LAMENESS AND LYING BEHAVIOUR IN GRAZING DAIRY COWS

by

Alexander John Thompson

B.Sc., The University of British Columbia, 2015

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Applied Animal Biology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

March 2018

© Alexander Thompson, 2018
Abstract

Lameness is a serious welfare issue for dairy cows. To date, the majority of studies have focused on its effect on health and behaviour at the herd-level. The objectives of this study were to identify firstly, between-cow and secondly, within-cow changes in lying behaviour associated with consistent and changing lameness status in grazing dairy cows. Previous studies of lying behaviour in grazing dairy cows have not considered the effect of precipitation, thus a third aim was to determine the effect of precipitation on lying behaviour. A total of 252 dairy cows from 6 pasture-based farms in southern Brazil were gait scored weekly to assess lameness using a 5-point scale (1 – 5, numerical rating score [NRS]) for 4 consecutive wk. Cows were considered to have consistent lameness if they were scored as lame (NRS ≥ 3) on each of the 4 visits and considered to have a changing lameness status if scored as being non-lame (NRS < 3) on at least one of the 4 visits. Cows classified as having a changing lameness status were further classified as developed, recovered, or inconsistent. Lying behaviour was recorded continuously using leg-mounted accelerometers. Cow-level variables included parity, days in milk, and body condition score. Since only one primiparous cow was identified as lame at each of the 4 visits, the between-cow analysis of lameness was run on multiparous cows only. The overall prevalence of clinical lameness on the first visit was 39%, with a development and recovery rate of 16% and 10% over the 4 visits, respectively. The between-cow effect of consistent lameness status on daily lying time and number of lying bouts was dependent on precipitation. There was no within-cow effect of changing lameness status on any of the lying behaviours. Precipitation decreased daily lying time, increased mean lying bout duration, and decreased the daily number of lying bouts. The results of my research provide the first evidence that the effect of consistent lameness
status on lying behaviour is dependent on rainfall in grazing dairy cows. Future work measuring lying behaviour of grazing dairy cows should include precipitation as a co-variate.
Lay summary

This study set out to identify the effects of lameness (i.e., problems with locomotion) on lying behaviour in grazing dairy cows. The effect of lameness on lying time was dependent upon precipitation. Rainfall decreased total daily lying time, increased individual lying bout duration, and decreased the total daily number of lying bouts. The results of this work provide insights that can aid our understanding of the effects of lameness and rainfall on grazing dairy cows.
Preface

This thesis was completed under the supervision of Dr. Marina A.G. von Keyserlingk. My supervisory committee included Drs. Daniel M. Weary and William K. Milsom from the University of British Columbia. The research project described in this thesis was approved by the University of British Columbia Animal Care Committee (A15-0082) and the Federal University of Santa Catarina Ethics Committee of Research on Animals (PP00949).

The second chapter was prepared in collaboration with D.M. Weary, J.A. Bran, R.R. Daros, M.J. Hötzel, and M.A.G von Keyserlingk. These collaborators were involved in supervision, data analysis, and manuscript editing. The main ideas were developed in collaboration by all authors and data for the study was collected by J.A. Bran, R.R. Daros, and A.J. Thompson.
Table of contents

Abstract ........................................................................................................................................... ii
Lay summary.................................................................................................................................... iv
Preface .............................................................................................................................................. v
Table of contents ............................................................................................................................. vi
List of tables ................................................................................................................................... ix
List of figures .................................................................................................................................... x
List of abbreviations and symbols .................................................................................................. xi
Acknowledgements ......................................................................................................................... xii
Dedication ......................................................................................................................................... xiv

Chapter 1: Introduction .................................................................................................................. 1

1.1 What is lameness? .................................................................................................................. 1

1.1.1 Lameness detection ............................................................................................................. 2

1.1.2 The prevalence of lameness ............................................................................................... 4

1.1.3 Risk factors associated with lameness ............................................................................... 4

1.1.3.1 Herd-level risk factors ................................................................................................ 5

1.1.3.2 Cow-level risk factors ................................................................................................. 6

1.1.4 Effects of housing system .................................................................................................. 7

1.2 Lying behaviour in dairy cattle ............................................................................................ 8

1.2.1 Factors influencing lying behaviour .................................................................................. 9

1.2.1.1 Housing and management ......................................................................................... 9

1.2.1.2 Cow-level factors ..................................................................................................... 10
1.2.1.3 Disease .................................................................................................................. 10
1.2.1.4 Climate and weather ............................................................................................. 11
1.3 Research aims ............................................................................................................... 13

Chapter 2: Lameness and lying behaviour in grazing dairy cows ...................................... 15
2.1 Introduction ................................................................................................................... 15
2.2 Materials and methods .................................................................................................. 17
  2.2.1 Data collection .......................................................................................................... 18
    2.2.1.1 Animal measures ............................................................................................... 18
    2.2.1.2 Gait assessment ............................................................................................... 18
    2.2.1.3 Lying behaviour ............................................................................................... 19
    2.2.1.4 Temperature and precipitation ......................................................................... 19
  2.2.2 Statistical analysis .................................................................................................... 20
    2.2.2.1 Between-cow analysis: Consistent lameness status ....................................... 20
    2.2.2.2 Within-cow analysis: Changing lameness status ............................................ 21
  2.3 Results .......................................................................................................................... 23
    2.3.1 Lameness ................................................................................................................ 23
    2.3.2 Lying behaviour ..................................................................................................... 25
    2.3.3 Between-cow results: Consistent lameness status .............................................. 25
      2.3.3.1 Daily lying time ............................................................................................. 25
      2.3.3.2 Lying bout duration ....................................................................................... 26
      2.3.3.3 Number of lying bouts ............................................................................... 27
    2.3.4 Within-cow results: Changing lameness status ................................................... 29
  2.4 Discussion ..................................................................................................................... 30
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4.1</td>
<td>Conclusions</td>
<td>33</td>
</tr>
<tr>
<td>Chapter 3: General discussion</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>Thesis findings</td>
<td>34</td>
</tr>
<tr>
<td>3.2</td>
<td>Strengths and limitations</td>
<td>35</td>
</tr>
<tr>
<td>3.3</td>
<td>Future research directions</td>
<td>38</td>
</tr>
<tr>
<td>3.4</td>
<td>General conclusions</td>
<td>40</td>
</tr>
</tbody>
</table>

Bibliography | 42
List of tables

Table 2.1 The number of cows, prevalence of clinical and severe lameness, and percentage of cows that had consistent and changing lameness status on each of the six farms in Southern Brazil (n = 201 cows)............................................................................................................................................. 24

Table 2.2 The effect of consistent lameness status (NONLAME: n = 35; LAME: n = 32) on the daily lying time (min/d), natural logarithm of mean lying bout duration (min/bout), and daily number of lying bouts (no./d) of multiparous cows modelled using linear mixed-effects models with farm and cow as random effects............................................................................................................................................. 28

Table 2.3 The effect of parity (primiparous: n = 25; multiparous: n = 67) on the daily lying time (min/d), natural logarithm of mean lying bout duration (min/bout), and daily number of lying bouts (no./d) of NONLAME cows modelled using linear mixed-effects models with farm and cow as random effects. .................................................................................................................................................. 29

Table 2.4 The effect of changing lameness status (developed and recovered; n = 30 cows) on the daily lying time (min/d), natural logarithm of mean lying bout duration (min/bout), and daily number of lying bouts (no./d) of grazing cows modelled using linear mixed-effects models with farm, cow, and week as random effects ........................................................................................................................................... 30
List of figures

Figure 2.1 Bar plots showing consistent lameness status (NONLAME vs. LAME) in relation to (A) mean daily lying time (min/d), (B) mean lying bout duration (min/bout; untransformed), and (C) mean daily number of lying bouts (no./d) of multiparous cows on days with (light gray) and without precipitation (dark gray). Error bars represent ± 1 SEM (n = 67 cows).......................... 26

Figure 2.2 Bar plots showing parity (primiparous vs. multiparous) in relation to (A) mean daily lying time (min/d), (B) mean lying bout duration (min/bout; untransformed), and (C) mean daily number of lying bouts (no./d) of NONLAME cows on days with (light gray) and without precipitation (dark gray). Error bars represent ± 1 SEM (n = 59 cows).............................. 27
List of abbreviations and symbols

BCS: body condition score
C: Celsius
d: day
DIM: days in milk
kg: kilogram
ln: natural logarithm
min: minute
mm: millimeter
no.: number
NRS: numerical rating score
S: south
sec: second
SEM: standard error of the mean
THI: temperature-humidity index
W: west
wk: week(s)
°: degrees
’: minutes (coordinates)
%: percent
”’: seconds (coordinates)
Acknowledgements

Firstly, a wholehearted thank you to my supervisors. Thank you Dr. Marina (Nina) von Keyserlingk for opening the door and giving me the opportunity to work with this research group as an undergraduate student, for your unending support for every essay, poster, abstract, presentation, and defence since, and most of all for the incredible passion, guidance, and wisdom you have offered me throughout. I will always be grateful for this experience. Thank you Dr. Daniel (Dan) Weary for always being there to push me one extra step, even when I was sure I had reached my last, and for your contagious enthusiasm for all things, especially statistics. Thank you both for everything you have done for me, I feel lucky to have been under your guidance. Thank you Dr. William Milsom for your kindness, patience, and support and for always being there for me when I needed you. A big thank you to Dr. Maria J. Hötzel, who made my research possible through an international collaboration between the Laboratório de Etologia Aplicada e Bem-Estar Animal at the Universidade Federal de Santa Catarina and the Animal Welfare Program at the University of British Columbia and also for being an amazing supervisor during my time in Brazil.

