THESIS

DAIRY HEIFER HABITUATION TO THE MILKING ROUTINE: STRESS IN THE PRIMIPAROUS COW AND ITS IMPACTS ON BEHAVIOR AND PRODUCTION.

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2023

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ABSTRACT

DAIRY HEIFER HABITUATION TO THE MILKING ROUTINE: STRESS IN THE PRIMIPAROUS COW AND ITS IMPACTS ON BEHAVIOR AND PRODUCTION.

The transitional period surrounding parturition and onset of lactation is undoubtedly a stressful time in the life of a dairy cow. This is especially true for primiparous cows, who have no previous experience to the milking routine and must become accustomed to increased contact with human caretakers as well as the host of novel sights, sounds, smells and sensations in the milking parlor. Behaviors stemming from acute stress have the potential to increase risk of injury to parlor employees, who must be located close to the cows in order to perform their duties. Even so, the specific changes in cow behavior in the parlor over the course of the first lactation are not well documented, presenting a challenge to farm managers who wish to train employees in primiparous cow management. The main focus of this thesis is to present current research on this topic, as well as present new research regarding specific, daily changes in primiparous cow behavior during the first lactation.

Chapter one is a review of the current literature regarding sources of stress in first-lactation heifers and their impacts on various aspects of cow behavior and production, as well as on worker safety and wellbeing. The roles of precision livestock farming technologies on modern dairy farms are also discussed in this chapter, as well as the potential of these technologies for dairy cow welfare management and research.

The objective of chapter two is to describe the dynamics of milking unit kick-off in primiparous and multiparous cows during the first three months of lactation. Data were collected

from 199 primiparous (PRI) and 670 multiparous (MUL) cows who calved between August and November of 2020. From 3 days in milk (DIM) until 90 DIM, data were downloaded daily for each cow from the farm's software program. The main variables of interest were parity category and milking machine kick-offs (KO), which were reported by the milking system when an abrupt interruption in the milk flow occurred. KO events were used in our analysis as a proxy for habituation to the milking routine, and were analyzed by DIM. We found that proportions of KO were greater in PRI than in MUL throughout the monitoring period, and that when analyzed by DIM, first-lactation cows showed a non-linear trend of kick-offs. This indicated that changes in behaviors displayed during the habituation process are not linear, but instead are more complex.

Chapter 3 is an analysis of additional data that were collected during the study presented in chapter 2. Study participants were the same, but our goal in this chapter was to investigate any possible relationships between rates of machine kick off, daily changes in milk yield, and occurrence of mastitis during early lactation. Cows with varying frequencies of machine kick-offs were categorized into quartiles. Quartiles were then analyzed for potential interactions with milk yield and mastitis occurrence. Overall, we found no differences in milk yield between KO quartiles, but both primiparous and multiparous cows in the quartile with the highest KO rates had higher rates of mastitis.

ACKNOWLEDGEMENTS

This project would not have been possible without the help of my advisor, Dr. Temple Grandin, who first inspired me to study livestock behavior and welfare. She gave me the freedom to create my own project and encouraged me along the way to find a topic I was passionate about and pursue it. I am also deeply grateful for Dr. Pablo Pinedo, who provided me with the tools and contacts needed to put this project together, and who spent countless hours helping me understand the data programs, statistics, and general intricacies of putting together a project from scratch.

Words cannot express my gratitude to my family and friends for their encouragement throughout this journey, and especially my husband Caleb. Thank you all for grounding me, cheering me on, and insisting that I was capable even when I didn't feel that I was.

And of course, thank you to my sweet Kodi and Harvey. Your tail wags and snuggles were always exactly what I needed when I felt overwhelmed, and your persistent energy kept me from sitting at my desk for longer than was good for me.

DEDICATION

I would like to dedicate the work in this thesis to my grandfather, Don Wiechman, and my 4-H mentor, Glenn Sanger. I grew up watching you work your cattle with a gentleness, patience, and quiet understanding that I only later realized did not come naturally to all animal handlers. You instilled in me the curiosity and desire to understand that I needed to do this research, and I hope that in some small way, the words in this thesis can be used to instill curiosity and understanding in others who love and care for their own livestock.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: LITERATURE REVIEW

Importance of understanding of cow behavior

The dynamics between producer, animal caretaker, and cow on any dairy operation are complex yet integral to the success of the operation. Success of the producer relies on, among other things, efficient workers and healthy, productive cows; success of the employee relies on effective management and training by the producer and cooperation of the animals in routine handling events; success of the cow relies on the producer's ability to understand her needs and the employee's ability to carry out the activities that provide for those needs. Stress management is one aspect of cow care that has a strong influence on cow, worker, and producer. Low-stress handling practices are increasingly incorporated into standard operating procedures on modern dairy farms as producers recognize the benefits associated with this style of handling. Still, there are obstacles to the consistent practice of low-stress handling techniques among animal caretakers, many of which can be reduced through effective training. Language barriers and time constraints can inhibit effective training (Wilmes and Swenson, 2019), but a lack of training materials and general information regarding certain topics can also reduce a producer's ability to effectively communicate how to handle cows in specific scenarios (Sorge et al., 2014).

One of these under-studied topics is the behavior of first-lactation heifers during the periparturient period, a period during which both heifers and human caretakers experience situations that have the potential to decrease their welfare (Phillips et al., 2021). Although conversations with dairy employees indicate that handlers often consider training heifers to the milking routine to be both frustrating and dangerous, there is a surprising lack of information regarding the specific changes in cow behavior over the course of the first lactation, which limits the scope of training that animal caretakers can receive.

The goal of this literature review is to present the current research about primiparous cow behavior and welfare, specifically during the habituation process to the milking routine. In the first section, the many sources of stress experienced by the primiparous cow are thoroughly discussed, as well as some of the impacts of stress on both the animals and their human caretakers. Next, the current research involving the process of habituation to the milking routine is presented, as well as the gaps in research that have not yet been investigated. Finally, the applications, benefits and shortcomings of precision livestock farming technology in the management of dairy cow welfare are summarized.

Stress in the primiparous cow during early lactation

Acute stress (stress related to a negative affective state and stimulation of a fight-or-flight response) in production livestock has well-known, detrimental effects on animal welfare and productivity (Collier, 2017) as well as on the ease of handling and worker safety (Grandin, 1999; Williams, 1984). On commercial dairies, animal handling-related injuries comprise between 24% and 38% of all reported injuries, with approximately 20% of those involving moving animals to the parlor and 50% occurring in the milking parlor itself (Grandin et al., 1998; Douphrate et al., 2013; Lindahl et al., 2016; Edwards and Kuhn-Sherlock, 2021). Many behaviors that pose a risk to human handlers, such as kicking and crushing handlers against the pen, are in response to acute stress and most often occur while the handler is attaching the milking unit or completing other milking-related tasks (Douphrate et al., 2013; Edwards and Kuhn-Sherlock, 2021). Common handling-related injuries include soft-tissue injuries, fractures, breaks, and dislocation, all of which can decrease worker ability to complete required tasks (Edwards and Kuhn-

Sherlock, 2021) and indicate areas of concern related to animal and worker wellbeing. In addition to actual worker injury, stress-related behaviors can cause decreased production, increased contact with handlers as they try to manage stressed cows (such as reattaching the milking unit) and a subsequent increased risk of additional injury to the handler (Rushen et al., 1999; Munksgaard et al., 2001).

Though many unwanted behaviors will typically decrease over time due to repeated exposure to the milking routine, the initial weeks and months of the first lactation particularly stressful, and both animal and handler are at a risk for decreased wellbeing during this time (Phillips et al., 2021). During this period, primiparous cows experience a wide variety of novel situations and stressors, such as the physiological/metabolic stress caused by onset of lactation and parturition, introduction to a new housing system and social group, and increased human-animal interactions (Trevisi et al., 2012; Phillips et al., 2021). Primiparous cows are also introduced to the milking routine for the first time, during which they experience novel noises and smells, confinement in the milking stall, udder sanitization and stimulation, and attachment of the milking machine (Phillips et al., 2021).

Although anecdotes indicate that handling primiparous cows is generally considered by handlers to be frustrating at best (and dangerous at worst), there is relatively little information regarding the specific, day-to-day changes in behavior over the course of the first lactation. This information could be valuable for a variety of uses, including improved training for handlers, early identification of individual animals with a temperament ill-suited for a particular milking system, and a generally improved understanding of the interaction between stress, behavior and production over the course of the first lactation.

Physiological Stress

Though the physiology of parturition and onset of lactation is largely outside of the scope of this review, it is important to note that many of the physiological changes that occur during this time can have direct impacts on a cow's stress and behavior during early lactation. Dairy cows in the periparturient period are at an increased risk for potentially painful conditions, such as mastitis and udder edema, due to increased metabolic stress and a weakened immune response (Smith et al., 1985; Mallard et al., 1998; Fitzpatrick et al., 2013). Discomfort associated with udder and teat inflammation, infection, and injury are stressful to the animal, and have been shown to correlate with an altered affective state and behaviors commonly associated with pain and discomfort. A 2015 study found that mastitic cows displayed higher frequencies of kicking and tripping while being milked than control cows, and continued to kick more often even after the end of their treatment period (Fogsgaard et al., 2015). Other articles have reported an increase in stepping behavior during milking in cows showing signs of hyperkeratosis (a painful callusing of the teat) (Cerqueira et al., 2018), and a positive correlation between teat lesions and kicking behavior in the parlor (Rousing et al., 2004). Thus, it can be speculated that certain pain and discomfort in the udder and teats, of which cows are at an increased risk during the periparturient period, may alter cow behavior during milking.

