A Novel Approach to Understand the Impact of Dry-off on Dairy Cow Welfare in Automatic Milking Systems

by

Elizabeth C. McGuire

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Master’s Committee:

Advisor: Jennifer Van Os

Kate Creutzinger

Jimena Laporta

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ABSTRACT

The dry-off period is a critical transition in the dairy cow's lactation cycle, marking the switch from active milk production to a non-lactating state that allows mammary gland recovery. While a non-lactation period is essential for udder health and subsequent milk yield, an abrupt cessation of milking can negatively impact cow welfare. Modern dairy cows in conventional North American systems produce more milk at dry-off than cows in more natural or pasture-based systems. These differences are significant, with organic herds achieving 85% (range: 72 to 91%) of the yields recorded for conventional herds (Schwendel et al., 2015). High milk yield during late lactation leads to excessive milk accumulation in the udder at dry-off, increasing intramammary pressure and risk of udder engorgement, discomfort, milk leakage, and mastitis (Zobel et al., 2015; Gott et al., 2016).

Gradual dry-off protocols, which progressively reduce milking frequency before final milking cessation, have shown promise for easing this transition and improving dairy cow welfare by lowering milk yield and udder pressure before and after dry-off, respectively. However, implementation of gradual dry-off can be challenging in conventional milking parlors. Automatic milking systems (AMS), with their ability to control milking frequency individually, offer a novel solution to implement welfare-focused gradual dry-off protocols with minimal additional labor.

This thesis evaluates the effects of a two-week gradual dry-off protocol delivered via AMS, compared to abrupt dry-off, on milk yield, milking behavior, lying behavior, udder characteristics, pain sensitivity, and milk leakage in 30 Holstein cows monitored from 74 days before expected calving to 5 days post dry-off. Cows undergoing gradual dry-off had a significantly greater reduction in milk yield in the two weeks before dry-off, particularly from

day -5 to -1 before dry off, and they produced 11% less total milk (348.4 ± 25.8 kg) than abruptly dried-off cows (392.9 ± 17.9 kg) over the 2-week period. After dry-off, gradual cows had fewer AMS refusals than abrupt cows (1.08 ± 0.2 vs. 1.70 ± 0.3 refusals/day), indicating reduced motivation to be milked.

Three-dimensional imaging revealed that teat spacing increased post dry-off in both groups, but abruptly dried-off cows had significantly greater teat spacing on Day 3 compared to gradual cows, suggesting greater udder engorgement. Udder volume and surface area increased after dry-off in both treatments, with no significant differences between groups. No significant differences were detected between treatments in udder firmness, pain responses during palpation, or lying behavior before or after dry-off. Milk leakage post dry-off was significantly higher in abruptly dried-off cows on Days 1, 3, and 5.

These results demonstrate that AMS can support gradual dry-off protocols that reduce milk yield prior to dry-off, lessen motivation to be milked, and decrease udder engorgement and milk leakage after dry-off, without negatively impacting behavior or causing detectable pain. This approach offers a practical, scalable strategy to improve dairy cow welfare during the dry-off transition while maintaining operational feasibility.

By integrating gradual dry-off directly within the AMS, this study enables individualized control of milking frequency with minimal labor input. The application of 3D imaging technology to quantify dynamic udder changes provides a novel, non-invasive method for monitoring udder physiology. Additionally, using AMS refusal data as a behavioral indicator of milking motivation offers fresh insights into the cows' affective states during dry-off. These combined innovations advance precision dairy management by linking automated milking technology, advanced imaging, and behavioral measures to enhance animal welfare in a commercial setting.

Overall, this work contributes important evidence supporting gradual dry-off in AMS environments as a welfare-friendly alternative to abrupt dry-off, advancing sustainable and humane dairy production practices.

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LITERATURE REVIEW

Lactating and Non-Lactating (dry) Period in Dairy Cattle

Once dairy cows have reached maturity, they are typically bred approximately once per year to produce a calf and initiate lactation. In the U.S dairy industry, cows commonly have an average lactation length of 305 days and a 60-day non-lactation or "dry" period before their calving date. The transition between the lactation and non-lactation period is referred to as "dry-off". The non-lactation period allows for mammary involution, removal of senescent epithelial cells, and regeneration of the mammary epithelial cells (Capuco et al., 1997), which are of key importance for milk production and overall udder health (Vilar & Rajala-Schultz, 2020). The benefits of the non-lactation period for cattle have been acknowledged since the early 1800s (reviewed by Dix Arnold and Becker, 1936). Longer dry periods are associated with greater milk production in the subsequent lactation. For example, cows with a 60 day dry period had greater milk yield during the 14 weeks after calving than cows with a 30 or 0 day dry period (Van Knegsel et al., 2014). However, this production boost may come at a tradeoff with cow welfare. Cows with shorter or no dry periods often exhibit improved energy balance and fewer metabolic disorders in the subsequent lactation, likely due to reduced milk production and, thus, lower energetic demands (Olagaray et al., 2020).

In a natural setting, milk is produced by the cow to meet the nutritional needs of their calf. Milk production would rapidly increase for the first few weeks after calving before slowly declining. A gradual decline in milk production happens as the cow initiates fewer sucking attempts and the calf starts to consume more forage and less milk (Martin, 1984). Weaning from milk occurs gradually at approximately 7 to 14 months after calving (Reinhardt and Reinhardt, 1981).

For modern dairy cows, average milk production per cow has increased due to genetic selection and improvements in nutrition and management (Barkema et al., 2015). While the definitions of high-producing cows vary in the literature, production thresholds at dry-off include >17.8 kg/day (Odensten et al., 2007a), >20 kg/day (Bertulat et al., 2013), >21 kg/day (Dingwell et al., 2004), and up to 25–35 L/day (Silanikove et al., 2013). As a result, modern dairy cows often produce substantial volumes of milk even at the end of lactation compared to cows managed more similarly to a natural setting such as pasture raised beef cows.

Dairy cows on typical commercial farms are abruptly switched from being milked 2-3 times per day to none at the end of lactation. This abrupt cessation of milking has become an established management practice with 75% of farms in the United States and Europe using abrupt dry-off (Vilar and Rajala-Schultz, 2020). While providing a dry period is beneficial to milk yield and mammary gland health, abrupt dry-off may pose a threat to dairy cattle welfare from discomfort and increased risk of intramammary infection caused by an accumulation of milk in the udder (O'Driscoll et al., 2011; Dingwell et al., 2003; Zobel et al., 2015).

Dry-off Methods and Animal Welfare

Animal welfare is of key importance to the dairy industry for both ethical and sustainability reasons (Fraser et al., 1997; Von Keyserlingk et al., 2014). Animal welfare is defined by "the physical and mental state of an animal in relation to the conditions in which it lives and dies" (OIE, 2019). To consider an animal's welfare, various stakeholders consider 3 key components: biological functioning, the affective state the animal is experiencing, and the naturalness of its life (Fraser et al. 1997). Another way to assess animal welfare is to use the five domains, considering an animal's nutrition, environment, health, behavior and mental state (Mellor, 2017). Similar to the five domains, it is standard practice in some settings, such as

European on-farm welfare assessment programs, to use the five freedoms to assess animal welfare. That is, animals should have the freedom from hunger and thirst, from discomfort, from pain, injury, and disease, and to express normal behaviors (FAWC, 2009).

During the dry-off process, there are several areas of concern considering animal welfare. These concerns include, but are not limited to, pain or discomfort and the risk of intramammary infection. The best way to assess the impact of dry-off practices on these welfare concerns, whether in research or practical on-farm settings, is to use animal-based welfare measurements (Whay et al., 2013). However, the lack of animal-based welfare measurements in the dairy cow dry-off literature is a major knowledge gap. These potential welfare concerns are increasing as cows are continuously bred for higher milk yield, as higher-producing cows have more milk remaining and being secreted within the udder when milking abruptly ceases (Zobel et al., 2015; Cattaneo et al., 2023). To date, research studies have evaluated udder engorgement and pressure, as well as changes in cows' resting behavior, as potential welfare indicators surrounding dry-off (Franchi et al., 2022; Chapinal et al., 2014).

In the initial stages of mammary involution, continued milk production leads to engorgement of the cisterns, ducts, and alveoli (Oliver & Sordillo, 1989). In late lactation, when cows are dried off, they are often still producing substantial amounts of milk, and it is increasingly common for cows to yield more than 20–25 kg/day at this stage (Gott et al., 2016; Zobel et al., 2015; Stefanon et al., 2002). As more milk is produced and accumulates in the udder, it creates increased intramammary pressure. This pressure is significant when cows skip milking (O'Driscoll et al., 2011) or milking is ceased (Cousins et al., 1980; Oliver and Sordillo, 1989). Cows with a high milk yield at dry-off may have increased animal welfare considerations due to increased udder engorgement (Zobel et al., 2015).

Bertulat et al. (2013) found milk will continue to accumulate in the udder for days after the milking process has stopped. This accumulation of milk will cause udder engorgement and a rise in udder pressure, which has been shown to result in udder discomfort (O'driscoll et al., 2011). Since pain can be difficult to quantify directly in animals, udder engorgement has been used as an alternative measurement of udder pain (Chapinal et al., 2014; Zobel et al., 2015). Several measurement techniques have been used to quantify udder engorgement. Manual palpation scoring systems have been developed to assess udder firmness in a standardized way (Franchi et al., 2022), while dynamometers offer a more objective measure of internal udder pressure (Rees et al., 2014). More recently, researchers have explored the use of 3D imaging technology. Novel research has started to use 3D cameras to monitor changes in udder shape, symmetry, and volume (Montes et al., 2024).

Another way of measuring udder pain is by measuring the change in lying and standing behaviors (Medrano-Galarza et al., 2012). Albright (1987) indicated that increased standing time in cattle is a sign of discomfort. When a cow's udder is full, she may opt to stand for longer periods and have significant changes in her lying and standing behavior (Osterman and Redbo, 2001). This is in alignment with Stefanowska et al. (2000), who found a reduction in lying time when milkings were skipped. In addition, previous studies have found that milk production at dry-off and lying time are inversely related, with cows who are higher producing spending less time lying down after dry-off (Chapinal et al., 2014).

Intramammary Infections and Antimicrobial Use

Another health and welfare concern at the time of dry-off is the risk of intramammary infections (IMI), also referred to as mastitis. Mastitis is an inflammation of the mammary gland caused by microorganisms, such as bacteria , in the udder (Sharma et al., 2006). Intramammary infections affect animal health, welfare, and production. (Medrano-Galarza et al., 2012). While cows can have an IMI at any time during their lactation, multiple studies have shown that cows are at the greatest risk during the dry-off process (reviews by Dingwell et al., 2003; Bradley and Green, 2004). This is especially true for cows with high milk yield at dry-off (Rajala-Schultz et al., 2005). Cows producing in excess of 21 kg/d are 1.8 times more likely to experience a delayed teat canal closure compared to cows producing less than 15 kg/day at dry-off (Dingwell et al., 2004). Milk leakage will keep the teat end open and allow bacteria to enter the canal (Dingwell et al., 2004; Klass et al., 2005). In addition to the negative impact on cow welfare, mastitis has been estimated to cost the dairy industry $2 billion per year (Rollin et al., 2015). Milk losses, treatment costs, and early culling all contribute to the financial loss dairy farms face due to mastitis (e.g., Petrovski et al., 2006; Halasa et al., 2007; Huijps et al., 2008).

Due to the high risk of IMI with the abrupt cessation of milking, blanket dry-cow antibiotic therapies have become frequently recommended (e.g., Blowey and Edmondson, 2010). More than 90% of dairy cows in the United States receive antimicrobials after every lactation (USDA, 2016) to treat or prevent mastitis during the dry period. Non-judicious use of antimicrobials contributes to the development of antimicrobial-resistant pathogens (Abdi et al., 2021). An alternative method would be to reduce milk leakage, giving proper time for keratin plugs to naturally develop and block pathogens from entering the teat (Dingwell et al., 2004). Reducing production before dry-off can result in less milk leakage (Franchi et al., 2022), reduce the risk of new IMI before dry-off and through the dry period (Gott et al., 2016; Natzke et al., 1975), and reduce udder engorgement after dry-off (Larson et al., 2021).

