the dry period.
[3]BvsH = within-cow difference between baseline and HND treatment.
[4]BvsL = within-cow difference between baseline and LND treatment.
[5]T = treatment effect.
[6]Range = maximum – minimum.

Table 2.7 Effect of dietary treatments on blood parameters of Holstein dairy cows

	Baseline[1]		Treatment[2]			*P*-Value				
Serum concentration	Mean	SE	HND	LND	SE	BvsH[3]	BvsL[4]	T[5]	Day	T × Day
Glucose, mmol/L	3.5	0.04	3.7	3.7	0.07	0.02	0.08	0.55	<0.001	0.89
Natural log-transformed non-esterified fatty acid[6]	-2.26	0.060	-2.34	-2.38	0.109	0.16	0.19	0.46	<0.001	0.77
Non-esterified fatty acid, mmol/L[7]	0.10	-	0.10	0.10	-	-	-	-	-	-
BHB, mmol/L	0.5	0.02	0.42	0.39	0.026	0.01	0.003	0.06	<0.001	0.24
Natural log-transformed haptoglobin[6]	-1.73	0.043	-1.36	-1.40	0.067	<0.001	0.008	0.66	<0.001	0.13
Haptoglobin, g/L[7]	0.18	-	0.24	0.22	-	-	-	-	-	-

[1]Baseline = 16-d period in late lactation immediately prior to the start of dry-off.
[2]HND = higher nutrient density diet (1.55 Mcal/kg of DM; n = 24); LND = lower nutrient density diet (1.48 Mcal/kg of DM; n = 23). Treatment diets were fed over a 26-d period, beginning at the start of a 5-d dry-off and 21 d into the dry period.
[3]BvsH = within-cow difference between baseline and HND treatment.
[4]BvsL = within-cow difference between baseline and LND treatment.
[5]T = treatment effect.
[6]Non-esterified fatty acid and haptoglobin were log-transformed, given that they did not meet the assumption of normality.
[7]Back-transformed non-esterified fatty acid and haptoglobin data.

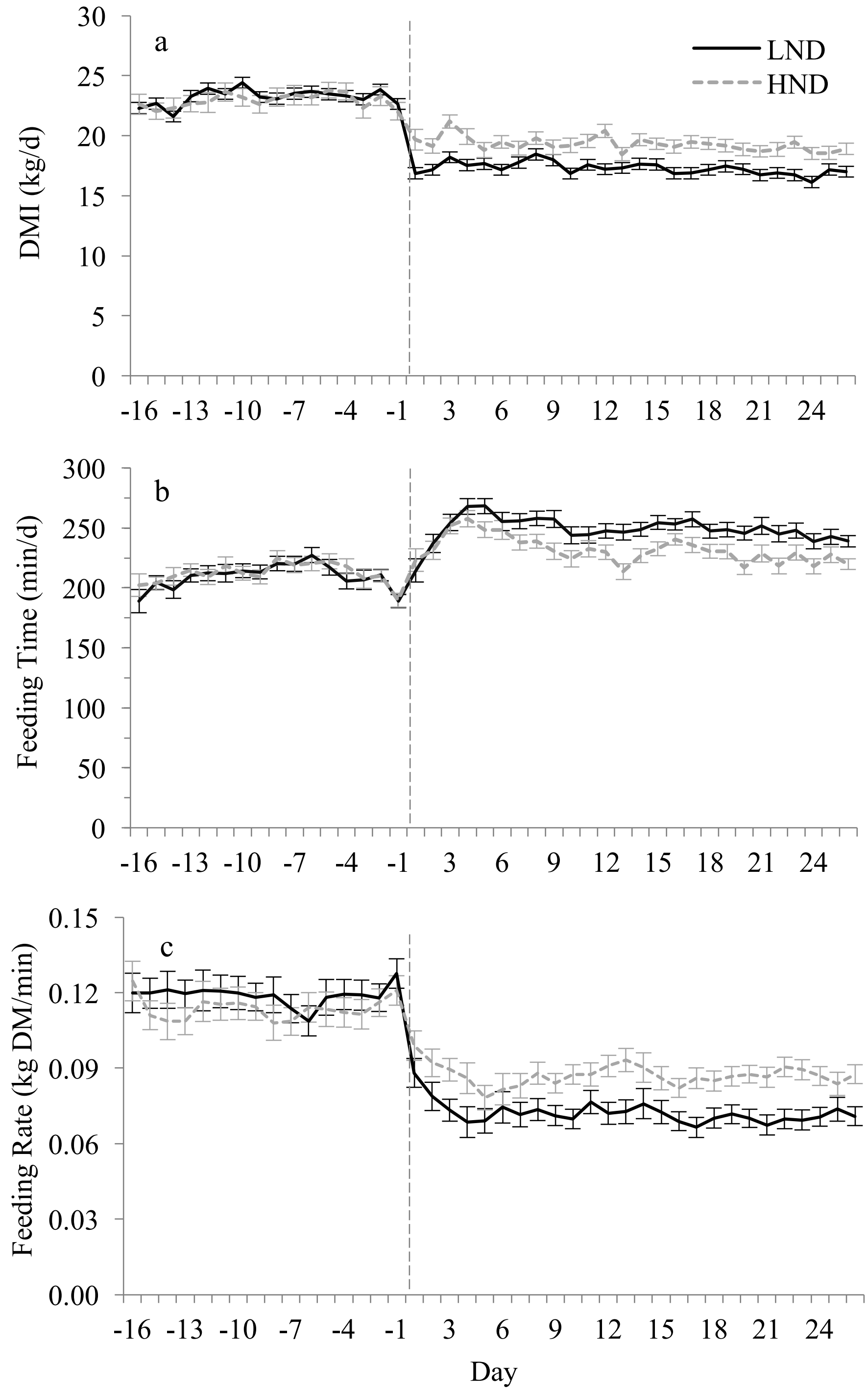

Figure 2.1 Daily (± SE): a) DMI (kg/d), b) feeding time (min/d), and c) feeding rate (kg DM/min) for cows fed 1 of 2 dietary treatments differing in ration nutrient-density (LND: lower nutrient-density; HND: higher nutrient-density) undergoing a 5-d dry off beginning at d 1 (dotted vertical line).