Environmental Stress

Environmental stress can be defined as "any stimuli that demand(s) a response from the animal to adapt to new circumstances" (Naqvi et al., 2012). Although there are many of these environmental stressors on a dairy operation, for the purposes of this literature review we will primarily discuss the those that are specific to the milking routine.

Fear in animals is an adaptive response intended to protect an individual from a specific threat (Boissy, 1995). Due to their prey nature, cattle and other species of livestock have heightened sensitivity to novel situations and stimuli, especially when perceived as a potential danger (Grandin, 1984; MacKay et al., 2014). Each step of the milking routine involves a multitude of novel sights, sensations, sounds, and smells that the primiparous cow must become habituated to, and that have the potential to elicit intense fear responses that have a negative impact on animal welfare, milking efficiency, and milk production (Andrea et al., 2015; Kutzer et al., 2015).

Typically, modern commercial dairy farms have standardized milking protocols designed to maximize milk removal, milk quality, cow comfort and health, and worker efficiency (*NADIS*, 2010; Ruegg et al., 2005). Though the specifics of individual milking routines vary, they typically consist of moving cattle to the milking parlor, restraining the animal, pre-milking preparation (i.e. cleaning the udder and stimulating milk letdown), attachment of the milking unit and subsequent milk removal, and post-milking care (such as a disinfectant dip) (Ruegg et al., 2005).

As prey animals, cattle rely heavily on vision for early detection of threats in their environment (Barbosa and Castellanos, 2005). Though they have 360^{o} vision, cattle also have poor depth perception, and tend to balk when there are abrupt changes in the environment until they are able to investigate and decide whether or not the change presents a threat. (Grandin, 1980). Entrance into the milking parlor typically involves changes in flooring material, amount of light (as cattle move from sunny outdoors to the darker interior of the parlor), and sudden motion as milking employees go about their tasks. To the first lactation-heifer, each novel sight likely presents a potential threat, increasing stress levels.

After entrance into the milking stall itself, cows are then exposed to the novel tactile sensations associated with the milking routine. These may include forestripping of the teats, pre-dipping, attachment/detachment of the milking unit (Toledo, 2021). Additionally, previous experience being in close proximity to human caretakers is usually limited in primiparous cows, so the human-animal bond has not yet been established. Kutzer et al. (2015) found that when milking staff exposed first-lactation heifers to tactile contact with the udder prior to onset of lactation, they showed fewer stress-related behaviors at milking than heifers who had never been touched (Kutzer et al., 2015). Other research indicates that cows who had previous experience being touched by a human had fewer stress reactions during a novel palpation procedure by a veterinarian (Schmied et al., 2010), and heifers that had previous experience with being brushed in a cattle chute responded more calmly to new people than inexperienced heifers (Ujita et al., 2021b). From these studies, it seems that the novelty of both tactile stimulation and human interaction are interrelated, and both are contributing factors to the stress associated with habituating to the milking routine

In addition to the visual and tactile stimulation, first lactation heifers must also become accustomed to novel sounds and smells. There is some evidence that among cattle breeds, Holsteins (the most prevalent breed used in commercial US dairies) are more sensitive and reactive to noise than beef-type cattle (Lanier et al., 2000). In one study, heifers in a controlled setting took approximately 12 days to begin habituating to parlor noises, though in a practical setting it may take longer due to the variability in noise from day to day (Arnold et al., 2007). There is also evidence that indicates that cows can differentiate between distinct, complex odors and react differently to them (Rørvang et al., 2017), and that certain smells may have positive impacts on cattle experiencing a change in environment or routine (Osella et al., 2018). In the author's experience giving tours of a dairy farm, humans who have not previously been in a dairy parlor are often overwhelmed by the variety of smells associated with the room; it seems likely that in cattle, who are thought to have a more sensitive sense of smell than humans, these novel smells might have an impact on stress levels during the habituation period (Padodara and Jacob, 2014).

With so many novel stimuli involving all five senses, it makes sense then that first lactation heifers would display stress-induced behaviors upon initial introduction to the milking routine, and would continue for some time after until the individual has habituated to the environment. How long this takes, however, has not been thoroughly described.

Animal-Animal Interactions

Establishment of the social hierarchy within the dairy herd is crucial to maintaining stable and low-stress dynamics between individuals, and influences the outcomes of competition for resources (Grant and Albright, 2001; Cook et al., 2007). Social regrouping and the subsequent disruption in the social hierarchy occurs regularly throughout the lactation cycle on commercial dairies to make certain management practices easier (such as moving late gestation cows to a fresh pen to allow the dairy to provide for the specialized needs of that group). However, with each regrouping the social hierarchy must be reestablished, and the increase in agonistic interactions between individuals and the associated elevation in stress levels can negatively impact animal wellbeing and performance, especially for middle ranking and subordinate individuals (Hasegawa et al., 1997). Decreases in some milk production parameters have been observed for at least a day following changes in animal grouping (Brakel and Leis, 1976; Hasegawa et al., 1997; Keyserlingk et al., 2008). Effects on social behavior can last even longer, with social stabilization occurring somewhere between 5 and 15 days (Kondo et al., 1984;

Hasegawa et al., 1997). Research also indicates that regrouping is associated with lower dry matter intake, increase in rate of feeding, decreased rumination, and increased competitive displacement at the feed bunk, as well as decreased lying bouts, lying times and initiation of allogrooming events, which is an indicator of positive animal interactions (Keyserlingk et al., 2008; Schirmann et al., 2011). Some research also indicates that cattle with a more dominant personality and who have more interactions with herd members have increased likelihood of being removed from the herd in the first 60 days postpartum due to uterine disease (Chebel et al., 2016). These negative effects are typically short lived, lasting between 3 and 7 days as dominance is established and the hierarchy stabilizes (Grant and Albright, 2001), but they are still present a potential factor in the stress experienced by cows during early lactation.

Human-Animal Interactions

Establishing a positive human-animal relationship is crucial for the wellbeing and productivity of both animal caretaker and cow. There are a number of events throughout a dairy cow's life where she is in close contact with human caretakers, including feedings and pen cleaning, movement from pen to pen, veterinary inspection/treatment, milking procedures, and other management procedures such as artificial insemination (AI). Though some of these simply require the handler to be in close proximity the animal, many others require physical contact and, in some cases, physical discomfort and stress to the animal. It has been shown that both the behavior and attitude of the handler, as well as the previous experiences of the cow, can have a great impact on the physiological stress indicators, behavior, and productivity of the animal in these situations (Hemsworth et al., 2000a; Waiblinger et al., 2003).

A thorough understanding of this human-animal interaction in intensive livestock operations is important for reducing animal stress and for increasing animal productivity and over-all wellbeing. Positive interactions reduce future stress for both human and animal, and negative interactions can increase risk of injury, difficulty of handling, and decrease production.

Though a direct causal relationship between handler interaction and cow productivity is unlikely to be established due to the complexity of the processes involved, several studies have indicated that there is an association between them. A study conducted in 2000 indicated a significant relationship between a cow's willing to approach a stockperson in close range and conception rates of first AI, as well as evidence of significant correlation between stockperson attitude/behavior towards the cows and cow behavior and milk productivity (Hemsworth et al., 2000a). More specifically, studies have shown that stockperson attitude can have an association with milk yield (Hemsworth et al., 2000a; Waiblinger et al., 2002; Hanna et al., 2009), and that aversive interactions with stockpeople are negatively correlated with milk protein and fat, as well as an increase in milk cortisol (Breuer et al., 2000; Hemsworth et al., 2000a).

In addition to having an effect on production, it is widely known and accepted that aversive handling on the part of humans leads to an increase in cow reactivity and especially in dangerous behaviors. Cows that are stressed are more likely to react in ways that can put handlers in danger, such as balking, kicking, pushing, etc. (Peters et al., 2010; Lindahl et al., 2016), and a large portion of animal handling injuries are related to these fearful and agitated behaviors (Grandin, 1999). In the parlor, cows perform a "flinch, step, kick" (FSK) response when stressed, which has been shown to be at least in part a response to the attending stockperson (Breuer et al., 2000). Conversely, studies have showed that cows that had positive handling experiences show less restless behavior, kick less when alone, and have lower heart rates during palpation when compared to cows that have average experiences (Waiblinger et al., 2003). Another study showed that that handling during calving has a positive impact on cow parlor behavior in the weeks following parturition (Hemsworth et al., 1989).

Facilitating quality relationships between workers and cows has the potential to positively impact a dairy at all levels: the producer (through improvements in productivity of cows), the welfare of animal caretakers (by reducing animal behaviors that put workers in danger), and the welfare of the cow herself (through reducing stress levels during routine handling events).

Effects of stress on cow productivity and behavior

Milk Production

The effects of stress on milk production has been extensively researched and, as with other production parameters in the livestock industry, research indicates a strong correlation between acute stress and undesirable changes in production. In reaction to aversive situations, such as a novel parlor or presence of a handler that has previously been rough with the cow, many cows experience increased levels of plasma cortisol, elevated heart rates, inhibition of oxytocin release, and increased residual milk and reduced total milk yield (Rushen et al., 1999; Van Reenen et al., 2002).

Tactile stimulation of the teat releases oxytocin, which in turn causes milk let-down and ejection. Interruption of tactile stimulation (i.e., kicking off of the milking unit) ceases the collection of milk, thereby reducing milk production and potentially causing incomplete extraction of milk. Similarly, inhibition of oxytocin in response to the increased outside stressors can disturb milk ejection, also reducing overall production (Bruckmaier and Blum, 1998). It is also important to note that repeated failure to completely extract milk from the udder is associated with reduction in future milk production rates, milk yield, and milk lactose, as well as an increase in somatic cell count (Schmidt et al., 1964; Penry et al., 2017).