To everyone that helped with my research, thank you very much. A special thank you to Ruan Daros and José Bran. Ruan thank you for making my time in Brazil such a fantastic experience and for your continued support and endless source of ideas for this project. José thank you for sharing your knowledge with me and your continued support with this project. Thank you to Melissa García and Guilherme Rodrigues for your help and friendship throughout my research. Also, thank you to the farmers in Southern Brazil who voluntary took part in this research.
Thank you to the Elizabeth R. Howland Fellowship that provided funding throughout my studies and to Mitacs that provided funding for my travel and collaborative research in Brazil through their Globalink Research Award.

Finally, thank you to my parents who have provided me with unconditional love and support in all of my endeavors. An especially heartfelt thank you to my mother who is an outstanding role model for me and to everyone around her - I love you so much. Thank you to my brothers, Spencer and Lucas, for your diverse interests and keeping me a well-rounded person.
To the cows.
Chapter 1: Introduction

This review has three main aims. Firstly, to critically evaluate the literature regarding lameness in dairy cattle, including a brief description of lameness prevalence in different housing systems, current methods for its detection, and commonly associated risk factors. Secondly, to describe the current literature on lying behaviour in dairy cattle, exploring the factors affecting lying behaviour, including housing and management, animal factors, and disease. Thirdly, to review the literature on the effects of weather on cattle behaviour. Throughout I will identify gaps in the literature where research is needed to better understand or provide solutions that minimize lameness.

1.1 What is lameness?

Lameness is widely regarded as one of the most important welfare problems for dairy cows. Despite decades of research, the prevalence of lameness remains high around the world (UK: Barker et al., 2010; North America: von Keyserlingk et al., 2012; China: Chapinal et al., 2014; Brazil: Bran et al., 2018). In addition to compromising the welfare of affected individuals, lameness also has widespread negative impacts on the productivity and longevity of dairy cattle (Huxley, 2013). Previous work has shown that lame cows have lower milk yield (Bicalho et al., 2007, 2008; Leach et al., 2012), reduced reproductive performance (Alawneh et al., 2011), and in some cases have an increased risk of being culled (reviewed by Huxley, 2013). This makes lameness one of the most costly disorders for the dairy industry (Kossaibati and Esslemont, 1997; Bruijnis et al., 2013).
For the purpose of this review I have adopted the definition supported by Flower and Weary (2009) where lameness is a deviation in gait resulting from one or more diseases or disorders of the claw (foot), leg, or nervous system that cause walking or standing to be uncomfortable or painful (Whay et al., 1998). To be clear, lameness is not considered a disease but rather an outcome that may arise from disease or from a disorder. The discomfort or pain associated with the underlying malady results in a change in locomotion, the evaluation of which is commonly used by researchers, veterinarians, and farmers to diagnose lameness (Flower and Weary, 2009).

Lameness is most often the result of lesions in the claw (Blowey and Weaver, 2011; DeFrain et al., 2013), which can be separated into two types: infectious and non-infectious (Mason, 2009). Some examples of infectious lesions include digital and interdigital dermatitis, heel erosion, and foot rot, examples of non-infectious lesions include sole hemorrhage, sole and toe ulcers, white line disease, and horizontal, vertical, and axial fissures in the claw wall (Mason, 2009). Minor lesions typically do not cause lameness (Whay et al., 1997), while major lesions can severely impair locomotion (Whay et al., 1998; Flower and Weary, 2006). However, both infectious and non-infectious lesions can be painful, affect locomotion, and thus impair the welfare of the affected animal (Whay et al., 1998).

1.1.1 Lameness detection

Regardless of housing system lameness is commonly detected using subjective observational methods, including locomotion or gait scoring (Flower and Weary, 2009). Though a number of scoring systems have been developed, the subjective scoring systems developed by
Sprecher et al. (1997), and modified by Flower and Weary (2006), have gained much traction both in terms of research with lameness but also in farm animal welfare assurance programs (e.g., Canada’s ProAction). I have relied on the locomotion scoring system proposed by Flower and Weary (2006) for the research summarized in this thesis. The scale follows a numerical rating score (NRS) based on a 5-point scale to assess gait and posture, in which a score of 1 represents a sound animal and a score of 5 represents a severely lame animal. The score is often further categorized into non-lame (NRS ≤ 2), moderately lame (NRS = 3), clinically lame (NRS ≥ 3), and severely lame (NRS ≥ 4) for analysis and interpretation (e.g., Ito et al., 2010; Weigele et al., 2017).

More recently, effort has been placed on finding automated and objective lameness detection strategies. In general, these systems use sensors and subsequent analysis with mathematical algorithms to assess the locomotion of dairy cows (Schlageter-Tello et al., 2014). These strategies have been broadly classified into three approaches: kinetic (e.g., measuring forces exerted on the ground while standing or walking; Rajkondawar et al., 2006), kinematic (e.g., tracking step length, step height, or back curvature with video or pressure sensitive flooring; Van Nuffel et al., 2013; Van Hertem et al., 2014), and indirect approaches (e.g., using lying behaviour to identify lame animals; Chapinal et al., 2009; Ito et al., 2010). The use of technology offers an objective and efficient means of lameness detection, however, more research is needed in how best to adapt these technologies to develop practical systems for use on farms.
1.1.2 The prevalence of lameness

The prevalence of clinical lameness differs between housing systems and among farms using similar housing systems. For example, estimates of the clinical lameness prevalence in freestall barns from different regions of North America varied from 27.9% (British Columbia) to 54.8% (Northeastern US) (von Keyserlingk et al., 2012). More recent estimates of clinical lameness in intensive systems in North America range between 7.2% and 21% (Solano et al., 2015b; Cook et al., 2016; Westin et al., 2016; Adams et al., 2017), which are generally lower than estimates in the UK (36.8% in Barker et al., 2010), China (31% in Chapinal et al., 2014), and Brazil (31.9% to 43.2% in Costa et al., 2017).

There has been comparatively little research on the prevalence of clinical lameness in pasture-based dairy herds. Despite temporary improvements in gait reported for cows given access to pasture (Hernandez-Mendo et al., 2007), published work reports clinical lameness prevalence at similar levels to recent estimates indoors, ranging from 8.3 to 31% (Ireland: Olmos et al., 2009; New Zealand: Fabian et al., 2014; Australia: Ranjbar et al., 2016; Brazil: Bran et al., 2018). The continued high prevalence is worrisome, particularly given the amount of research undertaken over the last decades (see Huxley et al., 2013), and suggests that there is room for improvement in housing and management to minimize lameness.

1.1.3 Risk factors associated with lameness

The etiology of lameness is complex. The risk factors associated with lameness can be broadly considered in two categories: herd- and cow-level risk factors. Herd-level risk factors
include aspects of housing and management, while cow-level risk factors include characteristics of individual animals (e.g., breed, age, and stage of lactation).

1.1.3.1 Herd-level risk factors

A number of studies have found associations between lameness and environmental factors such as the type and depth of bedding (Cook, 2003; Cook et al., 2004; Ito et al., 2010; Schirmann et al., 2013), flooring type and traction (Somers et al., 2003; Solano et al., 2015b), stall dimensions (Espejo and Endres, 2007; Dippel et al., 2009), use of alley scrapers (Main et al., 2010), and time spent away from the milking parlour (Espejo and Endres, 2007). Cows also decrease their lying time if lying areas are uncomfortable (e.g., narrow lying stalls, rubber mattresses; Cook and Nordlund, 2009), a known risk factor associated with lameness for herds housed with hard flooring (e.g., concrete) (Galindo and Broom, 2000). In contrast, previous studies have shown that access to pasture (i.e., a soft standing and lying surface) for herds housed indoors is associated with a decreased risk of lameness (Haskell et al., 2006; Olmos et al., 2009).