Alternatives to the Traditionally Implemented Abrupt Dry-off

Several ways to reduce milk production before the cessation of milking have been explored in dairy cattle production. Operations may choose to reduce production before dry-off through changes in milking frequency, feeding strategies, or medications. Gradual dry-off is a process in which daily milking frequency is gradually reduced before milking stops completely. As cows are milked it stimulates oxytocin and prolactin, signaling the mammary gland to produce and release milk. Thus, by reducing the milking frequency, it reduces milk protein and lactose synthesis, resulting in lower milk yield (Collier & Romagnolo, 2002).

Some negative effects of dry-off have been mitigated by drying cows off gradually. Previous research has found a 33% reduction of milk yield in the final week of lactation when milking frequency was reduced from twice to once daily (Gott et al., 2017). Similarly, Rajala-Schultz et al. (2018) found that reducing milk yield using gradual dry-off techniques resulted in cows showing longer lying bouts compared to abruptly dried-off cows. In addition, a gradual reduction in milking frequency will reduce the amount and duration of milk leakage, especially in high-producing cows (Zobel et al., 2013). This differs from Larsen et al. (2021), who found no evident signs of milk leakage after dry-off when comparing abrupt or gradual dry-off. However, this null result could be due to the evaluation taking place 2 hours after morning milking and not allowing enough time for milk to accumulate in the udder. To our knowledge, no study to date has found any negative health effects of reducing milking from twice to once daily on dairy cows. This method has been proven to reduce milk yield and udder engorgement without inducing a negative energy balance, in contrast a behavior even in the absence of an appropriate to reducing production through changes in feed (Larsen et al., 2021).

While gradual dry-off techniques have shown welfare benefits, there remains limited research evaluating the potential production consequences in the following lactation. Earlier findings suggest that dry period length can influence milk yield after calving (e.g., Van Knegsel et al., 2014), but it is unclear whether alternative dry-off methods such as gradual or voluntary dry-off compromise subsequent milk production. Thus, it is important to examine whether these welfare-oriented strategies maintain or reduce milk yield in the next lactation, particularly under commercial AMS conditions.

Another method to reduce milk yield before dry-off is to reduce dietary nutrient intake. With fewer nutrients available, a decrease in milk synthesis will occur (Larsen et al., 2021). A restriction of feed allocated will limit the energy supply, preventing the accumulation of milk and reducing udder firmness and intramammary pressure (Tucker et al. 2009). A reduced energy diet during the two weeks prior to dry-off can decrease milk production at dry-off but is most effective when combined with gradual dry-off (France et al., 2022). Larsen et al., (2021) found a 45% reduction in milk yield when both feeding level and milking frequency were reduced compared to a 30% reduction with only one treatment.

Ideally, we would reduce dietary nutrient intake without inducing hunger in cows, which can negatively affect cow welfare (Franchi et al., 2019; Zobel et al., 2015). Reduced feeding level has been shown to induce a negative energy balance, compromising animal welfare due to metabolic stress and hunger (Larsen et al., 2021). Further, a reduction of nutrients in late lactation and during dry-off can create metabolic challenges to cattle, resulting in greater blood non-esterified fatty acid (NEFA) and cortisol concentrations, which increase the risk of metabolic disease after calving and indicate a stress response, respectively (Odensten et al., 2007). A negative energy balance and metabolic stress has been replicated in low energy diets by feeding straw (Odensten et al., 2005) and hay (Ollier et al., 2015). In addition, this method has been shown to increase feeding motivation to make up for dietary changes, as shown by increased vocalization (Valizaheh et al., 2008; Tucker et al., 2009) and by increased maximum weight pushed to obtain concentrate in a feeding motivation test (Franchi et al., 2019).

A third method to reduce milk production is the use of dopamine agonists such as Cabergoline, and Hydrolysate. Cabergoline is typically injected intramuscularly after the last milking. It causes a rapid decrease in milk synthesis and milk accumulation in the first 48 hours after the injection then its effects decline (Larsen et al., 2021). Cabergoline injection also results in a decrease in dry matter intake (DMI) and lower prolactin concentration in the bloodstream (Ollier et al., 2013) (Boutinaud, et al., 2016). Larsen et al. (2021) found an abrupt reduction in DMI lasting approximately 24 hours. However, there is a literature gap surrounding the welfare implication related to this abrupt reduction in DMI. As a result of low DMI and milk synthesis, Cabergoline injections have been reported to reduce the risk of milk leakage (Bach et al., 2015; Bertulate et al., 2017: Hop et al., 2019). Similarly to other treatments that reduce milk production, cows given Hydrolysate will have decreased udder pressure and spend more time lying following dry-off (Leitner et al., 2007). This method has a synergistic effect when used with gradual dry-off and little effect when used with abrupt dry of due to the substantial difference in milk yield (Larsen et al., 2021). However, the finding of Larsen et al., (2021) differs from Bach et al. (2015) and Hop et al. (2019), which both found a decreased risk of milk leakage when abrupt dry-off cows were treated with Cabergoline. Overall, there are large gaps in the literature surrounding dopamine agonist use in the dry-off process, especially the effects on animal welfare and metabolism.

Motivation Testing as an Indicator of Milking Motivation

Motivation testing involves assessing the level of desire an animal has to perform a behavior (e.g., milking). Through motivation testing, we are better able to understand the needs of our animals and improve upon their welfare. It is important to ask ourselves two questions: (1) which behaviors are most important and (2) how do we best provide an outlet for them (Jensen & Pedersen 2008)?

Spinka (2006) outlined the importance of considering the consequences of preventing a behavior (e.g., cessation of milking) as a way to represent the need of said behavior. This is supported by Friend's (1989) concept of ethological needs: behaviors driven largely by internal motivation, where prolonged prevention can negatively impact animal welfare. This can be represented by an animal attempting to perform a behavior even in the absence of an appropriate environment or stimulus (Weeks and Nicol, 2006), such as cows entering the AMS with a lack of feed reward. Animals may also perform abnormal behaviors as stress responses when unable to fulfill their motivation (Jensen and Toates, 1993), such as cows spending a significant amount of time waiting to be milked.

Current literature already indicates that milking is a motivated behavior especially for high yielding cows and abruptly dried-off cows. Zobel et al. (2013) found that abruptly dried-off cows had 6.2 times greater odds of standing near the gate and spent 5.2 times longer near the gate compared to gradually dried-off cows in the days following dry-off. This is supported by Rathore (1982) who found high producing cows will enter the milking parlor earlier than lower producing cows, suggesting that milking is a motivated behavior for high yielding cows.

To address the second question, how do we best provide an outlet for cows to express their motivation to be milked, we propose an innovative approach using AMS to improve dry-off management. While we do not propose eliminating the dry period due to the negative impacts this would have on animal health and welfare, AMS technology presents an opportunity to gradually reduce cows' motivation to be milked as they approach dry-off. By tailoring milking frequency and intervals through AMS, cows experience a more gradual reduction in udder fill, potentially alleviating udder discomfort, reducing frustration, and minimizing abnormal behaviors associated with an unmet motivation to be milked. The study reported in this thesis specifically quantifies the relationship between AMS-driven gradual dry-off, on behavioral indicators of milking motivation.

Integrating Gradual Dry-off Protocols within AMS Management

Overall, it is important to note that the alternatives to abrupt dry-off, such as gradual dry-off or dopamine agonists injections, may require more attention to individual animals and extra labor. In conventional parlors, where cows are milked as a group, gradual dry-off can be less practical and economically disadvantageous compared to abrupt dry-off. As a result, abrupt dry-off remains the predominant method on commercial dairy farms in both the United States and Europe, largely because of its simplicity and low labor requirements (Vilar & Rajala-Schultz, 2020; Pattamanont et al., 2020). However, this constraint may be mitigated with the use of automatic robotic milking systems. AMS adoption is more widespread in parts of Europe than in the U.S., driven in part by smaller average herd sizes and different labor economics (Lage et al., 2024). European systems are often designed for free-flow traffic and precision feeding, making them well-suited to gradual dry-off protocols. In contrast, U.S. farms typically have larger herds, and while AMS adoption is growing, implementation remains limited by high upfront investment costs, and infrastructure compatibility.

Automatic milking systems are designed to allow cows to self-regulate the milking process without direct operator supervision. Cows in AMS facilities are often kept in a free stall barn with access to the robotic milkers. Feed, in the form of concentrate pellets, is provided to the cow upon entry into the robot and is postulated to be the main driving factor for cows to visit an AMS (Prescott et al., 1998). Operators can regulate a maximum number of visits and implement parameters around minimum amounts of visits per day. If cows fall below the minimum number of daily visits or exceed the allowed time in between visits, the operator will "fetch" or bring the cow into the AMS to be milked. This unique style of individual milking allows for easy implementation of new animal welfare-based dry-off practices.

Milking frequency and/or pelleted feed allotted to individual cows can be automatically adjusted through computer software without the need for extra labor or time that would be required from gradual dry-off in conventional milking parlors. A possible solution to improve cow welfare at dry-off is by programming in a deliberate, gradual dry-off schedule; cows could be housed in free flow AMS pens but barred (refused) from entering the milking box on a tiered-down system (e.g., 3 milkings/day to 2 milkings/days to 1 milking/day). This precision-based approach not only offers a feasible solution to reduce labor demands associated with gradual dry-off, but also enhances the ability to support individual cow welfare.

CONCLUSION

The dry-off period is a critical stage in the lactation cycle that allows the mammary gland to rest and repair in preparation for the next lactation. While abrupt cessation of milking is the industry standard, it has been associated with several negative welfare outcomes, including udder engorgement, milk leakage, behavioral frustration from unmet motivation to be milked, and increased risk of intramammary infections. Current research suggests that these risks are more pronounced in high-yielding cows, who may experience greater intramammary pressure and delayed teat canal closure. In response, gradual dry-off strategies have been explored as potential alternatives. These strategies can help reduce milk yield and associated discomfort, though they are rarely implemented in conventional systems due to added labor demands.

Automatic milking systems (AMS) provide an opportunity to apply gradual dry-off protocols at the individual cow level, with minimal labor input. Despite this potential, there remains a lack of research on how these systems can be leveraged to improve cow welfare during dry-off. Specifically, little is known about how gradual dry-off protocols in AMS affect behavior, udder physiology, and motivation to be milked.

To address these knowledge gaps, the study presented in this thesis evaluates the effects of a gradual dry-off protocol compared to abrupt dry-off, implemented in an AMS environment. The study focuses on milk yield, robotic milking visits and refusals, lying behavior, udder characteristics (including dimensions, firmness, leakage, and pain sensitivity), and motivation to be milked. Additionally, novel 3D imaging technology was used to objectively quantify changes in udder dimensions. By applying animal-based welfare indicators, this research aims to determine whether gradual dry-off can improve cow welfare and reduce health risks during this transitional period.

