Evaluating the Relationship between Hoof Trimming and Dairy Cattle Well-Being

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CHAPTER ONE: Systematic Literature Review

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1.1 Introduction

Lameness is an important concern for the dairy industry because of its high prevalence and the effect it has on the productivity and well-being of the animal. Worldwide estimates of lameness prevalence have varied from 20 to 55% (Espejo et al., 2006; Barker et al., 2010; Von Keyserlingk et al., 2012). Furthermore, the prevalence of hoof lesions at the time of hoof trimming (HT) has been shown to be even greater (Cramer et al., 2008; Holzhauer et al., 2008; Becker et al., 2014; Solano et al., 2016). The effects of lameness result in significant economic costs to producers and are estimated to range from $100-$220 per cow (Cha et al., 2010). These costs include decreases in milk production, (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005) reproductive performance,(Bicalho et al., 2007a; Peake et al., 2011; Hudson et al., 2014) and increased culling (Booth et al., 2004; Bicalho et al., 2007a). Lameness also causes changes in behavior (Chapinal et al., 2010a; Gomez and Cook, 2010; Navarro et al., 2013) that are an indication it causes pain (Tadich et al., 2013; Bustamante et al., 2015).

Despite all of the negative effects lameness has on the animal and the dairy industry there has been limited research on evaluating preventative practices in clinical trials (Potterton et al., 2012). A variety of risk factors have been identified in observational studies

(Holzhauer et al., 2008; Barker et al., 2010; Solano et al., 2016) as potential targets for interventions. One commonly recommended practice is hoof trimming (**HT**) (Raven, 1985; Shearer and van Amstel, 2001). Hoof trimming is a common practice in the US dairy industry with almost 85% of herds HT a percentage of their cows at least once a year (NAHMS, 2007).

Hoof trimming is thought to prevent lameness by restoring a more upright foot angle by shortening dorsal wall length and excessive thickness of the sole at the toe thereby distributing weight more evenly within the weight bearing surface of each claw and balancing weight bearing between the two claws (Raven ET., 1985). Several different hoof trimming techniques have been described in the peer reviewed literature, (Manske et al., 2002a; Ouweltjes et al., 2009; Tanida et al., 2011; Van Hertem et al., 2014) text books (Raven, 1985; Shearer and van Amstel, 2001; Greenough, 2007; Blowey, 2015) or at hoof trimming conferences (Siebert, 2005; Daniel, 2014). Hoof trimming techniques can be split into categories based on how the technique leaves the angle of the sole relative to the metatarsals. The majority of methods (Raven, 1985; Shearer and van Amstel, 2001; Manske et al., 2002a; Blowey, 2015) advocate a flat sole whereby the abaxial and axial walls are trimmed to be level and perpendicular to the axis of the metatarsals. The differences that exist between the flat hoof trimming methods are mainly procedural. This method advocated originally by Raven (1985) focuses on using specific measurement to achieve proper dorsal wall length and toe thickness. Other methods prefer to use hoof angle (Manske et al., 2002a) or sole reading methods (Blowey, 2015) to achieve appropriate length and thickness. The measurements for proper dorsal wall length and corresponding sole thickness have recently been called into question (Nuss and Paulus, 2006; Archer et al., 2015). Adaptations to the flat trimming method have been advocated and have focused on increasing the amount of horn removed underneath the flexor tuberosity to reduce pressure on the sole ulcer location (Ouweltjes et al., 2009; Gomez N.B. Cook, N. Kopesky, J. Gaska, and D. Dopfer, 2013).

Alternative trimming methods to the flat trimming method encourage a sloped sole whereby the abaxial wall is higher than the axial wall (Amstutz, 1979; Siebert, 2005). Proponents of this method consider this a more natural sole angle and the procedures to achieve this angle focus on reading the sole and stopping when dehydrated horn disappears. The majority of hoof trimmers use either one of these methods as a basis for their own personalized trimming techniques (Daniel, 2014). Data from our research groups show that out of 44 hoof trimmers attending a 2014 HT conference, 55% used functional trimming as described by Raven (1985), 17% used the white line method (a sole reading method described by Blowey (2015), 12% used the Kansas method (the method described by Siebert (2005) and 15% used a combination of methods (Scherping et al., 2015).

Unfortunately, limited data exist about the efficacy of these various HT methods. Since HT is a commonly recommended practice to prevent lameness there is a need to evaluate the efficacy, frequency and the physiological and behavioral responses of the animal to these different techniques. One method recently used to review current knowledge is to identify knowledge gaps and set directions for future research through a systematic evaluation of the current literature (Sargeant and O'Connor, 2016). The objective of this chapter is to critically evaluate the evidence that exists for HT techniques as it relates to efficacy, frequency, and associations with behavior and physiological parameters.

1.2 Methods

To achieve our objective, we carried out a narrative integrative review in June 2016 and attempted to reduce bias by incorporating aspects of a systematic review as described by Sargeant and O'Connor (2016). Databases searched included: Pubmed, Agricola, Google Scholar, and Web of Science. The search terms used for all databases were "cattle hoof trimming", and "cattle claw trimming". All languages were included in the searches. A total of 613 articles were retrieved from the results of the 4 databases and stored in RefWorks Software© (LLC, 2016). Of the 613 articles, 348 duplicates were removed leaving 265 left for further review. The abstracts of these 265 articles were retrieved and evaluated by the primary author (GS) according to the inclusion criteria below.

Inclusion Criteria:

1. Study animals were living adult dairy cows.
2. One group of cows underwent a HT procedure.
3. Study used either a before and after comparison or a separate control group.
4. Published in a peer reviewed journal and written in English.

A total of 16 articles met the inclusion criteria and were grouped into 4 categories: behavior (4), physiology (7), efficacy (2), frequency and timing (2).

1.3 Associations with Behavior

1.3.1 Behavior Background

Behavior is used as a surrogate to indicate the effect a procedure has on an animal's affective state (Dawkins, 2003). Common behavioral indicators used to evaluate lameness include, locomotion score (LS), lying time, activity and walking speed. Locomotion scoring is a subjective measure (Winckler and Willen, 2001; O Callaghan et al., 2003) that defines lameness based on an alteration in gait that develops due to pain. Locomotion scoring is commonly used to evaluate a herd's welfare status (O Callaghan et al., 2003; Flower and Weary, 2009). Unfortunately, a recent review (Schlageter-Tello et al., 2014) found 25 different manual LS systems and 15 different automatic LS systems described. This variation and the subjectivity of LS creates difficulty when comparing studies that use LS as the outcome. Other behaviors that have been associated with lameness are lying time, activity, and walking speed. Changes to these parameters are hypothesized to be an indicator of a pain response (O Callaghan et al., 2003; Cook et al., 2007; Ito et al., 2010).

1.3.2 Review of Behavior literature

Chapinal et al. (2010a) evaluated the general effect of routine HT on LS, walking speed, lying time, and the distribution of weight between the rear legs while standing. This study consisted of 48 lame and non-lame lactating Holsteins with daily data collection 1 week prior to HT and for 5 weeks after. Results from this study showed that LS was negatively impacted only in the 2 immediate days following HT with no long term impact. Additionally, walking speed was decreased and lying time increased following HT and these change persisted for the entire study period. Finally, only lame cows showed a change in rear leg weight distribution after trimming. Unfortunately, this study failed to describe the HT methods or treatments used and housed cows in groups of 12 limiting its generalizability.

A follow up study by Chapinal et al. (2010b) used a similar methodology and focused on the effect of using a non-steroidal anti-inflammatory (NSAID) flunixin meglumine (FLUMEG) before and 24 hours after HT. The study used 66 lactating Holstein cows allocated to 3 treatment groups. Group 1 (n=10) underwent a sham HT. Group 2 (n=28) cows were hoof trimmed and received FLUMEG. Group 3 (n=28) cows were hoof trimmed and received saline. Gait scores, and weight distribution were monitored, before HT, 2 hours after HT, and the day after HT. Lying time and frequency of steps were monitored in 3 time periods, 2 days before HT, 2 days after HT and 3 days after HT. For both the FLUMEG and saline groups there was a tendency ($p<0.10$) for an over 2 hour increase in lying time in the first 2 days post HT. This increased lying time only persisted past 2 days in the saline group. The sham trimmed group did not show this increase in lying time. No biologically significant changes were found for the other outcomes measured in this study. Due to the inclusion of a sham trimming group this study provides evidence that the HT process affects behavior. Unfortunately, the distribution of lame cows was not equal between groups since lame cows were not enrolled in the sham group. This makes it unclear if the relationships found in this study are due to the HT process, lameness, or treatment of the lameness. Similar to the first study this study did not adequately describe the HT technique.

A very small study by Tanida et al. (2011) evaluated the gait components of 17 cows post HT. The study measured vertical, lateral and forward acceleration 1 month before HT, and once monthly for the 2 months post HT. Acceleration and direction were measured using a motion sensor on the final thoracic vertebrae of the cow and by analyzing video recordings of cow movements. Results suggested that the cows gait was smoother in the month post HT as the variances of the lateral and vertical acceleration were lower than the baseline values. The utility of this study is very limited due to a small sample size, and a limited number of unclearly described measurement times. In addition, the lesion status of these cows was not described. Similar to the previous studies HT method was not described clearly.

A more recent study by Van Hertem et al. (2014) evaluated the relationships between HT and rumination, neck activity and LS in a 1,100 cow dairy. Cows in this study were evaluated at 19 different times for LS and had daily electronic rumination and activity data recorded. Locomotion scores were analyzed in several different manners including long term (up to 70 days post HT), and short term (1 week pre and post HT). Results from 152 cows with LS data from 6 different time periods ranging from 34 days before HT to 70 days post HT showed that lameness prevalence doubled significantly from 16% to 32% in first 2 weeks post trimming, but returned to immediate pre-HT levels by 70 days. An analysis of 288 cows that had complete data on LS, rumination, activity and milk yield for the week before and after HT was also completed. This analysis showed that LS increased in the week after HT, while neck activity decreased only on the first day post HT. The effect of HT on rumination was dependent on parity. Due to the larger sample size they were able to explore potential confounders such as lesion status, parity and DIM. This study provided adequate details about the HT equipment and technique used.

1.3.3 Summary of Behavior Literature

All 4 of the studies have several common experimental design issues that limit the conclusions that can be drawn about the association between HT and behavior. None of the studies fully describe the HT process making it difficult to judge differences in techniques and limits the external validity of each study. In addition, all 4 studies included lame cows making it difficult to determine if the effect was due to the HT process or the lesion treatment process. Only one study had a large enough sample size to attempt to control for this confounding (Van Hertem et al., 2014). Based on these 4 studies it is clear that the HT process is associated with a change in animal behaviors such as resting time and LS. The increase in LS from several studies (Chapinal et al., 2010a; Van Hertem et al., 2014) would indicate this is a negative change in the welfare status of the animal (O Callaghan et al., 2003; Flower and Weary, 2009) and supports the hypothesis that the increase in lying time is a compensatory response (O Callaghan et al., 2003; Flower and Weary, 2009; Gomez and Cook, 2010; Ito et al., 2010). However, to truly evaluate the impact of preventative HT on animal behavior there is a need for HT studies on cows without lesions.

1.4 Associations with Physiological Measures

1.4.1 Physiological Background

To assess the welfare impact of a procedure it is important to evaluate the impact it has on the animal physiologically (Fraser, 1993; Dawkins, 2003). Exposure to stressors challenges the homeostasis of the animal leading to the activation of the hypothalamic pituitary adrenal axis and the sympathetic nervous system. This subsequently leads to an increase in stress hormones in the blood stream (Harris, 2015) and can impact physiological functions such as heart and respiratory rate, milk production and reproductive functions. The HT process has the potential to expose the animal to various stressors including: restraint, handling, isolation, and pain due to treatment.

1.4.2 Review of Physiological Literature:

Nishimori et al. (2006) investigated the effect of HT on blood parameters, in addition to milk composition and production using 11 Japanese Holstein cows. Samples were taken before and after HT at time points that were not clearly specified. This study showed that after HT, milk fat %, protein %, and some blood parameters changed. However, due to limited sample size and unclear sampling times, the observations in this study may be difficult to replicate consistently. As a result, replication on a larger scale and improvement of methods are warranted.

Pesenhofer et al. (2006) compared the effect of different chute designs on fecal cortisol metabolites and milk yield before and after HT. This study included 207 lame and non-lame animals randomly assigned to the different chute designs that underwent HT according to the functional HT method (Raven, 1985). Milk yield was recorded 7 days before HT and until 13 days after HT. Fecal cortisol metabolites were sampled 12 hours before HT and until 7 days after HT. This study showed a significant decrease of 0.6 L in milk production on the day of HT and the day after. Fecal cortisol metabolites were significantly higher for up to 24 hours following HT for both chute designs. This study showed that HT caused a stress response and affected the productivity of the animal. The inclusion of lame animals, however, does not allow for evaluating if the physiological changes are due to HT or lesion treatment.

A smaller study by Rizk et al. (2012) evaluated the stress response to HT in lateral recumbency. This study used a paired 3 way crossover design with 6 Holstein cows. Treatment groups consisted of cows receiving either a saline or xylazine injection prior to HT. Sampling times started 15 minutes before HT and continued until 3.25 hours after HT. Changes in the cardiorespiratory system, stress hormones and metabolism were measured and compared to status pre-HT. Results from this study showed that HT causes changes in: blood diastolic blood pressure, mean arterial blood pressure, oxygen saturation, respiratory rate, cortisol, and lactate. Xylazine mitigated these effects but showed a depressive effect on respiratory parameters. Observed changes in this study indicate that the HT process resulted in a stress response in the animal. However, caution must be used when interpreting these results because the length of restraint in the HT chute was extremely long (30 minutes).

Korkmaz et al. (2014) completed a randomized clinical trial using 14 cows that evaluated the impact of dexketoprofen on cows that were trimmed in a squeeze chute. Outcomes evaluated included cortisol, nitric oxide, malondialdehyde, total antioxidant activity, and heart and respiratory rates. Data was collected 30 minutes before HT and again at 15 and 30 minutes after HT. Results of the study showed an increase from baseline to 15 minutes post HT for heart, respiratory rate and cortisol. None of the other parameters were different when compared to baseline. Similar to the previous studies, this study is limited by sample size and a short follow-up period; it does support the view that the HT process affects physiological measures.

The study by Van Hertem et al. (2014), described in the behavior section also investigated milk production in the week before and after HT. Unlike some of the behavioral parameters, no association with HT was found for milk production.

Using a randomized control trial Maxwell et al. (2015) evaluated the effect of an early lactation functional HT compared to no trimming in 281 primiparous Holstein cows. Outcomes in this study were: 305 day adjusted milk yield and conception rate at 100 DIM. No significant difference between the HT and no HT group was found for either outcome. However, an interaction with LS existed wherein HT lame animals resulted in higher milk production post HT compared to untrimmed non lame animals. Results of this study with limited sample size suggested that trimming all heifers would not provide an economic return. This is one of the few studies to provide a clear description of their HT method.

The most recent physiological study investigated the claw temperature changes that occur at the coronary band after HT (Alsaaod et al., 2015). Skin temperature can be increased due to inflammation (Van hoogmoed and Snyder, 2002; Alsaaod et al., 2014) or other metabolic activity (Stewart et al., 2007). This study consisted of 81 non lame and lame cows that had infrared temperature readings at the coronary band taken pre HT and 21 days post HT. Hoof trimming technique was not described. At 21 days after HT the mean change in hind feet temperature was 0.25^0C ($p=0.08$) cooler. However, this temperature change was different between medial and lateral claws and not present in the front feet. Although this study showed a trend for a change in coronary band temperature the biological significance of this change after 21 days is unclear.

1.4.3 Summary of Physiological Literature

These studies provided evidence that the HT process changes physiological parameters. Caution must be used when interpreting and comparing these studies as sample size was limited and HT technique was not adequately described in most of the studies. The design of the studies also makes it difficult to determine if it is the restraint or the actual removal of horn that is causing the change in physiological measures. Even though effects on physiological parameters can be found, there is still a knowledge gap on the exact cause and the biological significance of these changes.

1.5 Efficacy of Hoof Trimming

1.5.1 Background of Efficacy

In addition to knowing how HT affects the animal from a behavioral and physiological perspective it is important to know if HT is efficacious at preventing lameness and lesions. The goal of HT is to prevent lameness by restoring proper foot angle, removing excess horn growth, and re-distributing the weight of the animal over the two claws (Raven, 1985). As discussed previously, there are various hoof trimming methods and it is important to evaluate the efficacy of these methods in preventing lameness.

1.5.2 Review of Efficacy literature:

Van der Tol et al. (2004) evaluated the standing weight distribution and surface area between hind claws in 5 Holstein cows before and 2 weeks after functional HT (Raven, 1985) using force plates. Results from this study showed that the average pressure on the hind limbs decreased by 30% and improved the distribution of weight between the lateral and medial claws. The improvement in the weight distribution did not equalize weight bearing between the 2 claws. Additionally, HT did not show a significant change in the maximum pressure on the claws. Although this study showed an improvement in certain weight distribution parameters due to HT, the extent of the difference was much smaller than expected and left the authors to speculate changes in HT techniques are necessary. This study had a very small sample size and only used one follow up sampling time period. This makes it possible that cow level confounders could have influenced the result or the follow up period was inappropriate.

Using force plates in a different manner Carvalho et al. (2006) evaluated the weight distribution and pressure points on the sole between trimmed cows (14) and untrimmed cows (17). In this study the untrimmed group had significantly more total pressure applied to heel of the lateral claw of the hind limb than trimmed cows. At the claw level numerical differences were found for increased pressure on several other claw regions. Due to small sample size, and the lack of descriptive data about the cows and HT technique it is difficult to determine if these differences are due to confounding or HT.

As a follow up study to the Van der Tol et al. (2004) study Ouweltjes et al. (2009) investigated the efficacy of functional HT (Raven, 1985) to an adaptation that decreases the pressure in the typical sole ulcer region on both claws. Non-lame cows were randomized to either the functional method (33) or the adaptation (32) and observed for 3 months. Outcome measures included claw health, claw conformation, LS activity, and floor contact pressures. Results of the study did not show any significant differences between the two HT methods. There are several possible reasons for the lack of differences between treatments including a short follow-up observation period and limited sample size. Additionally, the fact that both the lateral and medial claws were trimmed with the adaptation could have masked an effect. A similarly designed unpublished study (Gomez et al., 2013) did show a difference in lesion prevalence when only the lateral claw was trimmed with the adaptation. The Ouweltjes et al. (2009) study had a very clearly described trimming method section and should be considered a model for how to describe HT techniques in future publications.

A study from New Zealand evaluated the efficacy of HT in New Zealand by randomly allocating 2,695 cows to functional HT (Raven, 1985) or no HT (Bryan et al., 2012). Outcomes evaluated included incidence and time to lameness as identified by trained farm staff. In this study HT did not change the cumulative incidence of lameness but did increase the median time to lameness in the 70 days post HT. This well designed study had several strengths including: accounting for confounders and the use of multiple farms with one hoof trimmer. Using farm staff to identify lameness leads to an underestimated level of lameness (Espejo et al., 2006), but this is a non-selective bias.

1.5.3 Summary of Efficacy Literature

These 4 studies evaluated HT efficacy from two different perspectives and 3 studies showed a benefit by reducing pressures in the claw or increasing time to lameness. All the studies used the functional HT (Raven, 1985) method, but several other methods have been described (Shearer and van Amstel, 2001; Manske et al., 2002a; Siebert, 2005; Greenough, 2007; Ouweltjes et al., 2009; Tanida et al., 2011; Daniel, 2014; Van Hertem et al., 2014; Blowey, 2015) that also need to be evaluated. In addition, several studies were small in size (Van der Tol et al., 2004; Carvalho et al., 2006; Ouweltjes et al., 2009) or their findings are likely environment specific (Bryan et al., 2012). Therefore, more studies evaluating the efficacy of HT are necessary.

1.6 Frequency and Timing of Hoof Trimming

1.6.1 Background of Frequency and Timing of Hoof Trimming

Hoof growth can vary with breed and genetics, but the net growth of dorsal wall horn is around 1-2 mm/month (Hahn et al., 1986; Vokey et al., 2001). Functional hoof trimming attempts to deal with this growth by restoring an upright foot angle and balancing the weight bearing between the two claws.(Raven ET., 1985) Some observational studies have found associations with more frequent HT and lower lameness prevalence (Fjeldaas et al., 2006; Espejo and Endres, 2007).

1.6.2 Review of Frequency and Timing of Hoof Trimming Literature

Manske et al. (2002a) conducted a two year study on 3444 dairy cattle on multiple Swedish dairy farms that were block randomized to a second HT in the autumn. Regardless of allocation, all cows were LS and trimmed at the spring trimming. Results indicated that the additional trimming in the autumn led to lower odds of lameness, and horn type lesions. This study was well designed and included a long observation period with block randomization to control confounding, and used multiple hoof trimmers and farms to increase generalizability to other farms in Sweden.

Hernandez et al. (2007a) evaluated the efficacy of a hoof health examination and a HT at mid-lactation compared to no evaluation at mid-lactation in 313 randomly allocated cows in one herd. This study showed that cows in the non-trimmed group had a 25% higher ($p=0.09$) lameness incidence and a 1.25 ($p=0.12$) higher risk of becoming lame compared to the trimmed group. Some problems in study design limit the interpretation of this study as the study was done in only 1 herd, did not evaluate control cows for lesions upon entry into the study and there was inadequate detail of their HT method.

1.6.3 Summary of Frequency and Timing of Hoof Trimming Literature

These 2 two studies showed that an additional HT can be beneficial in the herds studied. However, only one of the studies (Manske et al., 2002a) described the HT technique used in enough detail that it could be repeated. From these studies it is still unclear what is the most efficacious time to trim animals for a second time during lactation and if the additional HT would be beneficial in all situations.