Compared to intensive systems, there has been limited investigation into environmental factors associated with lameness in grazing systems. Identified risk factors include season (Wells et al., 1993), exposure to damaged or smooth concrete (Barker et al., 2010; Ranjbar et al., 2016), and walking speed to and from milking (Bran et al., 2018). However, given the variability inherent to how outdoor areas are managed (e.g., weather, distance to pasture, variation in pathway quality, and inclination of pathways), there is a need for more research to identify environmental risk factors for grazing herds in regions with different climates and terrain.
There have been few studies to investigate the association between precipitation and lameness in grazing dairy cows, but all report that higher amounts of precipitation are associated with lameness (Eddy and Scott, 1980; Williams et al., 1986; Ranjbar et al., 2016). The most recent study by Ranjbar et al. (2016) reported that higher average daily rainfall in the 30 d before assessment increased the odds of being diagnosed with lameness by 1.06 times. The winter season, commonly cooler and with more precipitation in higher and lower latitudes (Cook, 2003), has also been associated with claw lesions and lameness (Wells et al., 1993; Lawrence et al., 2011). Unfortunately, the majority of studies investigating the effect of season on lameness are confounded: herds are often given access to pasture in the summer, but kept indoors in the winter (e.g., Clarkson et al., 1996). Research exploring the relationship between rainfall and season and how they affect lameness in outdoor-housed dairy cattle is needed.

1.1.3.2 Cow-level risk factors

Risk factors at the cow level are generally thought to influence lameness independently of environment and include high milk yield (Alawneh et al., 2014), low or changing body condition score (Newsome et al., 2017; Bran et al., 2018), breed (Barker et al., 2010; Bran et al., 2018), increasing parity (Warnick et al., 2001; Espejo et al., 2006; Solano et al., 2015b), and increasing stage of lactation (Warnick et al., 2001; Solano et al., 2015b). Regardless of housing type Holstein cows, the most common breed observed in the research in this thesis, have been found to have higher odds of developing lameness than other breeds (Barker et al., 2010; Bran et al., 2018).
1.1.4 Effects of housing system

As mentioned in 1.1.3.1, previous research has shown that access to pasture for housed cows may have protective effects for lameness (Haskell et al., 2006). For example, Hernandez-Mendo et al. (2007) found that providing lame cows with access to pasture for one month improved gait score. These authors speculated that provision of a comfortable standing surface, in this case pasture, aided in the recovery from hoof and leg injuries, but these injuries were not assessed so the conclusion should be viewed with caution.

There has been some research that suggests there are differences in the types of lesions affecting herds kept indoors and outdoors; however, little consensus exits in the literature. For example, sole damage (e.g., sole ulcers) has been found to more commonly affect herds without access to pasture than herds kept permanently at pasture (Navarro et al., 2013). Lesions reported to be common in herds permanently maintained at pasture include white line disease, sole injury (e.g., sole hemorrhages and punctures, but not sole ulcers) and axial disease (Lawrence et al., 2011). However, others have found that cows maintained at pasture for a full lactation had fewer cases of white line disease, sole and white line haemorrhages, heel horn erosion, and digital dermatitis than when kept only indoors (Olmos et al., 2009). These discrepancies may be explained by permanent versus temporary housing on pasture or differences in the quality of pasture and walkways to and from the milking parlour. Clearly, this is an area in need for more research.
1.2 Lying behaviour in dairy cattle

Research has focused on understanding the lying behaviour of cattle over the past half-century, with an early review chapter published by Hafez and Schein (1962). These authors, after reviewing the available evidence, completed almost entirely on grazing dairy cattle, estimated that cattle spend between 9 and 12 h/d lying down. While these early studies were interested in lying behaviour as it relates to the grazing patterns of cattle, more recently lying has been used as an indicator of cow comfort in intensively housed herds (e.g., von Keyserlingk et al., 2012) and as indicators of maladies (e.g., lameness) (Chapinal et al., 2009; Ito et al., 2010). Lying behaviour is often broken into various measures, including total daily lying time, the number of individual lying bouts per day, and the duration of each individual lying bout.

There is evidence that dairy cows attempt to maintain a relatively stable amount of lying time. Munksgaard et al. (2005) showed that cows are willing to forfeit feeding and social contact time to maintain a consistent proportion of the day lying. Jensen et al. (2005) showed that heifers housed in a tethered system have an inelastic demand for lying of 12 to 13 h/d. However, numerous factors can affect lying behaviour at both the herd- and cow-level, including housing and management, animal factors, disease, and climate and weather. These factors are reviewed below.
1.2.1 Factors influencing lying behaviour

1.2.1.1 Housing and management

The total daily lying time of cows kept in loose-housed indoor systems is similar, ranging between 10.8 to 11.2 h/d in automated milking systems (DeVries et al., 2011; Deming et al., 2013) and between 10.6 to 11.3 h/d in parlour-milking systems (Wechsler et al., 2000; Ito et al., 2009, 2014). Bedding depth and quality have also been shown to affect the lying time of indoor housed cows, with cows spending more time lying on softer and deeper bedding (e.g., straw and sand) and less time on harder surfaces (e.g., rubber mats and mattresses; Tucker et al., 2009; Calamari et al., 2014).

The daily lying time of grazing dairy cows is generally lower and more variable, reported between 8.0 and 10.5 h/d (Krohn and Munksgaard, 1993; Legrand et al., 2009; Olmos et al., 2009; Sepúlveda-Varas et al., 2014). This difference may be partly explained by cows spending more time walking to and from the milking parlour when housed primarily on pasture compared to indoor systems where the parlour is normally much closer (Stafford and Gregory, 2008). Furthermore, differences in pasture quality and supplemental feed availability may also affect lying time; with cows housed on poor quality pasture likely having to engage in more time spent grazing than those cows provided higher quality pasture or supplemental feed (Hernandez-Mendo and Leaver, 2004). Cows at pasture also have fewer, but longer individual lying bouts than cows housed indoors (Olmos et al., 2009; Navarro et al., 2013).
1.2.1.2 Cow-level factors

Similar to cow-level risk factors associated with lameness, daily lying time has been shown to increase with stage of lactation (Deming et al., 2013; Ito et al., 2014; Solano et al., 2015a) and parity (Sepúlveda-Varas et al., 2014). To my knowledge, no research to date has looked at what is driving these associations. Future research might consider a potential link with rumination time, which most often occurs while lying (Kilgour, 2012; Schirmann et al., 2012), but has not been examined across parity or stage of lactation.

1.2.1.3 Disease

A number of diseases have been found to be associated with decreased lying time in dairy cows, including clinical ketosis (Itle et al., 2015), mastitis (Medrano-Galarza et al., 2012), and subclinical hypocalcaemia (Jawor et al., 2012).

Lameness has been reported to significantly increase daily lying time between 24 and 126 min/d in indoor systems (Ito et al., 2010; Navarro et al., 2013; Weigele et al., 2017) and between 91 and 105 min/d in outdoor systems (Walker et al., 2008; Navarro et al., 2013; Sepúlveda-Varas et al., 2014). While previous research has reported that lameness increases lying time, the effect of lameness on the number and duration of lying bouts is less clear. For example, in indoor freestall systems moderate and severe lameness have been reported to increase the duration of individual lying bouts on farms with deep bedded stalls, but not on farms with mattresses (Ito et al., 2010; Weigele et al., 2017). Outdoors, Sepúlveda-Varas et al. (2014) found that clinically lame cows at pasture have longer, but fewer lying bouts per day compared to sound cows, while
Navarro et al. (2013) reported that clinically lame cows at pasture have more lying bouts compared to sound cows. More research is needed to disentangle the effects of the quality of the lying surface (including pasture) and lameness to understand the effect of lameness on the number and duration of lying bouts in dairy cows.

The behavioural effects of lameness are often considered at the herd-level (e.g., Olmos et al., 2009; Ito et al., 2010; Sepúlveda-Varas et al., 2014; Weigele et al., 2017); however, little research has explored how lameness affects behaviour at the cow-level. Future research should investigate within individual differences; namely, how lying behaviour is affected when individuals develop or recover from lameness. How cows alter their lying behaviour in response to changing lameness status this could be used for earlier detection of lameness, potentially influencing management decisions and improving treatment for affected cows.