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Chapter 2- The Final Milk:

When Cows Know Best, Let Behavior Guide Dry-Off Practices

ABSTRACT

The abrupt cessation of milking is a well-established dairy management practice used to begin the dry period but it has documented short-term negative implications for dairy cow welfare. After an abrupt dry-off, milk accumulates in the udder, resulting in udder pressure, udder pain, and milk leaking from the teat ends, which increases the risk of intramammary infections. Improvements in dry-off management have the potential to enhance animal welfare and reduce lost profits caused by intramammary infections. In this study, an automatic milking system (AMS) was used as a novel approach to manage gradual dry-off at the individual cow level. Thus, the objective of this study was to evaluate the effects of a gradual dry-off protocol, implemented through an AMS, compared to abrupt dry-off on milk yield, milking behavior, udder characteristics, and indicators of welfare in dairy cows before and after dry-off. Thirty Holstein dairy cows were enrolled 74 ± 3 d before expected calving and assigned to a gradual (n = 15) or abrupt (n = 15) dry-off treatment. Cows in the gradual treatment experienced a stepwise reduction in allowable daily milkings over a two-week period, and cows in the abrupt treatment had no changes from standard farm management. All cows were dried off 60 d before their expected calving date. Data collection occurred from 14 d before to 5 d after dry-off. Measures included milk yield, robotic milking visits (successes and refusals by the AMS), lying and activity behaviors, and udder characteristics, including dimensions, firmness, pain responses, and milk leakage. Cows in the gradual treatment produced a total of 11% less milk across that 2 wk period (mean ± SD: 392.9 vs. 348.4 ± 25.8 kg, respectively) and showed a significant treatment x day interaction, driven by lower daily yield on d -5 to -1. Gradually dried-off cows had fewer

AMS refusals post-dry-off, suggesting a reduced motivation to be milked, with gradual vs. abrupt cows averaging (LSM ± SE) 1.08 ± 0.2 vs. 1.70 ± 0.3 refusals/day. There was limited evidence of differences between treatments in udder engorgement and pain. Teat spacing was greater in cows dried-off abruptly compared to gradually on day 3 (LSM ± SE: 61.0 ± 3.1 cm vs. 48.9 ± 3.2 cm), suggesting greater engorgement than in the gradual treatment. However, udder volume and surface area increased after dry-off regardless of treatment, and there were no differences between treatments for udder firmness, pain-related behaviors, and lying and activity behaviors. There was a significant treatment × day interaction with cows in the abrupt dry-off group leaking significantly more milk than those in the gradual group on 1, 3, and 5 d after dry-off and no cows exhibited leakage prior to dry-off. These findings demonstrate that using AMS to implement a gradual dry-off protocol can reduce milking motivation and perhaps udder pressure and milk leakage after dry-off, ultimately supporting better welfare outcomes and potentially reducing mastitis-related financial losses. This approach may be a practical and effective welfare-focused alternative to abrupt cessation of milking in an AMS.

Key words: animal welfare, robotic milking, milking frequency

INTRODUCTION

The transition between the lactating to the non-lactating ("dry") period is a critical phase in dairy cow management, with implications for udder health, productivity, and animal welfare. In conventional systems, the cessation of milking ("dry-off") is typically performed approximately 60 days before a cow's expected calving date to allow for mammary gland involution and epithelial regeneration, which support milk production in the next lactation (Capuco et al., 1997; Vilar & Rajala-Schultz, 2020). Abrupt dry-off is the transition, from cows being milked two to three times per day to not at all (i.e., milking stops) to induce milk stasis. An abrupt cessation of milking remains the predominant method used on commercial dairy farms, practiced by over 75% of farms in the United States and Europe (Vilar & Rajala-Schultz, 2020). Abrupt dry-off is commonly chosen for its convenience and minimal labor needs compared to other dry-off methods (Pattamanont et al., 2020). However, abrupt dry-off may pose a challenge to cow welfare, particularly for high-producing (20–25 kg/day; Zobel et al., 2015), due to increased milk accumulation, resulting in increased udder pressure, risk of milk leakage causing intramammary infection, and continued motivation to be milked (Bertulat et al., 2013; Chapinal et al., 2014). Although the rate of milk secretion declines over time after dry-off, milk continues to accumulate in the mammary glands after milking ceases, and without removal (milking), it results in internal udder pressure and volume increase (Oliver & Sordillo, 1989; Cousins et al., 1980).

Milk leakage is a common consequence of increased udder pressure following dry-off, particularly in high-producing cows, and occurs when intramammary pressure builds due to cessation of milking (De Prado-Taranilla et al., 2020). This leakage delays teat canal closure, increasing the risk of intramammary infection (IMI) by allowing pathogens to enter the udder

(Dingwell et al., 2004; Sharma et al., 2006). In quarters leaking milk, the odds of developing clinical mastitis tended to be two times higher compared with quarters not leaking milk (De Prado-Taranilla et al., 2020). IMI during the dry period not only compromises cow welfare but also contributes to significant financial losses for the dairy industry due to treatment costs, discarded milk, and potential culling (Halasa et al., 2007; Rollin et al., 2015).

Udder engorgement refers to visual external udder changes after dry-off and is generally quantified by a linear increase in teat spacing over time described by Mein et al. (1995). Cows experience discomfort as a consequence of udder engorgement (O'Driscoll et al., 2011), which has been documented as pain responses during udder palpation (Medrano-Galarza et al., 2012) and behavioral disruptions such as reduced lying time (Chapinal et al., 2014). Cows with full or engorged udders may avoid lying down to minimize pressure on the mammary gland, leading to increased standing time (Osterman & Redbo, 2001; Stefanowska et al., 2000). Additionally, research suggests that cows may remain motivated to be milked even after dry-off, particularly those with high milk yields (>24 kg/d), as indicated by increased standing near milking areas and behavioral frustration when access to milking is denied (Zobel et al., 2013). High-producing cows may continue to feel an internal drive to be milked, particularly when milking has been abruptly stopped. While in conventional milking parlors this motivation may manifest as cows loitering near the holding pen or parlor entrance, Automatic Milking Systems (AMS) present a unique opportunity to observe this behavior more directly. In AMS, cows can initiate milking attempts voluntarily, making it possible to track how often cows seek access to milking after dry-off. Motivation to be milked may be observed as an increase in AMS refusals, or attempted milking visits. However, little research to date has evaluated cows' motivation to be milked after dry-off using behavioral indicators, such as AMS refusals. Understanding cows' motivation to be milked and how it may be driven by udder engorgement and pain responses provides insight into cows' affective state and assess whether current dry-off protocols meet their behavioral needs.

Due to the concerns around abrupt dry-off, gradual cessation of milking by stepping down daily milking frequency has emerged as a promising strategy to reduce udder pressure and improve cow comfort at dry-off. Gradual reduction in milking frequency has been shown to reduce milk yield (Gott et al., 2017), udder engorgement (Larsen et al., 2021), and milk leakage at dry-off (Franchi et al., 2022) as compared to abrupt dry-off. These benefits have been found without negatively affecting energy balance at dry-off (Larsen et al., 2021) or health disorders during subsequent dry period (Gott et al.,2017). Most studies on dry-off have been conducted in conventional milking parlors, where the implementation of gradual dry-off can be challenging due to space or labor constraints for managing late-lactation cows with lower milking frequencies than the rest of the herd. Automatic milking systems, which enable individualized control of cow milking frequencies based on programmable parameters, offer a unique opportunity to facilitate gradual dry-off with minimal additional labor (Prescott et al., 1998; Zobel et al., 2013). Despite the potential of AMS to support individualized dry-off strategies, little is known about how cows are impacted by gradual and abrupt methods in these milking systems, highlighting the need for further research in this context.

The objectives of this study were to evaluate the effects of a two-week gradual dry-off protocol compared to a conventional abrupt dry-off in an AMS on milk production, pre and post dry-off milking robot visits, lying and activity behavior, and udder morphological characteristics and pain reception indicators. We hypothesized that cows subjected to a two-week gradual reduction in milking frequency before scheduled dry-off would experience a gradual decline in milk yield, thereby reducing udder engorgement, milk leakage, pain and discomfort, as well as reduced motivation to be milked after dry-off.

MATERIALS AND METHODS

Animals, Housing, and Feeding Management

This study was conducted at the Pioneer Dairy Farm, University of Wisconsin–Platteville, Platteville, WI, from June 2024 to May 2025. The study procedures received approval from the University of Wisconsin–Platteville Institutional Animal Care and Use Committee (IACUC; protocol ID#0515-2024).

Thirty Holstein dairy cows (primiparous = 10, multiparous = 20) were enrolled 74 ± 3 d before their expected calving date and monitored until 5 d post dry-off. Inclusion criteria included cows with an average milking frequency greater than 2.3 milkings/d, which represented the herd's median daily milking frequency, from 89 to 99 d before expected calving. At the time of enrollment, cows were required to have an absence of intramammary infection as determined by somatic cell count (< 500,000 cells/mL, obtained using Dairy Herd Improvement testing records), and to have a locomotion score below 3 on a 5-point scale (Sprecher et al., 1997) as assessed by author EM. Farm staff ensured a minimum of 2 milkings/d by fetching cows to the robot until 74 d before calving.

Cows were housed in a single free-stall pen with 47 lying stalls and a rolling average of 46 cows (± 4.84, SD), equipped with a Lely Astronaut A5 AMS (Lely Industries, Maassluis, the Netherlands) in a free-flow configuration with up to 6 daily milkings allowed prior to enrollment. An ad libitum partial mixed ration (PMR) consisting of haylage, corn silage, corn, and a protein/mineral mix, formulated to meet the dietary requirements of late-lactation cows (National Academies of Sciences, Engineering, and Medicine, 2021) was provided in

RIC2Discover feed stations at the feed bunk (24 per pen; RIC2Discover, Insentec B.V., Marknesse, the Netherlands). Pellet concentrate was provided in the AMS during milking, and the allowance per visit was determined by daily milk yield and the estimated number of daily milkings. The AMS concentrate was formulated for lactating dairy cows (18.0% crude protein, 4.69% crude fat, and 0.87% calcium). Upon study enrollment, all cows were programmed to receive a maximum of 2.27 kg of concentrate daily in the AMS, regardless of treatment.

Experimental Treatments

Cows were enrolled on a rolling basis and assigned to an abrupt or gradual dry-off treatment (n = 15 per treatment). Treatments were balanced for DIM, 10 d average milk yield prior to enrollment, parity (primiparous vs. multiparous), and daily voluntary milking frequency (Table 1). Before study enrollment, to identify the cows in the herd who voluntarily milked the most frequently, cows were allowed a maximum of six milkings per day, with an optimal yield setting of 10.89 kg milk yield per visit. The minimum allowable time between visits was calculated using the Lely T4C software (Time-for-Cows, Lely Industries, Maassluis, the Netherlands) by dividing the expected daily yield by the optimal milk yield per visit setting, and dividing that value by 24 to determine the hours between permitted milking visits. Upon study enrollment, cows in the abrupt dry-off group were limited to a maximum of 2.8 milkings/d, which was the farm standard maximum allowed daily visits for lactating dairy cows.

Cows in the gradual dry-off treatment were dried-off in a step-down fashion based on their 10 -d milking frequency average before enrollment (91 to 81 ± 3 d before expected calving). At enrollment (-14 d), cows' 10 -d average milking frequency was rounded down to the nearest whole number. Then, allowable daily visits were incrementally reduced during the two week period before dry-off. A schematic representation of the gradual dry-off protocol is shown in Figure 1. Cows in both treatments were prevented from entering the AMS after the 2-wk study protocol (60 ± 3 d before their expected calving date). All study cows remained in the lactating pen for five days post-dry-off to continue data collection. On day six after dry-off, cows were moved to a dry cow facility.

Measurements

Body weight and condition. Cows were weighed once weekly -9, -2 and 5 d relative to dry-off, using an electronic scale while restrained in a manual chute (Q-Catch 54 Series, Arrowquip, Woodlands, Manitoba, Canada). The chute scale was calibrated weekly immediately prior to data collection. Body condition score (BCS) was assessed -9, -2 and 5 d relative to dry-off on a 5-point scale (1 = emaciated to 5 = obese; Sprecher et al., 1997) by 2 trained observers simultaneously and the average of their scores was used. All BCS observers (n = 9) were trained by the same trainer until they could identify 20 cows within 0.5 points of the trainer. Percent change in BCS and body weight was calculated between -9 to Day -2 d and -9 to 5 d relative to dry-off.