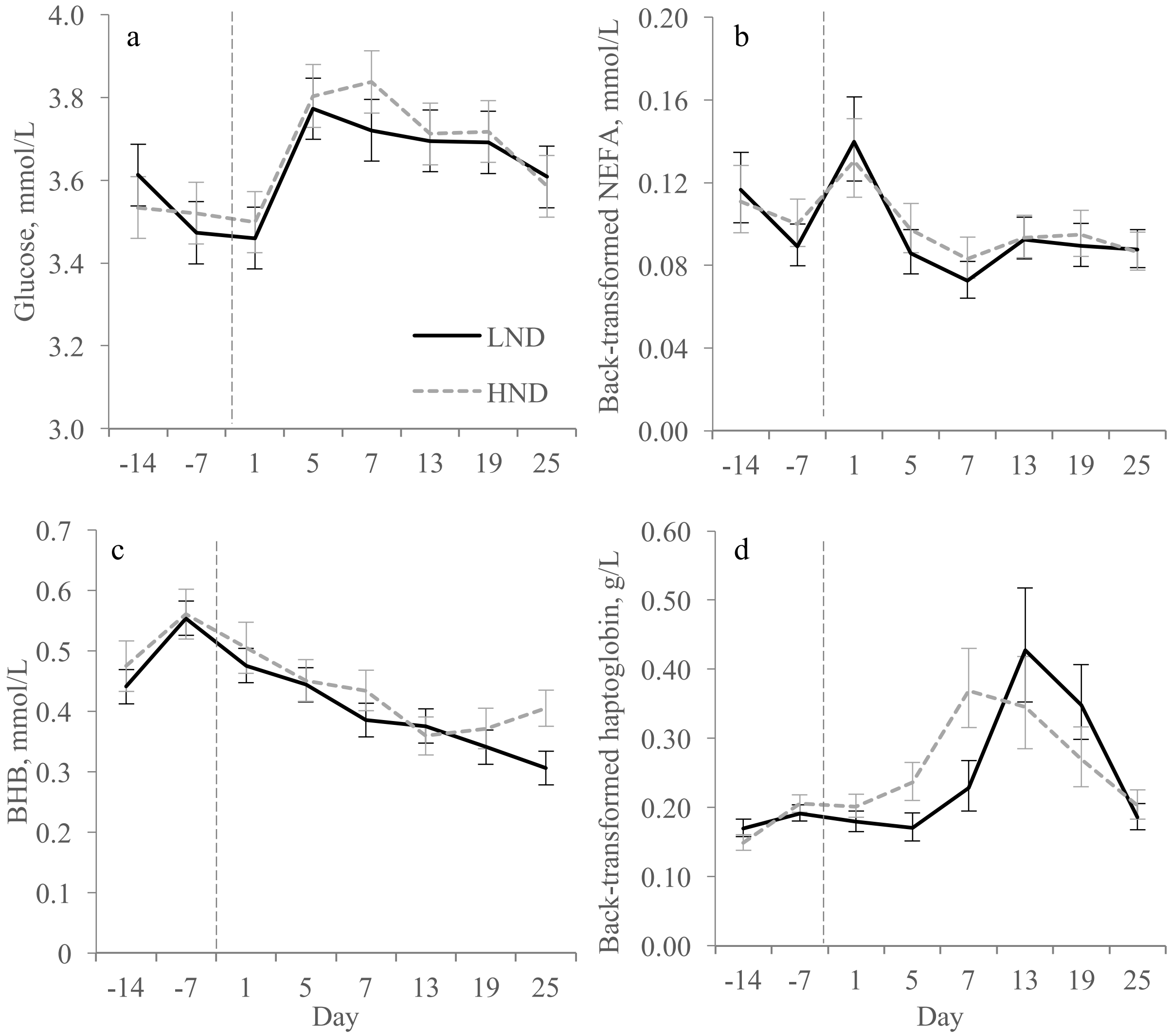

Figure 2.2 Average (± SE) for: a) serum glucose (mmol/L), b) serum non-esterified fatty acid (NEFA; mmol/L), c) whole-blood BHB (mmol/L), and d) serum haptoglobin (g/L) concentrations for cows fed 1 of 2 dietary treatments differing in ration nutrient-density (LND: lower nutrient-density; HND: higher nutrient-density) undergoing a 5-d dry off beginning at d 1 (dotted vertical line).

CHAPTER 3. GENERAL DISCUSSION

3.1 Important Findings

The use of abrupt cessation of milking to initiate dry off may have negative consequences for the welfare of dairy cows, especially those with greater milk production at the end of lactation. To reduce milk production and mediate cessation of milking, producers may opt to milk cows intermittently and/or provide cows with a lesser quality or quantity of feed before dry off (Bushe and Oliver; 1987; Skidmore et al., 1997). Although lower milk production at dry off is favorable from an udder health (Rajala-Schultz et al., 2005) and comfort (Bertulat et al., 2013) perspective, dry off strategies that excessively restrict feed quantity or quality may also negatively affect cow welfare, at least in the short term (Odensten et al., 2005; Valizaheh et al., 2008; Tucker et al., 2009). Furthermore, dietary transition itself may be stressful, thus limiting the number of dietary changes may be beneficial to cow welfare. With the extent that milk production has increased in recent decades, the practice of abrupt dry off, especially of high-producing cows, may not be appropriate. Thus, there is a need for specific recommendations for reducing milk production before dry off that promote cow health and welfare.

The objectives of this thesis were to evaluate the efficacy of using reduced dietary nutrient density and milking frequency as a strategy to decrease milk production before dry off and to simultaneously observe behavioral and physiological changes resulting from the dietary transition. Firstly, it was hypothesized that a greater magnitude change in dietary nutrient density would result in a greater reduction in milk production over a 5-d period of intermittent milking. Paired with a short period of intermittent milking, cows on both dietary treatments exhibited reduced daily milk production from the start until the end of the dry off period. Although it is likely reduced milking frequency was largely responsible for the observed reduction in milk yield, dietary nutrient density appears to have contributed to the change to some degree, as cows fed the LND diet produced on average 0.9 kg/d less milk during the dry off period compared with cows fed the HND diet. Although lying time was similar to baseline for LND cows, they had fewer, but longer, lying bouts per day, suggesting they did not experience much discomfort at dry off and differences between treatments in lying behavior were likely the result of management changes (i.e. dietary change and and lack of milking events) rather than differences in comfort level.