1.2.1.4 Climate and weather

Cows housed outdoors are exposed to a range of weather conditions including rain, snow, and wind, all of which can affect their behaviour and physiology (Tucker et al., 2007; Schütz et al., 2010). The thermoneutral zone is defined as the range of ambient temperatures over which an animal does not need to alter metabolic processes to maintain internal constancy (Moyes and Shulte, 2007); when outside of the upper and lower critical temperatures animals must engage in active cooling or thermogenesis, respectively (Polsky and von Keyserlingk, 2017). In high producing lactating dairy cows, the thermoneutral zone has been estimated to be between 2 and 27°C (Berman et al., 1985; Igono et al., 1992; Albright and Arave, 1997).
The temperature-humidity index (THI) index, normally used when measuring heat stress in dairy cows, was first introduced by Thom (1959). THI combines the effects of ambient temperature and relative humidity into a single unitless index. Though originally intended to describe the effect of environmental temperature on humans it is now used to describe heat stress in dairy cattle (Polsky and von Keyserlingk, 2017). Much of the literature on the behavioural effects of thermal distress in dairy cows cite an upper critical value of 72, which is equal to approximately 25°C at 50% humidity (Igono et al., 1992). When the THI remains within the thermal comfort zone (e.g., THI < 72), it does not influence the preference dairy cows show for an indoor or outdoor environment (Charlton et al., 2013). However, when THI exceeds the critical maximum during the day (e.g., THI > 72), Legrand et al. (2009) found that cows spend more time indoors, perhaps because shade was not available on pasture. For more discussion on the effect of heat stress on dairy cattle see Polsky and von Keyserlingk (2017).

Research on the effects of rainfall on lying behaviour of dairy cows is sparse, limited to a few experiments exposing cows to artificial precipitation while kept in outdoor pens with wood chip bedding (Tucker et al., 2007) or on rubber mats overtop of concrete (Schütz et al., 2010). In these studies precipitation decreased total daily lying time by 53% (Tucker et al., 2007) and 68% (Schütz et al., 2010). However, whether the bedding used in these studies adequately reflects the quality of pasture after rainfall remains unknown. A recent study by Chen et al. (2016) showed that cattle housed on an outdoor dirt pack reduced lying time by almost 75% when the lying surface was classified as severely muddy, with cows electing to spend a greater proportion of their lying time on concrete rather than in the mud. In indoor freestall systems, cows have also been shown to prefer to lie down on dry bedding compared to wet bedding (Fregonesi et al., 2007; Reich et al., 2010).
Some studies have measured the effect of precipitation on the preference for pasture use. For example, when given the choice, cows spend more time indoors than at pasture during periods of rainfall (Legrand et al., 2009; Falk et al., 2012). In beef cattle, Graunke et al. (2011) showed that the proportion of the herd observed lying down was dependant on both temperature and precipitation. At temperatures above freezing the proportion of the herd lying down was lower with precipitation compared to without, congruent with the findings of other studies with rainfall and wet lying surfaces (Tucker et al., 2007; Schütz et al., 2010). However, at temperatures below freezing, when precipitation turns to snow, the proportion of beef cows lying down was higher compared to without precipitation in frigid temperatures. Tucker et al. (2007) suggests that cows adopt lying positions that minimize heat loss (e.g., tucking legs underneath the torso) when the lying surface is wet. In contrast, snow acts as an insulator, likely explaining why cows spend more time lying during periods of snowfall. To date, no work has quantified the effect of precipitation on lying behaviour in grazing dairy cows. Given the strong and consistent effect of wet lying surface on lying behaviour, I predict that precipitation will have a similar effect on the lying behaviour of cows kept at pasture.

1.3 Research aims

The main objectives of this thesis are to identify, firstly, the between-cow effects of consistent lameness status on lying behaviour and, secondly, the within-cow changes in lying behaviour associated with changing lameness status. Between cows, I hypothesized that consistent lameness would be associated with increased daily lying time. Within cows, I hypothesized that individuals would adjust lying behaviour in response to changing lameness
status following the same directional trends predicted between cows. A third objective of this study is to describe the effects of precipitation on lying behaviour. Consistent with previous findings on wet lying surfaces (Fregonesi et al., 2007; Chen et al., 2016), I predicted that rainfall would decrease daily lying time, increase lying bout duration, and decrease the number of lying bouts per day.
Chapter 2: Lameness and lying behaviour in grazing dairy cows

2.1 Introduction

Lameness is the manifestation of one or more claw, leg, or nerve pathologies that can cause standing and walking to be uncomfortable or painful (Whay et al., 1997, 1998), and is typically presented and diagnosed by an abnormal gait (e.g., score 1 – 5, ≥ 3 as clinically lame) (Sprecher et al., 1997; Flower and Weary, 2006). In addition to compromising the welfare of individual animals, lameness has negative impacts on the productivity and longevity of dairy cattle. Previous work has shown that lame cows have lower milk yield, reduced reproductive performance, and in some cases have an increased risk of being culled (reviewed by Huxley, 2013). Lameness is also one of the most expensive production diseases that affects dairy cattle (Kossaibati and Esslemont, 1997). Despite its negative impact, lameness remains common on many commercial dairy farms (Bruijnis et al., 2013).

There has been limited research on the prevalence of lameness in pasture-based herds. The few studies published to date report a prevalence of clinical lameness ranging from 8.3% to 18.9% (e.g., Olmos et al., 2009; Fabian et al., 2014; Ranjbar et al., 2016). These estimates are generally lower compared to intensive indoor management systems (e.g., 27.9% to 54.8% in von Keyserlingk et al., 2012; 24% in Charlton et al., 2016), with some exceptions (e.g., 21% in Solano et al., 2015b; 13.2% in Cook et al., 2016; 7.2% in Adams et al., 2017).

Locomotion scoring is commonly used to identify lameness (Flower and Weary, 2009), with a variety of scoring systems used (e.g., Sprecher et al., 1997; Cook, 2003; Flower and Weary, 2006). However, these scores are subjective and time consuming to implement in large
herds, so much effort has been placed on finding more objective and efficient ways to detect lameness, such as kinematic video analysis of gait (Van Hertem et al., 2014) and step tracking from pressure-sensitive flooring (Van Nuffel et al., 2013; Thorup et al., 2014). Aside from changes in gait, lameness has been shown to also affect various behaviours in dairy cattle, such as lying (Ito et al., 2010; Sepúlveda-Varas et al., 2014; Weigele et al., 2017) and feeding (Weigele et al., 2017), which may serve as proxy measures for lameness detection.

With increased automated recording of cow behaviour (e.g., using wearable technology such as dataloggers) research exploring the effects of lameness on behaviour has gained traction. The majority of research has focused on the relationship between lameness and daily lying time (Walker et al., 2008; Ito et al., 2010; Chapinal et al., 2013), as modulated by the number and duration of individual lying bouts (Ito et al., 2010; Sepúlveda-Varas et al., 2014). The majority of studies have measured the behavioural effects of lameness at the herd-level, and less research has explored how lameness affects behaviour at the cow-level. To our knowledge no research has investigated the effect of changing lameness status on lying behaviour.

A challenge in using changes in lying behaviour to identify lameness is accounting for other factors that affect lying. In outdoor systems cows might be especially affected by environmental factors such as rain. Previous work has shown that dairy cows prefer to lie down on dry bedding (Fregonesi et al., 2007; Reich et al., 2010) and decrease daily lying duration when lying surfaces are wet (Tucker et al., 2007; Schütz et al., 2010; Chen et al., 2016); this response is thought to help reduce conductive heat loss, potentially more important in cooler temperatures (Tucker et al., 2007). When given the choice between staying indoors versus going to pasture, cows spent more time indoors during periods of rainfall (Legrand et al., 2009; Falk et al., 2012). At temperatures below freezing, when precipitation turns to snow, Graunke et al.
(2011) found that the proportion of beef cows lying down increased compared to periods with similar temperatures and no snow. To date, no studies have quantified the effect of precipitation on the lying behaviour of dairy cows housed at pasture.

This study had three main objectives. Firstly, to describe differences in lying behaviour between lame cows and sound cows, to test the prediction that lame cows would spend more time lying down. Secondly, to describe changes in lying behaviour when sound cows become lame and vice versa, testing if lying behaviours would change in relation to changing lameness status. Thirdly, this study aimed to document the effects of precipitation on lying behaviour. Consistent with previous findings with wet lying surfaces, I predicted that rainfall would decrease daily lying time.

2.2 Materials and methods

This study was approved by the University of British Columbia Animal Care Committee (A15-0082) and the Federal University of Santa Catarina Ethics Committee of Research on Animals (PP00949). Between June and August 2015, I evaluated a total of 252 lactating dairy cows from 6 pasture-based dairy farms with rotational grazing located in the west of Santa Catarina, Brazil (26°43′51″S 53°30′55″W). The farms were part of a larger study focused on risk factors associated with lameness, metritis, and ketosis in lactating dairy cows housed on pasture (see Daros et al., 2017; Bran et al., 2018). To be included in the study farms were required to house their cows on pasture for at least 16 h/d. On each farm, cows were milked twice daily at approximately 06:00 h and 16:00 h and were allowed access to supplemental silage and concentrate (an average of 21.3 ± 10.1 kg/cow/d and 6.8 ± 1.5 kg/cow/d, respectively) for
approximately 30 min immediately after each milking. Cows remained at pasture except when in the milking or feeding area.