AMS measurements. The Lely software automatically recorded individual cow data, summarized daily from 00:00 to 23:59 h. Daily AMS measures included milk yield (kg/d) and the number of successful milkings, unsuccessful milkings, and refusals from the AMS. A successful milking was defined as an instance in which a cow visited the AMS after her required minimum interval time had elapsed and was milked without any technical failure. An unsuccessful milking occurred when a cow visited the AMS after meeting the time eligibility criteria but was not milked, typically due to a system or attachment failure. Only one cow in the study (gradual treatment) experienced an unsuccessful milking event. Refusals were defined as visits in which a cow attempted to access the AMS before she was eligible for milking according to the minimal time interval. Before dry-off, the numbers of successful milkings, unsuccessful milkings, and refusals were recorded and summed daily for each cow. After dry-off for the 5 d cows remained in the lactating pen, refusals from the AMS were summarized by cow.

Lying and activity behaviors. Lying and activity behaviors were monitored using IceQube accelerometers (IceRobotics Ltd., Stirling, Scotland) attached to the right hind leg of each cow at enrollment. These devices, previously validated for measuring dairy cow activity (Finney et al., 2018), record at a frequency of 4 Hz and summarize data into 15-min intervals. The associated software generates daily summaries of lying time (min/d, converted to h/d for analysis to ease interpretation), number of lying bouts (number/d), average lying bout duration (min/bout), and step count (steps/d). An algorithm within the software also calculates a daily motion index based on step count and overall acceleration (Gladden et al., 2020). Data were downloaded three times per wk and summarized daily for each cow through the CowAlert Research Module (IceRobotics Ltd., Stirling, Scotland).

Udder measurements. Evaluations of the udder were conducted -9, -2, 0, 1, 3, and 5 d relative to dry-off. Assessments included characterization of milk leakage, udder firmness, udder pain response during palpation, and udder dimensions. Assessments were conducted by 9 trained observers. Observers were trained using a combination of 20 annotated training videos and live on-farm sessions . Following training, each observer was tested individually against a gold standard scorer (trainer EM) using a separate set of 34 video recordings. The training videos consisted of recordings of the (trainer EM) conducting an udder assessment as described below. For milk leakage, all observers achieved 100% agreement with the trainer (EM), resulting in a Cohen's Kappa of 1.00, indicating perfect reliability. For udder firmness during palpation, observers achieved a mean Cohen's Kappa of 0.78 ± 0.04 (SE; range: 0.75 to 0.88), reflecting substantial agreement. For udder pain assessment, the mean Cohen's Kappa was 0.84 ± 0.03 (SE; range 0.71 to 0.90), indicating substantial to almost perfect agreement across observers.

During each assessment, cows were placed in a chute and all measures were recorded while cows stood with all four hooves flat on the ground to ensure a consistent and safe position for udder examination. Milk leakage was recorded as present (1) or absent (0) during chute examinations, prior to the evaluator touching the udder so as not to stimulate the udder. Milk leakage was scored as present if milk was observed actively dripping or seeping from one or more teats without stimulation and absent if no milk was observed during visual inspection.

Udder firmness was evaluated through manual palpation following the method described by Franchi et al. (2022). To begin the assessment, a trained evaluator gently stroked the cow's flank for 5 to 10 s to allow the animal to become aware of their presence. The evaluator then assessed each udder quarter by visual inspection and palpating the rear of the udder to assess tissue characteristics, including the ease of separating quarters, quarter mobility, compressibility, and teat tip flexibility during palpation. Udders were scored as: soft (easily separable between quarters, moved readily upon touch, was compressible, and the teat tip followed the direction of palpation), normal (similar to soft but slightly firmer characteristics, with moderate tone and resistance), firm (little movement between quarters, greater resistance to compression, and a teat tip that did not follow palpation), or very firm (tense, non-compressible, and completely resistant to palpation, with no quarter movement and a rigid teat tip). For analysis, udder scores were collapsed into two categories: normal (soft and normal scores) and firm (firm and very firm).

Avoidance behavior in response to palpation has been used as an assessment of udder pain (Franchi et al., 2022). In this study, udder pain during palpation was evaluated immediately following the udder firmness assessment. Pain sensitivity was evaluated by gently palpating the upper and lower rear quarters of each udder half using 2 fingers to a depth of 2-3 cm for approximately 3 s. A pain response was recorded as a binary variable (present = 1 or absent = 0) based on the cow's behavioral reaction to palpation. A response was classified as present if the cow exhibited one or more of the following behaviors: leg lifting or kicking (a quick upward or backward movement of one or both hind legs), tail flicking (a rapid and forceful swish of the tail either upward or side-to-side), and withdrawal (the cow shifting her weight away from the evaluator's hand or stepping laterally or forward in response to palpation).

Manual measurement of teat distance was measured between the teat canal openings in each direction (front-to-front, right-to-right, back-to-back, and left-to-left) using a flexible, non-elastic measuring tape, and recorded in cm rounded to the nearest mm. Distance between each pair of teats was summed to calculate the total distance between teats (cm) and used as an assessment for udder engorgement (Larson et al. 2021).

A 3D camera system was implemented to capture additional udder dimensions and conformation, including volume, surface area, and distance between front-rear and right-left teats. To obtain these measurements, a ventral view of the udder was recorded for 10 s. An Intel RealSense 455 camera (30 frames/s, 848 x 480 pixels, capturing RGB and depth images concurrently) was held 10 cm from the ground via a block attached to the camera. To calculate udder dimensions, frames were extracted from the video recordings using custom python script and analyzed for image quality. A frame classification convolutional neural network (CNN) model (Montes et al., 2024) was used for image quality analysis, which classifies frames as good if the udder is portrayed and not occluded, and as bad otherwise, retaining those with >0.7 predicted probability of being good. Retained frames were then visually assessed, misclassified frames were discarded, and 10 good frames for each cow-timepoint were randomly selected, or all good frames if <10 were retained. A CNN segmentation model (Montes et al., 2024) was used to identify the udder region within each frame. To locate and identify each of the teats within the udder region, a key point detection CNN model (Montes et al., 2024) was used.

Next, the depth image, in which each pixel value represents the distance between the camera and the object in the scene, was used to reconstruct the 3D shape of the udder. Along with this, the camera intrinsics was used, a set of parameters that describe the camera's internal characteristics such as focal length and field of view. These intrinsics define how the 3D world is projected onto a 2D image and can be used to reverse that process, converting 2D image data into 3D coordinates. Using both the depth image and camera intrinsics, a 3D point cloud of the udder was generated. To remove points that did not belong to the udder, a manually fitted a plane was applied to the points on the border of the udder and removed the points lying above the plane. With a clean point cloud, functions from the Python library Video were used to estimate the volume (dm^3, i.e., L) and surface area (cm^2) of the udder. The distance between pairs of teats (cm) was calculated as the Euclidean norm of the line between their corresponding location in the point cloud.

Total teat distance measured by image analysis and hand-assessed measurements were compared, and observed a strong positive association ($R^2 = 0.764$) (Appendix 1). However, the slope (0.70 ± 0.03) was significantly different from 1 ($F_{(1,158)} = 96.52$, $P < 0.001$), and the intercept (6.56 ± 1.67) was significantly different from 0 ($F_{(1,158)} = 15.45$, $P = 0.0001$), indicating a systematic bias where the image-based measurements tended to underestimate compared to hand scores at higher values and overestimate at lower values.

Statistical Analysis

All statistical analyses were performed using R (Version 4.x; R Core Team, 2025) with the *nlme* package to fit linear mixed-effects models for all outcomes, including continuous, binary, and count data. This approach is appropriate for biological studies with relatively small sample sizes (Salinas Ruíz et al., 2023). The cow was treated as the experimental unit, and repeated measures were accounted for using appropriate correlation structures. Model fit was assessed through visual inspection of residual versus fitted value plots and quantile-quantile (Q–Q) plots. Estimated marginal means (EMMs) and pairwise comparisons were obtained using the emmeans package, with Tukey or Sidak adjustments applied to control for multiple comparisons. EMMs were plotted over time with emmip. Statistical significance was declared at $P < 0.05$ and tendencies when $0.05 \leq P < 0.10$.

Analyses were conducted separately for the pre-dry-off period (-15 to -1 d), and the post-dry-off period (0 to 5 d), unless otherwise specified. For lying behavior, steps, and motion index, the pre-dry-off period included Days −13 to −1. For count and binary outcomes, including milk leakage, pain behaviors, udder firmness, as well as udder dimension measures (volume, surface area, teat distance), the pre-dry-off period measurements -9 and -2 d relative to dry-off and the post-dry-off period was 0, 1, 3, and 5 d relative to dry-off.

For each outcome, fixed effects included treatment (abrupt vs. gradual), day relative to dry-off (categorical), and their interaction (treatment × day). A random intercept for cow identity was included in all models, with an autoregressive (AR1) correlation structure applied across days to account for repeated measures. Parity was included as a fixed effect to control for variance.

RESULTS

Body Weight and Body Condition Score

The mean percent change (± SD) in body weight before dry-off from -9 to -2 d was 1.83 ± 1.08%, whereas the change across the entire study period from -9 to 5 d was 2.35 ± 1.18%. For body condition score, the mean percent change during those time periods was −0.21 ± 1.46% and −0.40 ± 1.14% respectively based on a 5 point scale.

Milk Yield

Overall, from −14 to −1 d relative to dry-off, cows dried off gradually produced 11.3% less milk in total compared to those abruptly dried-off (mean ± SD: 348.4 ± 100.06 vs. 392.9± 69.23 kg). When evaluating daily milk yield, a significant treatment × day interaction was observed ($F_{14, 392}$ = 20.50, $P < 0.001$; Figure 2), driven by cows in the gradual dry-off group producing significantly less milk than abruptly dried-off cows from day −5 to −1 before dry-off ($P < 0.02$). There was a main effect of day relative to dry-off ($F_{1, 14} = 40.13$; $P = 0.001$) with milk yield gradually decreasing in both treatments, however there was no main effect of treatment group ($F_{1, 24} = 1.17$; $P = 0.29$)

AMS Visits

Across the 14 d prior to dry-off, overall visit frequency to the AMS did not differ between treatment groups ($F_{1, 27} = 0.47$; $P = 0.50$; Figure 3A). However, a treatment × day interaction was detected ($F_{14, 392} = 2.6$; $P = 0.001$), gradual dry-off cows visited the AMS significantly more often than abrupt dry-off cows on day −7 d($P < 0.001$) and–6 d relative to dry-off ($P = 0.009$). In addition, day itself had a significant effect ($F_{14, 392} = 3.36$; P =0.001) with cows in both treatments visiting the AMS less frequently near the end of dry-off.

Following dry-off, a significant treatment × day interaction was detected for AMS visits ($F_{5,140} = 5.79$; $P < 0.001$; Figure 3B). Cows in the abrupt dry-off treatment visited the AMS more frequently than those in the gradual group on 0 and 1 d relative to dry-off (both $P < 0.001$), no differences between treatments were observed 2 to 5 d after dry-off ($P > 0.05$). Overall, cows in the abrupt treatment visited the AMS more frequently than those in the gradual group (LSM ± SE: 3.95 ± 0.67 vs. 1.89 ± 0.70 visits/d; $F_{1,27} = 4.74$; $P = 0.038$). Regardless of treatment, visit frequency declined over time post dry-off ($F_{5,140} = 23.60$; $P < 0.001$), peaking at 6.65 ± 0.70 visits on day 0 and dropping to 0.55 ± 0.70 visits by day 5.

Activity

Lying behavior. Before dry-off, there were no significant treatment differences in daily lying time, number of daily lying bouts, or lying bout duration (all $P \geq 0.70$; Figures 4A, 4C, 4E) or no effect of day relative to dry-off was detected (all $P \geq 0.33$; Figures X-x). Likewise, after dry-off, there were no significant treatment differences for daily lying time, number of daily lying bouts, or lying bout duration (all $P \geq 0.28$; Figures 4B, 4D, 4F). Although a significant effect of day relative to dry-off was detected for post-dry-off lying time ($P = 0.022$), pairwise comparisons showed no significant differences between specific days (all $P \geq 0.08$). No treatment × day interaction was detected (all $P \geq 0.69$) before or after dry-off.