The difference in milk production between treatments was likely a reflection of the difference in DMI, wherein LND cows had consistently lower DMI than HND cows after the dietary change at the start of dry off. The LND diet was of higher NDF content than the HND diet, and would have promoted rumen fill and limited DMI to a greater extent (Mertens, 1997; Allen, 1996). The greater inclusion of roughage in the LND diet also resulted in increased feeding time and a slower feeding rate compared with the HND diet, as predicted. Meal-patterning results were also consistent with these findings, with LND cows having meals that were longer, but smaller, than those consumed by HND cows. Overall, both magnitude changes in dietary nutrient density resulted in differences in feeding behavior from baseline, with very few exceptions, and observed differences were consistent with my hypotheses.

Contrary to predicted, sorting against the long particles for both treatments did not differ significantly from baseline, nor did it differ between treatments. However, cows fed the LND diet did numerically sort against the long particles to a greater extent than HND cows. Had the difference in treatment diet formulation been greater, it is possible that a statistical difference in sorting of the long particles may have been detected. After dietary transition, the extent that cows sorted in favor of the short particles increased for both treatments compared with during the baseline period. Overall, cows fed the LND diet seemed to sort for the short particles more than cows fed the HND diet. Cows fed the LND diet also sorted in favor of the fine particles to a greater extent compared with baseline and with cows fed the HND diet. By sorting against the long particles and in favor of the short and fine particles, cows on both treatments were likely consuming a diet of higher nutrient density than intended. Given that both treatment diets were of relatively high nutrient density for far off dry diets (Dann et al., 2006), cows in this study may have been at greater risk of health disorders postpartum (Mann et al., 2015). Although cows on both treatments gained BW and BCS, cows fed the LND diet tended to gain less BW than cows fed the HND diet. Despite sorting activity, cows fed the LND diet spent similar time ruminating, with the exception of a few days, spent more time rumination per kg of DMI, on approximately three-quarters of days, and had higher mean reticulorumen pH than cows fed the HND diet.

Based on measured serum glucose and NEFA concentrations, neither treatment diet caused cows to experience NEB during the dry off period. Following the last milking at dry off, both LND and HND cows exhibited increased serum glucose concentration, likely due to decreased mammary uptake of glucose (Shennan and Peaker, 2000). Cows fed the LND diet tended to have increased NEFA immediately following the dietary change, however, this increase was transient and cows had similar or lower NEFA to baseline at each following measurement. The observed increase was likely because the LND diet was of lower palatability and limited DMI. Serum haptoglobin levels were elevated following dry off, indicating an inflammatory reaction in response to increased intramammary pressure with cessation of milking. This reaction differed slightly between treatments, with HND cows having elevated haptoglobin earlier than LND cows, although both HND and LND cows had haptoglobin concentrations similar to baseline levels by 2 wk into the dry period.

Although feeding a diet of lower nutrient density encouraged a greater degree of sorting activity, the results of this study indicate that this feeding strategy was more effective in reducing milk production during a gradual dry off, did not cause a substantial reduction in energy balance at dry off, and limited weight gain.

3.2 Future Research

It is well-established that nutritional management during the dry period has implications for DMI, health, and milk production in the subsequent lactation. Overfeeding during the far off dry period has been shown to negatively affect DMI postpartum, at least in the first 10 DIM (Dann et al., 2006) and feeding at a higher plane of nutrition during the dry period may put cows at risk of metabolic health disorders post-partum, especially ketosis (Mann et al., 2015). It is, therefore, recommended that future research investigating transitioning cows to the dry cow diet during a gradual dry off follow cows postpartum to observe effects on health, DMI and milk production in the next lactation. If future studies were to investigate the impact of dry off strategies on postpartum DMI, health and milk production, a larger sample size should be used, as that used in the present study would not have had sufficient power to detect statistical differences. Those studies should account for potential culls or deaths that may occur as a result of postpartum disease. Furthermore, the results of this study indicated that cows were likely overfeeding relative to requirements, and should future studies further explore manipulating diet nutrient density to mediate dry off, diets of lower nutrient density than in the present study should be tested.

In the present study, individual, automated feed bins were used to measure feeding behavior at the cow-level. Although useful for characterizing feeding behavior, restricting cows from accessing the feed of other cows does not account for the effects of competition that may occur on a commercial farm at the feed bunk. In settings where competitive pressure is high, subordinate cows may avoid visiting the feed bunk at peak feeding times, and may, therefore, consume a diet that has already been sorted and left nutritionally different than intended (Leonardi and Amentano, 2003; DeVries et al., 2004). Due to the degree of sorting activity observed in this study, especially by cows fed the LND diet, future work should evaluate if competition compromises the efficacy of similar feeding strategies at dry off and in the dry period.

Finally, within the current study, dietary transition was coupled with an intermittent milking schedule (i.e. gradual dry off). Researchers who have previously implemented different feeding strategies to reduce milk production prior to dry off have also used similar intermittent milking schedules preceding dry off (e.g. Odensten et al., 2005; 2007; Valizaheh et al., 2008). However, as abrupt dry off continues to be widely practiced, future research could also be focused on separating the effects of dietary transition and gradual dry off, by investigating the impact of magnitude of dietary transition following an abrupt dry off in addition to gradual dry off.