1.7 Conclusion

This review of 16 articles of HT as it relates to the efficacy, frequency, and associations with behavior and physiological parameters found several common study design issues that limit generalizability and conclusions. The majority of studies used a small sample size and lacked a clear description of the HT technique. In the reviewed studies, HT appears to initiate a stress response, change behavior, improve components of weight bearing and reduce lameness in specific environmental conditions.

There are still multiple knowledge gaps that need to be answered to create a more complete picture of the impact HT has on cows and lameness. Primarily, it is necessary to determine the most efficacious HT technique from a physiological, behavioral and productivity perspective. Furthermore, it is necessary to understand what the change in physiological parameters means to the animal and what is causing them, the restraint or the actual removal of horn. Additionally, to encompass all aspects of welfare (Fraser, 1993) it is necessary to understand the effect HT has on the behavior of a non-lame animal. Lastly, additional information is needed on appropriate timing and frequency of HT.

1.8 Aim of Thesis Research and Objectives

The overall aim of this thesis was to provide knowledge to the scientific community and producers about HT and to provide science based recommendations on if LIT or BIG model is more effective at preventing lameness and lesions. The following specific objectives will address this goal:

Objective One: *Evaluate the associations between preventative HT of cows with no lesions and activity, resting behaviors and milk yield in commercial dairy herds.*

Objective Two: *Compare and evaluate the duration of stress, pain, and inflammatory responses between cows restrained for hoof trimming and cows being hoof trimmed.*

Objective Three: *Compare the effects of the functional HT (LIT) method to an adaptation that results in increased modeling of the weight bearing claw (BIG) on risk of lameness and prevalence of new lesions at mid-lactation.*

Objective Four: *Compare the weekly milk production and culling risk for cows trimmed at dry-off with the functional HT (LIT) method or trimmed with the previously described adaptation (BIG) in chapter 4 of this thesis*

Objective Five: *Compare the 7-day milk yield post HT and the difference from baseline milk production between cows undergoing one of two HT techniques and cows that were only restrained in the HT chute.*

CHAPTER TWO: Observational study evaluating the associations of hoof trimming with dairy cattle behavior and milk yield in Canada and the United Kingdom

2.1 INTRODUCTION

Lameness is a costly (Cha et al., 2010; Liang et al., 2017) painful (Whay et al., 1998) disease in the dairy industry that affects the productivity (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005) and behavior (Chapinal et al., 2010a; Gomez and Cook, 2010; Navarro et al., 2013) of dairy cows. The herd level prevalence of lameness has been estimated to be between 20-55% in North America (Espejo et al., 2006; Von Keyserlingk et al., 2012; Solano et al., 2015). The cost of a case of lameness has been estimated to be around \$100 to \$220 per cow (Cha et al., 2010; Liang et al., 2017). The high cost of lameness is due to multiple factors, that include milk production loss (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005), decreased reproductive performance (Bicalho et al., 2007a; Peake et al., 2011; Hudson et al., 2014), increased culling (Booth et al., 2004; Bicalho et al., 2007a), and veterinary treatment and labor costs (Liang et al., 2017).

The behavior changes that are caused by lameness are part of a pain response (Whay et al., 1998; Tadich et al., 2013; Bustamante et al., 2015). Lameness causes a decrease in activity, increase in resting time, decreased walking speed and an increase in locomotion score (Chapinal et al., 2010a; Gomez and Cook, 2010; Navarro et al., 2013). These behavior changes indicate that the state of the animal's welfare is reduced (Dawkins, 2003) and there is a need for implementing preventative practices. A recommended preventative practice to prevent lameness is hoof trimming **(HT)** (Shearer and van Amstel, 2001) and it is estimated that 85% of US herds have cows trimmed at least once a year (NAHMS, 2007). If HT in non-lame cows prevents the onset of a future lameness event, then behavior changes associated with clinical lameness should not appear following the procedure.

However, a recent systematic review of the relationship between HT and dairy cattle welfare by Stoddard and Cramer, (2017) has shown that the scientific research surrounding the benefits of HT is limited to only 16 studies. The current data on HT can be broadly split into the following 4 categories: efficacy, frequency and timing, physiology and behavior. Hoof trimming slightly improves the weight distribution between the claws (van der Tol et al., 2004), but not as much as expected. In a grazing environment hoof trimming reduced heel height difference between the claws and increased the time to lameness, but it did not decrease the incidence of lameness between cows that were trimmed and not trimmed, respectively (Bryan et al., 2012). Increasing the frequency of HT has shown to be beneficial for reducing the odds of a lesion and lameness (Manske et al., 2002b; Hernandez et al., 2007b). Physiological data has shown that HT causes an acute stress response (Rizk et al., 2012; Korkmaz et al., 2014), as is indicated by a rise in respiratory and heart rates, as well as increases in plasma cortisol, lactate and glucose concentrations. It is possible that this acute stress response induces changes the behavior of the cow following HT, as observed in several studies (Chapinal et al., 2010a; b; Van Hertem et al., 2014). These previous behavioral studies have shown that lying time increases, activity decreases and locomotion score increases (Chapinal et al., 2010a; b; Van Hertem et al., 2014) following HT, as compared to before HT. This would suggest that the HT has a negative effect on the well-being of the animal, and the cow is showing compensatory behaviors following HT. This is concerning because HT has become a common practice in the dairy industry, and following the procedure the cow's behavior change is similar to the behavior changes that are associated with lameness. Unfortunately, all of the previously mentioned behavior studies included animals that were already lame prior to HT, so this could be influencing the observed changes. For HT to be beneficial as a preventive practice for a non-lame cow, one of the criteria is that the behaviors associated with lameness should not be present immediately following the procedure. The inclusion of lame cows in the currently available research makes it difficult to assess the impact HT has on the behavior and well-being of non-lame cows.

The objective of this study was to evaluate the associations between preventative HT of cows with no lesions and several behavior- and productivity related outcome measures including activity, resting behaviors and milk yield in commercial dairy herds. Our hypothesis was that preventative HT of healthy cows will have a positive or neutral association with cow behavior defined as an increase in activity level, increased number of lying bouts of shorter duration, no change in total lying time and no association with milk production.

2.2 MATERIALS AND METHODS

2.2.1 Herd Selection

The procedures in this observational study were approved by the University of Minnesota Institutional Animal Care and Use Committee (1411-32094A). The study used a convenience sample of 4 herds that were located the United Kingdom (n=2) and Ontario, Canada (n=2). Participating farms were required to use free-stall housing, have a regular trimming schedule, and use the AFI-PLUS or AFI-ACT2 pedometer system (Afimilk, Afikim, Israel) to allow for the collection of activity and resting time data (Borchers et al., 2016). Two of the farms used a mattress surface bedded with sawdust, one used deep bedded sand bedding and one used a combination of sand and sawdust bedding on a mattress surface. The average herd size was 565 cows, ranging from 100 to 1,260 cows. The average 305 day milk production for the herds ranged from 11,137 kg to 13,691 kg. Participating herds routinely trimmed cows either at dry off and as need (n=2) or at mid-lactation and dry off (n=2) during lactation

2.2.2 Cow Enrollment and Hoof Trimming

Cows were enrolled on an ongoing basis from April of 2015 until July of 2016, as they were trimmed per their farm’s protocol and schedule. Only cows that did not have a lesion present at their first appearance in the data set were included in the study. Data for all HT events at all 4 farms was recorded from the start of the study until the end of the study. Hoof trimming technique in this study was not controlled for, with hoof trimmers using their normal HT technique.

2.2.3 Data Collection Description The following behavioral parameters were collected daily: activity (steps/day), resting time (minutes/day) and resting bouts (bouts/day). In addition, milk yield (kg) data was collected at every milking. All data was captured automatically every time the animal entered the parlor for milking. Daily, this behavioral data was transferred from the AFI herd management software to a text based file. Hoof trimming data, describing the presence or absence of lesions, were collected from the farm's HT records. Cow level data such as days in milk (DIM) and parity was extracted from the farm's recording software DairyComp 305 (Valley Ag. Software, Tulare. CA).

2.2.4 Data Management and Statistical Analysis

All behavioral, milk yield, cow and lesion data management and analysis was performed using STATA 14.1 (Stata Corp., College Station, TX).

Behavior and milk yield data was collapsed to the day level and merged with HT and cow level data such as breed, lactation and DIM. Cows with any lesions already present at the first HT occurrence in the dataset were excluded from analysis. Only a cow's first non-lesion hoof trimming, whether that be at dry-off or during lactation, was included in the dataset. Season of HT was categorized into spring (March-May), summer (June-August), fall (September-November) and winter (December-February). Lactation number was categorized into parity 1 or parity 2 and greater. A time (days) from HT variable was created for each collapsed behavior and milk yield data point.

For each of the outcome variables: activity (steps/day), resting bouts (bouts/day), resting time (mins/day), and milk yield (kg/day) a general linear mixed model was built to compare the outcome for 7 days after HT to a baseline average of 5 days before HT. A 5 day average baseline was used for all of the outcome variables to reduce the impact of the natural variation that occurs in resting time and activity over a lactation (Maselyne et al., 2017). A 7 day follow up period was used for this study because of the focus on the acute effects HT has on the outcomes. The inclusion of dry off trims meant that data describing the outcomes shortly following HT would not be available on some of the cows. The decision to use a 7 day follow-up period was based on graphically evaluating the predictive margins of the univariate models. At day 7, most behaviors had returned to the 5 day baseline or formed a plateau.

For each outcome variable a final generalized mixed model was built by evaluating the association between the outcomes and the covariates: lactation number (1, 2+), season (spring, summer, fall, winter), farm ID (1, 2, 3, 4), and DIM in univariate models. If a covariate had a $P < 0.20$ in the univariate model, they were offered to the multivariable linear regression model. A stepwise backward elimination was then performed using $P<0.05$ as the final cutpoint for including a term in the model. Upon removal of non-significant covariates, the model AIC and change in coefficients were evaluated to determine if significant changes occurred. After variable selection, all biologically plausible interactions were investigated and included if significant interactions were present. Time (days) from trim was forced into all of the final models. Model outcomes presented in the results section are adjusted for the other significant covariates in the model. All models included an exchangeable correlation structure to account for the repeated measures on cow. The exchangeable correlation structure was chosen based on the high correlations between different time points. Other correlation structures were evaluated and compared using model AIC (Dohoo et al., 2009). The model assumptions of homoscedasticity, normality and a linear relationship were evaluated graphically for all final models.

2.3 RESULTS

A total 7,980 HT events were recorded and 2,652 of these were first HT events for the cow. Of the total 2,652 first HT events, only 1,572 cows did not have a lesion present and so were eligible for inclusion in the final analysis. The cows included in the analysis had an average DIM, and lactation of 174 ± 2.7 days, and 1.5 ± 0.01 respectively (Table 1). Descriptive statistics for the behavioral outcomes (activity, resting time, and resting bouts) and milk yield are presented in Table 1 and are based on the 5 day average prior to hoof trimming.

2.3.1 Activity (Steps per day)

The final generalized linear model for activity included lactation, season, DIM, and an interaction between farm and time (Table 2). To show the inverse relationship between activity and resting time, Figure 1 shows the farm-specific adjusted estimated mean activity levels and resting times, starting with the baseline prior to HT, and going until the end of the 7-day observation period. Figure 1 shows that activity increased numerically on the day of HT for all farms. The average magnitude of the change in activity for the 7 days following HT was dependent on farm and ranged from -17 to -39 steps.

2.3.2 Resting Time (Minutes per day)

The mean difference in resting time from baseline to following trimming was dependent on farm. In addition to the interaction term between herd and time, other variables remaining in the model were lactation and DIM (Table 2). After stratifying by herd, adjusted estimated mean resting times starting from baseline until the end of the observation period are plotted in Figure 1 with the corresponding activity data. On the day of HT cows had a decrease in resting time compared to baseline. Following HT resting time was elevated for cows for most of the farms, but again this was farm dependent. Following HT, an increase in resting times for the remainder of the observation period was observed ranging from 11 to 34 minutes above baseline.

2.3.3 Resting Bouts (Bouts per day)

The final model for resting bouts included time and farm ID with an interaction term between farm ID and time. Cows showed decreased resting bouts on the day of HT and returned to baseline the day after HT (results not shown).

2.3.4 Milk Yield (kg per day)

Milk yield was dependent on farm, with the final model including the covariates lactation, season and DIM (Table 2) in addition to an interaction between farm and time. Figure 2 shows the estimated adjusted mean milk yields for the entire observation period by farm. On the day of HT cows showed a change in milk production, but it varied in magnitude and direction among the four farms. Farms that had a decrease in milk yield on the day of HT ranged from 0.94 kg to 1.90 kg. Farm 2 had a 0.55 kg increase in milk yield on the day of HT.

2.4 DISCUSSION

This is the first study, to our knowledge, to investigate the relationship between preventative HT of cows without lesions and potential changes in short-term behaviors and milk production. Our results indicate that changes in the cows' behavior was associated with preventative HT, but the magnitude and direction of these associations was dependent on farm. These results indicate that on certain farms, HT can have varying impacts on the welfare of the animal.

On the day of HT an increase in activity and a corresponding decrease in resting time on all farms was the only consistent association with HT in our study. This is an expected observation due to the change in a cow's daily routine that HT creates. This increase in activity on the day HT has also been shown in previous research (Chapinal et al., 2010b; Van Hertem et al., 2014).

On the days following HT similar to previous research (Chapinal et al., 2010a; b; Van Hertem et al., 2014) cows on 2 farms had a decrease in activity. One farm however had an increase in activity following the day of HT. We hypothesize a decrease in activity on the day following HT is the cow compensating for an increase in activity on the day of HT. It is unclear what the biological impact of a decrease of (9-48) or increase (9-83) steps/day in activity has on the welfare of the cow, but this change in activity appears to be herd dependent. Based on the association of a decrease in steps on 3 farms following HT, we can hypothesize that the HT process caused this decrease. The exact reason as to why these cows would have this decrease in activity following HT is unclear. Possible reasons that HT is causing a change in behavior include the cow is acclimating to increased weight bearing between the claws or to possible pain or inflammation caused by the HT process. Determining what the HT process is causing that leads to this behavior change could help find ways to mitigate this change in behavior and potentially improve the welfare of the cow following HT.

Overall resting bouts showed very small variation on all 4 farms, but the association was dependent on farm ID. Based on there being little variation it appears that resting bouts are a behavior that is not easily influenced by HT.

On the day of HT, the association between milk yield and HT was variable in magnitude and direction, depending on the farm. Even though it was not statistically different from baseline on any of the farms, an economically important association was observed. The predicted milk yield for the 5 day baseline on farm 4 was 38.1 (95% CI: 35.5-40.7) kg and on the day of HT the predicted milk yield was 36.2 (95% CI: 33.6-38.8) kg, this is an approximately 2kg decrease in milk yield. This is different from the study by Van Hertem et al. (2014) were no difference in milk yield was observed. We hypothesize this association was caused by a decreased feed intake due to less time in the home pen or it was compensatory due to stress during the HT process. This hypothesis requires a future study.

This study did have some limitations due to study design. Since this study was an observational study and not a randomized controlled trial, we can only describe associations between HT and dependent variables of interest, and must be cautious ascribing causality. In addition, since a specific housing system was chosen (freestall barns), it limits the generalizability of these results to other housing systems. Cows were also not locomotion scored before entering the study. It could be possible that cows not displaying a lesion, but signs of lameness did enter the study. However, we expect this to apply to a small proportion of cows since lesions are a common cause of lameness (Leach et al., 2012; Green et al., 2014). Strengths of the study included the use of multiple farms that were located in different regions of the world were used. This study was carried out in two different countries with different hoof trimmers at each facility. Being able to use the data from multiple different farms allows for a more generalizable end result.

2.5 CONCLUSION

This study has shown that preventative HT of cows without lesions was associated with short-term changes in cows' behavior and milk production, but the direction and magnitude of these changes were dependent on farm. The impact of these behavior changes and milk production on the welfare of the cow is not completely clear. To minimize the impact of HT on the cow, various HT techniques and approaches to restraint should be investigated to determine what effect they have on behavior and milk production, and to determine what is most efficacious for the welfare of the cow.

Table 2.1: Descriptive statistics for the 1, 572 cows enrolled on 4 farms based on the 5 days period preceding hoof trimming.

Variable	Mean	Std. Dev	95% CI
DIM[*]	174	106	168-179
Lactation	1.5	0.5	1.4-1.5
Daily Yield (kg)	32.5	11.5	32.3-32.8
Total Activity (steps/day)	354	115	351-357
Total Rest (mins/day)	601	160	598-605
Total Rest Bouts (bouts/day)	11	4.1	10.9-11.1

*DIM = Days in milk.

Table 2.2: Results of mixed linear regression analyses of factors associated with outcome variables describing cow behavior or productivity.

Variable		Activity (steps/day)	Resting Time (mins/day)	Milk Yield (kg/day)
2+ Lactation[1]		-22.5 ± 5 (P<0.01)	58 ± 6.8 (P<0.01)	9.3 ± 0.5 (P<0.01)
DIM*		-0.05 ± 0.02 (P=0.027)	0.08 ± 0.03 (P=0.01)	-0.05 ± 0.02 (P<0.01)
Season[2]	Summer	12 ± 4.5 (P=0.006)	NI	0.3 ± 0.2 (P=0.21)
	Fall	21 ± 8.7 (P=0.015)	NI	-0.8 ±0.5 (P=0.11)
	Winter	-18 ± 7.8 (P=0.02)	NI	-1.4 ± 0.5 (P=0.001)
Time[3]		NR	NR	NR
Farm ID[4]		NR	NR	NR
Time X Farm ID[5]		NR	NR	NR

Outcome ± SE (P-Value)

[1] Lactation uses first lactation as the reference category compared to second lactation or greater.

[2] Spring is the reference category for season

[3] Days time from trim (Including: Day of hoof trimming and the individual days 1-7 post hoof trimming) with the 5 day baseline as the reference.

[4] Identifier for each farm included in the study with farm 1 as the reference farm.

[5] The interaction between farm ID and time is shown in figure 1 for activity and resting time and in figure 2 for milk yield.

*DIM = Days in milk

NI=Not included

NR=Not reported

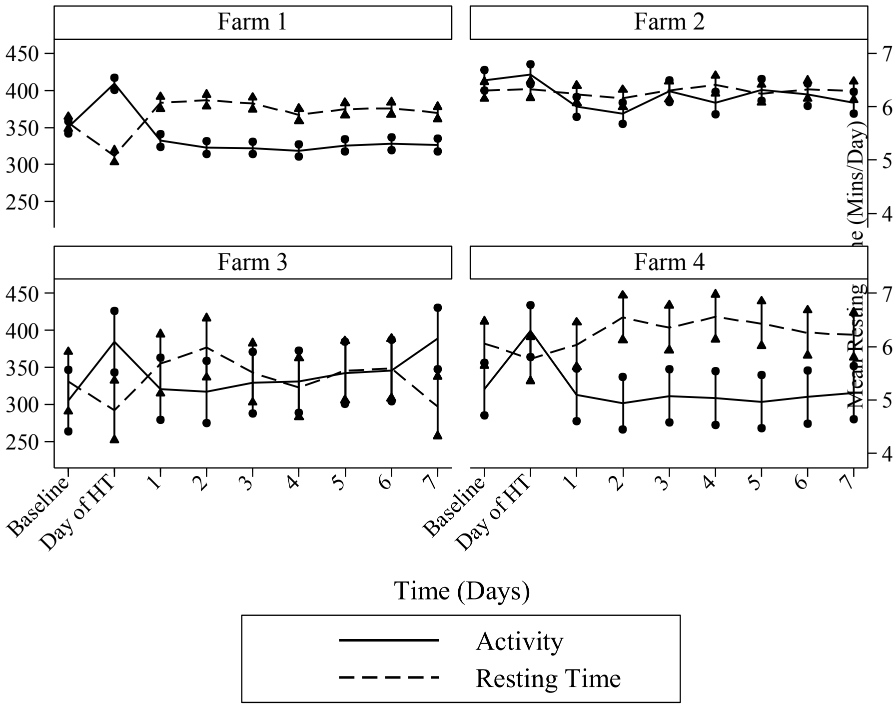


Figure 2.1: Estimated mean (95% confidence interval) for activity (steps/day) and resting time (mins/day), by farm, for 7 days following a hoof trimming (HT) event.

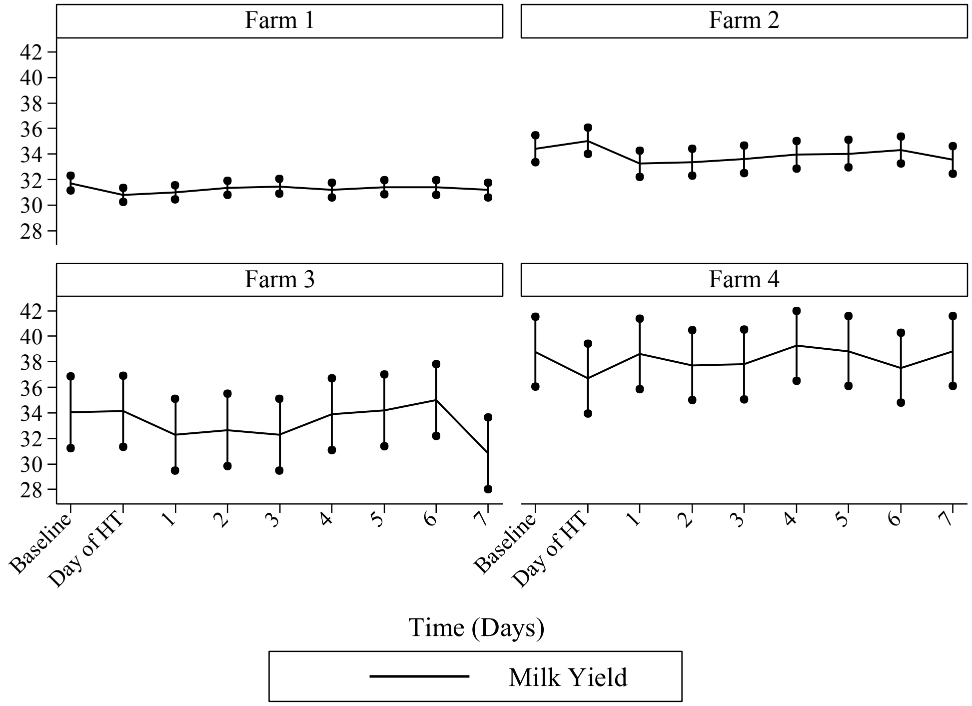


Figure 2.2: Estimated mean (95% Confidence Interval) milk yield (kg/day), by farm, for 7 days following a hoof trimming (HT) event.