Daily steps. The number of steps taken per day did not differ significantly between treatment groups prior to dry-off ($F_{1,23} = 0.61$; $P = 0.44$; Figure 5A). Day relative to dry-off significantly affected step count ($F_{12,249} = 4.15$; $P < 0.001$). Daily steps gradually increased, peaking around day –2, and then dropped sharply on day –1. No treatment × day interaction was detected ($F_{12,249} = 0.58$; $P = 0.85$).

Following dry-off, step count remained similar between cows in the gradual and abrupt dry-off groups ($P = 0.86$; Figure 5B). Step activity decreased over time post dry-off ($F_{5, 105} = 5.81$; $P = 0.0001$). No treatment × day interaction was observed ($F_{5, 105} = 1.92$; $P = 0.86$).

Motion index. Motion index was not significantly different between cows in the gradual and abrupt dry-off treatments prior to dry-off ($F_{1, 23} = 0.76$; $P = 0.39$; Figure 5C). However, motion index was significantly affected by day relative to dry-off gradually increasing and peaking on day –2 before declining sharply on day –1 ($F_{12, 249} = 5.70$; $P < 0.0001$), with no significant treatment × day interaction detected ($F_{12, 249} = 63$; $P = 0.81$).

After dry-off, there was no difference in motion index between cows in the gradual and abrupt dry-off treatment groups ($F_{1, 23} = 0.01$; $P = 0.91$; Figure 5D). Motion index declined over time ($F_{5, 105} = 4.83$; $P = 0.0005$), but no significant effects of treatment × day interaction ($F_{5, 105} = 0.20$; $P = 0.96$) were observed.

Milk Leakage and Udder Firmness Assessment

No cows exhibited milk leakage prior to dry-off; therefore, statistical analysis of pre-dry-off leakage was not performed. After dry-off, there was a significant treatment × day interaction ($F_{14,392} = 2.6$; $P = 0.001$; Figure 6A), with cows in the abrupt dry-off group leaking significantly more milk than those in the gradual group on 1, 3, and 5 d after dry-off (Day 1, $P = 0.01$; Day 3, $P = 0.001$; Day 5, $P = 0.028$). There was also a significant main effect of day ($F_{3,84} = 6.90$; $P < 0.001$), with leakage increasing after dry-off compared to day 0 and a significant effect of treatment ($F_{1,27} = 14.11$; $P < 0.001$), with abrupt cows being more likely to leak than gradual cows when averaged across all days (mean ± SE: 0.43 ± 0.06 vs. 0.13 ± 0.06; $P < 0.001$).

No significant differences were observed prior to dry-off for either udder firmness (treatment: $F_{1,27} = 1.55$, $P = 0.22$; day: $F_{1,28} = 0.00$, $P = 1.00$; treatment × day: $F_{1,28} = 1.30$, $P = 0.26$; Figure 6C) or pain response upon palpation (treatment: $F_{1,27} = 0.11$, $P = 0.74$; day: $F_{1,28} =$

0.08, $P = 0.79$; treatment × day: $F_{1,28} = 0.08$, $P = 0.79$; Figure 6B).

Following dry-off, udder firmness varied significantly over time (Day effect: $F_{3,84} = 3.85$, $P = 0.01$), but neither treatment ($F_{1,27} = 0.59$, $P = 0.45$) nor the treatment × day interaction ($F_{3,84} = 1.01$, $P = 0.39$) influenced firmness.

Pain response upon palpation did not differ significantly between treatment groups after dry-off ($F_{1,27} = 0.26$, $P = 0.61$), and the treatment × day interaction was nonsignificant ($F_{3,84} = 1.89$, $P = 0.14$). There was a trend toward a decrease in pain response over days post-dry-off ($F_{3,84} = 2.58$, $P = 0.059$).

Udder Dimensions

Before dry-off, total Euclidean teat distance as measured by image analysis did not differ significantly between treatments ($P = 0.45$; Figure 7A), day relative to dry-off ($P = 0.08$) or treatment × day interaction ($P = 0.85$). After dry-off, there was a significant interaction between treatment and day relative to dry-off ($P < 0.001$; Figure 7B). On the day of dry off (0 d), teat distances were similar between groups ($P = 0.87$), but cows in the abrupt dry-off treatment had significantly greater teat distances than cows in the gradual dry-off treatment on 3 d ($P = 0.025$) with similar tendencies on 1 and 5 d ($P = 0.066$ and $P = 0.088$, respectively). There was a significant main effect of day ($F_{3, 66} = 16.90$; $P < 0.001$), with Euclidean teat distance peaking on day 1 post dry-off before gradually decreasing, however there was no treatment main effect, ($P = 0.15$).

Before dry-off, udder volume did not differ significantly between treatment groups ($P \geq 0.50$; Figure 7C), day relative to dry-off ($P \geq 0.44$), or treatment × day interaction ($P \geq 0.33$) for either measurement. After dry-off, udder volume increased significantly over time ($P < 0.001$; Figure 7D), and a tendency for a treatment × day interaction was detected ($P = 0.05X$), but no pairwise comparisons between treatment group within day reached statistical significance (all $P > 0.14$). For udder surface area there was no effect of treatment ($P = 1$; Figure 7E, 7F) or treatment × day interaction ($P = 0.15$).

DISCUSSION

The purpose of this study was to evaluate the effects of a gradual versus abrupt dry-off protocol using an AMS on cow behavior, udder characteristics, and welfare outcomes before and after dry-off. Cows dried off gradually experienced a greater reduction in milk yield prior to dry-off and had fewer AMS refusals afterward, suggesting lower motivation to be milked than cows dried off abruptly. Gradually dried-off cows also had reduced udder expansion, as measured by an increase in teat distance, as well as fewer instances of milk leakage on d 1, 3, and 5 after dry-off, than abruptly dried-off cows. There were no treatment differences in other udder dimensions, lying behavior, or pain-related responses.

Responses Before Dry-off

Cows managed using a gradual dry-off protocol experienced greater reductions in daily milk yield during the final two weeks of lactation compared to those dried off abruptly. Specifically, cows in the gradual group had a total milk yield of 11% less than those in the abrupt group over this period, driven by reductions on d -5 to -1 before dry-off. This finding supports our prediction that reduced milking frequency would promote a decline in milk production leading up to dry-off and aligns with previous research demonstrating this effect in late lactation (Gott et al., 2017; Franchi et al., 2022). The idea of intentionally reducing milk yield before dry-off may appear concerning from a management standpoint; however, an 11% reduction, equivalent to drying cows off a couple days sooner, is relatively minor and may be outweighed by the potential welfare benefits. Additionally, previous studies suggest that higher milk yield at dry-off was associated with higher somatic cell score (SCS) in the following lactation, affecting cows' performance (Gott et al., 2017). Thus, while there is a modest short-term cost to gradual dry-off, it may be offset by longer-term gains in productivity, health, and longevity.

Prior to dry-off, there were no significant differences in lying behavior or pain response between cows in the gradual and abrupt dry-off treatment groups. Lying time, number of lying bouts, and average bout duration remained consistent across treatments, suggesting that the gradual reduction in milking frequency did not adversely affect resting behavior. On average, cows spent approximately 11 to 13 hours per day lying down, which aligns with expected values reported in healthy lactating dairy cows (Chapinal et al., 2014; Tucker et al., 2009). Similarly, activity measures such as step count and motion index did not differ by treatment. These findings indicate that neither strategy disrupted typical locomotor or rest patterns during the transition period, supporting the view that a gradual protocol can be implemented without negatively impacting cow comfort or behavior.

Udder characteristics measured before dry-off (firmness, volume, surface area, and teat distance) did not differ significantly between treatment groups. While cows in the gradual group produced less milk during this period, external signs of udder pressure or expansion were not detectably different. Importantly, the lack of difference in udder firmness suggests that gradual cessation did not increase udder discomfort prior to the final milking. Additionally, no milk leakage was observed in either group before dry-off, suggesting that gradual cessation did not put cows at greater risk of leakage before dry-off.

Responses After Dry-Off

Motivation to be milked. When intramammary pressure builds, it may not only cause discomfort (O'Driscoll et al., 2011) but also reinforce the behavioral drive to access the milking unit, particularly in high-yielding cows (Zobel et al., 2013). Following dry-off, cows in the abrupt treatment group attempted to access the milking robot more often than gradually dried-off cows. Attempted visits peaked on the day of dry-off and declined thereafter, a pattern likely reflecting persistent milking motivation in cows abruptly denied milking. In contrast, gradually dried-off cows had fewer refusals than cows dried off abruptly, supporting the hypothesis that cows managed with a gradual dry-off experience were less motivated to be milked after milking permissions are removed. As described by Spinka (2006) and Friend (1989), the prevention of internally driven, ethological behaviors, such as milking in high-producing cows can negatively affect animal welfare. The frustration of not being milked is shown by increased unsuccessful attempts and is in alignment with previous research that has similarly shown that reducing milking frequency in a parlor before dry-off leads to cows spending less time near the milking area, suggesting lower motivation to be milked (Zobel et al., 2013).

Indicators of pain or discomfort. We did not detect significant differences in pain-related behaviors between treatment groups. Overall pain response to udder palpation remained low before and after dry-off and did not differ significantly by treatment. These results may also reflect limitations in our measurement context, as behavioral scoring was performed in a restraint chute setting where cows may not have had full freedom to express subtle discomfort-related responses.

On average, healthy lactating dairy cows spend approximately 11 to 13 hours per day lying down (Tucker et al., 2009). In our study, however, post-dry off average lying time was slightly lower than this range. One possible explanation is increased time spent waiting outside the AMS, particularly for high-producing cows; future studies should examine waiting behavior to better understand how AMS access affects lying opportunity during the dry-off transition. Waiting behavior has been evaluated only in parlor-milking contexts post-dry off (Zobel et al., 2013), or in AMS systems during the lactating period (Solano et al., 2022). Despite these potential disruptions to routine, lying behavior including daily lying time, number of daily lying bouts, and lying bout duration did not differ significantly between abruptly and gradually dried-off cows. While we hypothesized that cows experiencing greater udder distension would spend more time standing or showing signs of restlessness, the lack of significant behavioral differences suggests that either discomfort was minimal or not sufficiently severe to alter lying patterns. These findings align with those of Rajala-Schultz et al. (2018), who reported that high milk yield before dry-off, rather than treatment, was the primary factor associated with reduced lying time and shorter lying bouts. Prior studies have reported changes in lying behavior associated with udder discomfort and skipped milkings (e.g., Stefanowska et al., 2000; Chapinal et al., 2014). Additional research using more sensitive behavioral metrics or continuous monitoring of more specific behaviors (e.g., AMS waiting time, interaction with the AMS) may help to clarify whether cows experience mild but undetected discomfort during the dry-off process.