2.2.1 Data collection

2.2.1.1 Animal measures

On the first farm visit the breed, parity, and days in milk (DIM) of each cow were retrieved from farm records or directly reported by the farmer. While cows were in the milking parlour I assessed body condition score (BCS; 5-point scale with 0.25 increments from 1 to 5; Ferguson et al., 1994). The average lactating herd size was 42 cows (range: 29 – 57 cows) with a total of 83.5% Holstein, 9.0% Jersey, and 7.5% Holstein-Jersey crossbred cows with an average parity of 2.6 (range: 1 – 6), mean DIM of 192 d (range: 7 – 737 d), and mean BCS of 3.17 (range: 2.25 – 4.5).

2.2.1.2 Gait assessment

For 4 consecutive weeks all lactating cows were locomotion scored by one of the researchers (J.A. Bran), an experienced veterinarian with training in gait scoring. Cows were scored as they exited the milking parlour using a 5-point numerical rating score, where 1 = sound and 5 = severely lame (Flower and Weary, 2006). Cows were categorized as non-lame (NRS ≤ 2), clinically lame (NRS ≥ 3), or severely lame (NRS ≥ 4).
To determine inter-rater reliability, a second observer (A.J. Thompson), trained using video and live-scoring, locomotion scored all lactating cows from one farm for each visit (n = 57 cows). The coefficient of determination ($R^2$) between the mean NRS values for the two observers was determined using linear regression ($R^2 = 0.51$).

### 2.2.1.3 Lying behaviour

For 3 consecutive weeks, beginning immediately after the initial gait scoring session and ending immediately before the final gait scoring session, lying behaviour was recorded at 1-min intervals using dataloggers (Hobo Pendant G, Onset Computer Corporation, Bourne, MA) attached to the hind leg of each cow. Dataloggers were attached using a self-adherent bandage (Co-Flex, Andover Healthcare Inc., Salisbury, MA) and padded to minimize risk of chafing. Once per week, loggers were replaced and the data downloaded. Standing or lying data were extracted following the methods outlined by Ledgerwood et al. (2010), using a 2-event filter to correct for bouts of standing or lying less than 3 min in duration (UBC Animal Welfare Program, 2013).

### 2.2.1.4 Temperature and precipitation

This study took place during the winter season in Southern Brazil, a period when rain is frequent and temperatures are mild. Hourly regional temperature and precipitation were retrieved from the Automatic Meteorological Station in São Miguel do Oeste, Santa Catarina (National Institution of Meteorology, Ministry of Agriculture; 26°46'S 53°30'W). The average daily
temperature throughout this study was 18.1 ± 4.6 °C (± standard deviation; range: 2.9 – 29.8 °C); it rained for 31 of 70 d during the study (average rainfall 17.7 ± 27.0 mm/d with rain).

2.2.2 Statistical analysis

Statistical analyses were performed in R (version 3.4.3; R Core Team, 2017) with linear mixed-effects models using R packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2016). Cows missing datalogger information for more than 1 wk were not included in the analysis. A total of 51 cows were excluded, leaving 201 cows in the final analyses.

2.2.2.1 Between-cow analysis: Consistent lameness status

Daily lying time, mean lying bout duration, and the daily number of lying bouts were summarized by cow. In total, 64 cows were scored as non-lame (NONLAME; NRS ≤ 2) and 39 cows were scored as lame (LAME; NRS ≥ 3) in each of the weekly lameness scoring sessions over the 3-wk study period. I was unable to test for differences in the behaviour of severely lame cows due to insufficient power: only 5 cows were scored as severely lame on each of the visits. To normalize the distribution, the mean lying bout duration (min/bout) and total daily precipitation (mm/d) were transformed using the ln function. Unconditional linear mixed-effects models with farm and cow as random effects were used to screen the fixed effects of parity (categorical: primiparous and multiparous), DIM (continuous), BCS (continuous: 5-point scale with 0.25 increments between 1 and 5), and total daily precipitation (continuous: mm/d), one variable at a time. Visual inspection of the relationship between daily precipitation and lying
time revealed a quadratic relationship, so a quadratic term was included in all analyses with daily lying time. The addition of the quadratic term improved model fit. Variables with $P$ values $\leq 0.2$ were considered as potential explanatory variables. These variables were checked for collinearity with lameness status, considered so if the odds ratio (OR) was greater than 10 (Dohoo et al., 2012). Parity was found to be highly collinear with lameness status (OR = 55), so the effect of lameness was considered separately for primiparous and multiparous cows. Since only one primiparous cow was classified as LAME in our study, only the multiparous model was used (NONLAME: $n = 35$ cows; LAME: $n = 32$ cows). The effect of parity on lying behaviour for NONLAME cows was also considered (primiparous: $n = 24$; multiparous: $n = 35$). Total daily precipitation and DIM were included in all final models, while BCS was included only in the final models for daily lying time. Linear mixed-effects models including farm and cow as random effects were used to test for the main effect of lameness on each lying behaviour for multiparous cows. Linear mixed-effects models including farm and cow as random effects were used to test for the main effect of parity on each lying behaviour for NONLAME cows. Normality and homoscedasticity of model residuals were assessed graphically. All possible two-way interactions were tested and reported if significant ($P < 0.05$).

2.2.2.2 Within-cow analysis: Changing lameness status

Cows were considered to have developed lameness if their weekly gait score changed from non-lame (NRS $\leq 2$) to clinically lame (NRS $\geq 3$) and remained clinically lame for the remainder of the observational period. Cows were considered to have recovered from lameness if their weekly gait score changed from clinically lame (NRS $\geq 3$) to non-lame (NRS $\leq 2$) and they
remained sound for the remainder of the observational period. Cows were considered to have an inconsistent lameness status if their weekly lameness status was not consistent. In total, 98 cows had changing lameness status, of which 22 cows developed or recovered from lameness on the final visit to the farm. Since there was no lying data available for the period following the final gait score, these cows were omitted from final analyses. Of the 76 remaining cows, 16 developed lameness, 14 recovered, and 46 showed an inconsistent pattern across the 4 visits. Given the small number of cows that changed lameness status, cows that developed and recovered from lameness were considered together (n = 30 cows).

Daily lying time, mean lying bout duration, the daily number of lying bouts, and total daily precipitation for cows with changing lameness status were summarized by cow and day (n = 1,340 observations [76 cows with 18 d of observation each = 1,368 possible observations]). Datalogger values were missing on 28 cow days. Lying bout duration (min/bout) and precipitation (mm/d) were both transformed using the $\ln$ function. Visual inspection of the relationship between precipitation and lying time indicated a quadratic relationship, so a quadratic term was included in the analysis. The addition of the quadratic term improved model fit. Linear mixed-effects models including farm, cow, and week as random effects were used to test of the main effect of lameness on each lying behaviour for cows that developed or recovered lameness. The two-way interaction between lameness status and precipitation was tested and retained if $P < 0.05$. 
2.3 Results

2.3.1 Lameness

At the first visit, the average prevalence of clinical lameness was 39.2% across the 6 farms (range: 25.7 - 60.6%), including 11.4% severe lameness (range: 0 - 25.5%). The mean proportion of the herd that developed and recovered from lameness were 15.5% (range: 0 – 23.5%) and 10.1% (range: 6.1 – 14.6%), respectively. Additionally, 32.2% of cows were never scored as lame, 18.9% of cows were consistently clinically lame, and 23.3% of cows were inconsistent in their lameness status throughout the study (see Table 2.1).
Table 2.1  The number of cows, prevalence of clinical and severe lameness, and percentage of cows that had consistent and changing lameness status on each of the six farms in Southern Brazil (n = 201 cows)

<table>
<thead>
<tr>
<th>Farm</th>
<th>Number of lactating cows*</th>
<th>Prevalence of clinical lameness (%)**</th>
<th>Prevalence of severe lameness (%)**</th>
<th>Consistent lameness status</th>
<th>Changing lameness status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Non-lame (%)</td>
<td>Consistently lame (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Developed (%)</td>
<td>Recovered (%)</td>
</tr>
<tr>
<td>1</td>
<td>42</td>
<td>25.7</td>
<td>7.1</td>
<td>20.0</td>
<td>8.6</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>44.0</td>
<td>20.0</td>
<td>48.0</td>
<td>16.0</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>60.6</td>
<td>25.5</td>
<td>11.8</td>
<td>44.1</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>30.8</td>
<td>6.9</td>
<td>34.6</td>
<td>11.5</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>39.6</td>
<td>8.8</td>
<td>39.6</td>
<td>20.8</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>34.4</td>
<td>0</td>
<td>39.4</td>
<td>12.1</td>
</tr>
</tbody>
</table>

* Based on total number of cows surveyed, total of 252 cows.

** Calculated using gait scores from the first visit to each farm.
2.3.2 Lying behaviour

Across the study, cows had a mean daily lying time of 10.9 h/d (range: 7.1 – 14.4 h/d),
mean lying bout duration of 80.8 min/bout (range: 46.8 – 132.7 min/bout), and mean daily
number of lying bouts 9.7 no./d (range: 5.4 – 15.2 no./d).