CHAPTER THREE: Pilot study evaluating acute stress, inflammation and pain responses associated with the hoof trimming process in non-lame dairy cattle

3.1 INTRODUCTION

Lameness is an important economic (Cha et al., 2010; Liang et al., 2017) and animal welfare concern for the dairy industry because of the impact it has on productivity and the pain (Whay et al., 1998; Shearer et al., 2013) this disease causes. This disease is estimated to cost producers between $100-$220 (Cha et al., 2010; Liang et al., 2017) per case and affects a large number of cows with an estimated prevalence between 20-55% in North America (Espejo et al., 2006; Von Keyserlingk et al., 2012; Solano et al., 2015). The cost associated with lameness is based on the decrease in milk production (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005), decreased reproductive performance (Bicalho et al., 2007a; Peake et al., 2011; Hudson et al., 2014), increased risk of culling (Booth et al., 2004; Bicalho et al., 2007a), and treatments costs (Liang et al., 2017) due to lameness. More important than the economic losses, lameness is detrimental to the welfare of the cow. This disease is painful (Whay et al., 1998) for the cow, as is made evident by the way lame cows change their behavior (Chapinal et al., 2010a; Gomez and Cook, 2010; Navarro et al., 2013). Some of the behavior changes that occur indicate lameness causes a pain response (Tadich et al., 2013; Bustamante et al., 2015). Considering the impacts lameness has on the cow, it is clear that the behavior, biological functioning and health of the cow is challenged, these being the three components of animal welfare (Fraser, 1993). This illustrates the importance of preventing and treating lameness to ensure that animal welfare is maximized and that milk production on the dairy operation is optimal.

Hoof trimming (**HT**) is a commonly recommended practice to prevent and treat lameness (Raven, 1985; Shearer and van Amstel, 2001). It is estimated that 85% of herds in the US have cows trimmed at least once a year (NAHMS, 2007). Even though HT has become a frequently used procedure, the scientific research surrounding this procedure is limited (Stoddard and Cramer, 2017). The current research has been focused on 4 categories: efficacy, frequency and timing, behavior and physiology. It is important to understand the effect HT has on both behavior and physiology, as behavior changes have been used to determine the impact a procedure has on the welfare status of the animal (Dawkins, 2003). Changes in physiological measures are likely responsible for the cow changing its behavior.

Current behavior research has shown an association between HT and behavior changes following HT including a decrease in activity and an increase in resting time (Chapinal et al., 2010a; b; Van Hertem et al., 2014). Similar findings for changes in activity and resting time were reported in chapter 2 of this thesis. However, these findings were from observational studies that did not measure any physiological measures. What remains unclear from the current behavior research is what physiological mechanisms are activated that result in these observed behavior changes.

Changes in the concentrations of physiological measures have been used to assess what effect a potentially adverse procedure or condition has on an animal (Coetzee et al., 2008; Rizk et al., 2012; Van Engen et al., 2014; Bustamante et al., 2015). Some of the physiological measures used in these studies included cortisol, lactate, glucose, haptoglobin and substance P. Increases in the plasma concentrations of cortisol, lactate and glucose have been used to indicate if the animal is stressed during a procedure. This is because of the association between these measures and the activation of the hypothalamic pituitary adrenal axis and sympathoadrenal system (Gregory, 2004). Haptoglobin is a positive acute phase protein that is released by hepatocytes into the blood stream in response to the immune system detecting an injury or trauma that results in inflammation (Murata et al., 2004). Lastly, substance P is a neuropeptide that regulates the firing of neurons from the spinal cord in response to a painful event (Coetzee et al., 2008; Bustamante et al., 2015).

Physiological measures can potentially be altered during the HT process in non-lame cows because cows are subjected to both restraint and removal of horn from the hoof. Currently the HT process has been shown to be a stressful event for the cow, by increased plasma concentrations of cortisol, glucose and lactate (Rizk et al., 2012; Korkmaz et al., 2014). In both of these studies blood samples were taken after the HT process and compared to baseline concentrations (Rizk et al., 2012; Korkmaz et al., 2014). However, both studies lacked a comparison to cows that were simply restrained (no HT), making it unclear if the stress response observed was due to restraint or the actual removal of horn during trimming. Furthermore, it is unclear if the cause of the stress response is attributed to pain or inflammation. It could be possible that the restraint during HT is causing an inflammatory or pain response due to the pressure placed on the body of the cow due to restraint or the application of uncomfortable limb and body positioning. Conversely the shaking of the grinder during the actual removal of horn could result in the corium being shaken in the horn capsule, thereby causing an inflammatory or pain response. Determining the origin of the stress response could aid in determining what part of the HT process results in the behavior changes following HT, and potentially lead to development of new HT methods designed to reduce this stress.

The first objective of this study was to compare the stress, pain, and inflammatory response between cows undergoing restraint for hoof trimming as compared to cows undergoing both restraint and hoof trimming. Our hypothesis for this objective was the stress response would be the same for both treatments, but that cows undergoing restraint and HT would have a different inflammatory and pain response as compared to cows undergoing restraint only. The second objective was to evaluate the duration of these responses within each treatment. Our hypothesis was the duration of these responses would last no longer than 24 hours.

3.2 MATERIALS AND METHODS

3.2.1 Study Design and Cow Enrollment

The procedures in this study were approved by the University of Minnesota Institutional Animal Care and Use Committee (1702-34604A). The study was carried out on the campus dairy facilities in St. Paul, Minnesota. A crossover study design was used with cows randomized to the order of treatment. The two treatments in this study were a sham trim where cows were restrained in a fashion to mimic the actual hoof trimming and a second treatment with cows being both restrained and hoof trimmed. Only cows that did not have a horn lesion in the current or previous lactation and were not lame based on a score of 2 or less using a 5 point locomotion scoring system (Sprecher et al., 1997) were eligible for inclusion in the study. To ensure that the cows were functioning in the normal range for physiological parameters a week prior to the study, a blood sample was taken using an EDTA vacutainer (Becton Dickinson Co., Franklin Lakes, NJ) from the coccygeal vein for a complete blood count (CBC) analysis using a VetScan HM2 analyzer (Abaxis North America, Union City, CA).

3.2.2 Training of Animals

After cows were selected for the study, they were individually trained for 7-days to help reduce stress of isolation and handling. Daily for 7-days before the start of the study protocol cows were walked through the parlor areas and exit lane they would take during the protocol. To simulate the time in the HT chute cows were restrained in the exit lane with a head gate for 10 minutes each day.

3.2.3 Jugular Catheter Placement 1

After the 7-day training session 2 jugular catheters were placed. Neck hair in an approximate 5 x 5 cm square around the jugular vein was clipped and the exposed hide cleaned aseptically with a thorough betadine scrub followed by an isopropyl alcohol (70%) rinse. At this time 3 to 5 ml of 2% lidocaine was administered subcutaneously to numb the area. The hide and jugular were punctured with a 12 Ga, 7.62 cm angiocath (Becton Dickinson Co., Franklin Lakes, NJ), the needle was removed, and 25 cm of gas sterilized tygon 0.040 ID tubing (Saint Gobain Performance Plastics, Minneapolis, MN) was placed into the jugular vein through the Teflon catheter before the catheter itself was removed from the jugular. An adhesive tape butterfly was wrapped around the exposed end of the tubing and super-glued to the neck to hold the catheter in place. The catheter was then flushed with sterile saline, filled with heparinized saline (200 IU/mL) and capped. The exposed end was secured under vet-wrap (Johnson and Johnson, New Brunswick, NJ) that was wrapped around the neck to protect the catheter from damage and disruption. This procedure was repeated for the second catheter on the other jugular vein. Catheters were left in the animal until the final blood collection 24 hours after the initial blood sample (Rouleau et al., 2003)(B. Crooker, personal communication, August 23, 2016).

3.2.4 Change in Catheter Protocol

On the first day of treatment after placing the jugular catheters, one cow had removed both of its catheters before the first scheduled blood collection (figure 1). By the end of the first day most cows only had one catheter that was still in place and an additional cow did not have any catheters remaining.

3.2.5 Jugular Catheter Placement 2

Cows were re-catheterized 14-days later from the initial catheterization using the same procedure as before, except with two changes: An equine halter was used on the cow and sutures were used instead of super glue. The equine halter was attached to the tie stall collar to pull the collar away from the catheter site. The second change was to use non-absorbable monofilament (0 Ga) suture (Ethicon US, LLC., Bridgewater, NJ) to attach the catheters to the skin of the cow (Rouleau et al., 2003)(A. Desrochers, personal communication, April 4, 2017).

3.2.6 Treatments

All cows in this study received both sham HT and actual HT in an upright chute (H-Series, Comfort Hoof Care Inc., Baraboo, WI) 48 hours after catheterization. For the sham HT legs were restrained in the same order and approximately for the same time that they would be for actual HT. This resulted in each of the front legs being restrained for 90 seconds and each of the rear legs being restrained for 180 seconds. The order for the sham HT started with restraining the rear left leg and then restraining the front left leg for 90 seconds and then releasing it. The rear left leg would be restrained for an additional 90 seconds before it was released and then the right rear leg would be restrained. After restraining the rear right leg the front right leg would be restrained for 90 seconds and then released. The rear right leg was restrained for an additional 90 seconds after the front right leg was released. When cows were actually trimmed an adaptation of functional HT method that was similar to the method described by Gomez et al. (2013). This resulted in modeling up to 18mm from the abaxial wall on the weight bearing claw.

3.2.7 Behavior Observations

While cows were in the HT chute, behavior was recorded using a video camera (SDH-B84040BF, Samsung Wisenet, Teaneck, NJ) and reviewed at a later time by an individual that was blinded to the treatment. Specific behaviors investigated included: vocalization, head swaying, weight shifting, loss of posture and pulling away while being restrained (Table 1).

3.2.8 Blood Sampling Frequency and Procedure

Cows were located in their tie stall for 9 of the blood samples and at the entrance of the HT chute for 1 of the blood samples (Figure 1). During the sampling process heparinized saline and blood was removed (5 mL) using a 10 ml sized syringe (Covidien, Minneapolis, MN) to clear the catheter and a 20 mL sample was collected using an 20 ml sized syringe (Becton Dickinson Co., Franklin Lakes, NJ). Upon finishing the blood sampling the catheter was flushed with saline (5 mL) and then filled with heparinized saline (15 IU/ml) and capped. At the 6 hour sampling period a higher concentration of heparinized saline (30 IU/ml) was used to ensure that catheters did not clot before the 24 hour sample was collected. Samples collected for Substance P were transferred into a chilled vacuum tube that contained EDTA (Becton Dickinson Co., Franklin Lakes, NJ) and benzamidine. Cortisol and haptoglobin samples were transferred into a vacuum tube containing sodium heparin (Becton Dickinson Co., Franklin Lakes, NJ). All three of these samples were then put on ice until processing where they were centrifuged for 15 min at 1,400 x g at 0 ºC. Once the samples were centrifuged, plasma was pipetted into cryovials they were frozen at -80 ºC until they were shipped to the University of Iowa PHAST lab for analysis. Blood glucose and lactate tests were done cow side by immediately dripping blood from the 20 ml syringe onto the testing strips for the hand held meters.

3.2.9 Analysis of Plasma Substance P, Haptoglobin and Cortisol Concentrations

Plasma concentrations of substance P, haptoglobin and cortisol were determined by the University of Iowa PHAST lab. All samples were run in duplicate with assays that were validated for bovine blood samples. Substance P samples were analyzed using a radioimmunoassay (RIA) double antibody system as described by Liu et al. (2008). A coefficient of variation for the substance P intra-assay variability was 9.5% and the inter-assay variability was calculated at 4.5%. Haptoglobin samples were analyzed using a commercially available enzyme linked immunosorbent assay (HP ELISA kit, MY Biosource Inc., San Diego, CA). The coefficient of variation for the haptoglobin intra-assay variability was 5.7% and an inter-assay variability of 4.8% was calculated. For Cortisol samples were analyzed using a commercial RIA kit (Corti-Cote, MP Biomedical, Irvine, CA), previously used for bovine samples (Kleinhenz et al., 2017). For cortisol the coefficient of variation intra-assay variability was 11% and the inter-assay variability was 6.3%.

3.2.10 Analysis of Blood Lactate and Glucose Concentrations

Lactate samples were analyzed using a Lactate Scout handheld device (SensLab GmbH, Leipzig, Germany). The Lactate Scout was validated for use in cattle by Burfeind and Heuwieser (2012). Glucose samples were analyzed using the technique described by Pineda and Cardoso (2015) using a Precision Xtra glucose meter (Abbott Laboratories, Abbott Park, Illinois).

3.2.11 Statistical analysis

All statistical analysis was performed using STATA 14.1 (Stata Corp., College Station TX) at the cow level. A significance level of $\alpha < 0.05$ was used for this study.

The a priori sample size calculation used in this study was based on the difference for haptoglobin concentrations observed in a lameness challenge model (Bustamante et al., 2015). With an expected difference of 3.85 ng/ml and a standard deviation of 1.50 with an 80% power, and an alpha of 0.05 a total sample size of 4 was calculated. This was inflated by 4 for each treatment to account for potential losses if jugular catheters lost function resulting in missing blood samples from a cow.

Blood level outcomes were analyzed using a multivariable mixed linear regression. Before any models were constructed the distribution of the data was visually assessed for normality and no transformations were needed. Individual mixed linear regression models were created for the outcomes substance P (pg/ml), cortisol (ng/ml), haptoglobin (ng/ml), glucose (mg/dl), and lactate (mmol/L) to determine if there was a difference between sham and actual HT.

Mixed linear regression models were also used again in separate models for each of the previous stated outcomes to determine if a difference from the 30 minute baseline sample occurred. If there was no difference between sham and actual HT for the outcome blood samples were combined into one group.

Missing lactate data due to the meter reporting a result of ‘low’ were imputed as 0.05 mmol/L, given that this was the lowest concentration the meter could detect reported by the manufacture. All of the linear regression models included an exchangeable correlation structure to account for repeated measures. The model assumptions of homoscedasticity, normality and a linear relationship were evaluated graphically for all final models.

The total number of behaviors is based on combining the frequency of all of behaviors observed for a treatment group. This means the observed behaviors were treated as equal importance to the cow. The total amount of behaviors displayed in both groups was compared using a repeated measures ANOVA analysis. Total chute time and how long each individual leg was restrained was compared between the two treatments using a repeated measures ANOVA analysis.

3.3 RESULTS

Eight lactating Holsteins were enrolled, however only five cows had both sham and actual HT data available, due to the previously described catheter loss. One additional cow had complete data for the actual HT since she was not able to be successfully re-catheterized for sham HT. The five cows that had complete data were used to investigate if there was a difference between sham and actual HT. If no significant differences between sham and actual HT were present, then results from the one cow that only had data for actual HT was included in the analysis to investigate the duration of change from baseline. Behavior data was investigated using the five cows that had complete data only. The five cows that had both sham and actual HT data had an average DIM of 193 (95% CI: 72-313) and a lactation distribution of four second lactation and one third lactation animals. During sham HT cows were in the hoof trimming chute on average 418 (95% CI: 408-428) seconds. The average time cows were restrained during the actual HT was 429 (95% CI: 357-501) seconds. A total of 57 out of the possible 110 (52%) lactate readings were read as low by the meter and imputed as 0.05 mmol/L.

3.3.1 Differences between Sham and Actual HT

Table 1 shows the estimated differences between sham and actual HT for each of the outcomes investigated in this study. The plasma haptoglobin concentration was decreasd by 58 ng/ml (95% CI: -84- -32) during actual HT compared to sham HT. However, this data included several outliers (concentration>10,000 ng/ml), based on values previously reported by Smith et al. (2010). After excluding the outliers, the plasma haptoglobin analysis considered only three cows that received both sham and actual HT.

3.3.2 Duration of Change from Baseline

There were no observed statistical differences from baseline for plasma haptoglobin, substance P, lactate and glucose during the study (Table 2). Estimates of the mean plasma cortisol concentration by treatment are shown in Figure 2. Regardless of treatment, plasma cortisol concentrations were increased from baseline for 3 hours after the cow entered the chute (Fig 2). The increase ranged from 34 (95% CI: 26-42) to 74 (95% CI: 66-82) ng/ml. The estimated mean concentrations of each physiological outcome for each time point are listed in table 2.

3.3.3 Behavior Frequency

The total amount of behaviors displayed by the sham group in this study was 28 and 34 total in the actual HT group. The frequency of behaviors was not different between sham and actual HT (P= 0.32). The frequency and a description of the displayed behaviors are shown in table 3.

3.4 DISCUSSION

Our results indicate that a similar type of acute transient stress occurs when a cow is restrained for HT and when she undergoes actual HT. This suggests that the horn removal process does not change the stress response. The only parameter that changed from baseline for either sham or actual HT was plasma cortisol. This elevation in cortisol lasted 3 hours after restraint or actual HT, indicating it was likely a result of an acute episode of stress.

Observed plasma cortisol concentrations are similar to studies by Rizk et al. (2012) and Korkmaz et al. (2014). In both of the previous studies they showed an increase in cortisol concentration following HT when compared to baseline concentrations. In our study plasma cortisol returned to baseline by 3 hours post HT, similar to the 3.25 hours observed in the study by Rizk et al. (2012). In addition the maximum concentration of cortisol (70-72 ng/ml) observed in the previous studies (Rizk et al., 2012; Korkmaz et al., 2014) is similar to the maximum concentration (85 ng/ml) observed in our study. The study by Korkmaz et al. (2014) only followed the cows to 30 minutes post HT and never observed a return to baseline. Based on the current study and the previous study by Rizk et al. (2012), it would seem 30 minutes is not enough time for cortisol to return to baseline concentrations following the HT procedure.

The maximum observed concentration of cortisol in our study is numerically higher than studies that have evaluated a painful procedure or condition such as dehorning (Heinrich et al., 2009; Stock et al., 2015) or lameness (Bustamante et al., 2015). Dehorning of calves resulted in a maximum cortisol concentration between 30-35 ng/ml, that remained elevated for 24-48 hours post dehorning (Heinrich et al., 2009; Stock et al., 2015). Inducing lameness caused an elevated cortisol concentrations starting at 6 hours after induction and lasted until the final blood sampling 48 hours later with an observed peak in concentration of 63.5 ng/ml (Bustamante et al., 2015). Even though these studies had numerically lower peak concentrations of cortisol, the duration of elevated cortisol concentrations in these studies (Heinrich et al., 2009; Bustamante et al., 2015; Stock et al., 2015) was much longer than our observed 3 hour increase. This illustrates that these stressful events caused a different stress response than the HT process. A likely explanation is these stress events are different because dehorning or inducing lameness is causing stress due to pain or inflammation.

The observed changes in plasma haptoglobin and substance P indicate that neither restraint nor the actual removal of horn results in a pain or inflammatory response. Even though the concentration of haptoglobin was significantly different between the treatments, we are not confident that this difference was biologically important. Previous research using these physiological measures to indicate pain or inflammation following an induced lameness (Bustamante et al., 2015) or castration (Coetzee et al., 2008) observed a much larger increase in concentration. Following an induced lameness baseline concentrations of haptoglobin approximately increased by 20 fold and substance P increased by approximately 6 fold (Bustamante et al., 2015). Post castration calves had an increased level of substance P by approximately 1.5 fold when compared to calves that were sham castrated (Coetzee et al., 2008). A large increase in the concentration similar to what was observed in the previous studies did not occur in the current study. Therefore it is likely that our observed difference in plasma haptoglobin concentration between restraint and the actual removal of horn was not biologically important to the cow.

The lack of an increase in blood glucose and lactate in our study is surprising. A normal response to an increase in cortisol (Forslund et al., 2010) is an increase in glucose and lactate. Evidence for this relationship was shown in the study by Rizk et al. (2012), when lactate and glucose concentrations were elevated from baseline an hour and 15 minutes following the HT process with a corresponding increase in cortisol concentration. A possible explanation for not observing a rise in glucose and lactate in the current study is the cows were lactating cows. By comparison, the previous research (Rizk et al., 2012) used non-lactating cows. Glucose and lactate are important components needed for milk production (Herdt, 2000) and based on the baseline concentration of glucose in the current study being approximately 20 mg/dL less than those in the study by Rizk et al. (2012), potentially less glucose was available to direct to a stress response. This relationship between low blood glucose levels (<50 mg/dL) and stress was shown in the study by Mudron et al. (2005). In the latter study, when cows that had a lower glucose concentration were exposed to a stressor an increase in the concentration of glucose and lactate was observed, but the increase was not as large as what was observed for cows that had higher levels of glucose available before the stressor. If less glucose was available and it led to a smaller change in concentrations of glucose and lactate, the small sample size of this study would have limited our power/ability to detect such a small change. Based on the largest change from baseline for glucose, a post-hoc power calculation suggested that a sample size of 12 cows would have been needed to maintain statistical power and observe a difference.