Udder characteristics. Our hypothesis that gradually dried-off cows would show fewer signs of milk accumulation in the days following dry-off was partially supported by our results. Udder firmness was measured as another potential indicator of discomfort relating to pressure build-up shortly after the final milking (Bertulat et al., 2013). Post-dry-off udder firmness was significant effect of day with increased udder firmness on d 1 after dry-off, regardless of treatment. The lack of consistent treatment differences could be due to individual variation in udder conformation or limited sensitivity in our manual scoring system to detect subtle changes. Manual palpation scoring, while cost-effective, is inherently subjective and may lack the precision required to quantify minor changes in udder pressure or discomfort (Franchi et al., 2022). Alternative methods to assess udder firmness or internal pressure include the use of dynamometers and emerging 3D imaging technologies. Dynamometers offer a more quantifiable assessment of internal udder pressure (Rees et al., 2014), although this technique also requires training, and may be more costly due to the specialized equipment, which requires calibration. Three-dimensional imaging is a promising non-invasive approach to monitoring changes in udder shape, symmetry, and volume. This technology allows for repeated measurements without handling the animal, and may be particularly useful for tracking dynamic changes in udder size following dry-off (Montes et al., 2024)

Teat-to-teat distance, udder surface area, and volume, measured by 3D images, increased significantly after dry-off, with greater teat spacing observed in abruptly dried-off cows on 1 and 3 d after dry-off. This visible udder distension was likely driven by milk accumulation and increased intramammary pressure. Increased udder distension is consistent with previous reports that intramammary pressure rises and peaks within the first two days after dry-off (Bertulat et al., 2013). Similar patterns of udder distension have been previously quantified using both palpation scores and dynamometers (Zobel et al., 2015; Franchi et al., 2022). The fact that abruptly dried-off cows exhibited significantly greater teat spacing than gradually dried-off cows on 3 d suggests that gradual cessation of milking may mitigate some of the physical expansion associated with engorgement. However, regardless of treatment, we found that udder surface area and volume showed similar patterns following dry-off, with both metrics increasing from d 0 to 1. After d 1, gradually dried-off cows began to decrease, whereas abruptly dried-off cows continued to increase until d 3 before decreasing by d 5. These increases suggest that physical expansion of the udder occurred regardless of dry-off strategy and may reflect tissue distension associated with retained milk.

Milk leakage. The instances of cows leaking milk on d 1, 3, and 5 after dry-off were significantly reduced with gradual dry-off, consistent with previous research indicating that high-producing cows are at elevated risk for milk leakage and intramammary infections when milking is stopped abruptly (Dingwell et al., 2004; Zobel et al., 2015). Future studies should consider more precise methods to assess milk leakage, such as continuous visual monitoring in the home pen or automated udder imaging, to better capture timing, duration, and volume of leakage events. In addition, animals should be monitored in their next lactation for intramammary infections to evaluate long-term effects of milk leakage on udder health. Animals should also be evaluated for milk yield and longevity in the following lacation This approach merits further examination, and future work could evaluate the animal into their next lactation to determine changes in milk yield, mastitis rates, and longevity

Body Weight and Condition. As part of the experimental design, cows were housed in the AMS for 5 d post dry-off to determine their motivation to access the AMS. During this time, cows remained on a lactating cow diet, which is not ideal for cow health (National Academies of Sciences, Engineering, and Medicine, 2021). To monitor the potential for over-conditioning under these conditions, we descriptively tracked changes in body weight and body condition score. No cows exhibited changes in body condition greater than 0.75 (on a 5 point scale) which would be considered concerning by animal care staff.

CONCLUSION

This study demonstrates that AMS can support gradual dry-off protocols that effectively reduce pre-dry-off milk yield and post-dry-off motivation to be milked, as evidenced by fewer AMS visits. Gradually dried-off cows exhibited less udder engorgement after dry-off, as indicated by Euclidean teat distance, suggesting lower intramammary pressure. As well as fewer instances of milk leakage following dry-off. No significant treatment differences were observed in pain-related behaviors, or lying patterns, though subtle effects may have been undetected due to low behavioral response to udder palpation, likely influenced by the restraint of the chute limiting cows' ability to freely express discomfort, and limited sample size. These findings indicate that gradual cessation of milking can ease the transition to the dry period without compromising cow comfort or behavior. This study highlights the novel integration of gradual dry-off within an AMS framework, demonstrating how automated systems can facilitate welfare-conscious management without requiring significant labor input.

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Chapter 3- CONCLUSION

This research highlights the potential of using automatic milking systems (AMS) to facilitate gradual dry-off protocols that are both welfare-oriented and operationally feasible. Compared to abrupt dry-off, gradual reduction in milking frequency led to lower milk yield leading up to dry-off, reduced milking motivation post-dry-off (as indicated by fewer AMS refusals), less milk leakage and closer teat placement, suggesting reduced udder pressure and improved cow comfort. While no significant differences were found in lying behavior or pain responses, the use of AMS refusals as a novel indicator of milking motivation proved to be a valuable metric.

Additionally, measures of udder volume, surface area, and teat spacing supported the hypothesis that high producing cows will experience increased udder pressure in the days following dry off. Gradually dried-off cows exhibited narrower teat spacing post-dry-off, and while udder volume and surface area increased in both groups, there was only limited evidence suggesting treatment-related differences in overall udder measurements. Importantly, this study provided novel data demonstrating that measurements from 3D imaging closely aligned with traditional manual teat distance measurements, validating 3D imaging as an accurate and reliable tool for evaluating udder physiology during dry-off. These findings collectively suggest that gradual dry-off using automatic milking systems can improve cow welfare outcomes without requiring additional labor, making this a potentially promising and scalable strategy for farms already equipped with robotic milking systems.

Despite the encouraging results, several limitations of the study must be acknowledged. First, the study was conducted at a single university-affiliated research facility with a relatively small sample size (n = 30). While treatments were balanced by parity and production level, results should be interpreted cautiously when generalizing to larger herds or commercial operations. Additionally, due to the limited number of cows and the capacity of the research pen, data collection occurred on a rolling basis across the calendar year. While this approach may have introduced some environmental or seasonal variability, treatment groups were enrolled in a balanced manner over time, ensuring that any such variation was equally distributed. This design also enhances external validity by allowing results to be more representative of varying seasonal conditions.

Another important limitation is the short observation window following dry-off. While continuous daily monitoring over the full dry period would have provided more insight, data were collected only on three days across the five-day post-dry-off period due to facility access and scheduling constraints. This limits our ability to detect longer-term effects, such as the onset of clinical mastitis, gradual changes in udder health, or sustained behavioral adaptations. Future studies should incorporate daily behavioral and physiological monitoring throughout the entire dry period to capture potential delayed effects, including changes in lying behavior, teat condition, or udder firmness, as well as the development of intramammary infections. Future studies should incorporate a longer observation period with consistent daily data collection, which could strengthen interpretation of post-dry-off dynamics. Furthermore, the present study did not include physiological indicators of metabolic status, which could have provided additional context on cow health during this transition. Including measures such as non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), and cortisol concentrations in future work would help assess whether gradual dry-off affects metabolic load, energy balance, or stress levels in cows. In the absence of such measures, we cannot draw conclusions about whether this strategy avoided metabolic stress or changes in energy balance.

In terms of methodology, animal-based welfare indicators such as pain responses and lying behavior did not significantly differ between treatments. This could reflect a true lack of difference, or it may highlight limitations in the sensitivity of our behavioral measurements and the short duration of post-dry-off monitoring. Since assessments of the udder were conducted in a chute, cows may not have been able to fully express behaviors indicative of discomfort or pain, potentially masking subtle treatment effects. The lack of difference in lying time may indicate that any discomfort experienced by cows was minimal or not severe enough to alter resting behavior. Alternatively, lying patterns could have been more strongly influenced by individual cow factors such as milk yield before dry-off, aligning with observations by Rajala-Schultz et al. (2018), who identified high milk production as a primary predictor of reduced lying duration and shorter lying bouts.

Moving forward, several avenues for future research are warranted. Longer-term studies following cows through the entire dry period and into the next lactation would be valuable for understanding the full impact of gradual dry-off on health, productivity, and welfare. Additional research should also explore the effects of combining gradual dry-off with other strategies such as feed restriction or dopamine agonist use, while carefully considering potential welfare trade-offs. Moreover, the integration of motivation testing methodologies (e.g., push-gate or operant tasks) could further illuminate the affective experiences associated with different dry-off strategies. An especially novel approach would be to evaluate a voluntary dry-off model, in which cows are allowed to self-regulate cessation of milking within an AMS. Unlike conventional systems, AMS does not operate on fixed milking times (e.g., twice daily) but rather monitors hours since the last milking on a per-cow basis. This enables a more dynamic and individualized approach to managing dry-off, allowing for the gradual reduction in milking frequency to be based on each cow's recent milking behavior and production level. This design could offer a unique window into cow preferences and internal motivations during the dry-off period, while potentially improving welfare through a more individualized approach.

Lastly, while this study was conducted in an AMS environment, many commercial dairies still operate with conventional milking parlors. Research is needed to develop labor-efficient protocols for gradual dry-off that are compatible with conventional systems. The potential for 3D imaging technology to monitor udder changes also deserves further refinement and exploration, particularly its use in real-time on-farm applications.

In conclusion, this study demonstrates that AMS can be used to implement gradual dry-off protocols that reduce milking motivation and udder engorgement. These findings suggest that gradual dry-off is a promising, welfare-focused alternative to abrupt cessation of milking, and opens new avenues for innovation in dairy cow management during the critical transition to the dry period. By including measures from all three of Fraser's circles, this project gave a broad and unique look at cow welfare during dry-off. Even if not all measures showed treatment differences, this well-rounded approach strengthens the findings and highlights the value of looking at welfare in a holistic way.

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TABLES AND FIGURES

Table 1. Descriptive statistics for treatment group characteristics at enrollment (15 d before dry-off). Cows assigned to the abrupt (n = 15) and gradual (n = 15) dry-off treatments did not differ significantly in average daily milking frequency, average daily milk yield, or DIM during the 10 d prior to enrollment. Parity distribution (primiparous vs. multiparous) was also balanced between groups. Values are presented as mean ± standard deviation except where otherwise specified. Statistical comparisons were made using t-tests for continuous variables and a chi-square test for parity.

Treatment	Abrupt n=15	Gradual n=15	t / χ^2	*P-value*
10 d average milkings/day	3.8 ± 0.2	4.0 ± 1.5	0.30	0.77
10 d average milk production (kg/d)	30.8 ±8.2	29.5 ± 5.0	0.20	0.84
DIM	317 ± 48	295 ± 34	1.44	0.16
Primiparous (10)	6	4	0.15[1]	0.70
Multiparous (20)	9	11		

[1]Chi-square test for parity distribution between treatment groups.

Figure 1. Gradual dry-off protocol based on the 10 d daily average milking frequency prior to enrollment. Cows assigned to the gradual dry-off treatment had their allowable daily automatic milking system visits reduced incrementally over a 14 d period prior to dry-off based on their baseline milking frequency.

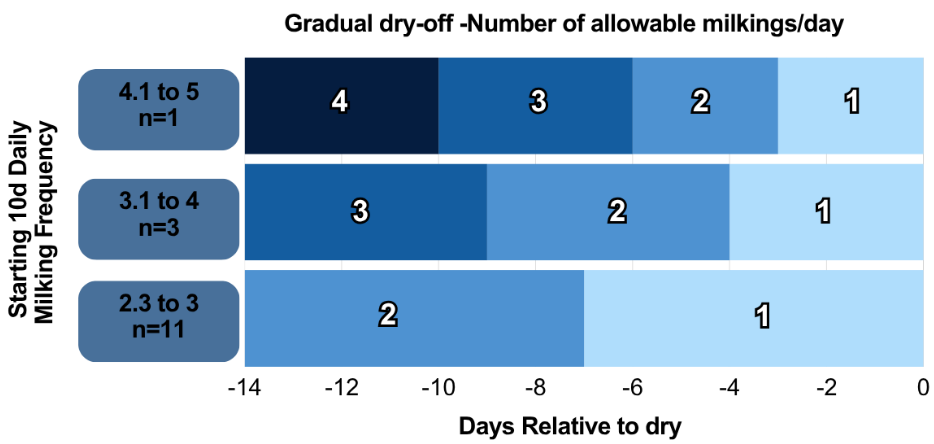


Figure 2. Daily milk yield (kg/d) from -15 to -1 d before dry-off for cows assigned to either the abrupt (red line and circles, n= 15) or gradual (blue line and triangles, n=15) dry-off treatment over the 15 d leading up to scheduled dry-off (-15 to -1 d; 60 ± 3 d before expected calving date). Least squaremean values are shown with standard error bars. Statistical differences ($P < 0.05$) between treatments within day are indicated with asterisks.