Reproduction

In addition to milk production, reproductive efficiency is crucial to farm profitability. The effects of handling stress on cattle reproduction has not been studied as extensively as on milk production, but there is evidence to suggest that positive handling experiences and calm cattle temperaments can have a positive influence on future reproductive performance. Hemsworth et al. (2000) found that negative handling interactions was correlated with increased flight distance between cows and humans, and that smaller flight distances were associated with higher rates of conception to first AI (Hemsworth et al., 2000b). Another study found that heifers that were habituated to a handling facility for four weeks post-weaning reached puberty quicker than those who had not been habituated to human handling. The same study also found that when temperament was assessed using chute scoring systems, cows who showed a more excitable/aggressive temperament had a decreased rate of pregnancy and calving than those with a milder temperament. They speculated that this might be due to changes in the mechanisms that control ovulation and conception (Cooke, 2012). A 2014 study confirmed these findings, with excitable cows having lower AI conception rates than calm cows. In that study, it was also shown that facility design influenced temperament, indicating that differences in temperament (and possibly reproductive efficiency) were at least in part due to handling stress (Kasimanickam et al., 2014). While not confirmation of a direct causal relationship between handling stress and reproduction, these studies do indicate an important relationship between the two.

Parlor Behavior

While the effects of stress on economically important variables like production and reproduction primarily impact the producer, the effects of stress on cow behavior directly impact the safety and overall welfare of animal caretakers, especially when it comes to kicking during milking.

Characterization of the Flinch-Step-Kick (FSK) response to milking was first described in an article by Willis et al. (1983) as a series of behaviors (flinching, stepping, and kicking) displayed by dairy cattle in response to stressful situations in the milking parlor. According to Willis et al., it was standard procedure at that time to cull dairy cows who displayed higher rates of the FSK behaviors due to reduced worker efficiency and safety associated with those behaviors; the study, however, found that cows that displayed FSK responses may have higher milk production, and so culling based on this behavior was potentially not economically justifiable (Willis, 1983). Since then, the FSK response has been used in many studies to determine cow affective state and stress levels during milking, though kicking in the parlor is likely correlated with many different factors. Stage of lactation, udder health, parlor style and environment, and parity/experience of the cow may all impact her behavior during the milking routine.

At different stages of lactation and parity, cows may be at an increased risk for painful udder conditions that lead to discomfort and restlessness at milking. Udder edema, for example, is a painful swelling of the mammary tissues that is most common in primiparous cows and during early lactation (Emery et al., 1969; Morrison et al., 2018), which may increase kicking in the parlor (Okkema and Grandin, 2021). As parity increases, some studies have found an increased risk of mastitis (Uzmay et al., 2003; Jingar et al., 2014), which in turn may also increase the frequency of stepping and kicking during milking (Fogsgaard et al., 2015; Cerqueira et al., 2017). Other conditions, like teat lesions and hyperkeratosis have also been correlated with the FSK behaviors (Rousing et al., 2004; Cerqueira et al., 2018).

Environmental factors such as temperature of the room during milking and parlor design can impact frequency of stepping and kicking (Wenzel et al., 2003; Gygax et al., 2008; Cerqueira et al., 2017), as well as presence of handlers and the quality of the relationship with those handlers (Rushen et al., 1999). For primiparous heifers, introduction to the milking environment is a very stressful event with a host of novel sights, smells, sensations, and sounds that are associated with the milking environment. First lactation heifers are notorious for increased frequency of stress behaviors during early lactation milking, and though this causes an increased risk of injury to the handlers as well as decreased welfare for the cows, there are still significant gaps in research about heifer behavior during this time period. In the rest of this literature review, this time period will be referred to as the "habituation period", and the following sections will describe the habituation process and current research into dairy cow habituation, as well as the need for more research in this subject area.

Habituation of first-lactation heifers to the milking routine

Habituation is a behavioral phenomenon that has been extensively studied in a wide range of species and contexts. Traditionally, it is described as a decrease in behavioral response to a stimulus due to repeated exposure to that stimulus (excluding a decrease in response due to fatigue or adaptation), and is thought to be a basic form of learning that allows an individual to filter out stimuli that does not merit a response in favor of focusing on more important stimuli (Thompson and Spencer, 1966; Rankin et al., 2009).

Thompson and Spencer's 1966 article on habituation was one of the first papers to attempt to describe the process of habituation, and continues to be used as a foundation for current research on the topic. In the paper, nine criteria are described and defined in order to distinguish habituation from other causes of decline in behavior, which were more recently reviewed and affirmed by Rankin et al. in 2009. Grissom et al. (2009) further summarizes those nine characteristics into four main themes:

1. "Habituation occurs to repeated stimuli. This theme encompasses both Criterion 1, the basic phenomenon of habituation, and Criterion 9, habituation of responses to dishabituating stimuli, as the latter can be viewed as a special case of the former (Thompson and Spencer, 1966)."
2. "Habituation is reversible. Criterion 2, spontaneous recovery, and Criterion 8, dishabituation, are both examples of the reversibility of habituation under different circumstances."
3. "Habituation can be improved by modifying certain parameters. Habituation is stronger in magnitude and/or more rapid when it is relearned following a period of spontaneous recovery, (Criterion 3), when stimuli come with increasing frequency (Criterion 4), and when stimuli are relatively weak (Criterion 5)."
4. "Habituation can progress beyond experimental expectations. Habituation can progress to responses that are lower than baseline (Criterion 6), and can generalize to stimuli other than the original habituating stimulus (Criterion 7)." (Grissom and Bhatnagar, 2009)

Some methods of describing habituation over time include average number of responses, frequency of responses, rate of rehabituation, and magnitude of initial response (Rankin et al., 2009).

Current Research

An understanding dairy cow behavior during the habituation period is particularly relevant to handlers working in the milking parlor, as they spend a great deal of time in close contact with their animals. The behavioral responses that dairy cows often display to novel stimuli (such as kicking) pose significant risk of injury to the worker, creating a need for realistic expectations regarding the changes in behavior over the habituation process. Furthermore, exposure to a given stimulus changes over the course of the lactation and dry periods, potentially necessitating a "rehabituation" period at the onset of a new lactation. This rehabituation period may cause a temporary increase in behavioral responses during early lactation even in older cows as compared to the end of the previous lactation, but the differences in the rate of habituation between primiparous and multiparous cows is largely unknown.

In relation to dairy cattle, there have been relatively few studies that focus on describing the habituation process regarding introduction to the milking routine. To date, the majority of these studies focus on methods of desensitizing cattle to noises and handling prior to calving in order to reduce stress at the onset of lactation. Most of those studies are in agreement that exposing primiparous dairy cows to aspects of the milking routine prior to lactation reduces reactive behaviors once lactation and regular milking begins (Kutzer et al., 2015; Phillips et al., 2021; Ujita et al., 2021a). Similar studies have also been performed in non-cattle dairy species like donkeys and buffalo, with similar results (Polikarpus et al., 2014; De Palo et al., 2018).

Implications for workers

There is a significant lack of research regarding the challenges that workers face when handling first-lactation heifers. Anecdotally, workers face higher levels of frustration and increased risk of injury when moving and milking primiparous cows as opposed to multiparous cows, and yet there remains a lack of training and research concerning low-stress handling and risk-management in regard to primiparous cows.

Interestingly, while many people might assume reactivity and fearfulness have a positive linear relationship, there is evidence that this is not always the case, with both extremely fearful and extremely calm animals showing less reactive behaviors during milking than those in-between (Bremner, 1997; Sutherland et al., 2012; Sutherland and Dowling, 2014). Furthermore, a relationship has been shown between the behavioral response of cows to humans and the performance of FSK behaviors during milking (Rushen et al., 1999; Waiblinger et al., 2002; Rousing et al., 2004). Negative handler attitudes (such as frustration) when moving cattle are especially detrimental to use of low-stress handling techniques (Waiblinger et al., 2002), and anecdotally, dairy workers find handling first-lactation heifers to be one of the more dangerous and difficult parts of their job. It can be assumed, then, that workers who are not trained in the more complex patterns of stress behavior are at an increased risk of becoming frustrated when a cow reacts to a novel situation in a way that is unexpected, in turn increasing the risk of worker injury and eroding the human-animal bond that is so crucial to low-stress handling. A deeper understanding of both stress behaviors and the behavioral trends associated with habituation over the course of the first lactation have the potential to equip caretakers to better handle nervous cows calmly and patiently, in turn reducing the cattle behaviors that cause frustration and impatience.

Role of precision livestock farming technology in cow management

It seems clear that there are important benefits to increasing our understanding and awareness of stress in the primiparous cow and the associated habituation process to the milking routine. There is even a potential for improving worker and animal welfare by exposing first-lactation heifers to the milking parlor prior to the onset of lactation. However, we have not yet discussed an important barrier to implementation of pre-lactation training (and other types of reducing handling stress in heifers)- the practicality of individual-based animal training on large, commercial dairies. In this section, we will review precision livestock technologies and their role in dairy cow production and welfare. In the next chapter of this thesis, research generated from one of these technologies in the monitoring of habituation is presented, in the hope that increased understanding of this process will be a positive addition to the management of first-lactation dairy farms.

History of precision livestock farming

The consolidation of small dairy farms to large-scale operations over the last 50 years has led to a shift in management strategies and adoption of new technologies. With this change in the structure of the dairy industry, there has also been a shift in the relationship of stockperson and animal. For most of history, farmers took a “close-up” approach, where they were able to monitor individual animals for stress, disease, etc. However, since the dramatic increase in ratio of cows to handlers, this is no longer practical or, in many cases, even possible. To better manage large operations at the cow level, a large amount of time and money has been invested into the development of technologies and associated algorithms that can aid in remote, real-time monitoring of animals on an individual basis (Clay et al., 2020; Eckelkamp and Bewley, 2020). The integration of these technologies and their respective algorithms into the management and decision-making of an agriculture operation is known as precision livestock farming (PLF), and is now common practice on many modern dairy farms world-wide (Kleen and Guatteo, 2023). The use of PLFs has become integral to the success of commercial livestock operations, and are a vital tool in maintaining high levels of welfare in the herd as they allow for early detection of problems and establish a baseline for production and behavior.