3.3. Implications

Despite growing concerns for the welfare of dairy animals (von Keyserlingk et al., 2009), there is a surprising lack of specific recommendations for dry off. In the Canadian Code of Practice for Dairy Cattle, the sole recommendations for dry off are that cows have a BCS of 3.25-3.75 and that cows be treated "with an approved intramammary dry cow preparation, as recommended by [the] herd veterinarian" (DFC-NFACC, 2009). Due to the aforementioned welfare concerns with abrupt dry off of high-producing cows and restrictive feeding to reduce milk production, research is necessitated to inform recommendations that promote cow welfare around the time of dry off. The results of this study contribute to our knowledge of how dairy cows are affected by dry off, and may be used to inform such recommendations. To date, this is the only study, of which the author is aware, to compare total mixed rations of differing nutrient densities as a feeding strategy for dry off, rather than low quality forages with or without supplementation. The results of this thesis indicate that feeding a lower nutrient density diet ad libitum at dry off did not substantially alter energy balance, and facilitated a reduction in milk production when paired with a gradual cessation of milking. Additionally, this thesis builds on our understanding of how feeding behavior, rumination behavior, and rumen health are impacted by the transition from a lactating diet to a lower nutrient density dry diet.

CHAPTER 4. REFERENCES

Abuelo, A., J. C. Gandy, L. Neuder, J. Brester, and L. M. Sordillo. 2016. Markers of oxidant status and inflammation relative to the development of claw lesions associated with lameness in early lactation dairy cows. J. Dairy Sci. 99:1-9. doi:10.3168/jds.2015-10707.

Agenäs, S., K. Dahlborn, and K. Holtenius. 2003. Changes in metabolism and milk production during and after feed deprivation in primiparous cows selected for different milk fat content. Livest. Prod. Sci. 83:153-164. doi:10.1016/S0301-6226(03)00096-4.

Allen, M. S., and D. R. Mertens. 1988. Evaluating constraints of fiber digestion by rumen microbes. J. Nutr. 118:261–270.

Allen, M., and M. Oba. 1996. Fiber digestibility of forages. Pages 151–171 in Proc. 57th Minnesota Nutr. Conf. Protiva Tech. Symp. Ext. Special Programs, Bloomington, MN. Univ. Minnesota, St. Paul.

Allen, M. S. 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74:3063-3075.

Allen, M. S. 2014. Drives and limits to feed intake in ruminants. Anim. Prod. Sci. 54:1513-1524.

Alsemgeest, S. P., H. C. Kalsbeek, T. Wensing, J. P. Koeman, A. M. van Ederen, and E. Gruys. 1994. Concentrations of serum amyloid-A (SAA) and haptoglobin (HP) as parameters of inflammatory diseases in cattle. Vet. Q. 16:21–23. doi:10.1080/01652176.1994.9694410.

Bachman, K. C., and M. L. Schairer. 2003. Invited review: Bovine studies on optimal lengths of dry periods. J. Dairy Sci. 86:3027-3037. doi:10.3168/jds.S0022-0302(03)73902-2.

Beauchemin, K. A., L. Eriksen, P. Nørgaard, and L. M. Rode. 2008. Salivary secretion during meals in lactating dairy cattle. J. Dairy Sci. 91:2077-2081. doi:10.3168/jds.2007-0726.

Beever, D. E. 2006. The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. Anim. Reprod. Sci. 96:212-226. doi:10.1016/j.anireprosci.2006.08.002.

Bernier-Dodier, P., C. L. Girard, B. G. Talbot, and P. Lacasse. 2011. Effect of dry period management on mammary gland function and its endocrine regulation in dairy cows. J. Dairy Sci. 94:4922-4936. doi:10.3168/jds.2010-4116.

Bertulat, S., C. Fischer-Tenhagen, V. Suthar, E. Möstl, N. Isaka, and W. Heuwieser. 2013. Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yields. J. Dairy Sci. 96:3773–3787. doi:10.3168/jds.2012-6425.

Blowey, R., and P. Edmondson. 2010. Mastic control in dairy herds, 2nd ed. CAB International, Oxfordshire, UK.

Bos, M. E. H., F. J. Taverne, I. M. van Geijlswijk, J. W. Mouton, D. J. Mevius, and D. J. J. Heederik. 2013. Consumption of antimicrobials in pigs, veal calves, and broilers in the Netherlands: Quantitative results of nationwide collection of data in 2011. PLoS ONE 8:e77525. doi:10.1371/journal.pone.0077525.

Bradley, A. J., and M. J. Green. 2004. The importance of the nonlactating period in the epidemiology of intramammary infection and strategies for prevention. Vet. Clin. Food Anim. 20:547-568. doi:10.1016/j.cvfa.2004.06.010.

Bushe, T. and S. P. Oliver. 1987. Natural protective factors in bovine mammary secretions following different methods of milk cessation. J. Dairy Sci. 70:696-704. doi:10.3168/jds.S0022-0302(87)80060-7.

Capuco, A. V., R. M. Akers, and J. J. Smith. 1997. Mammary growth in Holstein cows during the dry period: quantification of nucleic acids and histology. J. Dairy Sci. 80:477–487.

doi:10.3168/jds.S0022-0302(97)75960-5.

Chapinal, N., D.M. Veira, D.M. Weary, and M.A.G. von Keyserlingk. 2007. Technical note: Validation of a system for monitoring individual feeding and drinking behaviour and intake in group housed dairy cows. J. Dairy Sci. 90:5732–5736. doi:10.3168/jds.2007-0331.

Chapinal, N., G. Zobel, K. Painter, and K. E. Leslie. 2014. Changes in lying behavior after abrupt cessation of milking and regrouping at dry-off in freestall-housed cows: A case study. J. Vet. Behav. 9:364-369. doi:10.1016/j.jveb.2014.07.008.

Cole, J. B., D. J. Null, and A. De Vries. 2011. Short communication: Best prediction of 305-day lactation yields with regional and seasonal effects. J. Dairy Sci. 94:1601–1604. doi:10.3168/jds.2010-3865.

Cook, N. B., K. V. Nordlund, and G. R. Oetzel. 2004. Environmental influences on claw horn lesions associated with laminitis and subacute ruminal acidosis in dairy cows. J. Dairy Sci. 87:E36– E46. doi:10.3168/jds.S0022-0302(04)70059-4.

Coppock, C. E. 1977. Feeding methods and grouping systems. J. Dairy Sci. 60:1327-1336.

Crossley, R. E., A. Harlander-Matauschek, and T. J. DeVries. 2017. Variability in behavior and production among dairy cows fed under differing levels of competition. J. Dairy Sci. 100:3825-3838. doi:10.3168/jds.2016-12108.