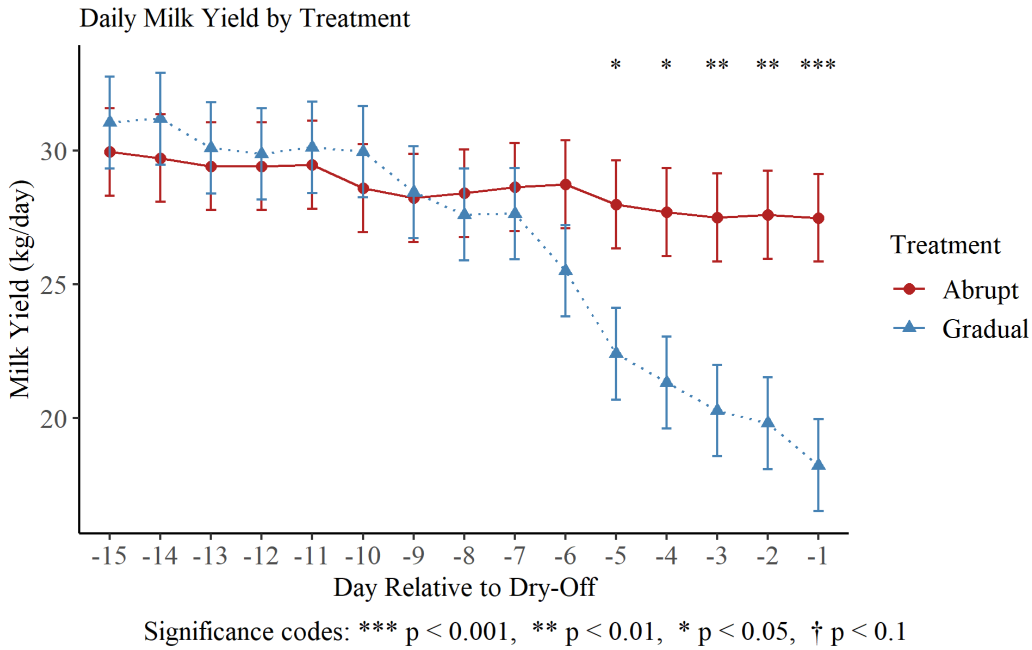


Figure 3. Daily number of Automatic Milking System (AMS) visits for dairy cows assigned to either the abrupt (red line and circles, n = 15) or gradual (blue line and triangles, n = 15) dry-off treatments. Panel (A) shows AMS visits in the 15 d before scheduled dry-off (-13 to -1 d; 60 ± 3 d before expected calving), and panel (B) shows visits in the 6 d following dry-off (0 to 5 d). Least square mean values are shown with standard error bars. Statistical differences (P < 0.05) between treatments within day are indicated with asterisks.

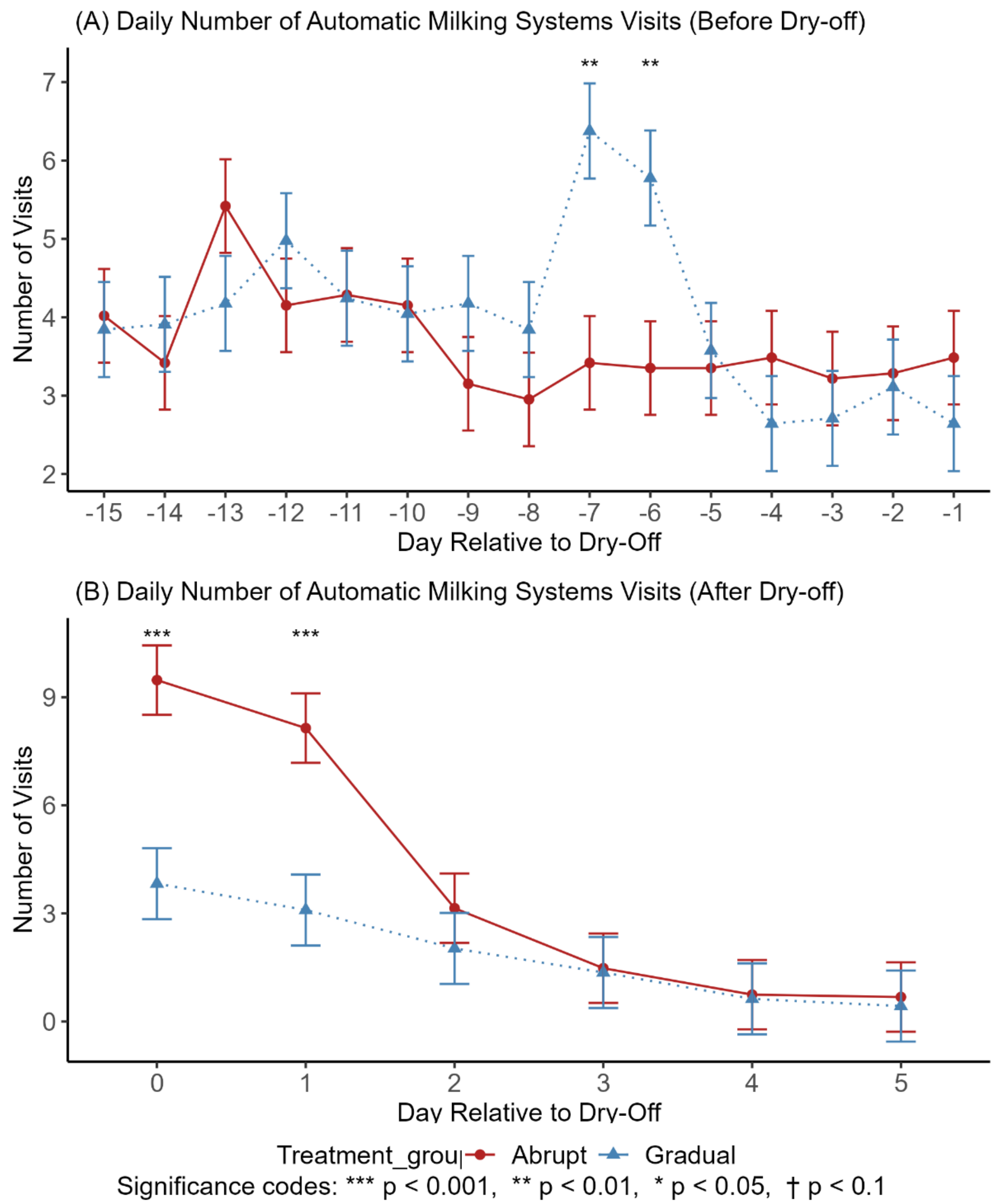


Figure 4. Daily lying behavior measures for cows assigned to either the abrupt (red line and circles, n = 15) or gradual (blue line and triangles, n = 15) dry-off treatments. Panels (A, C, and E) show average daily lying time (h), count of daily lying bouts, and average lying bout duration (m), respectively, during the 13 d before scheduled dry-off (-13 to -1 d; 60 ± 3 d before expected calving). Panels B, D, and F show the same measures during the 6 days following dry-off (0 to 5 d). Least square mean values are shown with standard error bars.

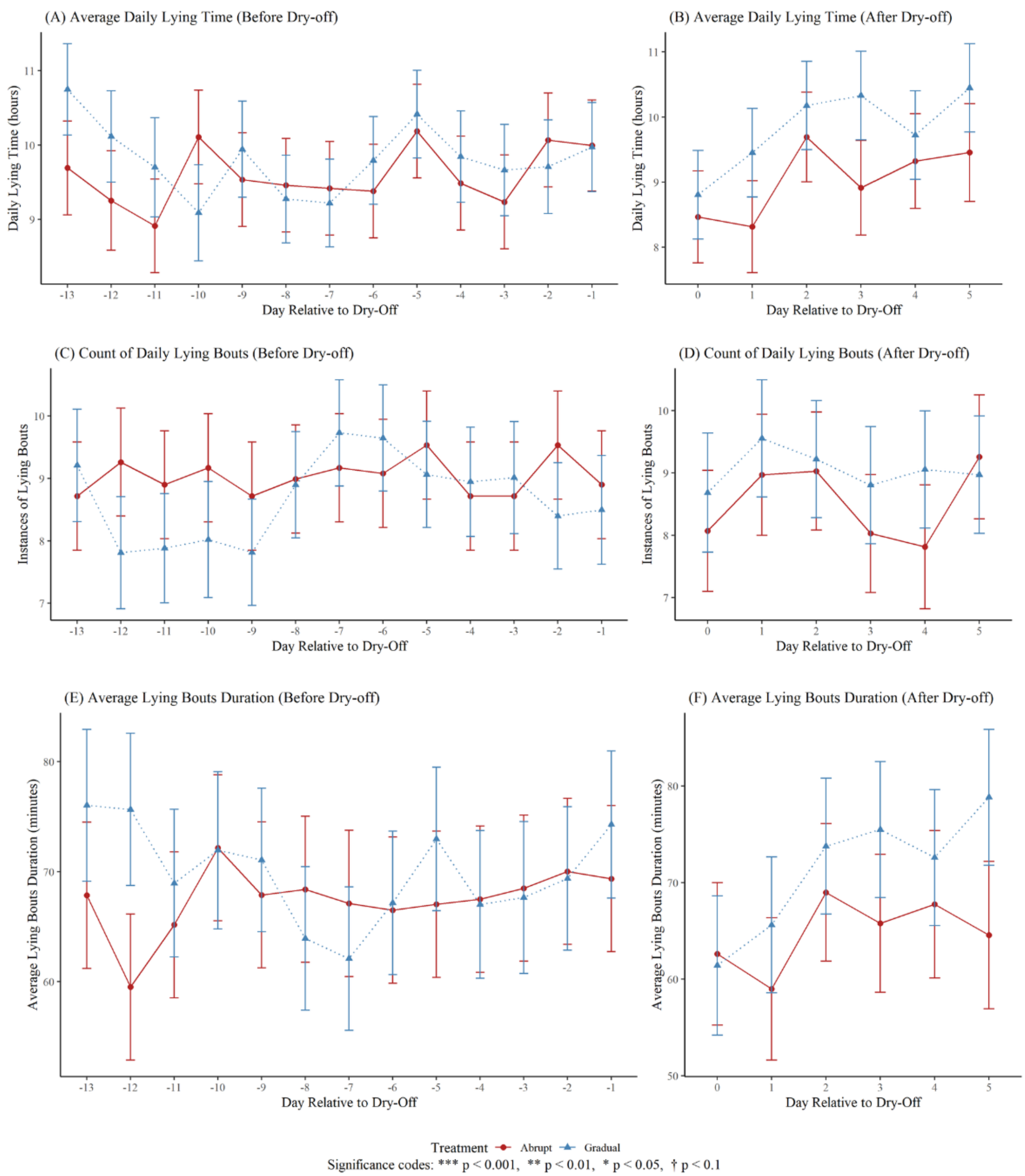


Figure 5. Daily sum of motion for cows assigned to either the abrupt (red line and circles, n = 15) or gradual (blue line and triangles, n = 15) dry-off treatments. Panels A and C show the steps and motion index respectively during the 13 d before scheduled dry-off (-13 to -1 d; 60 ± 3 d before expected calving), while panels B and D show steps and motion index respectively during the 6 days following dry-off (0 to 5 d). Least square mean values are shown with standard error bars.