From the results of our study we hypothesize that the changes in activity in the days following HT shown in chapter 2 of this thesis and previous studies (Chapinal et al., 2010a; b; Van Hertem et al., 2014) is not due to the stress from restraint. This is based on observing an acute increase in cortisol concentration for 3 hours following restraint or actual HT and no biologically relevant changes in behavior frequency or indicators of inflammation or pain. If this stress response was the cause of the change in behavior following HT, we would have expected a longer duration stress response. As such, it is still unclear why the behavior changes observed in Chapter 2 are occurring based on these results. Considering the other events that occur on the day of HT (e.g. sorting out of her home pen, movement to the HT chute area, waiting for HT, waiting to return to her home pen), it could be possible that the disruption of the cow's daily routine is causing these behavior changes, and not the actual event itself. Previous research has shown that when cows are intentionally deprived of lying time they will compensate by increasing their lying time when they are able to lay down again (Metz, 1985; Cooper et al., 2008; Ouweltjes et al., 2011). However, this compensation in lying time appears to dissipate after 10 hours. It could be possible that the combination of this stressful event and disruption of the cow's day result in longer behavior changes. Future research should consider what affect the disruption of the cow's daily routine due to HT has on the behavior of the cow.

3.5 CONCLUSION

This study showed that the restraint for HT in sound cows causes an acute 3 hour cortisol response. This acute cortisol stress response was not associated with a corresponding increase in plasma haptoglobin, substance P, glucose, or lactate. It is therefore unlikely that this stress response alone is responsible for the behavior changes observed post-HT.

Further research should focus on determining the impact the disruption of the cow’s daily routine and the stress due to HT has on the behavior of the cow.

Table 3.1: Mixed linear regression model estimates of the difference between sham hoof trimming and actual hoof trimming for the physiological outcomes post hoof trimming

Physiological Outcome	Parameter Estimate (95CI)*	P value
Haptoglobin ng/ml (n=3)	-58 (-84- -32)	<0.001
Substance P pg/ml (n=5)	-0.81 (-4.01-2.37)	0.61
Cortisol ng/ml (n=5)	1.3 (-12-15)	0.57
Glucose mg/dl (n=5)	-1.8 (-5.1-1.4)	0.24
*Lactate mmol/L (n=5)	0.073 (-0.11-0.25)	0.43

Change compared to sham hoof trimming
*95% Confidence Limit

Table 3.2: Mixed linear model estimates of changes from baseline for concentrations of plasma haptoglobin, substance P, cortisol, lactate and glucose from baseline, at each time based on when the cow entered the hoof trimming chute for Sham hoof trimming or actual hoof trimming

		Time from cow entering hoof trimming chute									
Variable	Treatment	-30 mins	-15 mins	0 mins	Release	15 mins	30 mins	1 hour	3 hours	6 hours	24 hours
Cortisol ng/ml (n=6)	Sham and Actual**:	10.9 (4.3-17.5)	18.9 (12.4-25.6)	14.7 (8.12-21.3)	*74.9 (68.3-81.5)	*83.4 (76.8-89.9)	*85.4 (78.8-92.0)	*44.9 (38.3-51.5)	5.83 (1.76-12.4)	7.14 (0.54-13.7	8.17 (1.54-14.7
Glucose mg/dl (n=6)	Sham and Actual**:	56.3 (50.6-61.9)	61.7 (56.1-67.4)	62.6 (57.1-68.3)	64.1 (58.4-69.8)	63.8 (58.2-69.5)	66.3 (60.6-71.9)	67.5 (61.8-73.1)	65.3 (59.6-70.9)	62.4 (56.7-68.0)	62.3 (56.6-67.9)
Lactate mmol/l (n=6)	Sham and Actual**:	0.55 (0.39-1.49)	0.60 (0.08-1.30)	0.85 (0.31-1.16)	1.68 (1.30-2.31)	1.15 (0.60-1.44)	0.70 (0.15-1.03)	0.92 (0.31-1.31)	0.60 (0.09-1.27)	0.64 (0.03-0.98)	0.66 (0.3-1.06)
Haptoglobin ng/ml (n=3)	Sham:	465 (400-530)	446 (381-511)	492 (427-558)	475 (410-540)	462 (397-527)	459 (394-525)	432 (366-497)	440 (375-505)	485 (420-551)	485 (420-551)
	Actual HT:	407 (341-472)	388 (323-453)	434 (369-499)	417 (352-482)	404 (339-469)	401 (336-466)	373 (308-439)	382 (316-447)	427 (362-492)	427 (362-492)
Substance P pg/ml (n=5)	Sham and Actual:	84.2 (64.6-104)	81.1 (61.5-101)	82.4 (62.8-102)	82.2 (62.5.4-102)	81.7 (62.0-101)	84.8 (65.1-104)	82.9 (63.3-103)	79.1 (59.5-99.8)	83.4 (63.7-103)	81.5 (61.8-101)

Mean (95% Confidence Interval)

*Change from baseline P <0.05 (-30 mins blood sample)

**Sham and actual HT results combined, due to no difference between treatments.

Table 3.3: Type, description and frequency of behaviors observed in videos from 5 cows undergoing sham or actual hoof trimming

Behavior	Description	Sham Hoof Trimming	Actual Hoof Trimming
Vocalization	Cow opens mouth and a sound louder than a grunt is heard and sustained for 3 seconds this was noted chute side during the treatment.	1	0
Head Swaying	Head of the cow goes from side to side in a fast motion while in the headlock. One bout is complete when the cows head has returned to its normal position in the chute and has remained in the general area for 10 seconds.	5	5
Weight Shifting	The chest of the cow is visibly moving or one of the legs that is not restrained is lifted in the air.	15	20
Loss of Posture	Three or more of the legs are no longer supporting the animal.	2	1
Pulling away while restrained	The cow attempts to pull the leg away that is restrained.	4	7

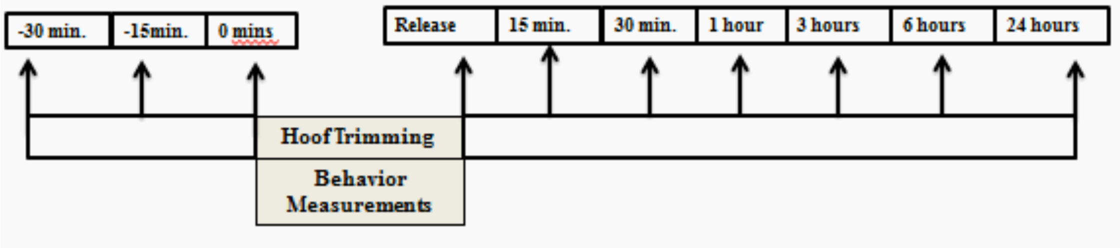


Figure 3.1: Timeline for collection of blood samples and behavior outcomes in relation to sham or actual hoof trimming events.

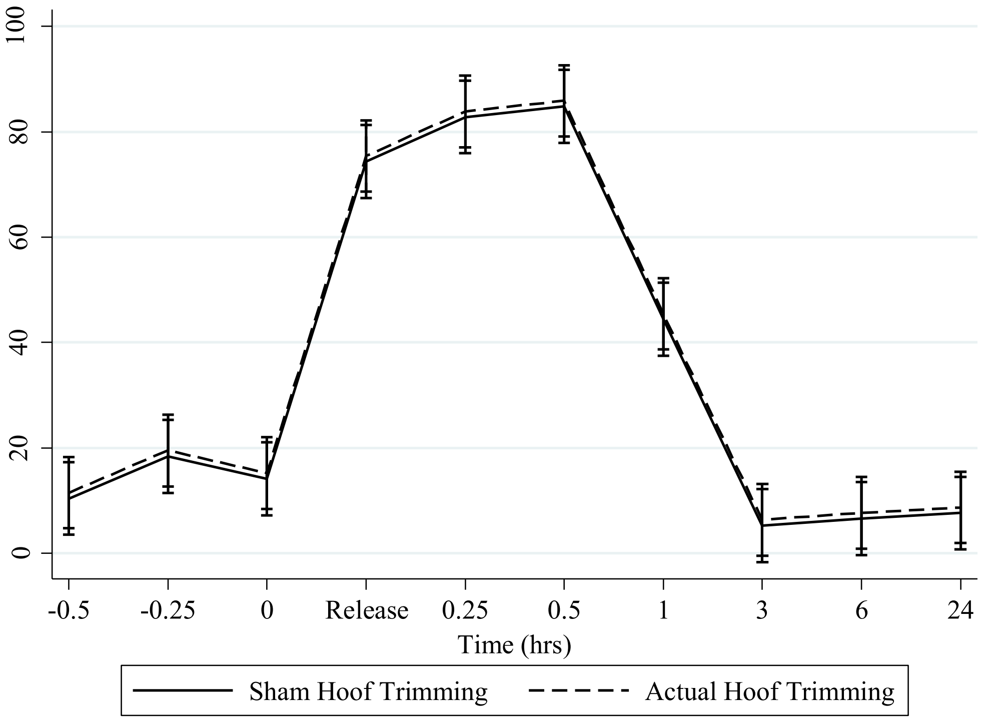


Figure 3.2: Estimated mean cortisol concentrations based on final mixed linear regression with 95% confidence intervals at each blood sampling time point from 5 cows undergoing sham or actual hoof trimming.

CHAPTER FOUR: Randomized controlled trial to evaluate the effect of two different hoof trimming techniques on time to lameness and lesion prevalence at mid-lactation in dairy cattle

4.1 INTRODUCTION

Lameness is a painful disease (Whay et al., 1998) that can be visually diagnosed by observing alterations in the gait of affected cows. The change in gait is a compensatory action for the pain and discomfort caused by this disease (Tadich et al., 2013; Bustamante et al., 2015). This debilitating disease affects a large portion of the industry with an estimated prevalence ranging from 20-55% at the herd level in North America (Espejo et al., 2006; Von Keyserlingk et al., 2012; Solano et al., 2015). The estimated cost incurred by the producer due to a case of lameness has been estimated to range between \$100 and \$220 (Cha et al., 2010; Liang et al., 2017). This estimated cost is based on the decreased milk production (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005), decreased reproductive performance, (Bicalho et al., 2007a; Peake et al., 2011; Hudson et al., 2014), increased culling risk, (Booth et al., 2004; Bicalho et al., 2007a) and associated veterinary treatment and labor costs (Liang et al., 2017). Not only is lameness costly for producers, it is detrimental to the welfare of the cow because of the pain this disease causes and the behavior changes (Chapinal et al., 2010a; Gomez and Cook, 2010; Navarro et al., 2013) that occur. Given the cost to producers and the clear indication that lameness decreases the welfare of the cow by affecting several aspects of welfare including natural behavior, health and biological functioning (Fraser, 1993), prevention is essential for sustaining the cow and the industry. However, preventing lameness is complex because of the large number of factors that can influence the severity and prevalence of lameness. The prevalence of lameness can be impacted by a variety of herd level risk factors that have been identified by previous research. Lameness prevalence is influenced by bedding depth, access to pasture, time away from the home pen, hoof trimming timing, hoof trimming of heifers prior to calving, frequency of alley cleaning, and footbathing frequency (Cook, 2003; Espejo and Endres, 2007; Cramer et al., 2009a; Chapinal et al., 2013). However, there has been limited investigation into the efficacy of procedures to prevent lameness.

A recommended procedure to prevent lameness is hoof trimming **(HT)** (Raven, 1985; Shearer and van Amstel, 2001). Currently there are several HT techniques being used in the industry with the main difference between these techniques being the walking surfaces has a flat sole (Raven, 1985; Manske et al., 2002b; Blowey, 2015) or sloped sole (Amstutz, 1979; Siebert, 2005). Regardless of technique, it is estimated that 85% of US herds trim cows at least once a year (NAHMS, 2007). Despite a large portion of the industry trimming cows, hoof lesions present at the time of HT are common (Cramer et al., 2008; Becker et al., 2014; Solano et al., 2016). This could be because the HT techniques being used are not the most effective techniques for preventing lesions. The scientific evidence for the effectiveness of HT techniques preventing lesions and lameness is currently limited (Stoddard and Cramer, 2017).

Currently there have been five controlled trials investigating the efficacy of HT preventing lameness (Manske et al., 2002; Hernandez et al., 2007; Gomez et al., 2013; García-Muñoz et al., 2017; Mahendran et al., 2017). All of these studies compared cows that had been trimmed using a flat sole technique to cows that were not trimmed. Three of these studies (Hernandez et al., 2007b; García-Muñoz et al., 2017; Mahendran et al., 2017) used the functional HT method (Raven, 1985; Shearer and van Amstel, 2001). The remaining two studies used an adaptation of the functional HT method, that focused on the angle of the toe instead of claw length (Manske et al., 2002b) or increased the modeling on the weight bearing claw (Gomez et al., 2013). The results of these studies were equivocal, indicating either no statistical difference (Hernandez et al., 2007b; García-Muñoz et al., 2017; Mahendran et al., 2017) or that HT was protective against lameness or lesions (Manske et al., 2002; Gomez et al., 2013). Taken as a whole, the conclusions of these studies suggest that HT has the potential to be beneficial for the cow. However, research is limited in indicating which HT technique is most effective.

Current research comparing techniques has focused on the modeling process, the removal of horn adjacent to the interdigital space on the medial and lateral claws to remove horn under the flexor tuberosity. Focusing on the modeling step seems logical since the goal is to prevent excess pressure from being applied at the corium to prevent hoof horn lesions. The effect of different HT techniques was investigated by Ouweltjes et al. (2009) when the functional HT method was compared to an adaption that resulted in unrestricted modeling of both claws for lesion and lameness incidence. At the end of the 3 month study there was no statistical difference in lesion or lameness incidence. However, this study was carried out over a short duration and with a small sample size. In addition, the adaptation used in this study could have resulted in more weight bearing on the lateral claw because the surface area on the medial claw would have been reduced.

An alternative to the technique described by Ouweltjes et al. (2009) could be more effective at preventing lesions and lameness was described by Gomez et al., (2013). This technique increases the modeling of the weight bearing claw only. Increasing the modeling on the weight bearing claw could prevent excess pressure to the common sole ulcer location (van der Tol et al., 2004) which could lead to a reduction in lesions and lameness.

The objective of our study was to compare the effects of the functional HT (LIT) method to an adaptation that results in increased modeling of the weight bearing claw (BIG) on risk of lameness and prevalence of new lesions at mid-lactation. Our hypothesis was cows trimmed with the BIG method will have fewer lesions, and less risk of lameness than cows trimmed with the LIT method.

4.2 MATERIALS AND METHODS

4.2.1 Herd Selection

The procedures in this randomized controlled trial were approved by the University of Minnesota Institutional Animal Care and Use Committee (1412-32099A). Farms were recruited from a convenience sample of contacts provided from industry personnel such as hoof trimmers and veterinarians. Farm inclusion criteria included: located within 2 hours drive of the University of Minnesota, free-stall housing, recycled sand bedding, and trimming cows routinely at dry off and mid-lactation. In total 10 farms were approached and 3 of them agreed to participate in the study. Farms were located in Wisconsin (n=2) and Minnesota (n=1). Two hoof trimmers were included in this study, with one o hoof trimmer servicing 2 of the farms. The average herd size was 1,333 cows, ranging from 892 to 1,928. The average 305 day milk production ranged from 11,195 kg to 13,483 kg.

4.2.2 Cow Enrollment

Cows were enrolled between December 2015 and December 2016. Cows in their first lactation or greater were considered for enrollment when they received their regularly scheduled HT prior to drying off per farm protocol. Only cows without hoof horn lesions such as sole ulcers, white line disease, and thin soles at the time of enrollment were eligible to be enrolled in the study. Cows with digital dermatitis **(DD)** were enrolled in the study after receiving standard farm treatment. A coin was flipped to determine that the BIG technique was the first treatment to be administered at the week level, with the treatment allocation alternating (BIG/LIT/BIG/LIT) every week thereafter.

4.2.2 Hoof Trimmer Training and Treatments

Each farm's hoof trimmer was trained on the two techniques and lesion identification on farm by Dr. Gerard Cramer. Little model was the functional trimming method as described by (Raven, 1985). Big model was the adaptation to the functional trimming method that results in unrestricted modeling of the weight bearing claw that was similar to the technique used in Gomez et al. (2013). To standardize the BIG model treatment cows were modeled up to 18mm away from the abaxial hoof wall on the weight bearing claw as shown in Figure 1. Hoof trimmers were provided a gauge to standardize the modeling size for both the BIG and LIT model treatments as shown in Figure 1. In addition, hoof trimmers were provided with a lesion guide (Figure 2) to help standardize the recording of lesions at the mid-lactation evaluation. To evaluate adherence to allocation protocol investigators visited the farms every 4-6 weeks at the time of HT to observe as cows were being enrolled in the study.

4.2.3 Data Collection

Cows were locomotion scored by one of the authors (GS) before their scheduled dry off trim and then every other week until they were trimmed a second time or reached 165 DIM in the subsequent lactation, which ever came first. Two of the three farms were visited every other week for locomotion scoring using a 4 point system (modified from Cook, 2003; Cramer, 2007; Thomas et al., 2015) shown in table 1. One investigator performed all of the locomotion scoring. Prior to the start of the study this investigator was trained on the 4-point system by Dr. Gerard Cramer by scoring live cows and pre-recorded videos. To determine the consistency of scoring, the investigator locomotion scored one pen twice for separate milking's at the beginning and end of the study, in order to generate a Kappa calculation. A Kappa score of 0.60 was calculated for each scoring comparison. During the study, if lameness was identified by farm personnel it was treated per farm protocol. Lesion data was collected from either DairyComp 305 (Valley Ag. Software, Tulare. CA) as entered based on hoof trimmer records or from the hoof trimmer directly from their Hoof Supervisor (KS Dairy Consulting Inc., Dresser. WI) records.

4.3.4 Statistical Analysis

All data management and statistical analysis was performed at the cow level using STATA 14.1 (Stata Corp., College Station. TX).

Sample Size

The a priori sample size calculation was based on preliminary data generated by Gomez et al. (2013) to estimate a sample size that would allow us to detect a 7-8% difference in lesion prevalence at mid-lactation between treatments (assumes alpha of 0.05%; power of 80%; control group lesion incidence of 30%). The sample size was inflated from 588 animals to 700 in each treatment group to account for loss to follow-up, given the assumption that 20% of the cows would be culled before mid-lactation.

Survival to lameness

The outcome for the survival analysis was time from the start of the next lactation following enrollment to lameness or censoring. A cow was considered lame once two successive locomotion scores of 3 or the occurrence of one locomotion score of 4 occurred (scale of 1 to 4). A similar technique of successive locomotion scores was used in the study by Thomas et al. (2015). The date associated with the diagnosis of lameness was the date of the second scoring of 3 or the date of a score of 4. Cows were censored when they received their mid-lactation trim or reached 165 DIM in the lactation following enrollment. Cows were not locomotion scored during the dry period. Only cows that had a locomotion score prior to HT and were not identified as lame prior to dry off were included in the analysis.

The difference in time to lameness between the LIT and BIG technique was investigated using a Kaplan-Meier analysis with the median time to lameness being calculated and evaluated using the Wilcoxon test. A two stage model building process was then used to build a multivariable Cox proportional regression model. In the first stage univariate Cox proportional regression models were constructed to investigate the association between lameness and the covariates: lactation, DIM at enrollment, breed, season, DD at enrollment and whether or not the cow was identified as lame prior to enrollment (yes/no). Lactation number was categorized into parity 1 or parity 2 and greater. Season was categorized into spring (March-May), summer (June-August), fall (September-November), and winter (December-February). Cows were categorized as lame pre-trim if they had a single locomotion score of 3 or greater immediately prior to enrollment HT.

In the second stage, covariates with a $P < 0.20$ in stage one were put into a multivariable Cox proportional regression model. A manual stepwise backward elimination was then performed. When covariates were eliminated the change in the remaining coefficients were observed and model AIC was compared to determine if a significant change occurred. Final significance was set at $P < 0.05$. In the final model herd was accounted for as a fixed effect. After the final main effects model was determined, biologically plausible interactions between treatment and other covariates were evaluated. If an interaction term was significant with a $P < 0.05$ the data was stratified on the interaction term.

The proportional hazard assumption was assessed in the final Cox proportional regression model using the Schoenfeld residuals test. Goodness of fit was assessed using the Gronnesby and Borgan omnibus test. Outliers and influential points were identified through using deviance and scaled scored residuals (Dohoo et al., 2009).

Lesion prevalence at mid-lactation

The main outcome was the risk difference between BIG and LIT technique for any lesions at mid-lactation (100-165DIM). In addition, subset analyses compared the risk of lesions by specific categories of lesions. For these subset analyses the lesions were categorized into hoof horn lesions (sole ulcer, white line, toe ulcer and thin sole), infectious (DD and foot rot), and other (corkscrew, leg injury, and other). Only cows that were trimmed between 100-165 DIM in the lactation following enrollment were included in the analysis.