Dann, H. M., N. B. Litherland, J. P. Underwood, M. Bionaz, A. D’Angelo, J. W. McFadden, and J. K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. J. Dairy Sci. 89:3563–3577. doi:10.3168/jds.S0022-0302(06)72396-7.

Davis, S. R., V. C. Farr, and K. Stelwagen. 1999. Regulation of yield loss and milk composition during once-daily milking: A review. Livest. Prod. Sci. 59:77-94. doi:10.1016/S0301-6226(98)00204-8.

DeVries, T. J., K. A. Beauchemin, and M. A. G. von Keyserlingk. 2007. Dietary forage concentration affects the feed sorting behavior of lactating dairy cows. J. Dairy Sci. 90:5572-5579. doi:10.3168/jds.2007-0370.

DeVries, T.J., Dohme, F., Beauchemin, K.A., 2008. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feed sorting. J. Dairy Sci. 91, 3958-3967. doi:10.3168/jds.2008-1347.

DeVries, T. J., M. A. G. von Keyserlingk, D. M. Weary, and K. A. Beauchemin 2003.Technical note: Validation of a system for monitoring feeding behavior of dairy cows. J. Dairy Sci. 86:3571–3574. doi:10.3168/jds.S0022-0302(03)73962-9.

DFC-NFACC (Dairy Farmers of Canada and the National Farm Animal Care Council). 2009. Code of Practices for the Care and Handling of Dairy Cattle. Dairy Farmers of Canada, Ottawa, Ontario, Canada.

Dingwell, R. T., D. F. Kelton, K. E. Leslie, and V. L. Edge. 2001. Deciding to dry-off: Does level of production matter? Pages 69–79 in Proc. Natl. Mastitis Counc. Ann. Mtg. National Mastitis Council, Madison, WI.

Dingwell, R. T., D. F. Kelton, and K. E. Leslie. 2003. Management of the dry cow in control of peripartum disease and mastitis. Vet. Clin. Food Anim. 19:235-265.

Dingwell, R. T., K. E. Leslie, Y. H. Schukken, J. M. Sargeant, L. L. Timms, T. F. Duffield, G. P. Keefe, D. F. Kelton, K. D. Lissemore, and J. Conklin. 2004. Association of cow and quarter-level factors at drying-off with new intramammary infections during the dry period. Prev. Vet. Med. 63:75–89. doi:10.1016/j.prevetmed.2004.01.012.

Eckersall, P. D., F. J. Young, C. McComb, C. J. Hogarth, S. Safi, A. Weber, T. Mcdonald, A. M. Nolan, and J. L. Fitzpatrick. 2001. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. Vet. Rec. 148:35-41.

Erb, H. N., R. D. Smith, R. B. Hillman, P. A. Powers, M. C. Smith, M. E. White, and E. G. Pearson. 1984. Rates of diagnosis of six diseases of Holstein cows during 15-day and 21-day intervals. Am. J. Vet. Res. 45:333–335.

Falk, M., A. Münger, and F. Dohme-Meier. 2016. Technical note: A comparison of reticular and ruminal pH monitored continuously with 2 measurement systems at different weeks of early lactation. J. Dairy Sci. 99:1951–1955. doi:10.3168/jds.2015-9725.

Fregonesi, J.A., and J. D. Leaver. 2001. Behaviour, performance and health indicators of welfare for dairy cows housed in strawyard or cubicle systems. Livest. Prod. Sci. 68:205–216. doi:10.1016/S0301-6226(00)00234-7.

Friggens, N. C., B. L. Nielsen, I. Kyriazakis, B. J. Tolkamp, and G. C. Emmans. 1998. Effects of feed composition and stage of lactation on the short-term feeding behavior of dairy cows. J. Dairy Sci. 81:3268–3277. doi:10.3168/jds.S0022-0302(98)75891-6.

Greter, A.M., T.J. DeVries, and M.A.G. von Keyserlingk. 2008. Nutrient intake and feeding behavior of growing dairy heifers: Effects of dietary dilution. J. Dairy Sci. 91:2786–2795. doi:10.3168/jds.2008-1052.

Greter, A. M., and T. J. DeVries. 2010. Effect of feeding amount on the feeding and sorting behaviour of lactating dairy cattle. Can. J. Anim. Sci. 91:47–54. doi:10.4141/CJAS10067.

Halasa, T., K. Huijps, O. Østerås, and H. Hogeveen. 2007. Economic effects of bovine mastitis and mastitis management: A review. Vet. Q. 29:18–31. doi:10.1080/01652176.2007.9695224.

Halasa, T., O. Østerås, H. Hogeveen, T. van Werven, and M. Nielen. 2009. Meta-analysis of dry cow management for dairy cattle. Part 1. Protection against new intramammary infections. J. Dairy Sci. 92:3134-3149. doi:10.3168/jds.2008-1740.

Huxley, J. N., M. J. Green, L. E. Green, and A. J. Bradley. 2002. Evaluation of the efficacy of an internal teat sealer during the dry period. J. Dairy Sci. 85:551-561.

Huzzey, J. M., M. A. G. von Keyserlingk, and D. M. Weary. 2005. Changes in feeding, drinking, and standing behavior of dairy cows during the transition period. J. Dairy Sci. 88:2454-2461. doi:10.3168/jds.S0022-0302(05)72923-4.

Jiang, F. G., X. Y. Lin, Z. G. Yan, Z. Y. Hu, G. M. Liu, Y. D. Sun, X. W. Liu, and Z. H. Wang. 2017. Effect of dietary roughage level on chewing activity, ruminal pH, and saliva secretion in lactating Holstein cows. J. Dairy Sci. 100:2660-2671. doi:10.3168/jds.2016-11559.

Kanz, P., M. Drillich, D. Klein-Jöbstl, B. Mair, S. Borchardt, L. Meyer, I. Schwendenwein, and M. Iwersen. 2015. Suitability of capillary blood obtained by a minimally invasive lancet technique to detect subclinical ketosis in dairy cows by using 3 different electronic hand-held devices. J. Dairy Sci. 98:6108–6118. doi:10.3168/jds.2014-8957.