By collecting large amounts of data specific to each cow on a continuous schedule, farmers have instantaneous access to more detailed records than ever before, which has allowed for improvements in many areas such as disease management, heat detection, and production trends. That being said, there are some notable disadvantages to a PLF management style, which will be discussed later on in this section. While these technologies are not necessarily specific to acute stress management, they are an important part of modern dairy management and have the potential to improve animal welfare, worker welfare, and production. Furthermore, they provide researchers with access to vast amounts of data both more quickly and more objectively than some conventional data collection methods.

PLF for disease management

One of the main goals of a PLF system is to provide timely warnings when an individual animal begins to exhibit signs of disease. While visual observation of animals is crucial to diagnosis and treatment of disease, lack of training, time, and labor continue to be barriers preventing timely, accurate detection and diagnosis of health issues on commercial dairy farms (Rai et al., 2023). Thus, remote monitoring systems can be powerful tools in the early detection of health issues. Additionally, in the research of animal behavior and welfare, awareness of disease that might influence behavior results is crucial, and these technologies offer a way for researchers to control for these variables.

On farm, PLF technologies are often used in early detection of a wide variety of changes in behavior and physiology that indicate infections, metabolic disorders, and other diseases. Mastitis, for instance, is one of the most frequent diseases afflicting the modern dairy cow and is a leading cause of economic loss and decreased welfare on commercial operations (Ruegg, 2017; Puerto et al., 2021). While cows experiencing acute infection will display clear visual signs (such as changes in milk texture and consistency and inflammation of the mammary gland), subclinical mastitis often goes unnoticed due to a lack of obvious symptoms (Khan and Khan, 2006; Cheng and Han, 2020). However, even in subclinical cases there is a substantial decrease in milk production and quality, and these cases may cause more financial loss than clinical cases (Sinha et al., 2014; Romero et al., 2018). Somatic cell counts (SCC) have been the gold standard for monitoring udder health and mastitis cases, but traditional methods of testing include manual collection of milk samples that are then shipped to a laboratory for testing. Because this method of SSC analysis can be costly in both time and money, many dairies only perform these tests every few weeks (Deng et al., 2020). Automated somatic cell count (SCC) monitors offer a way to detect sub-clinical mastitis cases before symptoms are shown by testing each cow individually while milking is in progress, which has huge potential for improvements in animal welfare and farm profitability (Deng et al., 2020; Rai et al., 2023).

Utilization of accelerometers and pedometers in disease detection is less specific, but can also be analyzed for changes in behavior consistent with sick behavior in cows, allowing veterinarians to separate and evaluate cows with potential health problems. Cattle experiencing common transition diseases like ketosis and hypocalcemia typically display changes in the proportion of time spent lying vs standing (Sepúlveda-Varas et al., 2014; Itle et al., 2015). Changes from a baseline of behavior can be tracked via activity monitors, and the ability to flag cows who might be suffering from both sub-clinical and acute disease is a very powerful in disease management.

PLF technology has also been used to identify lame cows. Lameness, defined as a change to the gait and posture of an animal due to pain associated with the locomotor system, is another condition responsible for significant economic losses on modern dairy farms, and is known to have a significant impact on production, reproduction, and premature culling (Yanga and Jaja, 2021; Kofler et al., 2022). Furthermore, lameness is often intrinsically linked with pain, meaning that the condition can have significant impacts on animal welfare (Flower and Weary, 2009; Whay and Shearer, 2017). The traditional method for assessing cattle for lameness was in-person observation of cattle with the use of a numerical scale to assess the severity in changes in gait and posture. The benefit of in-person scoring is that no additional technologies are required; however, it is both labor intensive and tends to vary in accuracy from observer to observer. Furthermore, changes in gait/posture tend to not become apparent until later stages of lameness, increasing treatment expense, recovery time, and animal discomfort. (Flower and Weary, 2009; Nejati et al., 2023; Rai et al., 2023). PLF technologies offer farms an objective method of detecting lameness more quickly and accurately than human observers.

The most common PLF technologies used for this purpose typically fall under one of three categories: kinetic, kinematic, or accelerometric (Bradtmueller et al., 2023). Kinetic technologies, also known as force and pressure platforms, are among the most common technologies for assessing lameness, and involve measuring the force, pressure, and weight of each limb as a cow either stands or walks on them. Included within this category of technology are force plates, pressure mapping systems, and weight distribution platforms. Kinematic technologies, also known as vision-based technologies, are another popular method of lameness detection. As the name-implies, these technologies utilize recorded videos or sequential images of a cow walking to analyze her motion and posture and identify any abnormalities. Finally, accelerometers can be used to measure both activity data (steps taken, time spent standing vs lying, etc.) as well as a gait pattern and symmetry to identify abnormal locomotion in a particular cow. (Bradtmueller et al., 2023; Nejati et al., 2023).

PLF for reproduction

Perhaps more than any other sector, the dairy industry relies on efficient reproduction to maintain not only a steady source of replacement animals, but also for the initiation of milk production. As the pressure for efficient, sustainable milk production has increased, dairy farmers have placed extra emphasis on genetic selection for maximizing milk yield. However, the hyper-focus on selecting for high milk production has had unintended consequences- namely, an adverse effect on reproductive traits such as days open and number of services to conception (Berger et al., 1981; Mokhtari et al., 2015; Kgari et al., 2020; Brito et al., 2021).

With artificial insemination being the primary method of reproduction in the modern dairy cow, accurate detection of estrus is essential for reproductive efficiency. Traditionally, heat detection occurred via visual observation of changes in behavior, such as standing to be mounted by other cows. However, as the herd size increases, visually monitoring individual cows becomes a labor-intensive and impractical way of detecting estrus (Crowe et al., 2018).Precision technologies have offered dairy farmers a way to mitigate some of these issues.

At the onset of estrous, cows display significant changes in behavior, including standing to be mounted and mounting other cows, as well as a dramatic increase in moving around the pen- as much as 4x the amount of a cow not in estrous (Kiddy, 1977; Santos et al., 2022). Activity monitors such as pedometers and accelerometers are a commonly used PLF on dairy farms, and have been adapted to aid farmers in heat detection. These sensors are typically placed on the leg or around the neck, or are integrated into an ear tag, and provide a continuous, real-time record of a cow's activity. When used as a heat detection tool, activity monitors provide a high level of precision in detecting above-average activity for a given cow, and are able to determine whether or not these activity increases occur in the intervals typical for estrus-related movement. (Silper et al., 2015a). By transmitting this data to a computer database that is monitored by farm staff, dairy workers are able to accurately schedule insemination of individual cows without having to visually monitor them (Rorie et al., 2002; Silper et al., 2015b).

Radiotelemetry pressure detectors, also known as mount detectors, that are placed on the hindquarters or tail are another tool commonly in estrous identification, though their accuracy has been reported to be lower than some other methods (Saint-Dizier and Chastant-Maillard, 2018). Other examples of PLF technologies that are either currently used or are being investigated for use in detection of estrus include rumination collars, artificial intelligence operated video recordings, and infrared thermography. Furthermore, many of the above technologies have been adapted for use in identifying other important reproductive events, such as the onset of parturition, which adds to their value on commercial dairy farms (Santos et al., 2022; Riaz et al., 2023).

PLF in the milking parlor

Perhaps the most frequently used application of PLF technology occurs in the milking parlor of commercial dairy farms. These types of technologies tend to fall into one of two categories: those that reduce the labor required to operate a parlor and those that record data as cows are being milked (Yang et al., 2021).

Milking parlor jobs demand a lot from employees, both mentally and physically. Hours are typically long and inflexible, tasks are often repetitive and/or require considerable physical exertion to complete, flooring is often wet and unstable, and there is an inherent danger in working closely with large animals and heavy machinery (Douphrate et al., 2009, 2016; Lunner Kolstrup et al., 2013; Panikkar and Barrett, 2021). PLF technologies offer a way to help mitigate some of these issues by decreasing the workload on milking staff and increasing worker efficiency and safety (Gargiulo et al., 2018; Edwards et al., 2020; Lundström and Lindblom, 2021). For example, employees are at particular risk of injury when cows are being actively milked due to close proximity to the animals (Edwards and Kuhn-Sherlock, 2021), so automation of various tasks such as cluster removal, sanitization, etc. can help reduce various injuries associated with these tasks such as back and wrist injuries from bending and tissue injuries from being kicked. Many farms are converting to new parlor styles, such as the rotary or a fully automated milking system (AMS), which greatly reduce the labor needed to operate a milking parlor. In the rotary, cow stalls rotate around a center axis, allowing milking employees to remain relatively stationary, reducing both physical exertion and the risk of slipping or tripping. In AMS, milking is fully robotized, which means that workers do not need to perform any of the traditional tasks associated with injury (sanitizing the teats, attaching milking cups, etc).

In addition to technologies that reduce labor, farms also use PLF technologies for data-recording and management. Automatic milking meters are used to record information such as milk yield, flow rate, and time spent milking for each individual cow. RFID tags and scanners allow automatic syncing of the milking machine with a specific cow's data "profile" online. Management computer software and predictive algorithms allow for automatic data entry and analysis of information collected in the milking parlor, making it easier to keep track of more information about individual cows in large herds.