Separate logistic regression models were constructed for each outcome. In each separate logistic regression model only that category of lesion and cows without any lesions were included. A two-step procedure was used to build the final multivariable logistic regression models. The first step assessed univariate logistic models at $P<0.20$ between covariates and lesion outcome at mid-lactation. Covariates that were investigated included: breed, season, lactation, DD at enrollment and DIM at enrollment. All of the variables were defined the same as they were defined in the lameness analysis. Covariates significant at $P<0.20$ in a univariable model were offered into a multivariable logistic regression model and a manual stepwise backward elimination was then performed. When covariates were eliminated, the change in the remaining coefficients and model AIC were compared to determine if a significant change occurred. In all final models, herd was accounted for as a fixed effect. Biologically plausible interactions between treatment and other significant covariates were assessed and data was stratified on the interaction if the interaction term was significant at $P<0.05$. The adjusted odds ratio, risk and risk differences were estimated from the multivariable logistic regression model according to methods by (Norton and Miller, 2009). Model fit was assessed by comparing the proportions predicted by the model to the actual proportions in the data set. Assessing for outliers and influential points was done by graphing the residuals from the model (Dohoo et al., 2009).

4.4 RESULTS

In total 1,562 cows were enrolled in this study, with 789 cows in the LIT treatment and 773 cows in the BIG model treatment. Descriptive statistics for the enrolled population are presented in table 2.

4.4.1 Lameness Analysis

In total 1,449 cows were enrolled in the lameness portion of this study, of which 1,074 were included in the final analysis. In total 375 cows were excluded because they were missing a baseline locomotion score (n=206) or they were identified as lame using locomotion scoring by the investigator before the start of the next lactation (n=169; LIT = 41; BIG = 64). Of the cows excluded because of being identified as lame prior to the start of next lactation following enrollment in the LIT model group 59% (41/69) of them were identified as lame prior to enrollment HT. In total 100 cows were excluded from the BIG model group, 64% (64/100) of them were identified as lame prior to enrollment.

The flow of cows starting from the time of enrollment until the start of next lactation that were included in the lameness analysis is shown in figure 3. In the end, 263 (44.5%) and 165 (34%) of the LIT and BIG model group respectively were identified as lame in the next lactation.

Based on the Kaplan Meier survival analysis there was a difference (P=0.006) between treatments for time to lameness: The BIG model treatment group never went below 50% lameness during the observation period of 165 days, while the LIT model treatment group reached 50% lameness at 122DIM. The final Cox regression model included lameness status pre-trim, breed, farm ID. The model was stratified by lactation category because of an interaction between treatment and lactation. In the group of cows trimmed at the end of their first lactation, 81/275 (29%) in the LIT model and 40/234 (17%) in the BIG model groups were identified as lame, respectively [Hazard $\text{Ratio}_{\text{BIG}}$=0.5 (95% CI: 0.3-0.7)]. However, in second lactation or greater cows there was no treatment effect, with 182/316 (57%) cows in the LIT model and 125/249 (50%) cows in the BIG model groups identified as lame, respectively [Hazard $\text{Ratio}_{\text{BIG}}$ = 0.90 (95% CI: 0.72-1.13)]. Adjusted survival curves by treatment stratified on lactation are shown in Figures 4 and 5. The estimated associations between the other covariates and time to lameness are described in Table 3.

4.4.2 Lesion Analysis

In total 1,163 cows were used in the lesion analysis. A total of 399 of the original 1,562 cows enrolled in this study were excluded because they did not receive a subsequent trim following enrollment (n=270) or the trim occurred before 100DIM (n=126) or after 165 DIM (n=3). Based on the 129 cows excluded that were trimmed early or late, 84 cows were from the LIT model treatment, with 43 (48%) of these having a lesion when they were trimmed. In the BIG model treatment 45 cows were excluded, with 25 (55%) of these having a lesion at the time of trimming. Due to the small (BIG=5, LIT=5 lesions) prevalence of other or lame lesions at mid-lactation the results for this analysis were not reported.

Of the 1,163 cows included in the final analysis, 132 cows had 1 lesion and 4 cows had 2 lesions in different categories. The final logistic regression model for presence of any lesion included the covariates: DD at enrollment, lactation category, and farm ID. When the outcome was presence of any lesion there was no difference in the odds of a lesion occurring between the BIG and LIT model [Odds $\text{Ratio}_{\text{BIG}}$ = 0.92 (95% CI: 0.63-1.33)]. The odds ratio, adjusted risk of having a lesion by treatment and the adjusted risk difference are shown in Table 4. The estimated associations between other covariates and having any lesion are reported in Table 5.

The final logistic regression model for hoof horn lesions included: DD at enrollment and farm ID. It was stratified by lactation category because of a significant interaction between treatment and lactation category. The odds for presence of a horn lesion when cows were trimmed at the end of their first lactation with the BIG model was significantly lower [Odds $\text{Ratio}_{\text{BIG}}$ 0.3 (95% CI: 0.1-0.7)] than for first lactation cows trimmed with the LIT model. The adjusted risk difference between the BIG model and LIT model for cows trimmed at the end of their first lactation was 4.6% (95% CI: 1.2-8.1). This risk difference can be interpreted as 5 fewer cows per 100 that undergo HT using the BIG model technique having a hoof horn lesion, as compared to the LIT model treatment. There was no effect of treatment on odds for a hoof horn lesion for cows in their second lactation or greater [Odds $\text{Ratio}_{\text{BIG}}$ = 1.11 (95% CI: 0.66-1.85)]. The odds ratio, adjusted risk and risk difference for having a horn lesion by treatment stratified by lactation are listed in Table 4. The estimated associations between other covariates and having a horn lesion at mid-lactation are reported in Table 5.

The final logistic regression model for infectious lesions included DD at enrollment and farm ID. There was no effect of treatment on odds for an infectious lesion [Odds $\text{Ratio}_{\text{BIG}}$ =1.24 (95% CI: 0.62-2.50)]. The adjusted risk and odds ratio of having an infectious lesion by treatment and the adjusted risk difference are shown in Table 4. The estimated association between other covariates and presence of infectious lesions is shown in Table 5.

4.5 DISCUSSION

This was the first study conducted in adult dairy cows in multiple herds to compare the effect of BIG versus LIT modelling techniques on subsequent risk of lameness and lesion prevalence at mid-lactation. Our results indicate the effect of the BIG model on the hazard of lameness and risk of hoof horn lesions, relative to LIT model, was dependent on lactation number. For cows trimmed at the end of their first lactation, using the BIG model HT technique it was protective for subsequent development of lameness and hoof horn lesions. However, there was no difference between the two treatments in cows second lactation or older.

The overall lesion results of this study are similar to those found in the study by Ouweltjes et al., (2009), since an overall difference between trimming techniques was not observed. However, there was a difference when the outcome was restricted to only hoof horn lesions. In the study by Ouweltjes et al. (2009), the sample size was too small to be able to analyze the data by lesion type. It also makes sense biologically that we found a difference for hoof horn lesions, because the adaptation we used in the current study was focused on preventing excess pressure from being applied to the common sole ulcer location (van der Tol et al., 2004). Our results are similar to the study by Gomez et al., (2013), in that a reduction in the risk of hoof horn lesion was observed for first lactation cows. Based on the results of the current study it appears that the BIG model is more effective for younger cows.

Since this is the first study investigating the difference between these two techniques to include cows first lactation and greater, we can only hypothesize as to why there was not a difference in treatment for second lactation and greater cows. It could be due to a lower prevalence of lesions than expected, which would reduce the statistical power of the study. The lesion prevalence in this study overall was 11%, which is much lower than 25%-45% lesion prevalence that has been reported in previous studies (Fjeldaas et al., 2006; Cramer et al., 2008; Becker et al., 2014). An alternate hypothesis is that the benefits of using this modeling adaptation could have been masked by events that happened before enrollment of the second lactation and greater cows. Events such as presence of hoof horn lesions (Enevoldsen et al., 1991; Foditsch et al., 2016) or low body condition score (Randall et al., 2015) have been shown to be associated with an increased risk of a hoof horn lesion in the subsequent lactation. In addition, the study by Newsome et al., (2016) showed that if a cow had a lesion, increased bone growth occurs on the distal phalanx. If the second lactation and greater cows in the current study had an increased distal phalanx size, then the biological principles of the BIG model preventing pressure to the corium potentially would be minimized. To be able to determine if this is what happened, cows without lesions over their entire lifetime would need to be enrolled.

The reduction in the risk of horn lesions for cows trimmed using the BIG model was a subset analysis; this increases the potential that a type 1 error occurred. However, we did find supportive evidence when looking at the risk of lameness between the two treatments.

The risk of lameness was also dependent on lactation category, whereby the BIG model was protective for cows trimmed at the end of first lactation, but not for older cows. This is consistent with the previously discussed finding that the BIG model technique reduced the risk of horn lesions at mid-lactation, given that hoof horn lesions are a common cause of lameness (Leach et al., 2012; Green et al., 2014).

The presence of DD at enrollment had a significant association with the risk of a hoof horn, infectious, or overall lesion at mid-lactation, irrespective of trimming method used. This is concerning because DD is commonly found on dairy farms (Cramer et al., 2008; Solano et al., 2016) and it is an infectious disease that is transmitted through the environment (Biemans et al., 2017). This suggests that DD is an important risk factor that potentially has a greater effect on risk for lesion presence at mid-lactation than HT technique. Being lame pre-trim was also significantly associated with the risk of being lame after HT. These cows were most likely chronic, and thereby continued to show signs of lameness, regardless of HT used (Green et al., 2014; Randall et al., 2017). It was for this reason that cows that were identified as lame prior to the start of the next lactation following enrollment were excluded from the time to lameness analysis, given that it would be unclear if their outcome was due to treatment or chronic lameness. However, when the cows identified as lame prior to the start of their next lactation were included in the analysis, it did not change the final interpretation of the results. This was similar to what was observed when the cows excluded from the lesion prevalence at mid-lactation were included in the analysis. The overall interpretation did not change, but they represent a population of cows that were identified as lame by farm staff or did not receive their mid-lactation trim at their scheduled time. Not including these cows' results in an underestimation of the occurrence of new lesions in this study.

The findings in this study are specific to sand bedded freestall barns. This housing environment was chosen because they have been reported to have lower levels of lameness than other systems (Cook, 2003; Espejo and Endres, 2007). If a difference for the risk or lameness was observed in an environment that has low levels of lameness, then we would expect the treatment difference to be even larger in other environments that have elevated levels of lameness.

4.6 CONCLUSION

This study showed that trimming cows with the BIG model technique did not have an effect on overall lesion prevalence in older animals, but it did reduce the hoof horn lesion prevalence at mid-lactation and risk of lameness for cows trimmed at the end of their first lactation. Future work should investigate what other factors, such as presence of a lesion in a previous lactation, have on the relative effectiveness of the BIG (vs LIT) modeling HT technique in second lactation or greater cows. Additionally, future studies should investigate the effect this technique has on future milk production and culling risk of the cow.

Table 4.1: Four point locomotion scoring system used to evaluate the gate of the animal during the study. Combination and adaption of Thomas et al., (2015); Cramer, (2007); and Cook, (2003)

<u>4-Point Locomotion Scoring System</u>

1. **Not lame:** Cow walks comfortably and at the same speed as the rest of the herd. Animal has even stride lengths and weight-bearing on all limbs, with a straight back.

2. **Slightly lame**: A change in walking speed or stride length may be shown but gait is still fluid and change in stride length is similar for both legs. May have a slight back-arch. Cannot easily or quickly identify which limb is affected.

3. **Moderately Lame**: These cows may walk slower than normal with an irregular walking rhythm and shortened stride-length on one or both hind limbs. Cows may have an obvious head bob or back arch. Limb is identifiable due to decreased weight bearing during stance phase of locomotion. May also notice sinking of dewclaws and increase in flight-phase of non-affected limb. Cow may also appear to be walking stiff or non-fluid or placing legs rigidly and have shortened strides if both legs are affected.

4. **Very lame**: Easy to identify the affected limb and the cow is reluctant to bear weight on it. Cows walk much slower than the rest of the herd and have severe slower shortened stride lengths and a prominent back arch.

Table 4.2: Descriptive statistics for the 1,562 cows enrolled from all 3 farms at dry-off, overall, and by treatment when comparing two hoof trimming techniques on the time to lameness and lesion prevalence at mid-lactation.

Variable	LIT[2]	BIG[3]	Overall
Lactation at enrollment	1.92 (1.85-2.00)	1.94 (1.86-2.02)	1.93 (1.87-1.98)
DIM[1] at enrollment	308 (304-313)	312 (307-317)	310 (307-314)
Holstein	607	587	1,194
Crossbred	182	186	368
N	789	773	1,562

[1] DIM = Days in milk.

Lactation and DIM at enrollment are reported as the mean (95% Confidence interval)

[2] LIT= Functional HT method (modeled up to 42mm of abaxial wall)

[3] BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall)

Table 4.3: Cox proportional hazard model estimates of the associations between covariates and time to lameness based on the final Cox proportional hazard model comparing the effect of two hoof trimming techniques on time to lameness (n=1,074).

Variable	Hazard Ratio Lactation 1	Hazard Ratio Lactation 2
BIG model[1]	0.49(0.34-0.73)	0.90(0.72-1.1)
Lame Pre-trim[2]	2.5(1.7-3.7)	2.2(1.8-2.8)
Crossbred[3]	1.8(1.1-1.8)	1.6(1.2-2.1)
Farm[4]	1.3 (0.8-2.1)	1.3(1.0-1.7)

[1] LIT= Functional HT method (modeled up to 42mm of abaxial wall) is the reference category BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall). LIT model is the reference category

[2] Lame pre-trim is based on the lameness status before being trimmed for the study, not being lame before enrollment is the reference category.

[3] Holstein is the reference category

[4] Farm 1 is the reference category

Hazard ratio (95% confidence interval)

Table 4.4: Lesion prevalence at mid-lactation of the 1,163 cows included in the lesion analysis, by hoof trimming technique and overall.

Lesion		LIT[2]	BIG[3]	Overall
Hoof Horn Lesions	White Line	21 (3.66%)	14 (2.36%)	35 (3.00%)
	Thin Sole	20 (3.50%)	15 (2.53%)	35 (3.00%)
	Sole Ulcer	10 (1.75%)	12 (2.02%)	22 (1.89%)
Infectious Lesions	Digital Dermatitis	18 (3.14%)	20 (3.37%)	38 (3.26%)
Lame/Other		5 (0.875%)	5 (0.844%)	10 (0.86%)
Lesion total		74 (53%)	66 (47%)	140
Total Cows (N)		571	592	1,163

[1] Count (% of total lesions)

[2] LIT= Functional HT method (modeled up to 42mm of abaxial wall)

[3] BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall)

Table 4.5: Adjusted odds ratio, risk and risk difference between BIG and LIT model by lesion category, based on separate final logistic regression models for different lesion categories.

Dependent Variable		Odds Ratio	Risk LIT[2] Model (%)	Lesion Count	Risk BIG[3] Model (%)	Lesion Count	Risk Difference (%)	Lesion Total
Horn Lesion	Lactation 1 (n=536)	0.3(0.1-0.7)	6.8(3.9-9.8)	18	2.2 (0.5-3.9)	6	4.6(1.2-8.1)	24
	Lactation 2+ (n=583)	1.1(0.6-1.8)	11(7.5-16)	33	12(8.5-16)	35	1.0(-4-6)	68
Infectious Lesion (n=1,065)		1.2(0.6-2.5)	3.2(1.8-4.6)	18	3.9(2.3-5.4)	20	0.6(-1.5-2.8)	38
Overall (n=1,163)		0.9(0.6-1.3)	12(9.5-15)	72	11(8.7-14)	64	0.8(-4.4-2.8)	136

[1] Outcome (95% CI)

Adjusted for other covariates in the logistic regression model (see Table 6)

[2] LIT= Functional HT method (modeled up to 42mm of abaxial wall)

[3] BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall)

Table 4.6: Estimated associations between covariates and having a lesion at mid-lactation, by lesion category and overall, displayed as odds ratio based on final logistic regression models.

Variable		Horn Lesions Lactation 1	Horn Lesions Lactation 2	Infectious Lesions	Overall lesions
DD at Enrollment		7.8(2.1-30)	1.4(0.17-12)	21(9.6-47)	8.3(4.4-16)
Lactation[1]		NI	NI	NS	2.1(1.4-3.1)
Farm[2]	Farm 2	7.2(2.5-21)	2.8(1.5-5.0)	1.1(0.29-3.6)	2.8(1.8-4.3)
	Farm 3	11(3.2-40)	1.7(0.55-5.1)	4.5(1.6-12)	3.6(1.9-6.8)

[1] Lactation one is the reference category.

[2] Farm 1 is the reference category

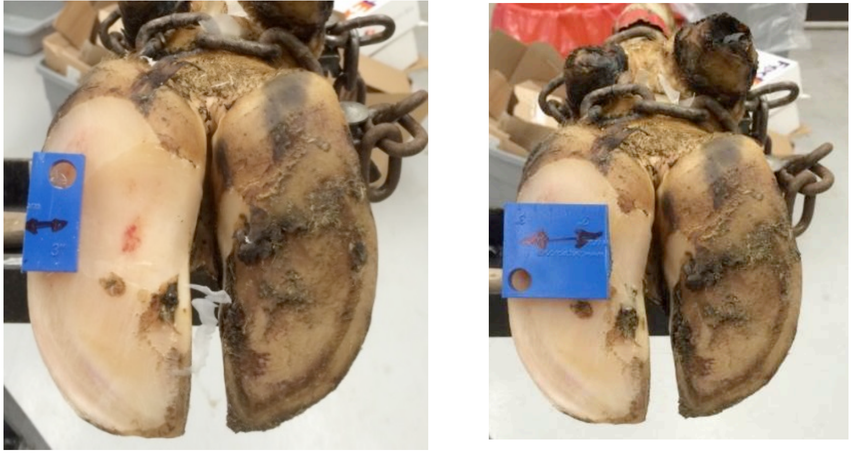
Odds ratio (95%CI)

Adjusted for main effects in the logistic regression model (see Table 5 for main effects)

NI= Not included

NS= Not Significant

Example of the placement and size of the gauge used for each treatment:



BIG Model (18 mm) **LIT Model (42mm)**

Figure 4.1: Examples of the placement and sizes of the gauges provided for the big model and little model treatment evaluated in the study.

White Line Disease

Separation of the white line, which may result in abscesses (puss filled cavity) in the white line region

If severe can be accompanied by swelling of the affected claw

Digital dermatitis (DD)/Warts

Acute is painful, raw and has bright-red, lesion > 2 cm

Typically in interdigital cleft

Chronic lots of skin/ hair around lesion, but not painful

Sole Ulcer

Localized defect in sole horn that exposes corium

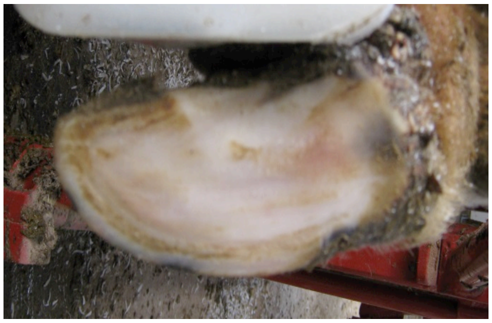
Can also be a haemorrhage that is painful to hoof testing

Typically occurs on inner side of sole, but can occur in the heel.

Thin Soles

Sole moves when thumb pressure is applied at the toe.

Dorsal wall length < 3 inches 7.5 cm



Overgrowth

Claws are at least 4 inches/ 10 cm long

Extensive overgrowth at sole ulcer site

Toe Ulcer/Necrosis

Penetration or separation of the horn in the toe triangle that results in exposure or infection of the corium

Foot rot
Symmetrical swelling of tissue above the claws
Can occur with dead, smelly skin between the claws.

Other
Other claw defects that are not included in previous categories for example: corkscrew, axial wall cracks and interdigital hyperplasia (korns)

Injured Cow
Cow is lame, and no physical lesion present.
Suspect shoulder/hip or some other non-foot origin lameness

Figure 4.2: Lesion guide provided to all hoof trimmers in the study.