Katoh, N. 2002. Relevance of apolipoproteins in the development of fatty liver and fatty liver-related peripartum diseases in dairy cows. J. Vet. Med. Sci. 64:293–307.

Kiesner, K., N. Wente, O. Volling, and V. Krömker. 2016. Selection of cows for treatment at dry-off on organic dairy farms. J. Dairy Res. 83:468-475. doi:10.1017/S0022029916000662.

King, M. T. M., K. M. Dancy, S. J. LeBlanc, E. A. Pajor, and T. J. DeVries. 2017. Deviations in behavior and productivity data before diagnosis of health disorders in cows milked with an automated system. J. Dairy Sci. 100:8358-8371. doi:10.3168/jds.2017-12723.

Klaas, I. C., C. Enevoldsen, A. K. Ersbøll, and U. Tölle. 2005. Cow-related risk factors for milk leakage. J. Dairy Sci. 88:128–136. doi:10.3168/jds.S0022-0302(05)72670-9.

Kobayashi, Y., C. K. Boyd, B. L. McCormack, and M. C. Lucy. 2002. Reduced insulin-like growth factor-I after acute feed restriction in lactating dairy cows is independent of changes in growth hormone receptor 1A mRNA. J. Dairy Sci. 85:748–754. doi:10.3168/jds.S0022-0302(02)74132-5.

Kok, A., R. J. van Hoeij, B. J. Tolkamp, M. J. Haskell, A. T. M. van Knegsel, I. J. M. de Boer, and E. A. M. Bokkers. 2017a. Behavioural adaptation to a short or no dry period with associated management in dairy cows. Appl. Anim. Behav. Sci. 186:7-15. doi:10.1016/j.applanim.2016.10.017.

Kok, A., A. T. M. van Knegsel, C. E. van Middelaar, B. Engel, H. Hogeveen, B. Kemp, and I. J. M. de Boer. 2017b. Effect of dry period length on milk yield over multiple lactations. J. Dairy Sci. 100:739-749. doi:10.3168/jds.2016-10963.

Krohn, C. C. 2001. Effects of different suckling systems on milk production, udder health, reproduction, calf growth and some behavioral aspects in high producing dairy cows—a review. Appl. Anim. Behav. Sci. 72:271-280.

Ledgerwood, D. N., C. Winckler, and C. B. Tucker. 2010. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. J. Dairy Sci. 93:5129-5139. doi:10.3168/jds.2009-2945.

Leitner, G., S. Jacoby, E. Maltz, and N. Silanikove. 2007. Casein hydrolyzate intramammary treatment improves the comfort behavior of cows induced into dry-off. Livest. Sci. 110:292-297. doi:10.1016/j.livsci.2007.02.016.

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557-564. doi:10.3168/jds.S0022-0302(03)73634-0.

Leonardi, C., F. Giannico, and L. E. Armentano. 2005. Effect of water addition on selective consumption (sorting) of dry diets by dairy cattle. J. Dairy Sci. 88:1043-1049. doi:10.3168/jds.S0022-0302(05)72772-7.

Lim, G. H., K. E. Leslie, D. F. Kelton, T. F. Duffield, L. L. Timms, and R. T. Dingwell. 2007. Adherence and efficacy of an external teat sealant to prevent new intramammary infections in the dry period. J. Dairy Sci. 90:1289-1300. doi:10.3168/jds.S0022-0302(07)71617-X.

Loiselle, M. C., C. Ster, B. G. Talbot, X. Zhao, G. F. Wagner, Y. R. Boisclair, and P. Lacasse. 2009. Impact of postpartum milking frequency on the immune system and the blood metabolite concentration of dairy cows. J. Dairy Sci. 92:1900–1912. doi:10.3168/jds.2008-1399.

López, S., J. France, N. E. Odongo, R. A. McBride, E. Kebreab, O. AlZahal, B. W. McBride, and J. Dijkstra. 2015. On the analysis of Canadian Holstein dairy cow lactation curves using standard growth functions. J. Dairy Sci. 98:2701-2712. doi:10.3168/jds.2014-8132.

MacDonald, P.D.M., and P.E.J. Green. 1988. User's Guide to Program MIX: An interactive program for fitting mixtures of distributions. Release 2.3, January 1988. Ichthus Data Systems, Hamilton, Ontario, Canada.

Madsen, T. G., M. O. Nielsen, J. B. Andersen, and K. L. Ingvartsen. 2008. Continuous lactation in dairy cows: Effect on milk production and mammary nutrient supply and extraction. J. Dairy Sci. 91:1791-1801. doi:10.3168/jds.2007-0905.

Mann, S., F. A. Leal Yepes, T. R. Overton, J. J. Wakshlag, A. L. Lock, C. M. Ryan, and D. V.

Nydam. 2015. Dry period plane of energy: Effects on feed intake, energy balance, milk production, and composition in transition dairy cows. J. Dairy Sci. 98:3366-3382. doi:10.3168/jds.2014-9024.

Maulfair, D.D., M. Fustini, and A.J. Heinrichs. 2011. Effect of varying total mixed ration particle size on rumen digesta and fecal particle size and digestibility in lactating dairy cows. J. Dairy Sci. 94:3527–3536. doi:10.3168/jds.2010-3718.

Maulfair, D.D., and A.J. Heinrichs. 2013. Effects of varying forage particle size and fermentable carbohydrates on feed sorting, ruminal fermentation, and milk and component yields of dairy cows. J. Dairy Sci. 96:3085–3097. doi:10.3168/jds.2012-6048.

Medrano-Galarza, C., J. Gibbons, S. Wagner, A. M. de Passillé, and J. Rushen. 2012. Behavioral changes in dairy cows with mastitis. J. Dairy Sci. 95:6994-7002. doi:10.3168/jds.2011-5247.

Mellado, M., J. M. Flores, A. de Santiago, F. G. Veliz, U. Macías-Cruz, L. Avendaño-Reyes, and J. E. Garcías. 2016. Extended lactation in high-yielding Holstein cows: Characterization of milk yield and risk factors for lactations > 450 days. Livest. Sci. 189:50-55. doi:10.1016/j.livsci.2016.05.004 1871-1413.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481. doi:10.3168/jds.S0022-0302(97)76075-2.