Challenges to implementation of PLF technologies

Despite the prevalence and importance of all of these technologies in commercial dairy farming, there are several barriers that prevent farms from utilizing PLFs to their full potential. Some of the biggest barriers to implementation of these technologies on modern dairy farms seem to be the cost of the equipment and software, the time required to learn how to operate them, and practicality/reliability (Hartung et al., 2017; Schillings et al., 2021; Bianchi et al., 2022). While these technologies can be labor and cost-saving in the long run, they usually require a significant investment both in terms of money and time spent training employees. The vast amount of raw data generated by these programs can be overwhelming, and technological glitches and lack of training can make it difficult for busy dairy workers to utilize the data in a meaningful way (Butler et al., 2012; Eckelkamp and Bewley, 2020). Furthermore, different PLF technologies often do not work in conjunction with one another, making it difficult to combine multiple pieces of data into a useable and useful tool for the average farm worker (Schillings et al., 2021).

In addition to these logistical barriers to use, it is important to recognize the change in the human-animal relationship that these technologies can bring. With a greater focus on managing animals remotely, some farmers have expressed concern regarding over-reliance on technology and a loss of traditional skills in managing cows, as well as a negative shift in the human-animal bond (Butler and Holloway, 2016; Werkheiser, 2018; Barrett and Rose, 2022). In one study, a farmer that was interviewed about his perceptions of automatic milking systems said "... I don't think you can ever replace a human looking at an animal and seeing that she's bright, alert, you know cudding all things like that. A robot will pick up milk drop or mastitis in the milk; it can't sort of look at the general health of a cow" (Butler and Holloway, 2016). Other dairy farmers have expressed concern that the increased pressure to perform caused by constant monitoring of key performance outcomes could negatively impact mental health, and that the 24/7 monitoring and notifications would result in an increase in stress levels for some farmers (Butler and Holloway, 2016; Islam, 2020).

These are all valid concerns and experiences. However, other farmers who have already implemented PLF technologies note that the technologies simply add to the tools a farmer has when managing cows, and that users of PLF needed to be even more skilled in their relationship with the cows. These farmers say that robotic milking, for example, required even more knowledge, discipline, and 'stockmanship' than conventional milking, and that for those willing to learn and implement these technologies, the role of the stockperson was enhanced (Butler and Holloway, 2016). In fact, a 2021 survey reported that one of the most difficult parts of running an automated milking system was finding employees who had a skilled "stockperson's eye", not just those who knew how to run a computer (Lundström and Lindblom, 2021).

The general consensus seems to be that while PLF technologies are incredibly advanced and robust in the tasks they are able to perform, they are still just a tool meant to enhance the role of dairy farmers rather than replace them. Farmers who are skilled animal caretakers, and who are disciplined and eager to learn often report that they are glad they converted to a PLF management style (Butler and Holloway, 2016).

Final remarks

There are many challenges facing modern dairy farmers regarding cow welfare, some old and some new. Periparturient cows still experience many different sources of stress, first-lactation heifers must still get used to the novelty of being milked, and milkers are still at risk of being injured when handling heifers. However, the drastic increase in herd size on modern dairies means that there are inevitable changes to the human-animal relationship. Some of these changes present challenges, such as the feasibility of farmers personally monitoring each cow and training her to the milking routine. Other changes present new opportunities, such as the ability to objectively and continuously monitor cows remotely using new technologies. When used responsibly, precision livestock technologies can be an incredible asset to farmers and can aid in maintaining high levels of welfare for both animals and humans. Furthermore, these technologies provide researchers with vast amounts of data so that more topics can be explored and studied. That being said, just like any other tool, PLF technology is not meant to be a replacement for humans. Farmers still have to care deeply about their animals and employees, and have an active hand in dairy operations in order for these tools to be beneficial. Researchers also still need to have a practical understanding of cattle in order to responsibly and ethically draw conclusions from the data generated through PLF.

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CHAPTER 2: PATTERNS OF MILKING UNIT KICK-OFF AS A PROXY FOR HABITUATION TO MILKING IN PRIMIPAROUS COWS

SUMMARY

The onset of lactation and the subsequent habituation to the milking routine is a stressful period, particularly for primiparous cows. The objective of this study was to describe the dynamics of milking unit kick-off (KO) behavior in primiparous (PRI) cows during the first three months of lactation, considering multiparous (MUL) cows as a reference for comparison. A total of 869 cows (PRI=199; MUL=670) on a dairy farm in northern CO were included in the analysis. Cows calving between August and November 2020 were enrolled from 3 DIM until 90 DIM. Participants were milked 3x/day in a 60-unit rotary parlor and data from each milking session were downloaded from DelPro Farm Manager software. Milking unit kick-off was used as a proxy for habituation to the milking procedure. Kick-off events were reported by the milking system and defined as an abrupt interruption in the milk flow during the milking session. Occurrence of KO was analyzed by grouping the three consecutive milking sessions in each day and categorized as yes or no, indicating whether or not an individual kicked at least once in a given day. Data were analyzed by repeated measures ANOVA and logistic regression, including parity category, calving season, occurrence of dystocia, and their potential interactions in the models. Overall, the odds of KO were greater for PRI vs. MUL cows (OR [95% CI] = 2.07 [1.58-2.73]). When KO was analyzed by DIM, proportions of KO were greater in PRI than in MUL during the whole monitoring period. In PRI, proportions of KO increased from 0.10/d to 0.20/d between 3 DIM and 15 DIM, to start decreasing around 30 DIM and remaining above MUL up to 90 DIM. On the contrary, in MUL cows, proportions of KO remained close to 0.05/d

during the 90 d period. We concluded that the relationship between days in milk and the proportion of primiparous cows displaying KO was not linear, but rather KO increased during the first two weeks postpartum before decreasing after the first month of lactation. First parity cows had greater levels of KO than multiparous cows, which is most likely associated with the process of habituation to milking during their first lactation.

INTRODUCTIONS

Acute stress related to a negative affective state and stimulation of a fight-or-flight response in dairy cows has well-known, detrimental effects on welfare and productivity and can have an impact on cattle handling and worker safety (Grandin, 1984; Williams, 1984). On commercial dairy operations, animal handling-related injuries comprise between 24% and 38% of all reported injuries, with around 20% of those involving moving animals to the parlor and 50% occurring in the milking parlor itself (Grandin et al., 1998; Lindahl et al., 2016; Edwards and Kuhn-Sherlock, 2021). Many behaviors that pose a risk to human handlers, such as kicking and crushing handlers against the pen, are in response to agitation and acute stress and most often occur while the handler is attaching the milking unit or completing other milking-related tasks (Grandin et al., 1998; Douphrate et al., 2013; Edwards and Kuhn-Sherlock, 2021).

The onset of the first lactation and the subsequent period of habituation to the milking routine is a particularly stressful period in a dairy cow's life, with an increase in interaction with human caretakers, new social groups, and a host of novel stimuli during the milking routine. Handling primiparous heifers during the transition period can also negatively impact human handlers, with increased difficulty of performing milking tasks and risk of cattle-related injuries (Sorge et al., 2014; Edwards and Kuhn-Sherlock, 2021; Phillips et al., 2021). Despite this, there is a gap in research regarding the specific changes in cow behavior over the course of the first lactation, which limits the scope of training that animal caretakers can receive.

Previous studies on heifer habituation to the milking routine indicate that primiparous cows tend to be more excitable than multiparous cows at various stages of the milking process (Andrea et al., 2015). The majority of research has primarily focused on shortening the habituation process through the use of pre-lactation exposure to the milking routine and/or contact with caretakers on early lactation behaviors and heifer habituation (Bremner, 1997; Arnold et al., 2007; Eicher et al., 2007; Kutzer et al., 2015). However, to the authors' knowledge, no studies describe daily changes in stress behaviors during the habituation period or differences in those daily behaviors between primiparous and multiparous cows during the first months of lactation.

The behavioral responses that dairy cows display to novel stimuli (such as kicking) pose significant risk of injury to the worker, creating a need for realistic expectations regarding the changes in behavior over the habituation process. Because of this, a better understanding of the habituation process is especially relevant to handlers working in the milking parlor, as they come in close contact with the animals on a daily basis. We hypothesized that milking unit kick-off (**KO**) would be most frequent at the beginning of lactation of primiparous cows, declining as the lactation advances. The primary objective of this study was to describe the dynamics of milking unit kick-off behavior in primiparous cows during the first three months of lactation and to compare these changes to those of multiparous cows over the same period of time.

MATERIALS AND METHODS

Behavioral data were collected from an organic-certified dairy farm in northern Colorado using the DeLaval activity meter system (DelPro Farm Manager software; DeLaval International AB, Tumba, Sweden). A total of 869 Holstein cows (199 primiparous and 670 multiparous) that calved between August and November 2020 were enrolled in the study at 3 days in milk and monitored until 90 DIM. As all the data used in this study were collected from on-farm software, no CSU Institutional Animal Care and Use Committee approval was required. Study cows were milked 3 times per day in a 60-unit rotary parlor (DeLaval International AB). The main variable of interest was milking unit kick-off (categorized as yes or no), monitored with the electronic on-farm milk meters in conjunction with the software program. Milking unit kick-off was identified by the milking system as an abrupt interruption in the milk flow during the milking session and considered in this study as a proxy for habituation to the milking process.

Data pertaining to individual cows, such as parity, calving date, and calving ease score were exported from PCDART herd management software (Dairy Records Management Systems). Reports were then generated and downloaded via a remote server once weekly for the duration of the study, which lasted until the last cow enrolled had completed her first 90 DIM.

Initial univariable models using only parity category (Primiparous=**PRI**; Multiparous = **MUL**) as explanatory variable were followed by multivariable models that added calving season and calving difficulty (1 = no assistance; 4 = extreme force) as covariables. Odds ratios (**OR**) for KO were calculated for primiparous and multiparous cows using PROC GLIMMIX (SAS 9.4; SAS institute Inc., Cary, NC) for the whole study period and by weekly periods.