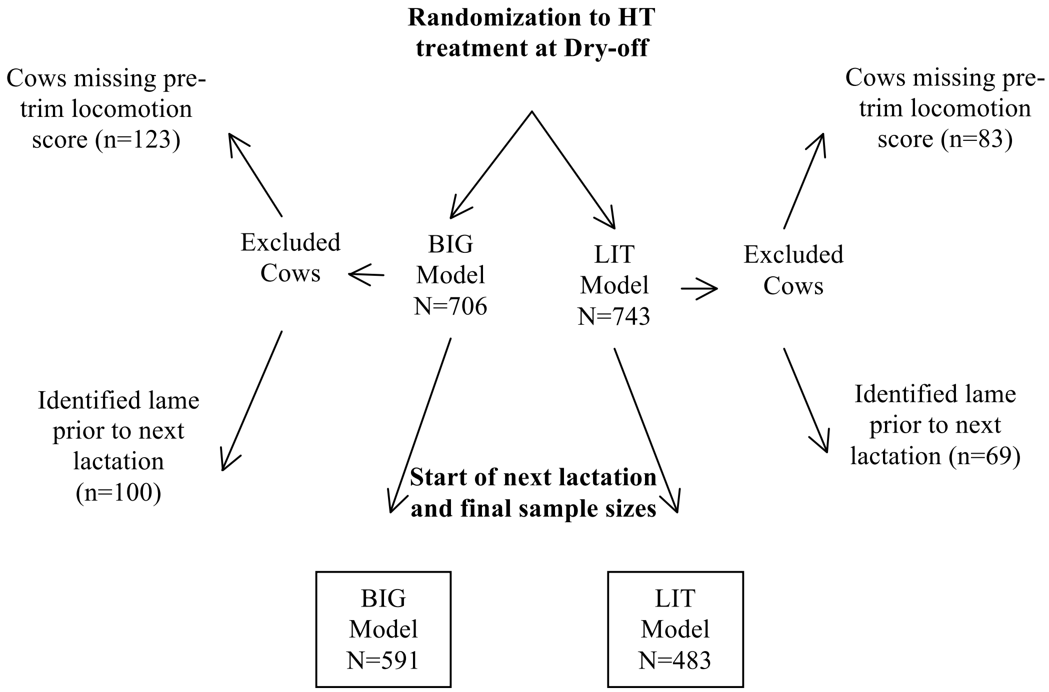


Figure 4.3: Flow diagram starting from the time of enrollment until cows started their next lactation and were included in the lameness analysis. Cows that were excluded from the original sample size for missing baseline locomoiton score or were identifed as lame before the start of next lactation are identified by treatment. (LIT= Functional HT method (modeled up to 42mm of abaxial wall), BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall))

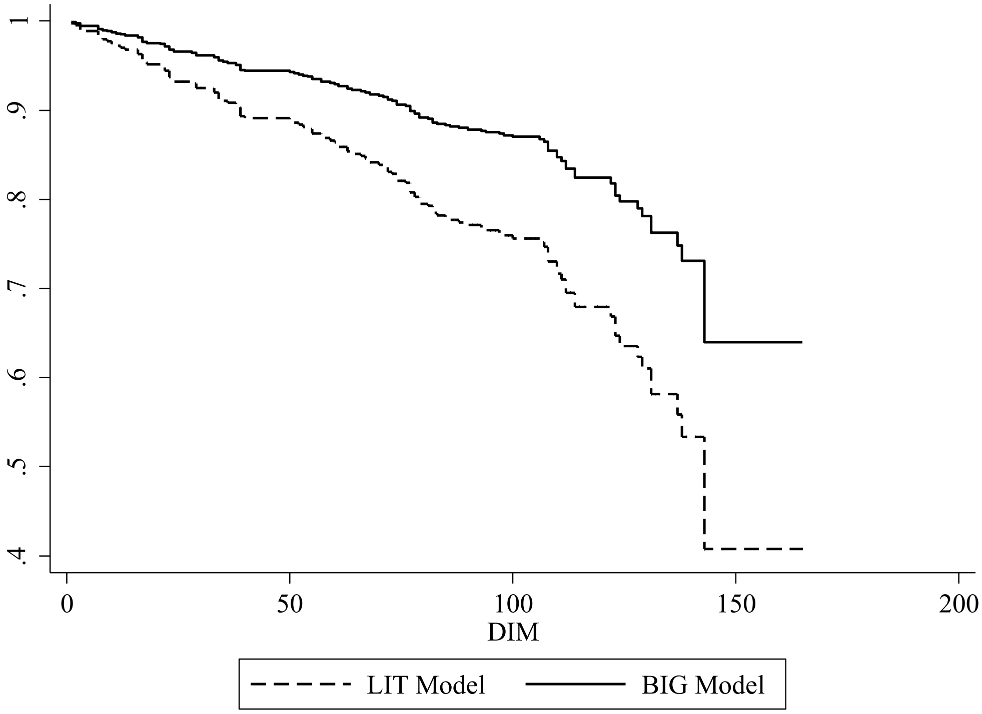


Figure 4.4: Effect of BIG vs LIT Modeling hoof trimming techniques on the adjusted hazard for lameness in the lactation following enrollment for cows enrolled at the end of their first lactation. (LIT= Functional HT method (modeled up to 42mm of abaxial wall) BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall)). Censoring occurred at 165 DIM in the lactation following enrollment.

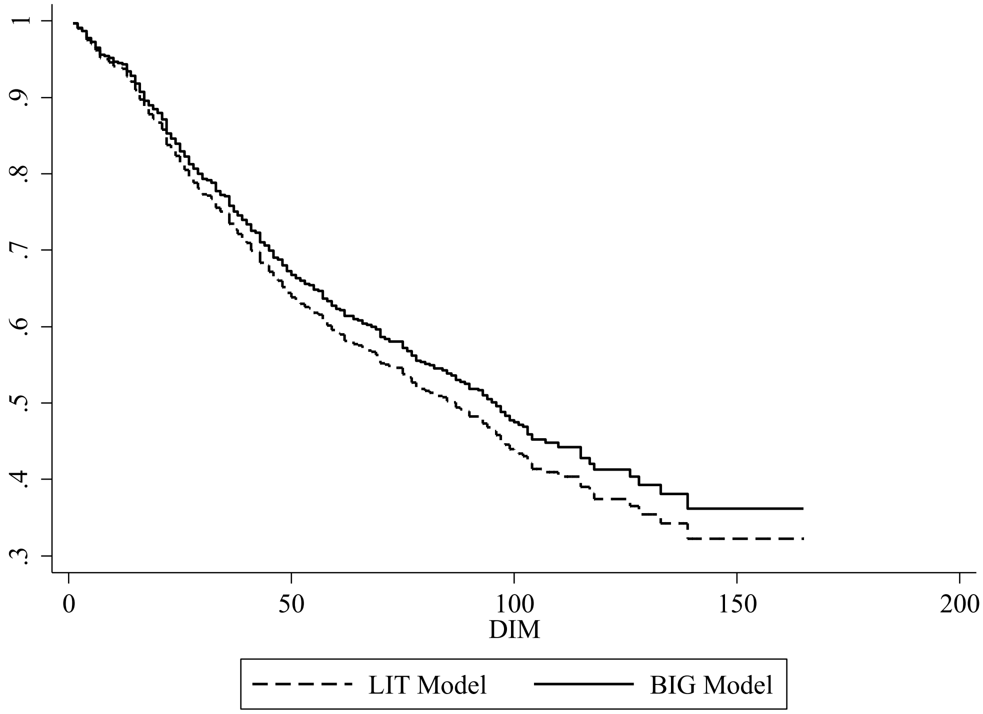


Figure 4.5: Effect of BIG versus LIT modelling hoof trimming technique on the adjusted hazard for lameness following enrollment for lactation 2 and greater cows. (LIT= Functional HT method (modeled up to 42mm of abaxial wall), BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall)). Censoring occurred at 165 DIM in the lactation following enrollment.

CHAPTER FIVE: Randomized controlled trial to evaluate the effect of two hoof trimming techniques on milk yield and culling risk of lactating dairy cows

5.1 INTRODUCTION

Lameness is a chronic painful disease (Whay et al., 1998) that has a significant negative impact on productivity of the cow (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005) , and causing a significant economic burden for producers (Cha et al., 2010; Liang et al., 2017). Lame cows experience decreased milk production, (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005), decreased reproductive performance (Bicalho et al., 2007a; Peake et al., 2011; Hudson et al., 2014), increased risk of culling (Booth et al., 2004; Bicalho et al., 2007a), and behavior changes (Chapinal et al., 2010a; Gomez and Cook, 2010; Navarro et al., 2013). These changes in productivity and behavior illustrate that lameness is challenging the natural behavior, health and biological functioning of the cow and decreasing its welfare (Fraser, 1993). The economic burden of lameness to producers has been estimated to range from $100-$220 per case and this based on estimated decreases in productivity and treatment costs (Cha et al., 2010; Liang et al., 2017). Furthermore, lameness affects a large proportion of cows, with the herd level prevalence estimated to vary from 20-55% in North American dairy herds (Espejo et al., 2006; Von Keyserlingk et al., 2012; Solano et al., 2016).

Hoof trimming **(HT)** is one of the recommended procedures to prevent lameness (Raven, 1985; Shearer and van Amstel, 2001). It is estimated that 85% of US herds trim cows at least once a year (NAHMS, 2007). However, the scientific research surrounding HT is limited overall and a recent review has shown that the current research typically does not consider the longer term impact on milk production or longevity (Stoddard and Cramer, 2017). Hoof trimming has the potential to improve longevity and milk yield through the reduction of lesions and lameness. This is because the occurrence of lameness or a lesion has been linked to a decrease in milk yield (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005) and increase in culling risk (Booth et al., 2004; Bicalho et al., 2007b; Cramer et al., 2009b). It is important to understand what effect HT has on milk production and longevity of a cow, since they affect farm profitability and impact the health and biological functioning of the cow, two of the three components of the cow's welfare.

Currently there are two studies that looked at the effect preventative HT had on long-term milk production (Maxwell et al., 2015; Mahendran et al., 2017). Both studies described the HT technique used as the functional HT method as described by Raven, (1985). Maxwell et al. (2015) compared the 305-day adjusted milk yield between first lactation cows undergoing HT at 50-80 DIM to an untrimmed control group. The estimated impact of HT on the 305-day adjusted milk yield was 144 liters (95% CI:-189 to 477 P=0.39). Similarly, Mahendran et al. (2017) found no effect of HT on the 4% fat corrected 305-day milk yield of heifers that were either trimmed before or after calving. Based on these studies it appears that preventative HT does not influence milk yield. However, both studies used a similar HT technique. It is possible that a different HT technique could be more effective at preventing lesions and lameness, and therefore result in increased milk yield.

Similar to milk production, there are no studies that have evaluated the effect HT technique has on the longevity of the cow. Previous research has shown cows that are lame or have a lesion are at an increased risk of being culled (Booth et al., 2004; Bicalho et al., 2007b; Cramer et al., 2009b). Similar to milk yield, if a certain HT technique is better at preventing lameness or lesions, then it would make sense that this would be reflected in increased longevity. In chapter 4 of this thesis, it was reported that an adaptation to the functional HT method that resulted in removing more horn under the flexor tuberosity reduced the risk of lameness and hoof horn lesions at mid-lactation in first lactation cows. Since this adaptation resulted in fewer horn lesions, it is expected that it would result in an increased longevity and milk yield when compared to the traditional functional HT method.

The objective of this study was to compare the milk production and culling risk for cows trimmed at dry-off using either the functional HT (LIT) method or trimmed with an adaptation (BIG). Our hypothesis was that cows in the BIG group would have a higher long term milk yield and less risk of being culled in the lactation following HT.

5.2 MATERIALS AND METHODS

5.2.1 Enrollment and Treatments

This study uses the same cows as were enrolled in chapter 4 of this thesis. A detailed description of the farm inclusion criteria, hoof trimmer training, and adherence to protocol can be found in that chapter. Briefly, 3 farms were enrolled in the study and 2 hoof trimmers were trained on the two HT techniques. At the dry off enrollment and HT event, cows in all lactations without hoof horn lesions were randomized at the week level to either receive a trim using the functional HT method (LIT) or the adapted method that results in increased modeling of the weight bearing claw up to 18mm away from the abaxial wall (BIG). Herd size of the three farms enrolled in the study ranged from 892 to 1,928 cows. Average 305 day milk production ranged from 11,195 kg to 13,483 kg. Herd size and average 305 day milk production for the one farm that was used for the milk yield analysis was 1,928 cows and 13,483 kg, respectively.

5.2.2 Data Collection

Culling data was available from all three farms enrolled in the study and daily milk yield data was only available for one farm. Data collection started from the time of enrollment in December 2015 until all cows received a second trim following enrollment or reached 165 DIM in the subsequent lactation. All culling and milk yield data was extracted from each farm's DairyComp 305 (Valley Ag. Software, Tulare. CA) record keeping software.

5.2.3 Statistical Analysis

All data management and statistical analysis was performed using STATA 14.1 (Stata Corp., College Station. TX) with outcomes evaluated at the cow level.

Milk Yield Analysis

Lactation data was collected on one farm with in-parlor milk meters, starting in the next lactation following enrollment until subsequent trim or censoring due to culling or reaching 165 DIM. Milk yield data was collected at the milking level for each cow, and then collapsed to the day level and subsequently the week level, to describe the daily average milk production per week (kg/cow/day). Before collapsing to the day level, milking level weights that were missing were imputed to the average between the two milk weights present for that cow on that day. If two milk weights on the milking level were missing the milk weight for that day was imputed for both missing milk weights. If the cow was missing all milk weights for a day, then that day of milking for that cow was excluded from the data set. Out of the total 119,621 milking days wherein cows were milked 3 times per day, 108,067 (90%) cows had all three milk weights available, while 8,697 (7.3%) were missing one milk weight, 1,383 (1.2%) were missing two milk weights and 1,474 (1.3%) were missing all three milk weights. The number of missing milk weights was not proportionally different between the two HT treatment groups.

To assess the association between milk yield and HT technique, a mixed linear model was constructed. Initially univariate mixed linear models were used to assess the association between milk yield (kg/day; dependent variable) and the covariates lactation, DIM at enrollment, breed, season, digital dermatitis **(DD)** status at enrollment, and 305-day projected milk yield (**M305**) from the previous lactation. Lactation number was categorized into parity 1 or parity 2 and greater. Breed was either Holstein or Crossbred. Season was categorized into spring (March-May), summer (June-August), fall (September-November), and winter (December-February). Associations between milk yield and the covariates at $P < 0.20$ in the univariate models were offered into a multivariable linear regression model. A manual backward stepwise elimination was performed to build the final mixed linear model. The treatment variable and a week variable based on the start of next lactation following enrollment were forced into the model. Upon removal of a covariate from the model, change in the AIC and change in coefficients were evaluated for significant changes in magnitude. After variable selection all biologically plausible interactions were investigated and an interaction term was included if the interaction term had a $P<0.05$.

All models included a random effect on cow to account for repeated measures of milk yield over multiple weeks, and an exchangeable correlation structure to account for the correlation between daily average milk production per week. Other correlation structures were evaluated in the final model and compared using model AIC. In the final model assumptions of homoscedasticity, normality and a linear relationship were evaluated graphically.

Culling Analysis

Culling analysis was performed using data from all three herds. The outcome for all survival analyses was time from enrollment HT to culling or censoring. Cows were censored when they received their subsequent trim following enrollment or reached 165 DIM in the next lactation following enrollment.

The difference in time to culling between the LIT and BIG treatment groups was investigated using Kaplan-Meir analysis. The difference between the treatments was tested using the Log-Rank test and the time to median culling was calculated for each treatment. A Cox proportional regression model was then constructed to determine the hazard ratio between the BIG and LIT model. The process of building the final Cox proportional regression started with assessing univariate Cox proportional regression models to investigate the association between culling and the covariates: lactation category, DIM at enrollment, breed, season, DD at enrollment, and enrollment ME305 (kg). These covariates were classified the same as previously described for the milk yield analysis. Herd effects were accounted for by forcing farm ID as a fixed effect into the final model. All covariates identified as $P<0.20$ in the univariate models were offered into a multivariable Cox proportional regression analysis. A manual stepwise backward elimination was then performed. Upon removal of a covariate from the model, change in the AIC and change in coefficients were evaluated for significant changes in magnitude. Biologically plausible interactions between the treatment and significant covariates were included in the final model if an interaction term had a $P<0.05$.

The proportional hazard assumption was assessed in the final Cox proportional regression model using the Schoenfeld residuals test. Goodness of fit was assessed using the Gronnesby and Borgan omnibus test. Outliers and influential points were identified through using deviance and scaled scored residuals (Dohoo et al., 2009).

5.3 RESULTS

5.3.1 Effect of hoof trimming method on milk yield

In total 1,183 cows were enrolled in the milk production section of this study from 1 farm. Of these, 39 cows were excluded after enrollment due to being trimmed (n=26) or culled (n=13) before the start of the next lactation, resulting in 1,122 cows that started a subsequent lactation following enrollment. Descriptive statistics for the cows included in the milk analysis are presented in Table 1. The final mixed linear model included the covariates lactation, breed, season and previous lactation ME305 milk yield. Cows trimmed using the BIG model technique produced 0.07 (95% CI:-1.03 to 0.89) kg/day less milk on a weekly basis when compared to the LIT model group. The predicted average daily milk yield on the week level by treatment, adjusted for other final model covariates, is shown in Figure 1. The estimates for other covariates in the final milk yield model are reported in Table 2.

5.3.2 Culling

A total of 1,562 cows from three farms were included in the culling analysis. Twelve cows were excluded from the analysis because a culling, trimming or fresh date following enrollment was not available. Descriptive statistics for the 1,550 cows used in the analysis are presented in Table 3.

In total 199 (12.8%) of the 1,550 cows included in this analysis were culled following enrollment before their next trim or reaching 165DIM in the subsequent lactation. This included 104 (13.1%) and 95 (12.5%) cows culled in the LIT and BIG model groups, respectively.

Kaplan-Meir analysis showed no difference (P=0.54) between the two treatment groups, and neither of the treatment groups went below 50% survival. The final Cox regression model included the covariates farm and lactation. There was no effect of HT treatment on risk of being culled [Hazard $\text{Ratio}_{\text{BIG}}$ of 0.92 (95% CI: 0.69-1.2)]. Survival curves by treatment, adjusted for other final model covariates, are shown in Figure 2. The estimates of the covariates in the final time to culling model are listed in Table 4.

5.4 DISCUSSION

This is the first study to compare the risk of being culled and long term milk yield between two HT techniques. Our results indicate that the BIG HT technique did not show evidence of an effect on long term milk production or the risk of being culled up to mid-lactation. This is important because to evaluate new HT techniques it is important to evaluate both long term effects on lameness, lesions and productivity.

This study is different from previous research because it compared the relationship between HT technique and milk. Previous studies evaluated the effect HT has on milk yield by comparing the milk yield between cows that had been trimmed and cows that were not trimmed (Maxwell et al., 2015; Mahendran et al., 2017). Similar to those studies we observed no difference in milk yield.

Not observing a difference in treatments for long term milk yield and risk of culling is surprising because earlier analysis (Chapter 4 of thesis) showed that BIG model reduced the risk of hoof horn lesions and lameness in cows trimmed at the end of their first lactation. Lameness and lesions have been associated with a decrease in milk yield (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005) and increase in culling risk (Booth et al., 2004; Bicalho et al., 2007b; Cramer et al., 2009b). Therefore it was expected that the protective effects would be manifested as an increase in long term milk yield and a decreased risk of culling. A key difference between our study and previous research is the observed prevalence or incidence of lesions and lameness: The prevalence of hoof horn lesions for LIT and BIG model for cows trimmed at the end of their first lactation was 7% and 2% respectively (Chapter 4). Similarly, the incidence of lameness for LIT and BIG model for cows enrolled at the end of their first lactation was 29% and 17% respectively (Chapter 4). Previous research that observed an increase in the risk of being culled associated with presence of a lesion reported a lesion prevalence between

46% and 68% for cows that were culled, and between 36% and 44% for cows that were not culled (Booth et al., 2004; Cramer et al., 2009b). Another study that observed an increase in the risk of being culled associated with lameness had an incidence of 42% (Bicalho et al., 2007b). The incidence of lameness in a different study that observed a decrease in milk yield was 70% (Green et al., 2002). What this indicates is the differences in the previous studies for either outcome of culling or milk yield was based on much larger differences in prevalence or incidence of lesions or lameness than the Chapter 4. Taking that into consideration and that a small numeric difference we observed between BIG and LIT HT techniques to prevent lesions and lameness, it is not surprising that we did not observe a significant effect of treatment on milk yield or risk of culling.

There are several differences in design between the current and previous studies that resulted in these large between-study differences in prevalence or incidence of lameness and lesions. These include study design, given that ours was a randomized clinical trial, while others were observational. (Booth et al., 2004; Bicalho et al., 2007b; Cramer et al., 2009b). Additionally, inclusion/exclusion criteria may have increased the prevalence of lameness in prior studies. Specifically, the population of cows included in the previous studies was a mixture of chronic and new cases of lesions and lameness since all cows were enrolled. By including all cows and potentially chronically lame cows, those studies could have created a larger difference in milk yield or risk of culling in the previous studies. A chronically lame cow would be at higher risk of having another lesion in the next lactation (Green et al., 2014; Randall et al., 2017), likely leading to decrease milk yield and a subsequent increase in risk of culling. The current study excluded cows with pre-existing lesions from enrollment, thereby reducing the possibility that a chronic cow would be enrolled. This was done so the study population would be truly representative of cows developing new cases of lameness or new lesions. In doing so, the current study excluded a high risk group of cows and reduced the overall incidence of lameness and lesions so HT technique could be investigated solely as a preventative measure on cows free of lesions at the time of HT. Currently, it is unclear what the effect HT has on a cow that chronically is affected by lesions or lameness and on its milk yield or risk of culling. This knowledge would be of great value in evaluating the effectiveness of HT techniques.

In summary, the smaller prevalence and incidence of new lesions and lameness, respectively observed in this study may have resulted in a lack of power, thereby limiting our ability to detect a significant effect of HT method on milk production or culling. Our a priori sample size calculations are described in Chapter 4. Post hoc sample size calculations were performed to determine what sample sizes would be needed to observe a biological important difference and still maintain 80% power with an alpha of 0.05%. The sample size needed to observe a difference of 1 kg/day on a weekly basis was 960 total cows. This study currently has 1,122 cows enrolled in the milk yield analysis. As such, we are confident that the lack of difference in milk yield was not due to a lack of power. The sample size needed to see a 5% reduction in the proportion of cows culled is 1816 total cows, this study enrolled 1,550 cows total. Based on this power calculation, the culling analysis was under powered to detect a biologically significant reduction in the risk of culling if it was present. This must be taken into consideration when assessing these results.

Since there was no evidence for a difference in the long term milk yield or culling risk, neither of the HT techniques in the long term appears to be better or worse for the cow based on these outcomes. However, to ensure that one of these HT techniques is not causing an immediate detrimental effect on the cow, effects on short term milk production, immediately after the HT event, would need to be assessed. A change in the short term milk production potentially could indicate one of the techniques is causing a compensatory reaction in the cow that would have been lost in the long term. Determining if a compensatory reaction occurs will help determine overall what affect the HT technique has on the welfare of the cow.

5.5 CONCLUSION

This study showed no evidence of an effect on the long term milk production or culling risk in the subsequent lactation when cows free of lesions were hoof trimmed with either the BIG model or the LIT model technique at dry off. To further evaluate the impact of these HT techniques future research is needed to determine if an immediate effect on the cow's milk production occurs that potentially would be lost in a long term comparison.