2.3.3 Between-cow results: Consistent lameness status

Model outputs for the association between lameness and lying behaviour in multiparous
cows can be found in Table 2.2. Model outputs for the association between parity and lying
behaviour in NONLAME cows can be found in Table 2.3.

2.3.3.1 Daily lying time

For multiparous cows, there was an interaction between the main effects of lameness
status and precipitation on daily lying time ($P = 0.01$). Overall, precipitation reduced total daily
lying time; however, the magnitude of this change was dependent on lameness status: LAME
cows decreased their lying time more than NONLAME cows on days with rainfall (Figure 2.1A).
Similarly, for NONLAME cows there was an interaction between the main effects of parity and
precipitation on daily lying time ($P = 0.001$). Again, precipitation reduced daily lying time
overall, but multiparous animals decreased their lying time less than primiparous animals on
rainy days (Figure 2.2A).
Figure 2.1 Bar plots showing consistent lameness status (NONLAME vs. LAME) in relation to (A) mean daily lying time (min/d), (B) mean lying bout duration (min/bout; untransformed), and (C) mean daily number of lying bouts (no./d) of multiparous cows on days with (light gray) and without precipitation (dark gray). Error bars represent ± 1 SEM (n = 67 cows).

2.3.3.2 Lying bout duration

For multiparous cows, there was no effect of lameness status on mean lying bout duration ($P = 0.160$), but precipitation increased mean lying bout duration regardless of lameness status ($P < 0.001$; Figure 2.1B). Similarly, for NONLAME cows there was no effect of parity on mean lying bout duration ($P = 0.432$), but precipitation increased mean lying bout duration regardless of parity ($P < 0.001$; Figure 2.2B).
Figure 2.2 Bar plots showing parity (primiparous vs. multiparous) in relation to (A) mean daily lying time (min/d), (B) mean lying bout duration (min/bout; untransformed), and (C) mean daily number of lying bouts (no./d) of NONLAME cows on days with (light gray) and without precipitation (dark gray). Error bars represent ± 1 SEM (n = 59 cows).

2.3.3.3 Number of lying bouts

For multiparous cows, there was an interaction between the main effects of lameness and precipitation on number of lying bouts ($P < 0.05$). Overall, precipitation decreased the lying bouts in both NONLAME and LAME cows ($P < 0.001$), but this effect was greater for LAME cows (Figure 2.1C). For NONLAME cows, there was no effect of parity on the number of lying bouts ($P = 0.502$), but precipitation decreased the number of lying bouts ($P < 0.001$; Figure 2.2C).
Table 2.2 The effect of consistent lameness status (NONLAME: n = 35; LAME: n = 32) on the daily lying time (min/d), natural logarithm of mean lying bout duration (min/bout), and daily number of lying bouts (no./d) of multiparous cows modelled using linear mixed-effects models with farm and cow as random effects.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Variable</th>
<th>Estimate</th>
<th>SEM</th>
<th>95% confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Daily lying time (min/d)</td>
<td>Intercept</td>
<td>563.58</td>
<td>70.73</td>
<td>0.04</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>DIM</td>
<td>0.22</td>
<td>0.09</td>
<td>0.04</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>BCS</td>
<td>27.35</td>
<td>20.83</td>
<td>-13.46</td>
<td>68.17</td>
</tr>
<tr>
<td></td>
<td>LAME</td>
<td>13.99</td>
<td>24.81</td>
<td>-34.64</td>
<td>62.61</td>
</tr>
<tr>
<td></td>
<td>ln(Precipitation)</td>
<td>-15.17</td>
<td>5.56</td>
<td>-26.06</td>
<td>-4.28</td>
</tr>
<tr>
<td></td>
<td>ln(Precipitation)^2</td>
<td>-41.96</td>
<td>6.56</td>
<td>-54.81</td>
<td>-29.10</td>
</tr>
<tr>
<td></td>
<td>LAME x ln(Precipitation)</td>
<td>-19.80</td>
<td>7.63</td>
<td>-34.76</td>
<td>-4.84</td>
</tr>
<tr>
<td></td>
<td>LAME x ln(Precipitation)^2</td>
<td>-0.53</td>
<td>9.25</td>
<td>-18.66</td>
<td>17.59</td>
</tr>
<tr>
<td>ln(Lying bout duration (min/bout))</td>
<td>Intercept</td>
<td>4.18</td>
<td>0.05</td>
<td>0.0003</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>DIM</td>
<td>0.0007</td>
<td>0.002</td>
<td>0.0003</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>LAME</td>
<td>0.06</td>
<td>0.04</td>
<td>-0.02</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>ln(Precipitation)</td>
<td>0.06</td>
<td>0.01</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>Daily number of lying bouts (no./d)</td>
<td>Intercept</td>
<td>10.00</td>
<td>0.44</td>
<td>0.0006</td>
<td>0.00009</td>
</tr>
<tr>
<td></td>
<td>DIM</td>
<td>-0.003</td>
<td>0.002</td>
<td>-0.006</td>
<td>0.0009</td>
</tr>
<tr>
<td></td>
<td>LAME</td>
<td>-0.26</td>
<td>0.39</td>
<td>-1.02</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>ln(Precipitation)</td>
<td>-0.81</td>
<td>0.13</td>
<td>-1.06</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>LAME x ln(Precipitation)</td>
<td>-0.38</td>
<td>-0.17</td>
<td>-0.72</td>
<td>-0.04</td>
</tr>
</tbody>
</table>
Table 2.3 The effect of parity (primiparous: n = 24; multiparous: n = 35) on the daily lying time (min/d), natural logarithm of mean lying bout duration (min/bout), and daily number of lying bouts (no./d) of NONLAME cows modelled using linear mixed-effects models with farm and cow as random effects.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Variable</th>
<th>Estimate</th>
<th>SEM</th>
<th>95% confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily lying time (min/d)</td>
<td>Intercept</td>
<td>526.92</td>
<td>58.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DIM</td>
<td>0.13</td>
<td>0.08</td>
<td>-0.01 - 0.28</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>BCS</td>
<td>34.91</td>
<td>16.86</td>
<td>1.86 - 67.96</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>Multiparous</td>
<td>19.83</td>
<td>19.97</td>
<td>-19.32 - 58.98</td>
<td>0.323</td>
</tr>
<tr>
<td></td>
<td>ln(Precipitation)</td>
<td>-40.84</td>
<td>5.58</td>
<td>-51.78 - 29.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ln(Precipitation)^2</td>
<td>-42.88</td>
<td>7.28</td>
<td>-57.16 - 28.60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Multiparous x ln(Precipitation)</td>
<td>25.33</td>
<td>7.70</td>
<td>10.23 - 40.43</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Multiparous x ln(Precipitation)^2</td>
<td>0.79</td>
<td>9.76</td>
<td>-18.34 - 19.92</td>
<td>0.935</td>
</tr>
<tr>
<td>ln(Lying bout duration (min/bout))</td>
<td>Intercept</td>
<td>4.23</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DIM</td>
<td>0.0006</td>
<td>0.0002</td>
<td>0.0002 - 0.001</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Multiparous</td>
<td>-0.03</td>
<td>0.04</td>
<td>-0.12 - 0.05</td>
<td>0.432</td>
</tr>
<tr>
<td></td>
<td>ln(Precipitation)</td>
<td>0.05</td>
<td>0.01</td>
<td>0.03 - 0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Daily number of lying bouts (no./d)</td>
<td>Intercept</td>
<td>9.80</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DIM</td>
<td>-0.004</td>
<td>0.002</td>
<td>-0.007 - 0.0004</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>Multiparous</td>
<td>0.28</td>
<td>0.41</td>
<td>-0.53 - 1.08</td>
<td>0.502</td>
</tr>
<tr>
<td></td>
<td>ln(Precipitation)</td>
<td>-0.94</td>
<td>0.09</td>
<td>-1.12 - 0.76</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

2.3.4 Within-cow results: Changing lameness status

There was no difference in daily lying time (P = 0.804), mean lying bout duration (P = 0.986), or the daily number of lying bouts (P = 0.874) for cows that changed their lameness status (i.e., developed or recovered from lameness; Table 2.4). Precipitation decreased total daily lying
time ($P < 0.001$), increased the mean bout duration ($P < 0.001$), and decreased the daily number of lying bouts ($P < 0.001$).