Least square means (LSM) for the daily proportions of KO in primiparous and multiparous cows were calculated using PROC GENMOD. A backward stepwise selection approach was used considering the 2-categories for parity, the covariables (COV), and their interaction in the initial model. Significant predictors were selected at *P* -value <0.05; interaction terms remained in the models at *P* -value ≤0.10.

RESULTS

A total of four study cows (1 primiparous, 3 multiparous) left the milking herd prior to completion of the monitoring period, but data collected from these cows prior to leaving the herd was still included in the analysis.

Figure 2.1 shows the daily proportions of primiparous and multiparous cows experiencing KO events during the study period. For the overall monitoring period, the odds of KO were greater for PRI vs. MUL cows (OR [95% CI] = 2.07 [1.58-2.73], P <0.0001). In addition, Table 2.1 presents the adjusted odds ratios (OR) and 95% CI for milking unit kick-off in primiparous vs. multiparous cows by weekly periods. When KO was analyzed by DIM, proportions of cows kicking were greater in PRI than in MUL during the whole monitoring period. In PRI, proportions of KO increased from 0.10/d to 0.20/d between 3 DIM and 15 DIM, before beginning to decrease around 30 DIM and remaining above MUL up to 90 DIM. On the contrary, in MUL cows, proportions of KO remained close to 0.05/d during the 90 d period (Figure 2.1).

DISCUSSION

As hypothesized in this study, milking unit KO was greater in PRI than in MUL during the whole monitoring period. This agrees with findings reported by Cequeira et al., 2017 where the odds of kicking were more than double in primiparous compared to multiparous cows (Cerqueira et al., 2017). This was an expected result, considering that primiparous cows had not been exposed to the milking routine previously and needed to habituate to the process.

However, our findings regarding the specific changes in kicking frequency during the first three months of lactation were unexpected. Some research suggests that both extremely fearful and extremely calm cows are less reactive during milking than those in-between (Bremner, 1997; Sutherland and Dowling, 2014). This is a possible explanation for the trends found in the present study, with inexperienced PRI cows (who are particularly stressed) displaying similar rates of KO compared to experienced MUL cows (who are often particularly calm) during the first days of lactation. Interestingly, occurrence of KO in PRI increased dramatically after the second week of lactation, while in MUL it remained close to 0.05/d throughout the 90-day monitoring period.

Anecdotally, it is often assumed that primiparous cows will display dangerous behaviors most often during the first week of lactation before steadily decreasing, but the data from this study indicates that this may not be the case. This finding contradicts Bremner, 1997 which found that primiparous cows move and kick more frequently during the first 7 milkings than during subsequent milkings (Bremner, 1997). It is possible that these differences in cow behavior were caused by outside factors, such as milking equipment and parlor style, but more research is needed to clarify why cows in the present study showed increases in KO frequency during the first week of milking. By expanding the information given to workers regarding dairy cow behavior during early lactation, more realistic expectations can be formed and improve the welfare of both workers and primiparous dairy cows during the milking routine.

Our findings are relevant for worker and cow welfare. With an increase in stress-related behaviors in primiparous heifers during the transition period, workers often experience greater difficulty performing milking tasks and a higher risk for injury (Sorge et al., 2014; Edwards and Kuhn-Sherlock, 2021; Phillips et al., 2021). It has been shown that both the behavior and attitude of the handler can have a significant impact on the physiological stress indicators and behavior in stressful situations (Hemsworth et al., 2000; Waiblinger et al., 2003), and inaccurate expectations of primiparous cow behavior has the potential to cause frustration, which can in turn further impact cow stress.

In addition to implications for worker welfare, increased frequency of milking unit kick-off can impact production and cow health. Each time a milking unit is removed, there is drop in vacuum and the cup must be reattached by a milking employee, interrupting the routine and decreasing efficiency of milking (Wieland et al., 2020). Additionally, it has also been shown that milking unit kick-off can increase cross contamination and risk of intramammary infection (Baxter et al., 1992; Thompson, 2019), which in turn can impact both cow comfort and milk production (Leslie et al., 2010; Rollin et al., 2015; Suarez et al., 2017). It is interesting to note that some research indicates a correlation between increased KO and high milk yield (O'Callaghan, 1996). However, despite multiparous cows typically producing a higher yield than primiparous, the research from the present study indicates that primiparous cows experienced more than twice as much KO than MUL during the whole monitoring period.

CONCLUSIONS

With implications for worker welfare, animal welfare, and productivity in the milking parlor, an increased understanding of the relationship between KO and parity is important to the industry as a whole. We concluded that primiparous cows evidenced greater levels of KO than multiparous cow throughout the first 90 days of lactation. Moreover, the relationship between days in milk and the proportion of cows displaying KO was not linear, but rather increased for the first several weeks before decreasing again. This has implications for training caretakers on what behaviors to expect from primiparous cows as they begin their first lactation, as well as on future research into reducing incidences of KO during early lactation.

TABLES AND FIGURES

Table 2.1. Adjusted odds ratios (OR) and 95% CI for milking unit kick-off in primiparous vs. multiparous (reference) cows by period of time[1].

Time period (DIM)	OR	95% CI	P value
Overall (3-90)	2.07	(1.58-2.73)	<0.0001
3-7	1.57	(1.07-1.22)	0.0202
8-14	2.05	(1.50-2.81)	<0.0001
15-21	2.74	(2.00-3.74)	<0.0001
22-28	2.62	(1.88-3.65)	<0.0001
29-35	2.48	(1.81-3.41)	<0.0001
36-42	2.22	(1.54-3.19)	<0.0001
43-49	1.79	(1.23-2.59)	0.0023
50-56	1.86	(1.27-2.74)	0.006
57-63	1.41	(0.94-2.13	0.1
64-70	2.53	(1.73-3.71	<0.0001
71-77	1.71	(1.11-2.62)	0.014
78-84	1.98	(1.29-3.03)	0.0016
85-90	2.09	(1.42-3.08)	0.0002

[1]The statistical models included parity (primiparous; multiparous), season of calving, and calving difficulty as fixed effects. Interactions tested were not significant and removed from the models.

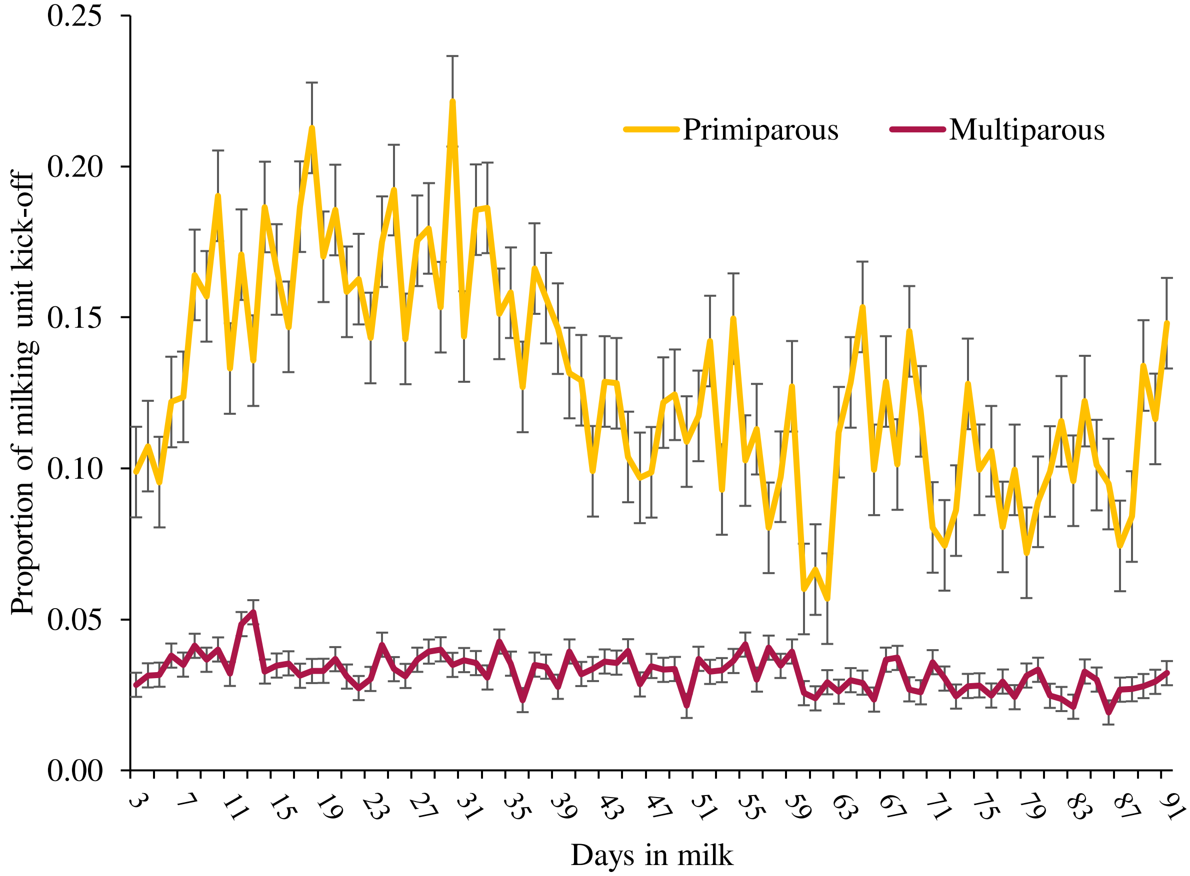

Figure 2.1. Proportions (SE) of milking unit kick-off events[1] during the first 90 DIM, separated by primiparous and multiparous cows[2].