Miller-Cushon, E. K., and T. J. DeVries. 2017. Feed sorting in dairy cattle: Causes, consequences, and management. J. Dairy Sci. 100:4172-4183. doi:10.3168/jds.2016-11983.

Murata, H., N. Shimada, and M. Yoshioka. 2004. Current research on acute phase proteins in veterinary diagnosis: An overview. Vet. J. 168:28–40. doi:10.1016/S1090-0233(03)00119-

9.

NMC (National Mastitis Council). 2006. NMC Factsheet—Dry Cow Therapy. Accessed Apr. 10, 2018. http://www.nmconline.org/wp-content/uploads/2016/09/Dry-Cow-Therapy.pdf.

Oba, M., and M. S. Allen. 1999. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci. 82:589–596. doi: 10.3168/jds.S0022-0302(99)75271-9.

Odensten, M. O., Y. Chilliard, and K. Holtenius. 2005. Effects of two different feeding strategies during dry-off on metabolism in high-yielding dairy cows. J. Dairy Sci. 88:2072–2082. doi:10.3168/jds.S0022-0302(05)72884-8.

Odensten, M. O., K. Holtenius, and K. Persson Waller. 2007. Effects of two different feeding strategies during dry-off on certain health aspects of dairy cows. J. Dairy Sci. 90:898-907. doi:10.3168/jds.S0022-0302(07)71573-4.

O'Driscoll, K., D. Gleeson, B. O'Brien, and L. Boyle. 2011. Does omission of a regular milking event affect cow comfort? Livest. Sci. 138:132-143. doi:10.1016/j.livsci.2010.12.013.

Oliver, S. P. and L. M. Sordillo. 1989. Approaches to the manipulation of mammary involution. J. Dairy Sci. 72:1647-1664. doi:10.3168/jds.S0022-0302(89)79277-8.

Ollier, S., X. Zhao, and P. Lacasse. 2014. Effects of feed restriction and prolactin-release inhibition at drying off on metabolism and mammary gland involution in cows. J. Dairy Sci. 97:4942-4954. doi:10.3168/jds.2014-7914.

Olofsson, J. 1999. Competition for total mixed diets fed for ad libitum intake using one or four cows per feeding station. J. Dairy Sci. 82:69–79. doi: 10.3168/jds.S0022-0302(99)75210-0.

Olsson, G., M. Emanuelson, and H. Wiktorsson. 1998. Effects of different nutritional levels prepartum on the subsequent performance of dairy cows. Livest. Prod. Sci. 53:279–290. doi:10.1016/S0301-6226(97)00022-5.

Pezeshki, A., J. Mehrzad, G. R. Ghorbani, H. R. Rahmani, R. J. Collier, and C. Burvenich. 2007. Effects of short dry periods on performance and metabolic status in Holstein cows. J. Dairy Sci. 90:5531-5541. doi: 10.3168/jds.2007-0359.

Pinedo, P. J., C. Fleming, and C. A. Risco. 2012. Events occurring during the previous lactation, the dry period, and peripartum as risk factors for early lactation mastitis in cows receiving 2 different intramammary dry cow therapies. J. Dairy Sci. 95:7015-7026. doi:10.3168/jds.2012-5398.

Plaizier, J.C., D.O. Krause, G.N. Gozho, and B.W. McBride. 2009. Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. Vet. J. 176:21–31. doi:10.1016/j.tvjl.2007.12.016.

Pomiès, D., B. Martin, Y. Chilliard, P. Pradel, and B. Rémond. 2007. Once-a-day milking of Holstein and Montbéliarde cows for 7 weeks in mid-lactation. Animal. 1:1497-1505. doi:10.1017/S1751731107000778.

Rabiee, A. R., and I. J. Lean. 2013. The effect of internal teat sealant products (Teatseal and Orbeseal) on intramammary infection, clinical mastitis, and somatic cell counts in lactating dairy cows: A meta-analysis. J. Dairy Sci. 96:6915–6931. doi:10.3168/jds.2013-6544.

Rajala-Schultz, P. J., J. S. Hogan, and K. L. Smith. 2005. Association between milk yield at dry-off and probability of intramammary infections at calving. J. Dairy Sci. 88:577-579. doi:10.3168/jds.S0022-0302(05)72720-X.

Rastani, R. R., R. R. Grummer, S. J. Bertics, A. Gümen, M. C. Wiltbank, D. G. Mashek, and M. C. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: Milk production, energy balance, and metabolic profiles. J. Dairy Sci. 88:1004-1014. doi:10.3168/jds.S0022-0302(05)72768-5.

Sanford, C. J., G. P. Keefe, I. R. Dohoo, K. E. Leslie, R. T. Dingwell, L. DesCôteaux, and H. W. Barkema. 2006. Efficacy of using an internal teat sealer to prevent new intramammary infections in nonlactating dairy cattle. J. Am. Vet. Med. A. 228:1565-1573. doi:10.2460/javma.228.10.1565.

Santscchi, D. E., D. M. Lefebvre, R. I. Cue, C. L. Girard, and D. Pellerin. 2011. Economic effect of short (35-d) compared with conventional (60-d) dry period management in commercial Canadian Holstein herds. J. Dairy Sci. 94:4734-4743. doi:10.3168/jds.2010-3596.

SAS Institute Inc. 2013. SAS version 9.4. SAS Institute Inc., Cary, NC.

Scherpenzeel, C. G. M., I. E. M. den Uijl, G. van Schaik, R. G. M. Olde Riekerink, J. M. Keurentjes, and T. J. G. M. Lam. 2014. Evaluation of the use of dry cow antibiotics in low somatic cell count cows. J. Dairy Sci. 97:3606-3614. doi:10.3168/jds.2013-7655.

Schirmann, K., et al. 2009. Technical note: Validation of a system for monitoring rumination in dairy cows. J. Dairy Sci. 92:6052–6055. doi:10.3168/jds.2009-2361.

Schlamberger, G., S. Wiedemann, E. Viturro, H. H. D. Meyer, and M. Kaske. 2010. Effects of continuous milking during the dry period or once daily milking in the first 4 weeks of lactation on metabolism and productivity of dairy cows. J. Dairy Sci. 93:2471-2485. doi:10.3168/jds.2009-2823.