### Table 2.4 The effect of changing lameness status (developed and recovered; $n = 30$ cows) on the daily lying time (min/d), natural logarithm of mean lying bout duration (min/bout), and daily number of lying bouts (no./d) of grazing cows modelled using linear mixed-effects models with farm, cow, and week as random effects.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Variable</th>
<th>Estimate</th>
<th>SEM</th>
<th>95% confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily lying time (min/d)</td>
<td>Intercept</td>
<td>695.06</td>
<td>23.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lameness status</td>
<td>1.56</td>
<td>6.25</td>
<td>-10.69 13.80</td>
<td>0.804</td>
</tr>
<tr>
<td></td>
<td>ln(Precipitation)</td>
<td>-26.06</td>
<td>3.35</td>
<td>-32.63 -19.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>ln(Precipitation)$^2$</td>
<td>-45.11</td>
<td>3.95</td>
<td>-52.85 -37.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ln(Lying bout duration (min/bout))</td>
<td>Intercept</td>
<td>4.35</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lameness status</td>
<td>0.0004</td>
<td>0.02</td>
<td>-0.04 0.04</td>
<td>0.986</td>
</tr>
<tr>
<td></td>
<td>ln(Precipitation)</td>
<td>0.05</td>
<td>0.009</td>
<td>0.03 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Daily number of lying bouts (no./d)</td>
<td>Intercept</td>
<td>9.25</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lameness status</td>
<td>0.03</td>
<td>0.18</td>
<td>-0.32 0.37</td>
<td>0.874</td>
</tr>
<tr>
<td></td>
<td>ln(Precipitation)</td>
<td>-0.94</td>
<td>0.07</td>
<td>-1.09 -0.80</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### 2.4 Discussion

The daily lying time of cows in my study is similar to lying times reported in previous studies with grazing dairy cows (Krohn and Munksgaard, 1993; Legrand et al., 2009; Olmos et al., 2009; Sepúlveda-Varas et al., 2014), as well as to cows kept in loose-housed indoor systems.
with either automated milking systems (DeVries et al., 2011; Deming et al., 2013) or parlour-milking systems (Wechsler et al., 2000; Ito et al., 2009). The daily number and duration of lying bouts reported here are also similar to results reported previously for indoor- and outdoor-housed cows (Ito et al., 2010; Navarro et al., 2013; Weigele et al., 2017).

In contrast to previous findings, I did not detect a direct effect of consistent lameness status on lying time in this study (Navarro et al., 2013; Sepúlveda-Varas et al., 2014; Weigele et al., 2017). Previous studies have reported that lame cows spend an additional 24 to 126 min/d lying down indoors (Ito et al., 2010; Navarro et al., 2013; Weigele et al., 2017) and 91 to 105 min/d lying down outdoors compared to non-lame cows (Walker et al., 2008; Navarro et al., 2013; Sepúlveda-Varas et al., 2014). Similarly, I did not detect a direct effect of consistent lameness status on the number or duration of lying bouts. Other work has shown that lameness influences lying bouts, but the effect differs depending on both the severity of lameness and the type of lying surface (Ito et al., 2010; Sepúlveda-Varas et al., 2014; Weigele et al., 2017). To understand how lying bouts are affected by lameness in grazing systems there is a need for future research to consider the quality of pasture as a lying surface.

To my knowledge no previous research regarding lameness and lying behaviour in grazing herds has accounted for precipitation (Olmos et al., 2009; Navarro et al., 2013; Sepúlveda-Varas et al., 2014). My findings indicate that precipitation causes a reduction in lying time in cows regardless of lameness status, but the reduction in lying time is greater in lame cows. This decline in lying time was likely driven by lame cows reducing their number of lying bouts more than non-lame cows during periods of rainfall. Rain may also increase the chance that cows lie on wet, muddy, and slippery surfaces, which may in turn compromise transitions from lying to standing and vice versa, perhaps especially affecting lame cows. Further research
addressing how well lame and non-lame cows transition from lying to standing and vice versa is warranted.

My results also showed that the non-lame multiparous cows reduced their lying time less than non-lame primiparous cows on days with rain. This result is in contrast to previous work that report greater reductions in lying time in dry cows compared to heifers when exposed to a muddy lying surface (Chen et al., 2016). These conflicting results suggest that more work is required to understand the effect of rain and wet lying surfaces on the lying behaviour of grazing cows with different parity.

Contrary to my prediction, I did not find within-cow differences in lying time or the number or duration of lying bouts associated with changes in lameness status (i.e., whether the same cow changed her lying behaviour before and after being scored as lame). In my study, cows were locomotion scored on a weekly basis, while lying behaviour was recorded every minute and summarized daily. Thus, a cow’s daily lying behaviour may have not accurately reflected her most recent locomotion score if she changed her lameness status in the days following the most recent assessment. Furthermore, there may have been error in the weekly gait estimates, with lower inter-rater agreement in this study compared to previous research using the same locomotion scoring scale (e.g., R² of 0.69 in Flower and Weary, 2006). This difference could be explained because in this study cows were live-scored but were assessed using video recordings in Flower and Weary (2006). Future research should consider including more frequent scoring of lameness, ideally on a daily basis, and for longer periods to allow for more meaningful comparisons between the lying behaviour of the same individual when sound and lame. Identifying whether lying behaviour changes before a change in gait would be an important first step.
This study is the first to quantify the effect of precipitation on lying behaviour in grazing dairy cows. Daily lying time decreased with rainfall, consistent with previous work that has shown reductions in daily lying duration associated with wet lying surfaces indoors (Fregonesi et al., 2007) and outdoors on wet dirt packs (Chen et al., 2016) and wet woodchip bedding (Tucker et al., 2007). Similarly, in agreement with the effect of muddy ground conditions found elsewhere (Chen et al., 2016), rainfall increased individual lying bout duration and decreased the number of lying bouts in this study. The results of this study show that precipitation is clearly an influential co-variate with lying behaviour in grazing dairy cattle.

The prevalence of lameness found in this study was similar to estimates previously reported for dairy cattle housed indoors (Solano et al., 2015a; Cook et al., 2016; Adams et al., 2017) and at pasture (Olmos et al., 2009; Fabian et al., 2014; Ranjbar et al., 2016). This research provides further evidence demonstrating that lameness remains a pertinent issue in both indoor and outdoor housing systems.

2.4.1 Conclusions

Lying time has the potential to be an effective and practical indicator of lameness in grazing systems, but more research is needed to identify key factors (e.g., precipitation) that also influence the lying behaviour of cows at pasture. Given the strong effect of precipitation on lying behaviour in this study, I recommend that future research include rainfall as a co-variate when working with grazing cattle.
Chapter 3: General discussion

In this chapter, I will discuss the findings of my research and how they contribute to the larger understanding of lameness and lying behaviour. Additionally, I will summarize the strengths and limitations of my research and consider topics for future research.

3.1 Thesis findings

In Chapter 1, I provided an overview of the available literature related to lameness and how it affects lying behaviour. Regardless of housing system lameness increases daily lying time (e.g., Ito et al., 2010; Sepúlveda-Varas et al., 2014), but may differently affect the daily number and mean duration of lying bouts (Ito et al., 2010; Sepúlveda-Varas et al., 2014; Weigele et al., 2017). In this chapter I also summarized the literature related to the effects of precipitation on lameness and lying behaviour in cattle. Although sparse, the work to date shows that precipitation is a risk factor for lameness (Ranjbar et al., 2016) and that wet or muddy lying surfaces decrease daily lying duration in dairy cows (Fregonesi et al., 2007; Tucker et al., 2007; Schütz et al., 2010; Chen et al., 2016).

The research undertaken for my dissertation (Chapter 2) expands our understanding of how lameness affects lying behaviour, and how precipitation affects this relationship, by showing that lying time and the daily number of lying bouts vary in relation to both precipitation and lameness. In addition, I also noted differing effects of precipitation on the lying behaviour of younger and older cows that were non-lame.
Precipitation caused a reduction in lying time regardless of lameness status, but the effect of precipitation was greater for lame compared to non-lame cows. Furthermore, lame cows had fewer lying bouts compared to non-lame cows on days with rainfall than on days without. The reduction of lying bouts for lame cows on rainy days likely explain the lower lying times observed for lame cows on these days. These results link previous work showing that lameness increases daily lying time (Weigele et al., 2017) and precipitation decreases daily lying time (Tucker et al., 2007).

The results of my study also provide evidence that parity affects lying behaviour differently depending on rainfall, with non-lame multiparous cows decreasing lying time less than non-lame primiparous cows on rainy days. Previous work has also found that other behaviours (e.g., social and feeding behaviour) differ between primiparous and multiparous cows (Neave et al., 2017). However, previous work with lying time and wet lying surfaces found the opposite relationship to the one found here: there were greater reductions in lying time resulting from muddy lying surfaces in dry cows (older) compared to heifers (younger; Chen et al., 2016). My work, and that of others, suggests that parity is an important factor that deserves consideration in its own right in future research.