[1]Milking kick-off data were analyzed by grouping the three consecutive milking sessions in each day and categorized as “yes” or “no”, indicating whether or not an individual kicked at least once in a given day.
[2] The statistical models included parity (primiparous; multiparous), season of calving, and calving difficulty as fixed effects. Interactions tested were not significant and removed from the models.

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CHAPTER 3: RELATIONSHIP BETWEEN KICKING, MILK PRODUCTION, AND MASTITIS OCCURRENCE IN PRIMIPAROUS AND MULTIPAROUS DAIRY COWS DURING EARLY LACTATION

SUMMARY

It is well known that both chronic and acute stress in production livestock animals can have detrimental effects on economically relevant parameters like milk production. In the primiparous cow, one of the most stressful periods of time is during the periparturient period, during which she experiences the onset of lactation and introduction to the milking routine for the first time. In previous studies, increases in behaviors such as kicking off the milking unit have been used to indicate both elevated stress levels and discomfort due to conditions of the mammary gland. The objective of this study was to investigate potential associations between rates of milking unit kick-off (KO) and differences in milk yield and mastitis presentation in both primiparous (PRI) and multiparous (MUL) during the first three months of lactation. 869 Holstein cows (PRI=199; MUL=670) from a certified organic dairy farm in northern CO were included in the analysis. Cows that calved between August and November 2020 were enrolled from 3 DIM until 90 DIM, and were milked 3x/day in a 60-unit rotary parlor. Cow KO events and milk production data were recorded automatically by the farm's milking system for each milking session, and then were downloaded from DelPro Farm Manager software. KO data were processed in the same manner as described in Chapter 2, and then cows were grouped into quartiles depending on their KO frequency. Subsequently, least square means (LSM) for daily proportions of KO were calculated considering the number of cows with KO events per day in the primiparous and multiparous categories. Odds ratios for the occurrence of KO in primiparous

vs. multiparous were calculated at multiple periods of time. Overall, the odds of KO were greater for PRI vs. MUL cows (OR [95% CI] = 2.07 [1.58-2.73]). No differences in milk yield were established among KO categories, while the percentage of cows affected with mastitis was greater in cows grouped in the quartile with more frequent KO events. We concluded that frequency of KO did not impact milk yield, though occurrence of mastitis may be correlated with increased amount of kicking in the parlor.

INTRODUCTION

There are many factors that influence milk production in dairy cows during early lactation. Genetics, parity, nutrition, disease, and many other factors can all impact economically relevant milking parameters such as flow rate and daily milk yield (Erb and Martin, 1980; Maréchal et al., 2011; Collier et al., 2017). While these factors have all been extensively studied and measured, less attention has been paid to the impact of acute stress specifically caused by the novel parlor environment on milk production in the periparturient cow.

In production livestock animals, acute stress caused by novel environments and poor handling techniques is widely accepted to be detrimental to production variables like meat quality, growth rates, and reproduction (Hemsworth et al., 1981; Hixon et al., 1981; Ligon, 2015). In market-weight beef cattle, acute stress due to transport, adverse handling, and holding pen environment is correlated with specific changes in meat quality (Van de Water et al., 2003; Romero et al., 2013; Peña et al., 2014; Njisane and Muchenje, 2017). Similar effects of rough handling and lairage conditions can be seen in pigs, such as increased levels of blood lactate and creatine phosphokinase, which subsequently increase the risk of poor quality pork after slaughter

(Warriss et al., 1994; Correa et al., 2010; Grandin, 2019). Acute stress and negative interactions with human caretakers have also been shown to have negative impacts on other economically-relevant variables in livestock, such as growth rates and rate of first-conception (Hemsworth et al., 1981; Hixon et al., 1981; Ligon, 2015).

As in other sectors of the livestock industry, the dairy sector has focused a large part of its research on the effects of environmental, nutritional, and physiological stressors on milk production parameters (Crowe and Williams, 2012; Ji et al., 2020; Pascottini et al., 2020). Research indicates that milk yield has a heritability of 0.25, meaning that 75% of the variability in milk yield observed between different cows is due to external factors like nutrition management, environment of the dairy, etc. (Collier et al., 2017). Thus, some of the more common topics of study include impacts of heat stress, nutritional stress (due to the physiological changes during the periparturient period), and disease occurrence. It is reasonable that much time has been spent to studying these topics, as they are relatively easy to study and quantify, and can be mitigated through specific changes to the management strategies on a given farm.

Also similar to other livestock sectors, the impacts on milk production of fear-based stress due to human interactions has gained traction as a topic for study in the last few decades, though there is still a need for further study. In reaction to aversive situations like novel environments and presence of handlers who had previously performed poor handling techniques, studies have found an increase in heart rate, elevated levels of plasma cortisol, and inhibition of oxytocin release; these physiological changes have been correlated with an increase in residual milk and a reduction in total milk yield (Rushen et al., 1999; Van Reenen et al., 2002). Another study found that about 19% of the differences in milk yield between cows was due to fear of human handlers and that some behaviors associated with fear (such as the number of flinch, step, kick responses and approaching stockpersons) were also correlated with changes in certain milk composition parameters like milk protein and milk fat (Breuer et al., 2000).

These studies have all been valuable in confirming the benefits of reducing acute stress in dairy cows; however, they generally focus on the impacts of sub-optimal conditions (i.e. obviously rough handling practices) on production parameters. Of course, these types of studies have been instrumental in changing the perception of the value of low-stress handling techniques. However, there have been relatively few studies that simply observe and describe the typical experience of dairy cows and their impact on outcomes such as milk yield. To date, there have only been a handful of studies that attempt to correlate the well-known specific, daily changes in production with the acute stress/fear experienced by the typical dairy cow during early lactation.

It has been established that there are predictable differences in milk production over the course of the lactation period (known as a lactation curve), as well as between primiparous and multiparous cows. These differences are typically attributed to physiological changes as a) lactation advances, and b) parity increases. Our main goal for this study was to investigate whether any of the differences in milk production between multiparous and primiparous cows, as well as the changes over the course of early lactation, can be specifically attributed to the stress of habituating to the milking routine (as opposed to normal physiological changes in the periparturient cow), with frequency of kicking behavior being a proxy for habituation.

That being said, one of the difficulties of using kicking behavior as an indicator of fear (especially when recorded via a computer system rather than on-farm observation) is the possibility for confounding factors that might also influence the likelihood of kicking. These primarily include poor attachment of the milking unit and painful conditions of the mammary gland that might result in an attempt to kick off the milking unit after it is applied. Common conditions such as mastitis, hyperkeratosis and teat lesions have also been associated with increased reactivity during milking (Rousing et al., 2004; Fogsgaard et al., 2015; Cerqueira et al., 2018). Additionally, these conditions are often associated with decreases in milk yield and quality, making it a significant factor when evaluating milk production trends. Thus, we also included occurrence of mastitis in our analysis to see if it had any correlation with milk yield and frequency of kicking.

MATERIALS AND METHODS

The materials and methods for this chapter were the same as for Chapter 2. Data collection occurred on an organic-certified dairy farm in northern Colorado using a parlor management software (DelPro Farm Manager software; DeLaval International AB, Tumba, Sweden). 199 primiparous and 670 multiparous Holstein cows that calved between August and November 2020 were enrolled in the study at 3 days in milk and monitored until 90 DIM. NO CSU Institutional Animal Care and Use Committee approval was required because of the remote nature of the data collection.

Study cows were milked 3 times per day in a 60-unit rotary parlor (DeLaval International AB). The main variable of interest was milking unit kick-off (categorized as yes or no), which was monitored with the electronic on-farm milk meters (DeLaval model MM27BC). The meter measured milk yield, milk flow, and milking duration through the use of infrared light technology. Kick-off occurrence, detected through these measurements, was then reported and stored in the parlor management software, where the records became available for transferring into Excel (Microsoft Corp.) spreadsheets in conjunction with the software program. Milking unit kick-off was identified by the milking system as an abrupt interruption in the milk flow during the milking process and considered in this study as a proxy for habituation to the milking routine.

Cow records outside of those collected in the milking parlor (such as parity, calving date, and mastitis occurrence) were exported from PCDART herd management software (Dairy Records Management Systems). Reports were then generated and downloaded via a remote server once weekly for the duration of the study, which continued until the last cow enrolled had completed her first 90 DIM.

Data exploration and descriptive analyses for daily average milk yield and daily average milk flow were performed using PROC MEANS and PROC GLM (SAS 9.4; SAS institute Inc., Cary, NC) and were separate for each parity category.

Cow KO events were recorded for each milking session. Subsequently, occurrence of KO was analyzed by grouping the three consecutive milking sessions in each day and categorized as yes or no, indicating whether or not an individual kicked at least once in a given day. In addition, numbers of days with a KO event were categorized for each cow into quartiles as Q1 (<2 d); Q2 (≥2 d to ≤3 d); Q3 (>3 d to ≤6 d) and Q4 (>6 d) (PROC UNIVARIATE) for milk yield and occurrence of mastitis comparisons and their potential interactions.

The logistic equation to investigate the effects of parity can be expressed as presented by de Mutsert et al. (2009):

$$\ln [p/(1-p)] = \beta_0 + \beta_1(\text{parity}) + \beta_2(\text{COV}) + \beta_3(\text{parity x COV})$$

where ln is the natural logarithm, p is the proportion of cows registering KO and $[p/(1-p)]$ are the odds of this outcome, β_0 is the model intercept for the study outcome, β_1, β_2, and β_3 are the regression parameters for parity category, the proposed covariables (COV), and the interaction term parity x COV.