Schukken, Y. H., D. J. Wilson, F. Welcome, L. Garrison-Tikofsky, and R. N. Gonzalez. 2003. Monitoring udder health and milk quality using somatic cell counts. Vet. Res. 34:579–596. doi:10.1051/vetres:2003028.

Schutz, M. M., L. B. Hansen, G. R. Steuernagel, and A. L. Kuck. 1990. Variation of milk, fat, protein, and somatic cells for dairy cattle. J. Dairy Sci. 73:484–493. doi:10.3168/jds.S0022-0302(90)78696-1.

Silanikove, N., U. Merin, F. Shapiro, and G. Leitner. 2013. Early mammary gland metabolic and immune responses during natural-like and forceful drying-off in high-yielding dairy cows. J. Dairy Sci. 96:6400-6411. doi:0.3168/jds.2013-6740.

Skidmore, A., K. Peeters, C. Sniffen, and A. Brand. 1997. Monitoring dry period management. Pages 171–201 in Herd Health and Production Management in Dairy Practice. A. Brant, J. Noordhuizen, and Y. Schukken, ed. Wageningen Pers, Wageningen, the Netherlands.

Sordillo, L. M., K. Shafer-Weaver, and D. DeRosa. 1997. Immunobiology of the mammary gland. J. Dairy Sci. 80:1851–1865. doi:10.3168/jds.S0022-0302(97)76121-6.

Sorensen, A., D. D. Muir, and C. H. Knight. 2008. Extended lactation in dairy cows: Effects of milking frequency, calving season and nutrition on lactation persistency and milk quality. J. Dairy Res. 75:90-97. doi:10.1017/S0022029907002944.

Sørensen, J. T., and C. Enevoldsen. 1991. Effect of dry period length on milk production in subsequent lactation. J. Dairy Sci. 74:1277–1283. doi:10.3168/jds.S0022-0302(91)78283-0.

Stefanon, B., M. Colitti, G. Gabai, C. H. Knight, and C. J. Wilde. 2002. Mammary apoptosis and lactation persistency in dairy animals. J. Dairy Res. 69:37–52.

Ster, C., M. C. Loiselle, and P. Lacasse. 2012. Effect of postcalving serum nonesterified fatty acids concentration on the functionality of bovine immune cells. J. Dairy Sci. 95:708–717. doi:10.3168/jds.2011-4695.

Suarez-Mena, F. X., G. I. Zanton, and A. J. Heinrichs. 2013. Effect of forage particle length on rumen fermentation, sorting and chewing activity of late-lactation and non-lactating dairy cows. Animal. 7:272-278. doi:10.1017/S1751731112001565.

Torres, A. H., P. J. Rajala-Schultz, F. J. DeGraves, and K. H. Hoblet. 2008. Using dairy herd improvement records and clinical mastitis history to identify subclinical mastitis infections at dry-off. J. Dairy Res. 75:240-247. doi:10.1017/S0022029908003257.

Tucker, C. B., D. E. Dalley, J-L K. Burke, and D. A. Clark. 2007. Milking cows once daily influences behavior and udder firmness at peak and mid lactation. J. Dairy Sci. 90-1692-1703. doi:10.3168/jds.2006-577.

Tucker, C. B., S. J. Lacy-Hulbert, and J. R. Webster. 2009. Effect of milking frequency and feeding level before and after dry off on dairy cattle behavior and udder characteristics. J. Dairy Sci. 92:3194-3203. doi:10.3168/jds.2008-1930.

Valizaheh, R., D. M. Veira, and M. A. G. von Keyserlingk. 2008. Behavioural responses by dairy cows provided two hays of contrasting quality at dry-off. Appl. Anim. Behav. Sci. 109:190-200. doi:10.1016/j.applanim.2007.03.001.

van Knegsel, A. T. M., G. J. Remmelink, S. Jorjong, V. Fievez, and B. Kemp. 2014, Effect of dry period length and dietary energy source on energy balance, milk yield, and milk composition of dairy cows. J. Dairy Sci. 97:1499-1512. doi:10.3168/jds.2013-7391.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharide in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Vitale, A. F., M. Tenucci, M. Papini, and S. Lovari. 1986. Social behaviour of the calves of semi-wild Maremma cattle, Bos primigenius taurus. Appl. Anim. Behav. Sci. 16:217–231.

Watters, R. D., M. C. Wiltbank, J. N. Guenther, A. E. Brickner, R. R. Rastani, P. M. Fricke, and R. R. Grummer. 2009. Effect of dry period length on reproduction during the subsequent lactation. J. Dairy Sci. 92:3081–3090. doi:10.3168/jds.2008-1294.

Wilde, C. J., C. V. P. Addey, P. Li, and D. G. Fernig. 1997. Programmed cell death in bovine mammary tissue during lactation and involution. Exp. Physiol. 82:943-953.

Wilde, C. J., C. H. Knight, and D. J. Flint. 1999. Control of milk secretion and apoptosis during mammary involution. J. Mammary Gland Biol. 4:129-136.

Wildman, E.E., G.M. Jones, P.E. Wagner, and R.L. Bowman. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495-501. doi:10.3168/jds.S0022-0302(82)82223-6.

Yang, W. Z., and K. A. Beauchemin. 2006. Physically effective fiber: method of determination and effects of chewing, ruminal acidosis, and digestion by dairy cows. J. Dairy Sci. 89:2618-2633. doi:10.3168/jds.S0022-0302(06)72339-6.

Yang, W. Z., and K. A. Beauchemin. 2009. Increasing physically effective fiber content of dairy cow diets through forage proportion versus forage chop length: Chewing and ruminal pH. J. Dairy Sci. 92:1603-1615. doi:10.3168/jds.2008-1379.

Zobel, G., K. Leslie, D. M. Weary, and M. A. G. von Keyserlingk. 2013. Gradual cessation of milking reduces milk leakage and motivation to be milked in dairy cows at dry-off. J. Dairy Sci. 96: 5064-5071. doi:10.3168/jds.2012-6501.