3.2 Strengths and limitations

The work described in this thesis builds on existing work that has shown lameness to increase lying time for cows housed indoors (Weigele et al., 2017) and outdoors (Sepúlveda-Varas et al., 2014). A noted strength of my work is that it has considered precipitation as a factor influencing lying behaviour. Previous research regarding lameness and lying behaviour in
grazing herds has not accounted for precipitation (Olmos et al., 2009; Navarro et al., 2013; Sepúlveda-Varas et al., 2014), despite evidence that this wet lying surfaces can reduce lying time (Tucker et al., 2007; Schütz et al., 2010; Chen et al., 2016). The results described here show a strong effect of precipitation on lying behaviour; this factor explained approximately one-sixth of the overall variation in lying behaviour. Furthermore, I found interactions between lameness and precipitation on lying time and the number of lying bouts as well as between parity and precipitation on lying time (see section 3.1 above). Clearly, precipitation is an important co-variate to be considered when investigating the behaviour of cattle kept at pasture.

Future research should give additional consideration to how best to capture precipitation data. In the current study I relied on regional weather information captured from the Automatic Meteorological Station in São Miguel do Oeste, Santa Catarina (National Institution of Meteorology, Ministry of Agriculture; 26°46'S 53°30'W), located in the city closest to the six farms visited in my study. Given the geographical separation between some of my focus farms (maximum of approximately 50 km) there is risk that the recorded precipitation values obtained from the meteorology station did not reflect what was happening at each farm. I suggest that future research measure rainfall in close proximity to the cows.

The number of animals that developed (n = 16) and recovered (n = 14) from lameness was insufficient to reliably test for the interaction between lameness and precipitation on lying behaviour separately for the two trajectories. To increase power I combined the two trajectories to test the two-way interaction between lameness and precipitation on the lying behaviour of cows that developed or recovered from lameness (n = 30), but this relationship may have differed between trajectories. For example, if the delay between changes in lameness status and lying behaviour differed when cows became lame versus when they recovered. I was unable to detect
differences in lying time, lying bout duration, or the number of lying bouts for cows that developed or recovered from lameness. To my knowledge, this was the first attempt to explore the effect of changing lameness status on lying behaviour in grazing dairy cattle. The absence of a relationship may also be a consequence of scoring lameness just once per week. This sampling frequency may not have been sufficient to relate to changes in lying behaviour summarized by day. Indeed this could be argued to be a major weakness, particularly when a single gait score taken once a week was used to assess its relationship with lying behaviour for 6 consecutive days following.

To determine lameness status, cows in this study were scored by a single trained observer. Previous research has shown that intra-rater reliability (i.e., one observer scoring the same animal repeatedly) and inter-rater reliability (i.e., two observers scoring the same animal) for locomotion scoring can be variable (O’Callaghan et al., 2003; Flower and Weary, 2006). Here, the inter-rater agreement of locomotion scores was 0.51 ($R^2$), which is lower than previously reported by researchers using the same scale ($R^2$ of 0.69 in Flower and Weary, 2006). However, in this study animals were live-scored, unlike in Flower and Weary (2006) where scorers had multiple opportunities to review video of each cow walking before locomotion scoring. In this study, almost half of the cows with changing lameness status were classified as having inconsistent lameness status (i.e., gait score changed on a weekly basis). Most of these changes in gait were likely accurate, but some ‘changes’ may have been due to error in lameness assessment during any one observation. Cows were scored for lameness as they exited the milking parlour. Although every effort was made to ensure that cows exited individually there were some occasions where they came out in small groups, which likely increased error in assigning a gait score to each individual. Furthermore, moderately lame cows (NRS = 3) are
difficult to identify because the changes in locomotion are slight. For example, in the scoring scale used here the only qualitative difference between being non-lame (NRS ≤ 2) and moderately lame (NRS = 3) is a slight limp while walking (Flower and Weary, 2006). The difficulty to identify moderately lame cows is reflected in that farmers consistently underestimate the prevalence of clinical lameness in their herds, both indoors and outdoors (Fabian et al., 2014; Cutler et al., 2017).

3.3 Future research directions

The research presented in this thesis suggests several additional questions. With growing public concern for the welfare of animals reared in intensive production systems (Cardoso et al., 2016), and citizens showing a preference for animals to be kept in natural environments (Schuppli et al., 2014), providing cows access to pasture may become more common in regions where currently little pasture is used. For example, in the US less than 10% of lactating cows are housed on pasture (USDA, 2014), but with increased public pressure this could increase. Given the dearth of information on the behaviour of cows housed on pasture, I see need for work to explore the use of behaviour to better understand the needs of pasture-based cows.

Lameness remains one of the greatest welfare challenges for dairy cattle (Huxley, 2013). The high prevalence of clinical lameness in my study indicates that this concern is not unique to indoor-housed cows but is also true for cows kept at pasture. This finding is echoed by a companion study that was done in the same region but on 44 farms; the prevalence of clinical and severe lameness was 31% and 14.4%, respectively (Bran et al., 2018). Research is needed to build upon the few studies regarding lameness in grazing herds.
Given that automated lameness detection systems often use subjective gait scores as their baseline for sensitivity and specificity (Schlageter-Tello et al., 2014), it is important to ensure that scorers are consistent within individual cows. While studies typically report intra- and inter-rater reliability (e.g., Flower and Weary, 2006), these are often based on only two locomotion scores per animal. This is because agreement is meant to ensure that scorers are accurate within and between themselves, but do not consider whether the score remained consistent across assessments of each cow. Future research should consider gait scoring animals more times to determine the number of subjective scores required to achieve a consistent classification of cows as sound (NRS ≤ 2), moderately lame (NRS = 3), and clinically lame (NRS ≥ 3).

As described in section 1.3, little is known regarding the prevalence of different types of hoof lesions in outdoor environments. Certain hoof lesions, such as sole ulcers, are more common in herds kept indoors (Navarro et al., 2013), but other types of hoof pathologies, such as white line disease, have been found to be both more (Olmos et al., 2009) and less prevalent (Lawrence et al., 2011) in herds kept outdoors. More information on the prevalence and incidence of these different types of hoof lesions in grazing herds is needed. For this thesis, I did not collect data about hoof lesions. However, this data would allow for investigation into the causal relationship between specific lesions and their effect on lying behaviour. I recommend related future research to include claw health in their study design. This type of work could include longitudinal intervention studies that follow cows over time before and after hoof trimming, an event that has been shown to influence lying time (Chapinal et al., 2010). Lying behaviour could be recorded throughout to measure (1) the effect of different lesions on lying behaviour in grazing dairy cows and (2) the effect of treatment (e.g., hoof trimming) on lying behaviour.
Primiparous cows have altered lying, feeding, and social behaviour in comparison to multiparous animals during the transition period (Neave et al., 2017), a time when cows are at greater risk for hoof lesions (Proudfoot et al., 2010). Similarly, the results from the research in this thesis and elsewhere (e.g., Chen et al., 2016) demonstrate that younger and older animals respond differently to precipitation. This suggests that animals of different ages may benefit from differing management strategies at pasture. Future research should investigate the effect of rainfall on other behaviours (e.g., feeding and social behaviour) in grazing herds and investigate potential interactions with parity.

Finally, to my knowledge, no work has addressed whether there are breed differences in gait attributes (i.e., differences between Holstein and Jersey cows). Head bob, which is one of the six gait attributes considered in the scoring system developed by Flower and Weary (2006), can range from steady and even head carriage to pronounced uneven head movement while walking. Though not quantified, I observed otherwise sound Jersey cows in this study to walk with an unsteady head carriage, while sound Holstein cows typically maintained steady and even head carriage during locomotion. Though a head bob is only one of six criteria considered when assessing lameness, the presence of an unsteady head carriage could predispose otherwise sound Jersey cows to higher gait scores than sound Holstein cows. Future research should consider potential differences in gait attributes for different breeds of cattle.

3.4 General conclusions

My thesis work contributes to the limited body of literature showing the effect of lameness on lying behaviour in grazing dairy cows and provides the first evidence of an
interaction between precipitation and lameness on lying behaviour in grazing dairy herds. The results of this thesis show that precipitation has a major effect on lying behaviour, but that this effect also varies with parity. Lying time has the potential to be an objective and consistent indicator of lameness in grazing systems, however future work to determine and include co-
variates that influence lying behaviour (e.g., lying surface quality of pasture and precipitation) is needed before lying behaviour can be used as an effective and practical indicator of lameness.
Bibliography


Bicalho, R.C., L.D. Warnick, and C.L. Guard. 2008. Strategies to analyze milk losses caused by
diseases with potential incidence throughout the lactation: A lameness example. J. Dairy Sci. 91:2653–2661.


Haskell, M.J., L.J. Rennie, V.A. Bowell, M.J. Bell, and A.B. Lawrence. 2006. Housing system, milk production, and zero-grazing effects on lameness and leg injury in dairy cows. J. Dairy


UBC Animal Welfare Program. 2013. UBC Animal Welfare Program: SOP - HOBO Data