Table 5.1: Descriptive statistics of the 1,122 cows enrolled from one farm in the study to compare the long milk yield between cows that underwent hoof trimming using two techniques by treatment and overall.

Variable	LIT[1]	BIG[2]	Overall
Lactation	1.9 (1.8-2.0)	2.0 (1.9-2.1)	2.0 (1.9-2.1)
DIM at enrollment	294 (290-298)	296 (291-300)	295 (292-298)
ME305*	14,235 (14,017-14,452)	14,188 (13,970-14,406)	14,211 (14,058-14,365)
Holstein	403	382	785
Crossbred	166	171	337
N	568	554	1,122

Lactation, DIM at enrollment, and M305 are reported as the mean (95% Confidence interval)

*ME305=305-day projected milk yield (kg) for the previous lactation

[1] LIT= Functional HT method (modeled up to 42mm of abaxial wall)

[2] BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall)

Table 5.2: Mixed linear regression model estimates of associations between covariates and milk yield based on the linear model comparing the effect of two hoof trimming techniques on milk yield (n=1,122).

Variable		Parameter Estimate	SE	95% Confidence interval
BIG model[1]		-0.071	0.49	-1.0 to 0.89
2+ Lactation[2]		-5.2	0.69	-6.6 to -3.8
Crossbred[3]		-1.2	0.55	-2.3 to -0.13
Previous ME305[*]		2.0	0.13	1.7 to 2.2
Season[4]	Summer	-1.3	0.69	-2.7 to 0.015
	Fall	-1.1	0.72	-2.5 to 0.32
	Winter	3.0	0.70	1.6 to 4.4

[1] LIT= Functional HT method (modeled up to 42mm of abaxial wall) is the reference category BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall). LIT model is the reference category

[2] Lactation uses first lactation as the reference category compared to second lactation or greater.

[3] Holstein is the reference category compared to crossbreds

[4] Spring is the reference category

[*] ME305=305-day projected milk yield per 1,000kg

Table 5.3: Descriptive statistics of the 1,550 cows enrolled from three farms to compare the time to culling between two hoof trimming techniques, by treatment and overall.

Variable		LIT[1]	BIG[2]	Overall
Lactation		2.0 (1.9-2.1)	1.9 (1.8-2.0)	2.0 (1.9-2.1)
DIM at enrollment		309 (305-314)	312 (307-317)	311 (307-314)
ME305*		13,786 (13,575-13,997)	13,725 (13,518-13,932)	13,756 (13,608-13,904)
Holstein		612	579	1,191
Crossbred		180	179	359
N	Farm 1	607	576	1,183
	Farm 2	129	126	255
	Farm 3	53	59	112
	Total	789	761	1,550

Lactation, DIM at enrollment, and M305 are reported as the mean (95% Confidence interval)

*M305=305-day projected milk yield (kg)

[1] LIT= Functional HT method (modeled up to 42mm of abaxial wall)

[2] BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall)

Table 5.4: Cox proportional hazard model estimates of the associations between hoof trimming technique, covariates and time to culling based on the final Cox proportional hazard model comparing the effect two hoof trimming techniques on time to culling (n=1,550).

Variable		Hazard Ratio	SE	95% Confidence Interval
BIG Model[1]		0.92	0.14	0.69-1.2
2 + Lactation[2]		1.5	0.22	1.1-1.9
Farm[3]	Farm 2	1.5	0.27	1.0-2.1
	Farm 3	1.9	0.44	1.2-3.0

[1] LIT= Functional HT method (modeled up to 42mm of abaxial wall) is the reference category BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall). LIT model is the reference category

[2] Lactation uses first lactation as the reference category compared to second lactation or greater.

[3] Farm 1 is the reference category

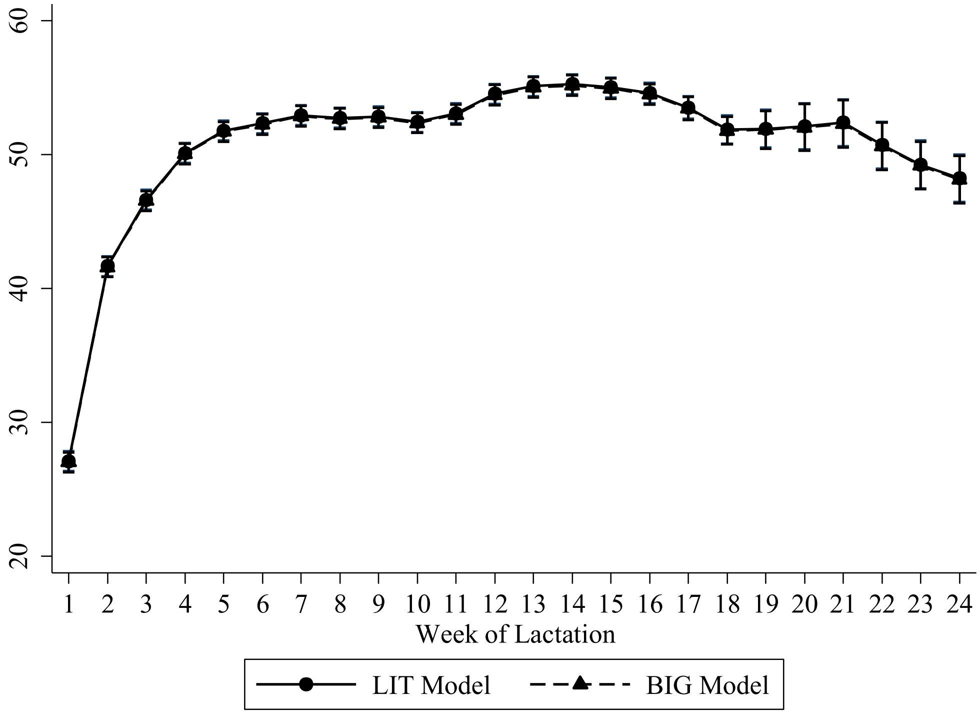


Figure 5.1: Predicted average daily milk yield for two HT techniques, by week of lactation, in the lactation after enrollment. Follow-up was discontinued at the subsequent hoof trim, or if censoring due to culling or reaching 165 DIM in the following lactation after enrollment. The values in this graph are adjusted for other covariates in the model. (LIT= Functional HT method (modeled up to 42mm of abaxial wall), BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall))

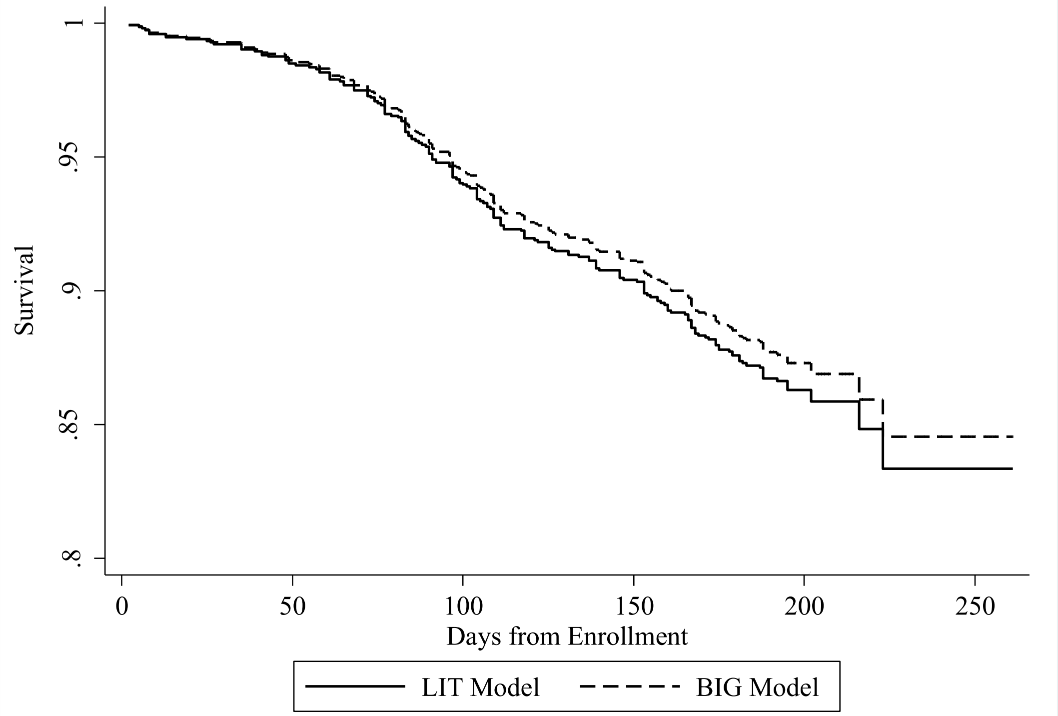


Figure 5.2: Cox regression graph of survival time starting from enrollment at scheduled dry-off trim until subsequent hoof trimming or reaching 165 DIM in the next lactation following enrollment. The model adjusts for other significant covariates in the model. (LIT= Functional HT method (modeled up to 42mm of abaxial wall), BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall))

CHAPTER SIX: Effect of hoof trimming technique and restraint on the milk production of dairy cows in following 7 days post hoof trimming

6.1 Introduction

Lameness is an important disease in the dairy industry because of the negative effects it has on the welfare (Whay et al., 1998; Shearer et al., 2013) and productivity of dairy cows (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005). Due to lameness cows experience decreased milk yield (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005), decreased reproductive performance (Bicalho et al., 2007a; Peake et al., 2011; Hudson et al., 2014) and increased culling risk (Booth et al., 2004; Bicalho et al., 2007a). Additionally, lameness causes behavior changes (Chapinal et al., 2010a; Gomez and Cook, 2010; Navarro et al., 2013) that are likely attributed to a pain response (Tadich et al., 2013; Bustamante et al., 2015). These changes in biological functioning, affective state and natural behaviors show that lameness impacts 3 components of the cow's welfare (Fraser, 1993), and are ample evidence that lameness needs to be prevented.

Hoof trimming (**HT**) is a commonly recommended practice to treat and prevent lameness (Raven, 1985; Shearer and van Amstel, 2001). It is estimated that 85% of US herds have cows trimmed at least once a year (NAHMS, 2007). However, the scientific research surrounding HT is limited, and investigations into the effects it has on the components of welfare so far have so far focused mostly on behavior and stress responses that occur following HT (Stoddard and Cramer, 2017). Based on previous research (Chapinal et al., 2010a; b; Van Hertem et al., 2014) and Chapter 2 of this thesis, we know that cows increase their resting time and decrease their activity short term following HT. Furthermore, HT has been shown to cause an acute stress response for 3 hours, shown by elevated concentrations of cortisol following HT (Rizk et al., 2012; Korkmaz et al., 2014, chapter 3).

In addition to these behavior and physiological changes, studies have reported equivocal results in that HT may decrease (Pesenhofer et al., 2006; chapter 2) or have no impact on milk production (Van Hertem et al., 2014). From Chapter 2 of this thesis, it is clear that the impact of HT on behavior and milk yield is farm dependent. There are several factors that could be responsible for this farm dependent effect including HT technique, daily routine, facility design and bedding type. Currently no studies have investigated what effect HT technique has on immediate milk production or what effect the restraint used during HT has on short-term milk production immediately following the HT procedure.

The objective of this study was to evaluate the effect of HT method (functional HT method (LIT) versus an adaptation (BIG) or just being restrained in the HT chute) has on milk production for 7 days following the procedure. Our hypothesis was cows that undergo removal of horn using the BIG model technique will have a higher milk production in the 7 days following the procedure, as compared to cows in the LIT model group or the restraint group. However, cows in all three study groups would experience a decrease in 7-day milk production following the procedure as compared to their 5-day average baseline milk yield before the procedure.

6.2 MATERIALS AND METHODS

6.2.1 Enrollment and Treatment

The mid-lactation cows used in this study were from one farm that was participating in a long term HT study that compared the effect of two HT techniques on the risk of lameness and prevalence lesions at mid-lactation in addition to evaluating the impact of a mid-lactation trim. The original enrollment activities at the previous dry-off event are described in Chapter 4. A detailed description of farm recruitment and training for the hoof trimmers can be found in Chapter 4. Herd size for the one farm included in this study was 1,928 cows and the average 305 day milk production was 13,483 kg.

Upon reaching approximately mid-lactation in the subsequent lactation (100 to 165 DIM), these cows exited the previous protocol (described in Chapter 4) and were enrolled into this new protocol at the time of the mid-lactation HT event. Cows were eligible to be included in the current study if overgrowth or a lesion was not present at their mid-lactation HT. Eligible cows with an even identification numbers were trimmed at mid-lactation using the HT technique they were originally randomized to at the previous dry-off (BIG or LIT), while cows with an odd cow identification number were only restrained and evaluated for lesions in the HT chute. The cows with an even identification number were trimmed using the functional HT method (Raven, 1985) (LIT) or an adaptation to the functional trimming method that results in unrestricted modeling of the weight bearing claw up to 18mm away from the abaxial wall (BIG). The farm milked three times daily, with milk production data from each milking, measured using in-parlor automated milk meters and captured automatically using on-farm record keeping software DairyComp 305 (Valley Ag. Software, Tulare. CA).

6.2.3 Statistical Analysis

All data management and statistical analysis was performed using STATA 14.1 (Stata Corp., College Station. TX) at the cow level.

Milk Yield Analysis

Milk yield data was collected starting 5-days before the cows were trimmed at mid-lactation until 7-days after. Milking level data was collapsed to the day level (kg/day). Before collapsing to the day level, missing milking level weights imputed. If one milking weight was missing on a given day, the average between the two present for that day was imputed for the missing milk weight. If two milk weights were missing on a given day, the milk weight present for that day was imputed for both of the missing milk weights. If the cow was missing all three milk weights for that day, it was excluded from the data set for that day. Out of the total 10,309 milking days, starting 5 days before HT until 7 days after HT, 9,492 (92.1%) had all three milk weights, 688 (6.7%) were missing one milk weight, 105 (1%) were missing two milk weights and 24 (0.2%) were missing all three milk weights. Missing milk weights were evenly distributed among the three treatment groups.

To determine if a there was a difference between the two HT techniques and restraint, a mixed linear regression model was built that compared the milk yield the day of and 7 days after HT to the 5 day average baseline milk production prior to the HT event day. A two-step model building process was used to build the final model. The first step was evaluating univariate linear models to assess the association between the milk yield and the covariates: lactation number (2, 3+), breed (Holstein and Crossbred), and season of mid-lactation HT (spring, summer, fall, and winter). In the second stage, covariates with an association of $P < 0.20$ in stage 1 were evaluated in a multivariable mixed linear regression analysis. Stepwise manual backward elimination was then performed. When covariates were eliminated the change in model AIC and the change in the remaining coefficients were observed to see if significant changes occurred. Treatment and days from trim were forced into the final mixed linear regression model. After variable selection all biologically plausible interactions were investigated and an interaction term in the model was included if an interaction term had a $P<0.05$. If no difference was observed between the BIG and LIT HT techniques, they were combined in a final model. The final mixed linear regression model was used to estimate the average milk production for each day in the study by treatment.

All models included a random effect on cow to account for repeated measures and an exchangeable correlation structure was included to account for the correlation between daily milk yields. Other correlations structures were evaluated in the final model and compared using model AIC. The model assumptions of homoscedasticity, normality and a linear relationship were evaluated graphically for the final model.

6.3 RESULTS

A total of 793 cows were enrolled into the study; 381 in the restraint group, 196 in the LIT model and 216 cows in the BIG model group. Descriptive statistics for the final sample size of the 793 cows are presented in Table 1. The final mixed linear regression model included the covariates breed and season. The 7-day change from baseline milk production was not different among the three treatment groups ($P=0.55$). Since the estimated difference in milk production between the BIG and LIT were not different

(P=0.37) from each other at 0.65 (95% CI: -0.69 to 2.0) and 1.3 (95% CI: -0.27 to 2.7) kg/day, respectively, they were combined in the final model shown in Table 2. Regardless of treatment group assigned, milk yield did decrease on the day of HT and day after HT. This decrease ranged from 0.60 (95% CI: -1.1 to -0.084) to 0.67 (95% CI: -1.2 to -0.16) kg/day for each of the treatment groups. A similar decrease in milk yield occurred again on days 5-7 post HT. The predicted mean milk yield by the categories of restrained and hoof trimmed starting from baseline until 7 days post HT is shown in Figure 3.

6.4 DISCUSSION

This is the first study to compare the impact that HT technique and restraint has on the immediate short-term milk production of dairy cows. The previous studies (Pesenhofer et al., 2006b; Van Hertem et al., 2014) and Chapter 2 of this thesis evaluated the whole HT process, whereas this study specifically evaluated HT technique and restraint separately. The results of this study indicate that having horn removed regardless of the technique had the same effect on the 7-day milk yield as being restrained and evaluated for lesions. This is different than our hypothesis when we thought cows in the BIG model group would have an increased milk yield when compared to the LIT model or restraint. However, similar to Chapter 5 these protective effects were not observed in long term milk production.

The magnitude of difference after HT (versus baseline milk production) observed in this study was similar to the previous randomized controlled trial (Pesenhofer et al., 2006), but slightly less than for the observational study described in Chapter 2 of this thesis.

However, the current study was carried out on only one farm, and in the former study (Chapter 2) reported a significant farm effect for the magnitude and duration of the milk yield decrease following HT. Regardless, a similar pattern of a decrease in milk yield on the day of HT and day after HT in the previous studies was observed in the current study. Another difference from previous studies is the decrease in milk production (from baseline) was observed again on day's 5-7 post HT in the current study. This decrease in milk yield on days 5-7 could potentially be attributed to a farm effect as well, in that this could have coincided with when the cows were sorted to a holding area following milking for a routine pen transfer on this facility.

Given that this study was conducted at only one facility, we cannot investigate if a farm effect was present. Furthermore, our findings are limited to a free-stall sand bedded herd. However, these results are similar to findings from previous studies (Pesenhofer et al., 2006; Chapter 2), increases our confidence that milk yield truly does decrease the day of and day after HT or restraint.

Since this study isolated the removal of horn from restraint, it provides evidence that the decrease in milk production that was observed in the current study following HT was more likely due to restraint, than the actual removal of horn during the HT process. Supporting evidence for this comes from Chapter 3 of this thesis when the same acute stress response of a 3 hour increase in plasma cortisol was observed for cows undergoing HT as compared to cows undergoing sham HT. This means the stress caused during the HT process was due to restraint and not the actual removal of horn.

Conversely it could be possible that the disruption of the cow's daily routine due to HT, and not the actual restraint, caused these observed changes in milk production. This is based on previous research that observed when the lying time of the cow was intentionally altered it made the cow compensate by increasing her lying time post challenge (Metz, 1985; Cooper et al., 2008; Ouweltjes et al., 2011). Since the lying time of the cow is going to be altered on the day of HT based on the previous research described in Chapter 2 of this thesis, it would be expected to also see a corresponding decrease in milk yield. However, one previous study reported that when a cow's daily routine is intentionally altered by limiting their lying time, milk production was not affected (Cooper et al., 2007). The difference between our study and the previous studies (Metz, 1985; Cooper et al., 2008; Ouweltjes et al., 2011) is that cows in our study had reduced access to feed on the day of HT when they are away from the home pen, while and in the previous studies when cows were intentionally prevented from lying down, feed access was still available.

This study is different than some previous studies (Metz, 1985; Cooper et al., 2008; Ouweltjes et al., 2011) because cows were exposed to an acute stressor (i.e. restraint) in the HT chute. Combining the effects of altering the lying time, decreased availability to feed and HT causing an acute stress response, it could be possible that these cumulative insults result in the observed decrease in milk yield over 1-2 days after HT. Based on this discussion we hypothesize that the observed decrease in milk production could have been attributed to a feed restriction alone or due to limiting lying time in addition to an acute stressful event. More research is needed to investigate and identify the potential multiple reasons an observed milk yield decrease occurs following HT. Determining what part of the HT process causes this decrease in milk yield is the first step in determining how to prevent the observed decrease in milk production following HT, thereby improving the welfare and performance of the cow.

6.5 CONCLUSION

This study showed that milk yield decreased on the day of and the day after HT, and again on day's 5-7 post HT, as compared to the 5-day baseline. This effect occurred regardless of whether the cow was restrained or hoof trimmed. The observed decrease in milk yield following HT was not due to the actual removal of horn or modeling technique, but more likely due to the management events surrounding the HT event (e.g. restraint, removal of cow from her home pen, and associated decreases in lying time or feed intake). Future research should attempt to determine if the short-term milk production loss associated with HT can be decreased by minimizing the disruption of the cow's time budget and by minimizing restrictions to feed or bunk access during the HT process.

Table 4.1: Descriptive statistics of the 793 cows from one farm used in the immediate milk yield analysis by treatment and overall.

Variable	Restrained	LIT[1]	BIG[2]	Overall
Lactation	1.8 (1.7-1.9)	1.9(1.7-2)	1.9 (1.8-2.1)	1.9 (1.8-2)
5 day Average Milk Yield (kg)	55.3 (55.1-55.5)	56.5(56.3-56.8)	56.3(56-56.6)	55.9 (55.7-56.1)
*DIM at Trim	112 (111-113)	111 (109-113)	111 (110-112)	111 (110-112)
Holstein	279 (73%)	138 (70%)	147 (68%)	564 (71%)
Crossbred	102 (27%)	58 (30%)	69 (32%)	229 (29%)
N	381	196	216	793

*DIM = Days in milk.