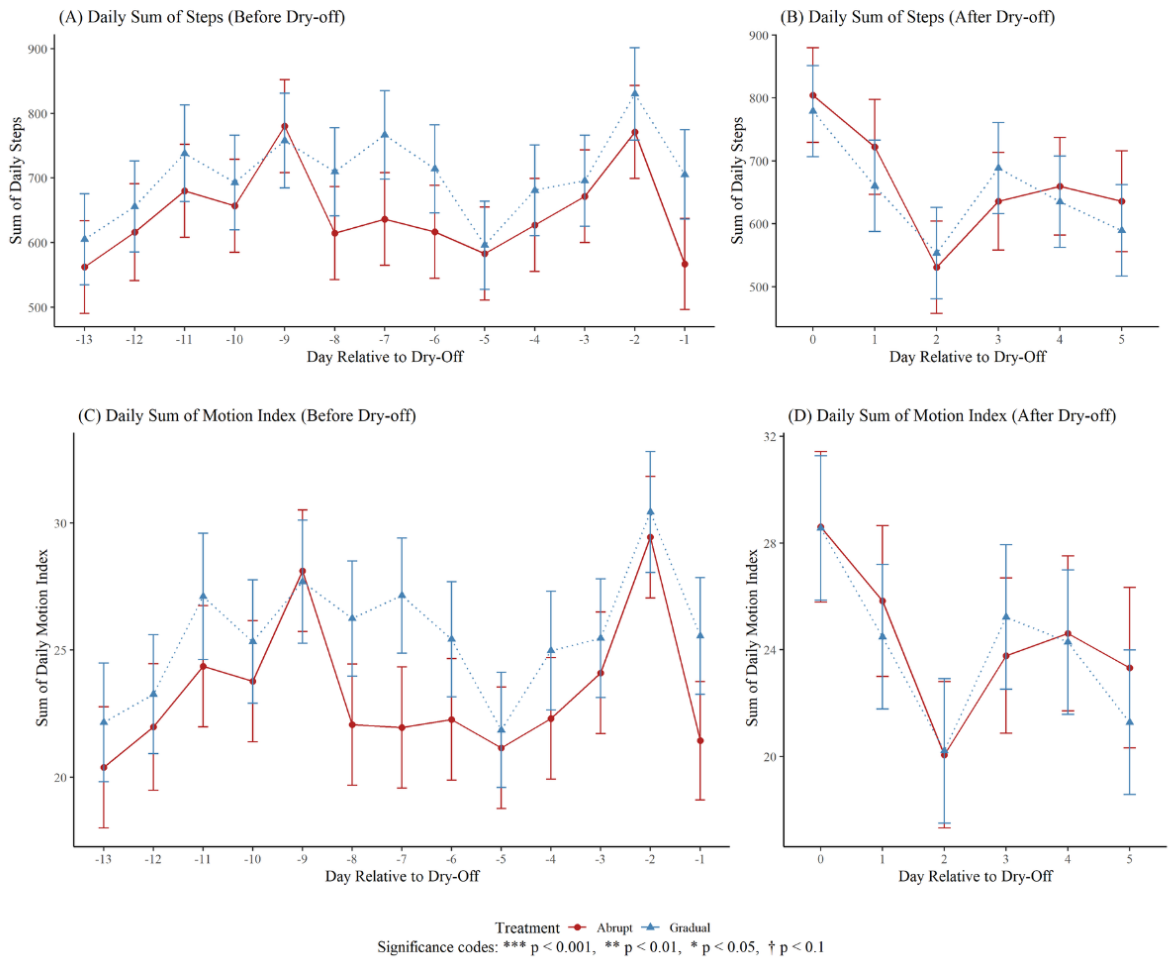


Figure 6. Indicators of milk leakage, udder discomfort and discomfort pressure in dairy cows before and after dry-off across abrupt and gradual treatment groups. (A) Number of cows exhibiting milk leakage on 0, 1, 3, and 5 d post-dry-off. (B) Number of cows displaying pain responses to udder palpation on -9 through 5 d relative to dry-off. (C) Udder firmness classifications (Soft, Normal, Firm, Very Firm) on each observation day for both treatments. Colored bars represent proportions of cows within each firmness score category, and overlaid lines indicate the proportion of cows classified as "Soft and Normal" or "Firm and Very Firm" in each group. Abrupt dry-off cows are shown in red and gradual dry-off cows in blue.

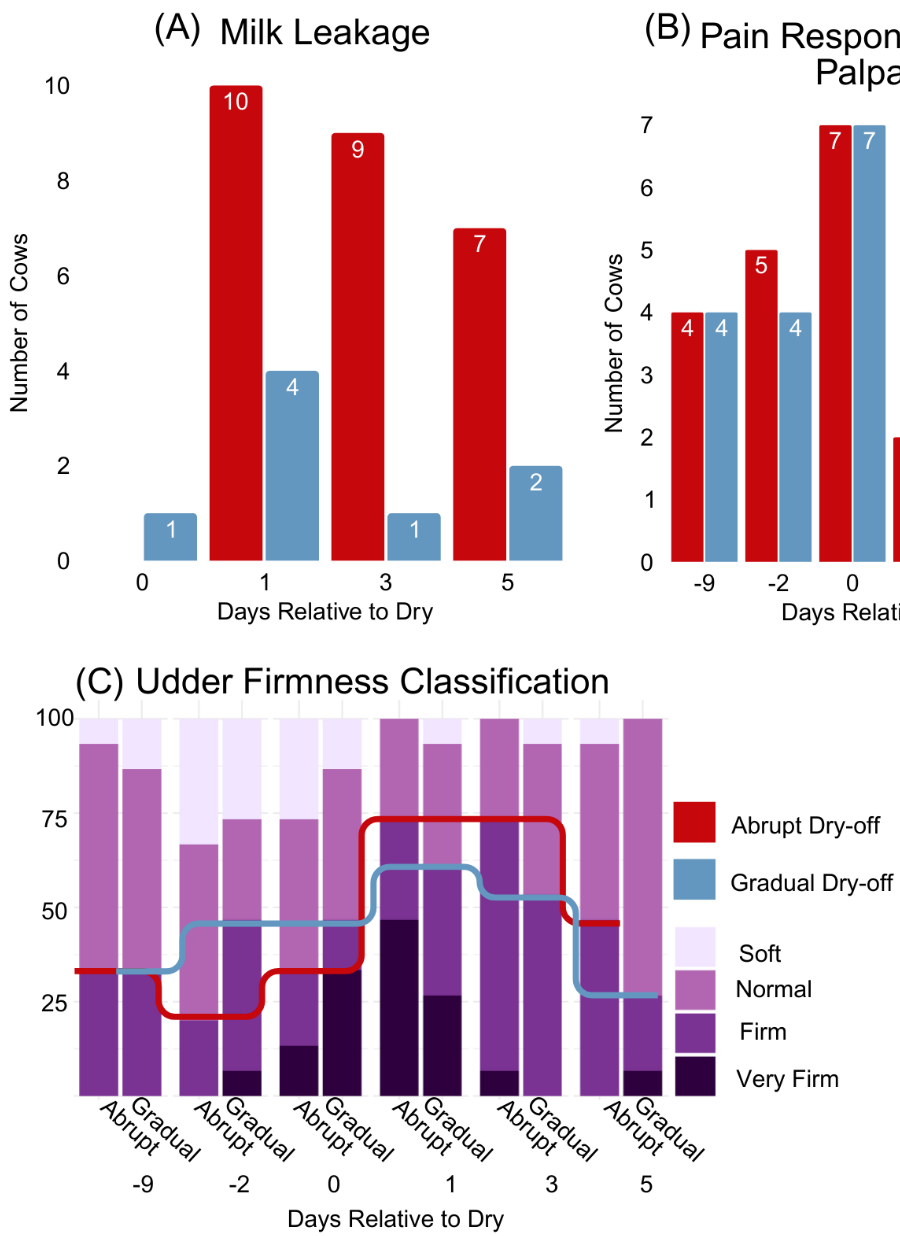
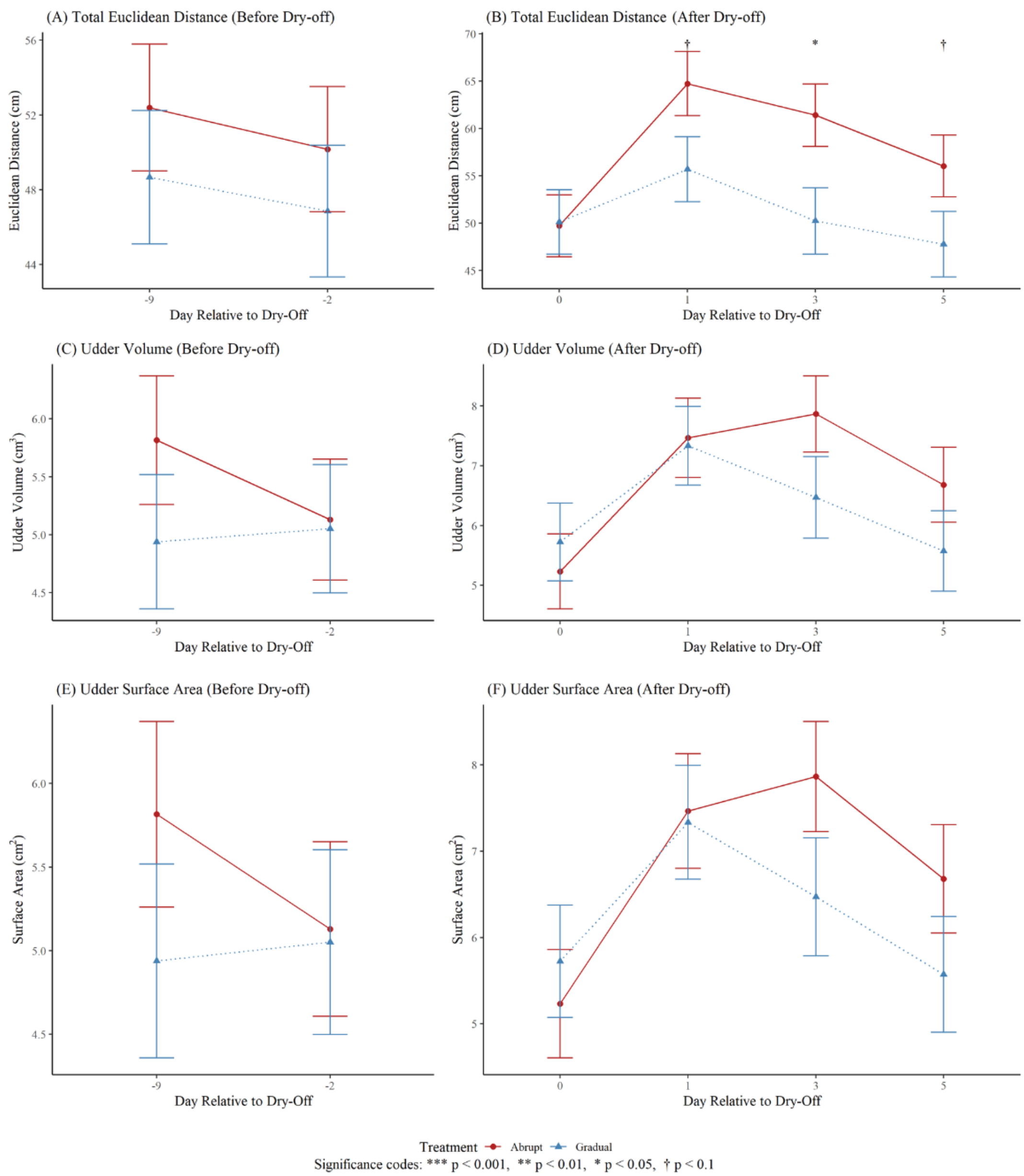


Figure 7. Udder characteristics for cows assigned to either the abrupt (red line and circles, n = 15) or gradual (blue line and triangles, n = 15) dry-off treatments. Panels A, C, and E show total Euclidean distance (cm), udder volume (cm^3), and udder surface area (cm^2), respectively, during the days before scheduled dry-off (-9 and -2 d). Panels B, D, and F show the same measures during the days following dry-off (0 to 5 d). Data are presented as least squares means ± standard errors. Statistical differences ($P < 0.05$) between treatments within day are indicated with asterisks; tendencies ($P < 0.1$) are indicated with daggers (†).



APPENDIX

Appendix Figure 1. Relationship between teat distance measured with 3D imaging vs. manually in the 7 days before scheduled dry-off for dairy cows (n = 30). Each purple point represents an individual teat measurement. The dashed black line indicates the linear regression fit with 95% confidence intervals (shaded gray area).