RESULTS

Out of the 869 cows enrolled in our study, 4 cows (1 primiparous, 3 multiparous) left the milking herd prior to completion of the monitoring period, but data collected from these cows prior to leaving the herd was still included in the analysis.

Figure 1 provides daily milk yield (kg/d) and average milk flow (kg/min) during the study period for the participant cows separated by parity category. Our findings were consistent with other research on the relationship between parity and milk production, with daily yield and flow rate being higher in MUL cows than PRI throughout early lactation (Erb and Martin, 1980; Firk et al., 2002; Lee and Kim, 2006; Ben Meir et al., 2019).

Average (SE) number of days with a KO event were 11.0 (0.77) d and 4.5 (0.36) d for primiparous and multiparous cows, respectively ($P < 0.001$). Average milk yield per day by quartile category of KO in primiparous cows were 30.6 kg (Q1), 27.4 kg (Q2), 29.5 kg (Q3), and 28.2 kg (Q4) ($P = 0.31$), while average milk yield in multiparous cows were 38.9 kg (Q1), 40.1 kg (Q2), 39.8 kg (Q3), and 38.3 kg (Q4) ($P = 0.22$). The percentage of cows who presented with at least one case of clinical mastitis during the study were 18.7% (Q1), 20.0% (Q2), 4.50% (Q3), and 24.4% (Q4) ($P = 0.10$) in primiparous cows, while in multiparous the percentage of affected cows were 9.56% (Q1), 7.45% (Q2), 8.75% (Q3), and 23.5% (Q4) ($P < 0.0001$) (Table 3.1).

DISCUSSION

In this study, we found no correlations between milk yield and kick-off frequency. This finding contradicts previous research that did find a correlation between increased chances of kicking and high milk yield (O'Callaghan, 1996).

Both parity groups showed a partial correlation between mastitis occurrence and kicking behavior, with greater percentages of clinical mastitis in the Q4 groups (the quartile with the highest number of days with a KO event) than in other quartiles (PRI= 24.4%; MUL= 23.5%, $P < 0.0001$). This is in agreement with previous research that indicated a correlation between udder discomfort and reactivity during milking (Baxter et al., 1992; Thompson, 2019).

It is important to note that although it is plausible to consider milking unit kick-off as an indication of adaptation to the milking routine, the lack of research supporting the assumption of KO as a proxy for habituation to the milking procedure is a limitation of the current study. While kicking behavior during the milking routine has been used to indicate acute stress, there are many potential sources of stress in the parlor aside from the habituation process. Sharp contrasts between shadows and brightly lit areas (Willson et al., 2021), high ambient temperatures in the parlor (Cerqueira et al., 2017), and the presence of pest flies (Renčínová et al., 2021) have all been shown to increase risk of stress behaviors in cows. Other factors that can influence cow behavior during milking include improper milking techniques (Cerqueira et al., 2017), improper vacuum levels (Meyer et al., 2021), and stray voltage (Appleman and Gustafson, 1985).

Additionally, to the authors knowledge, the level of correspondence between machine-detected KO and actual kicking during the milking has not been investigated. Moreover, there is possibility for variation in KO identification, depending on the definitions used by different manufacturers. Due to restrictions associated with the COVID-19 pandemic, the authors of this study were unable to be present in the milking parlor during data collection, so validation of this was not possible, nor was the occurrence of other potentially confounding factors such as udder edema or teat placement.

Most previous studies focused on cow behaviour in the parlor are based on visual observation (Rousing et al., 2004; Cerqueira et al., 2017). However, the advent of precision technologies creates opportunities for the monitoring of specific behaviors in large numbers of animals. In addition to the approach suggested in this study, further research into kicking behavior during the milking procedure could be analyzed by use of leg attached sensors or by the application of algorithms linked to video recording.

CONCLUSIONS

Overall, we found that there was no correlation between frequency of milking unit kick-off and changes in milk yield during the first three months of lactation. Thus, there was no indication that stress related to habituation to the milking routine impacted milk yield. Kick-off was, however, related to mastitis occurrence, with more cases of mastitis being found in groups of cows with a greater tendency to kick. With implications for worker welfare, animal welfare, and productivity in the milking parlor, an increased understanding of the causes of kicking behavior during milking is important to the dairy industry as a whole.

TABLES AND FIGURES

Table 3.1. Percentage of cows in each kick-off quartile[1] experiencing at least one occurrence of mastitis during the first 90 days of lactation.

	Parity Category	
KO Quartile (Q)	Primiparous (P=0.10)	Multiparous (P<0.0001)
Q1 (<2 d)	18.70%	9.56%
Q2 (≥2 d to ≤3 d)	20.00%	7.45%
Q3 (>3 d to ≤6 d)	4.50%	8.75%
Q4 (>6 d)	24.40%	23.50%

[1]Cows were categorized and placed into quartiles based on the number of days a KO event was recorded during the monitoring period. Results for multiparous cows were more significant than for primiparous cows, but both parity groups found higher proportions of mastitic cows in the quartile with the highest frequency of KO.

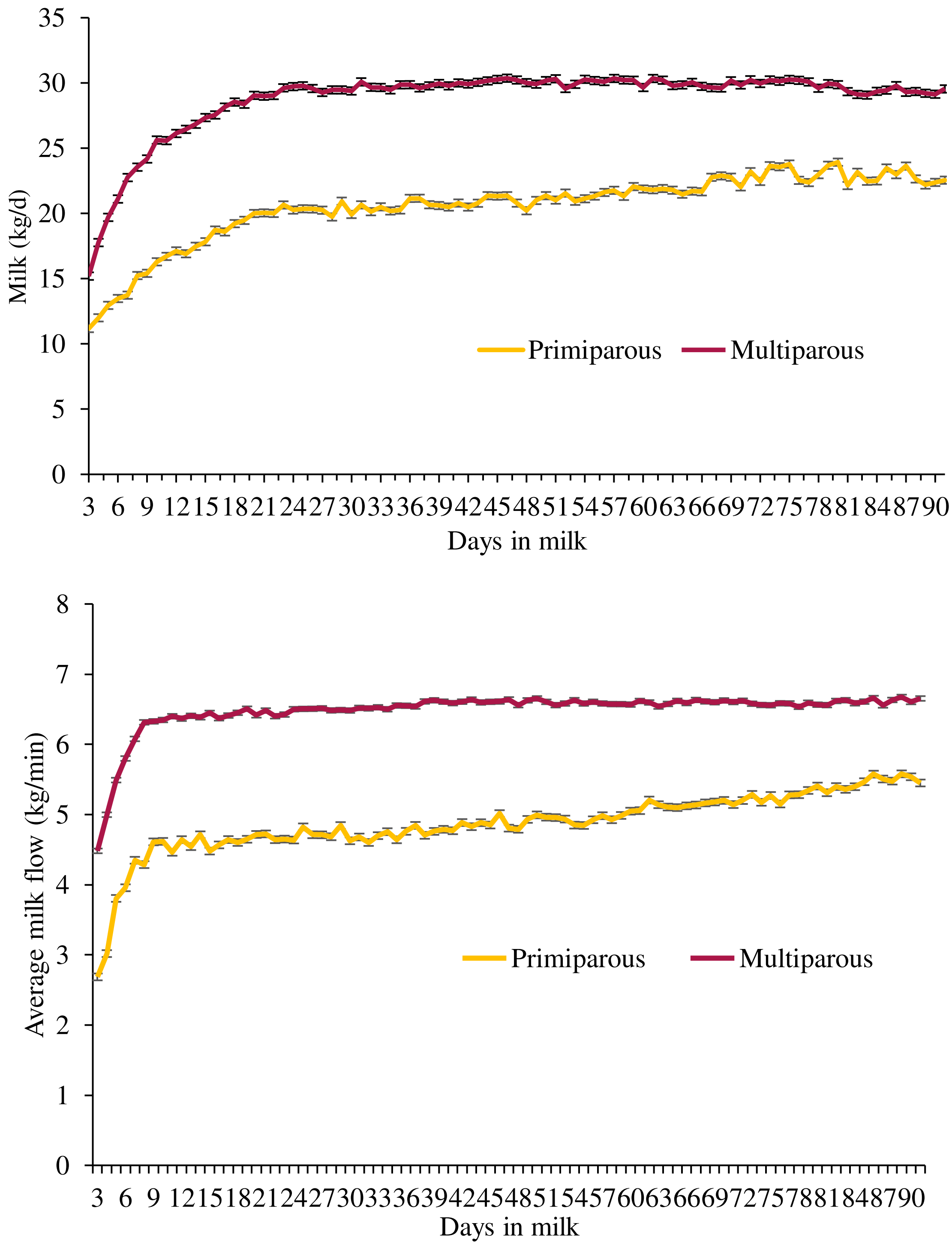

Figure 3.1. Daily milk yield (kg/d) and average milk flow (kg/min) of primiparous and multiparous cows during the first 90 DIM. Bars represent standard errors.

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CHAPTER 4: GENERAL CONCLUSIONS

The purpose of this thesis was to explore acute stress in the first lactation dairy heifer and its impacts on cows, their human handlers, and dairy operations as a whole. In Chapter 2, milking unit kick-off was greater in primiparous cows than in multiparous. Changes in kick-off for primiparous cows did not show a linear relationship with days in milk. In Chapter 3, there was no relationship between rates of kick-off and milk yield in either parity category, but there was evidence of a relationship between kick-off quartile and occurrence of mastitis. One of the main challenges in these studies was the lack of direct observation during milking. There are many factors that might impact the behavior of heifers, both physiological and environmental, and further research is recommended to fully understand the relationships between parity, days in milk, and kicking behavior. Still, the research presented in this thesis provides new insight into these dynamics, and is relevant for dairy farms that are interested in understanding the behavior of first-lactation heifers.