Lactation and DIM at enrollment are reported as the mean (95% Confidence interval)

[1] LIT= Functional HT method (modeled up to 42mm of abaxial wall)

[2] BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall)

Table 6.2: Mixed linear regression model estimates for the associations of hoof trimming technique and covariates with immediate milk yield starting on the day of hoof trimming until 7 days later (n=793)

Variable		Parameter Estimate	SE	95% Confidence interval
Horn removed[1]		1.0	0.57	-0.093 to 2.1
Crossbred[2]		-0.94	0.31	-1.5 to -0.32
Season[3]	Summer	0.56	0.39	-0.21 to 1.3
	Fall	-1.1	0.43	-1.9 to -0.26
	Winter	-0.51	0.39	-1.3 to 0.26

[1] Horn removed represents the combined hoof trimming technique group, reference category is restraint

[2] Breed uses Holsteins as the reference compared to Crossbreds.

[3] Spring is the reference category

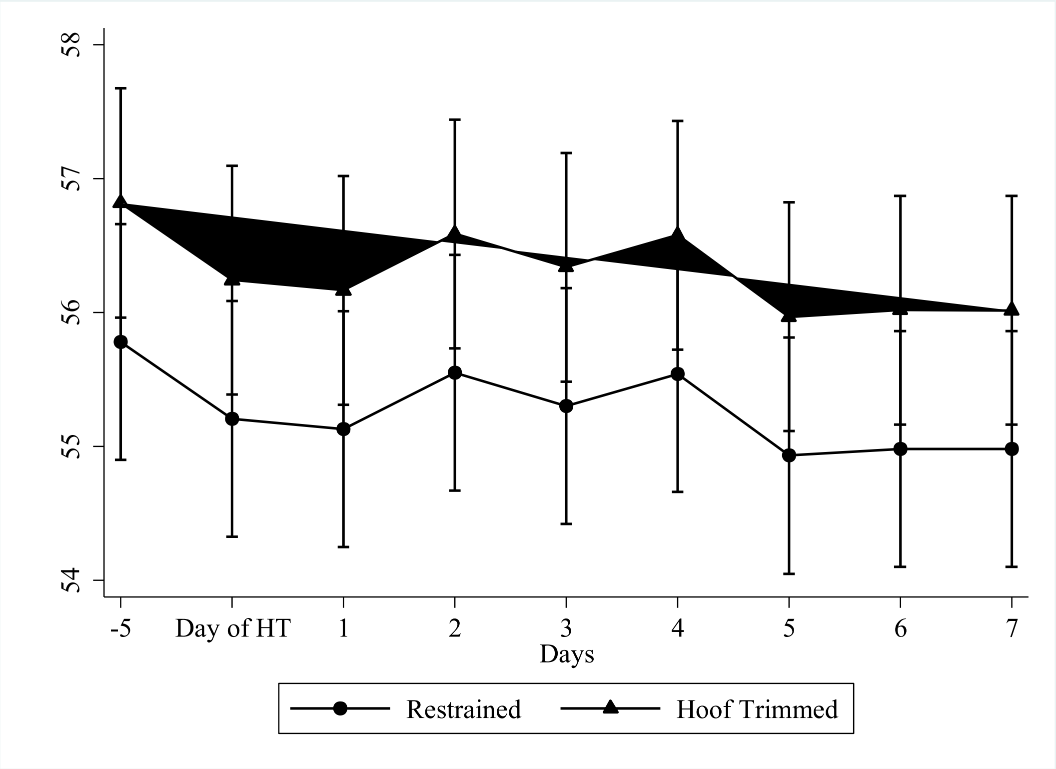


Figure 6.1: Predicted average daily milk with 95% confidence interval by restraint and a combined group for cows that had horn removed using either the BIG or LIT model, comparing the 5 day average (baseline) milk yield before hoof trimming until 7 days after hoof trimming. (LIT= Functional HT method (modeled up to 42mm of abaxial wall), BIG= Adaptation that results in increased modeling of the weight bearing claw (modeled up to18mm of abaxial wall))

CHAPTER SEVEN: Summary Chapter

7.1 INTRODUCTION AND OBJECTIVES

Lameness is an important disease to the dairy industry because of its high prevalence (Espejo et al., 2006; Von Keyserlingk et al., 2012; Solano et al., 2015) and the negative effects it has on cow productivity (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005) and well-being (Whay et al., 1998; Shearer et al., 2013). Hoof trimming **(HT)** is one of the recommended procedures to prevent lameness (Raven, 1985; Shearer and van Amstel, 2001) and it is estimated that 85% of US herds are having cows trimmed at least once a year (NAHMS, 2007). However, there has been a limited amount of scientific research (Stoddard and Cramer, 2017) about HT. Specifically it is unclear what impact HT has on the behavior of the cow, physiological responses, and what technique is the most efficacious for cows without lesions present at the time of trimming. The overall aim of this thesis was to provide knowledge to the scientific community and producers about HT and to provide science based recommendations on if LIT or BIG model is more effective at preventing lameness and lesions. Several objectives will be discussed in general terms in this chapter and areas for future research will be explored at the conclusion of the chapter.

7.2 SUMMARY OF RESEARCH ACTIVITIES AND RESULTS

Objective One: Evaluate the associations between preventative HT of cows with no lesions and activity, resting behaviors and milk yield in commercial dairy herds.

The first objective was to describe the associations between preventative HT of cows without lesions and activity, resting behaviors and milk yield. To do this we conducted a prospective cohort study on 4 farms in Canada (n=2) and the United Kingdom (n=2). Behavior data was collected using the Afi-PLUS or AFI-ACT2 pedometer system, milk yield data was retrieved from milking parlor data using the farms DairyComp 305 (Valley Ag. Software, Tulare. CA) and lesion data was collected from hoof trimmers directly or from DairyComp 305 records based on hoof trimmer records. Cows eligible for analysis in this study did not have a lesion present at their first appearance in the data collection. A generalized mixed model was then built for each outcome of behavior and milk yield. These models compared data from the day of HT until 7 days later to baseline data from the 5 days prior to HT. The results of this analysis showed that activity, resting time, and milk yield were associated with HT. The magnitude and direction of these associations were farm dependent. In general, the activity on the day of HT was elevated and corresponding resting time was decreased. On the days following HT, resting time increased and activity decreased. Milk yield decreased on the day of HT and day after on three of the four farms included in the study.

The associations found in in the results from this objective provide evidence that the HT process has an effect on the behavior and production of cows that don’t have lesions at the time of HT. It also provides evidence that the magnitude and duration of changes in these outcomes is farm dependent. This means some farms may not observe as large as change in milk production or behavior following trimming, and that farm routines might be more influential than HT on these outcomes. What is concerning about these results is the behavior and milk production changes observed following HT are similar to when a case of lameness occurs. This indicates that HT might have negative effect on the cow.

Objective Two: Compare and evaluate the duration of stress, pain and inflammatory responses between cows restrained for hoof trimming and cows being hoof trimmed.

The second objective of this thesis was to describe the physiological changes and duration of these changes that occur when a cow is sham hoof trimmed and when a cow is actually hoof trimmed. The physiological parameters chosen for this study can be grouped into a stress, pain or inflammatory response. To do this a crossover study was conducted as using 8 cows from the University of Minnesota St. Paul dairy barn. Due to jugular catheter failure only 5 cows had complete sham and actual HT data. In addition to physiological data, behavior data was collected when the cows were in the HT chute. Blood samples in this study were taken starting 30 minutes before trimming until 24 hours post HT.

To determine if there was difference in these outcomes for sham and actual HT a mixed linear regression model was then constructed for each physiological parameter that compared the difference between the two groups. The results of this analysis showed that there was no difference between sham and actual HT for the pain response (substance P) and stress response (cortisol, lactate and glucose). There was a significant difference between sham and actual HT for the inflammatory response (haptoglobin), in that cows that were actually trimmed had a lower concentration of haptoglobin than for sham trimming. However, the results for haptoglobin should be interpreted with caution, as the study had a small sample size and the haptoglobin concentrations observed in both groups was below a cut off for a healthy cow and much lower than haptoglobin concentrations reported by other studies that investigated painful procedures or conditions. There was not a difference between the treatments for behavior frequency.

Separate mixed linear regression models were then constructed to determine the duration of elevated concentrations for each physiological outcome using the 30 minutes prior to HT blood sample as the baseline. The results of these analyses indicated that there was no significant difference from baseline for pain (substance P), inflammation (haptoglobin) and certain stress responses (glucose and lactate). It did show that cortisol was elevated starting at the time the cow was released from the HT chute until 3 hours later.

This study found no difference between sham and actual HT for a pain response, inflammatory response and stress responses. It did show that restraint for HT in sound cows causes an acute 3 hour cortisol response, but this was not attributable the actual removal of horn. This acute cortisol stress response was not associated with a corresponding increase in haptoglobin or substance P. This indicates that the HT process is not causing a pain or inflammatory response. These results add to the limited knowledge of physiological changes that occur during HT and investigated several new measures that have not been investigated during the HT process.

Objective Three: Compare the effects of the functional HT (LIT) method to an adaptation that results in increased modeling of the weight bearing claw (BIG) on risk of lameness and prevalence of new lesions at mid-lactation.

The third objective of this thesis was to compare the efficacy of two HT techniques on lameness and lesion prevalence at mid-lactation. A randomized controlled trial was conducted using a convenience sample of 3 farms in Minnesota (n=1) and Wisconsin (n=2). Cows at the end of their first lactation or greater without horn lesions at their scheduled dry-off trim were randomized to either BIG or LIT model HT technique at the week level. Cows trimmed using the BIG model treatment were modeled up to 18mm from the abaxial wall on the weight bearing claw. Cows in the LIT model treatment received a trim using the functional HT method (Raven, 1985). A Kaplan Meier analysis and Cox proportional survival analysis was performed to investigate the difference in time to lameness for BIG and LIT model. For this analysis only cows that made it to their next lactation without being identified as lame and had a pre-trim lameness locomotion score were included. The results of the Kaplan Meier analysis showed a significant difference between the two treatments. Cows in the BIG model group never went below 50% survival and cows in the LIT model group went below 50% lameness at 122 DIM. The Cox proportional survival analysis results had a significant interaction with lactation. BIG model was protective against lameness for cows trimmed at the end of their first lactation with a hazard ratio of 0.5 (95% CI: 0.3-0.7). For second lactation and greater cows there was not a difference between the treatments.

To compare what effects the treatment had on the lesion prevalence at mid-lactation, only cows that were trimmed in the subsequent lactation between 100-165 DIM were included in the analysis. Lesion type was categorized by hoof horn lesion, infectious lesion, other/lame and overall, and separate logistic regression models were constructed to calculate the risk, risk difference and odds ratios between LIT and BIG model for each of these lesion types. The results for the infectious, other/lame and overall lesion types indicated no difference between the two HT techniques. However, the model for presence of a hoof horn lesion had an interaction between lactation and treatment: When cows trimmed at the end of their first lactation were trimmed using the BIG model it reduced the risk of hoof horn lesion at mid-lactation when compared to LIT model. For cows enrolled in second lactation or greater there was no difference between treatments for hoof horn lesions at mid-lactation. The risk difference between the BIG model and LIT model for horn lesions for cows trimmed at the end of their first lactation was 4.6% (95% CI: 1.2-8.1).

The results of this objective indicate that the BIG model technique was protective for cows trimmed at the end of their first lactation. It resulted in a decreased risk of a hoof horn lesion at mid-lactation and decreased risk of lameness. It is still unclear why these protective effects were not present in second lactation cows. Regardless these results contribute to the very limited investigation into HT techniques and provided scientific evidence that the BIG model technique should be considered for implementation Furthermore it provides an estimate of the prevalence of lesions at mid-lactation when cows were free of lesions at enrollment. This means the lesion prevalence in this study is a more accurate representation of the lesion prevalence for cows that are not chronically affected by lesions since the current prevalence estimates include chronically affected cows. This is important for future research that is developing new strategies to prevent lameness.

Objective Four: Compare milk production and culling risk for cows trimmed at dry-off with the functional HT (LIT) method versus an adaptation (BIG) HT method

The fourth objective of this thesis was to compare the long term milk yield and culling risk between the two HT techniques at dry-off, with follow-up concluding when cows received their second trim or reached 165 DIM in the subsequent lactation. Data from this objective will provide more evidence for if there is a difference in efficacy for these two techniques. The cows used in this objective were a subset of cows that were enrolled in objective four. Long term milk yield was assessed starting from the first day of the subsequent lactation until there next trim or 165 DIM using a mixed linear regression model that compared the average daily milk yield per week from cows enrolled on the farm located in Minnesota. Only cows that were not trimmed before the start of their next lactation following enrollment were included. The results from this analysis indicate there is a lack of evidence of a difference in milk yield between the two treatments.

Culling risk was investigated using all of the cows enrolled in for objective 4 starting from the day of enrollment until their next trim or 165DIM in their subsequent lactation following enrollment. This outcome was investigated using a Kaplan-Meier survival analysis and a Cox proportional survival analysis. Both of these analyses showed no evidence for a difference between BIG and LIT model for the risk of being culled.

The results of this objective provide evidence that neither of these two techniques impacted long term milk yield or culling risk. It was expected to a see an increase in long term milk yield and decreasing culling risk for cows trimmed using the BIG model because of the protective effects against lameness and lesion prevalence observed for first lactation cows in Chapter 4. The expected increase in long term milk yield and decrease in culling risk was also based on previous observational studies that reported a decrease in milk yield (Warnick et al., 2001; Green et al., 2002; Hernandez et al., 2005) and increase in culling risk (Booth et al., 2004; Bicalho et al., 2007b; Cramer et al., 2009b) when lameness or lesions occur. However, it is important to take into consideration the cows enrolled in this study are different than the cows enrolled in the previous research, in that some of the cows enrolled in the previous research had lesions present at the time of enrollment. This means that potentially cows chronically affected by lesions would have been included in prior studies. A chronically lame cow is known to be at a higher risk of having another lesion in the next lactation (Green et al., 2014; Randall et al., 2017), likely leading to an increased risk of culling and a decreased milk yield. By excluding these chronic cows from our study, this reduced the prevalence of observed lesions by mid lactation, as described in Chapter 4. This could have made it so the protective effects of the BIG (vs LIT) HT technique were based on a smaller numeric difference in lameness incidence or lesion prevalence, which could result in the observed lack of significant treatment differences in long term milk yield and culling risk.

Objective Five: Compare the 7-day milk yield post HT and the difference from baseline milk production between cows undergoing one of two HT techniques and cows that were only restrained in the HT chute.

The fifth objective of this study was to compare the short-term milk yield change that occurs following HT and determine if post-HT milk yield will be different between two HT techniques or simple restraint. Based on the finding that HT was a stressful event in Chapter 3 and HT was associated with behavior changes in Chapter 2, it was hypothesized that milk yield would decrease immediately following HT or restraint. The cows included in this objective were a subset of cows enrolled in objective 4 on one farm located in Minnesota. Once the cows reached mid-lactation, cows without lesions were enrolled into the current study. Cows with an even cow ID number were trimmed with the same technique that they received at initial enrollment at the previous dry-off event (BIG or LIT) and cows with an odd cow ID number were not trimmed, but were restrained in the chute. Cows assigned to HT groups were trimmed using the functional HT method (LIT) or the adaptation that results in modeling up to 18mm from the abaxial wall (BIG). A mixed linear regression model was constructed to compare the 5-day baseline milk yield prior to HT to the day of and 7-days following HT for each HT technique and restraint. The results of this analysis showed no difference in milk yield response between the HT techniques and restraint at any time. However, a decrease in daily milk yield was observed for all three treatments on the day of and the day after HT, and on days 5-7 post HT, as compared to the 5-day baseline milk yield.

The results from this study show that there was not a difference in milk yield response between cows that were restrained in a HT chute, and cows that were either trimmed with the BIG or LIT model. This suggests that milk yield decreases observed following HT are due to restraint, and not the actual removal of excess horn during the HT process. However, we are not certain that this decrease in milk yield was due solely to the restraint, given that on the day of HT the cow's daily routine, access to feed, and access to stalls is altered for part of the day. Future research needs to be done to determine what effect disruption in these other management related factors that are affiliated with the HT event have on milk yield.

7.3 IMPLICATIONS AND OPPORTUNITIES FOR FUTURE RESEARCH

The aim of this thesis was to provide knowledge to the dairy industry about HT and to provide science based recommendations on if LIT or BIG model is more effective at preventing lameness and lesions. Based on the results from this thesis it was shown that the HT process is associated with behavior changes and causes an acute stress response and decreases milk yield. Based on the results of these objectives the acute stress response is due to restraint, not the actual removal of horn in the HT process. The decrease in milk yield that was observed after HT needs further research to determine what effect disruption of the cow's day with no access to feed has on milk yield.

In addition, we compared a HT technique that is currently used in the industry to an adaptation that increased the modeling on the weight bearing claw in the typical horn lesion location (van der Tol et al., 2004). Based on a subset analysis, this adaptation was protective for hoof horn lesions and lameness for cows trimmed at the end of their first lactation. However, there was no difference between the two HT techniques for long term milk yield and culling risk. The final conclusion was BIG model is more effective at preventing hoof horn lesions than the LIT model, and there was no evidence that BIG model (vs LIT) was detrimental to the welfare of the cow based on milk yield and culling risk.

One surprising finding in our study was that BIG model was only protective for cows trimmed at the end of their first lactation. We hypothesized that this was due to previous lesions that occurred before enrollment in older cows. Based on previous research the occurrence of lesions (Enevoldsen et al., 1991; Foditsch et al., 2016) have been shown to be associated with increased risk of lesions in the next lactation. What this means is preventing lesions is important because our prevention method potentially becomes less effective if a lesion in the previous lactation isn't prevented.

Now that a decrease in milk yield following the HT process of cows free of lesions has been identified, further investigation needs to be done to determine how to prevent it. To do this, researchers should investigate what happens to the feed intake of the cow on the day off, and days shortly following HT. Without access to feed for some time during the day of HT, the feed intake for the cow could be decreased, potentially resulting in the short-term reductions in milk yield that we observed. However, it is unknown if the cow would increase its time at the bunk to compensate for this. Increasing time at the feed bunk seems unlikely because based on previous research (Metz, 1985; Cooper et al., 2008; Ouweltjes et al., 2011) and Chapter 2 of this thesis, given that the resting time of the cow was increased following HT. I hypothesize this would decrease the time the cow spends at the feed bunk. Determining if the cow compensates for this decreased feed intake by consuming more while at the feed bunk could indicate another potential reason why a decrease in milk production is observed. If the cows were to compensate by eating a large amount of feed in a short time it potentially could cause rumen acidosis (Owens et al., 1997). When rumen acidosis was induced in previous research the milk production of the cow was shown to decrease for the 2 days after induction (Krause and Oetzel, 2005). Determining if this occurs could provide insight into the effect restriction of feed on the day of HT has on the cow. Based on whether restricting access to feed on the day of HT has an effect on the immediate milk yield, I would want to investigate what effect providing feed while the cow is in the HT chute has on the immediate milk production. To do this I would design a system similar to the robotic milking systems that provide a concentrate feed while the cow is in the chute.

If reduced feed intake is ultimately discovered not to play a role in the observed decrease in milk yield associated with HT, then chute comfort would be the next area of research. Based on the results of this thesis the restraint during the HT process caused an acute stress response and a decrease in milk yield, not the removal of horn. Investigation into chute comfort to reduce the stress during the process could potentially result in a smaller or lack of an impact in milk yield following HT. The current research (Rizk et al., 2012; Korkmaz et al., 2014) has investigated possible pharmaceuticals to reduce stress during the HT procedure and have found the use of xylazine and ketoprofen a sedative and non-steroidal anti-inflammatory respectively mitigate the stress response. This is based on not observing as large of a cortisol response when the cows were treated and a faster return to baseline concentrations. However, they have not included a milk yield response. I think increasing chute comfort would be more effective for widespread use in the industry than the use of pharmaceuticals. This is because of not only cost, but regulations. The cost of changing a chute to increase comfort would be a one-time cost compared to the cost of administrating a pharmaceutical every time a cow enters the chute (i.e. drug and labor costs; meat and milk withhold times). The first part of chute comfort I would investigate is the restraint system used during HT. Currently the two chute designs found in the dairy industry are tilt tables and stand-up chute designs. Both of these designs have shown to cause stress for the cow in previous research (Rizk et al., 2012; Korkmaz et al., 2014)

The first step in stand up chute designs would be to identify what areas of the chute have the highest contact pressure between the cow and restraint. To do this I would use pressure sensors placed on the chute to determine if certain areas of the cow are feeling more pressure than others. I hypothesize the highest pressure will be on the belly band used in an upright chute. If more support can be given in this area I would expect the cow to have decreased level of plasma cortisol because the cow might feel it has more stability in the chute and have a smaller stress response.

Switching to future research on HT techniques, I would want to determine how the current HT techniques in the industry differ. By investigating at the claw level the weight distribution on the sole of the hoof between cows trimmed with these techniques. I would use a similar technique described by Van der Tol et al. (2004) that had cows stand on a pressure mat that would indicate the forces on the claw. Determining how the pressure is distributed between these techniques potentially would indicate if a technique is re-distributing the weight better and if it is putting the weight on the tissues designed for bearing weight. This could potentially guide future research to make even more adaptations that are more effective.

Lastly I would try to determine why cows in their second lactation or greater did not experience the protective effects of the BIG modeling technique, as was observed in cows trimmed at the end of their first lactation, for lesions and lameness. If possible I would re-run the experiment with only cows in their second lactation or greater that are free of lesions in the previous lactations. Since this would be very time consuming and expensive, going back into the current data and extracting the history of the cow potentially could be done. The only concern is the standardization of HT records would not be a possibility. This would provide evidence that lesions in previous lactations have a long term negative effect on the cow.

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