

Developing methods to improve welfare in periparturient dairy cows and pre-weaned calves

Turner Harrison Swartz

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Dairy Science

Christina S. Petersson-Wolfe, Committee Chair

Jeffrey M. Bewley

Hollie H. Schramm

Cynthia M. Wood

April 13th, 2018

Blacksburg, Virginia

Keywords: animal activity, periparturient disease, pre-weaned calf, meloxicam

Developing methods to improve welfare in periparturient dairy cows and pre-weaned calves

Turner Harrison Swartz

ABSTRACT

Animal behavior can be used to detect disease and well-being in dairy cattle. In this dissertation, we evaluated the accuracy of an accelerometer to measure step activity, lying time, and lying bouts in pre-weaned dairy calves. The output from the accelerometer was correlated with behavioral measurements taken from video footage. The accelerometer proved to be accurate in identifying step activity ($r = 0.99$), lying time ($r = 0.99$), and lying bouts ($r = 0.99$). The accelerometer was then used to detect behavioral changes occurring around respiratory disease events in pre-weaned calves. Activity declined 1 d prior to clinical disease onset, and this decline persisted for 3 d post-diagnosis. Furthermore, lying bouts declined beginning 2 d prior to diagnosis, and this effect persisted after diagnosis as well. However, aside from a slight reduction in milk intake, feeding behavior was not different between diseased and healthy calves. These data suggest that activity and lying behaviors may be a better measure than feeding behaviors for detection of respiratory disease in pre-weaned dairy calves.

Dystocia has detrimental effects on both periparturient dairy cows and newborn calves. We administered a non-steroidal anti-inflammatory drug, meloxicam to periparturient dairy cattle. Treatments included administration prior to calving (MEL-PRE, $n = 60$), post-calving (MEL-POST, $n = 69$), or a negative control (CTL, $n = 65$). We measured the length of labor to determine which cows had easy or difficult calvings. Eutocic MEL-PRE animals produced 6.8 kg/d more milk than eutocic CTL. Regardless of calving difficulty, MEL-PRE animals produced more milk fat, protein, and lactose (kg/d) than the CTL. Additional research is needed to determine appropriate treatments for dystocic calvings. Calves born during the above trial were monitored to determine if meloxicam administration prior to calving impacted newborn calf health and behavior. Calves born difficultly displayed fewer lying bouts for the first few days after birth when compared to calves born easily. No effect of treatment or calving difficulty was noted on calf health. Additional research examining intervention strategies aimed at improving well-being of calves born difficultly is needed.

Keywords: activity, periparturient disease, pre-weaned calf, meloxicam

Developing methods to improve welfare in periparturient dairy cows and pre-weaned calves

Turner Harrison Swartz

ABSTRACT

Public interest in animal welfare continues to grow, making it increasingly important that the dairy industry evaluates management practices to further advance animal well-being. Animal behavior can be used to detect disease and well-being in dairy cattle. We monitored activity and lying behaviors around respiratory disease events in calves. This was done to determine which behaviors were altered by respiratory disease, and if these behaviors could be used to detect respiratory disease events earlier. Activity and lying behaviors were measured using an accelerometer that works similarly to a pedometer. We were able to identify that calves that would manifest with respiratory disease would display a decline in activity prior to clinical disease diagnosis. These data suggest that activity measures could be a promising indicator for respiratory disease detection in calves, and allow for earlier detection.

Parturition, the act of a dairy cow giving birth, is a stressful, risky time period as disease incidences and death are high. Furthermore, an immense amount of inflammation occurs after calving due to parturition as well as metabolic stress associated with milk production. Therefore, in this study, we administered a non-steroidal anti-inflammatory drug (meloxicam) to alleviate inflammation. Treatments included administration prior to calving (MEL-PRE), post-calving (MEL-POST), or a negative control (CTL). We measured the length of labor to determine which cows had easy or difficult calving events. Animals that received meloxicam prior to calving and calved easily produced 6.8 kg/d more milk than CTL animals that calved easily. Additional research is needed to determine appropriate treatments for animals that calve difficultly. Calves born during the above trial were monitored to determine if meloxicam administration prior to calving impacted newborn calf health and behavior. No effect of treatment or calving difficulty was noted on calf health. Additional research examining intervention strategies aimed at improving well-being of calves born difficultly is needed.

Keywords: activity, periparturient disease, pre-weaned calf, meloxicam

ACKNOWLEDGEMENTS

“Do not fear failure but rather fear not trying.”

Roy T. Bennett

These words speak true to me today. When I first began my journey as a PhD student at Virginia Tech, my biggest fear was failure. So much so that I avoided that ‘wretched’ lab bench as much as possible. Even so, my research project was still supposed to fail – as similar studies failed before it. And as I read the past studies that had failed, and listened to other scientists insisting upon the upcoming failure of my study – I attempted to persuade my advisor to make a change in experimental design. She resisted. And we persisted. And if there is one thing I’ve learned from my advisor, it is that research should test boundaries and challenge conventional thought. And now I get it. It is amazing how much a person can learn, change, and realize over the course of a few years. This experience allowed me to tap into something in me that I didn’t think existed, and probably more importantly, this experience gave me self-worth. And maybe, just maybe that lab bench probably isn’t so wretched after all... Thank you, Dr. Petersson-Wolfe.

To Drs. J. Bewley, K. Leslie, M. McGilliard, H. Schramm, R. White and C. Wood: Whether it was experimental design, statistical analyses, writing, to looking at my research from another viewpoint – you made me a better researcher, and gave me a more malleable mind. Thank you.

To Mom, Dad, and my family – thank you all so much for your support and more importantly, instilling a work ethic in me that is unmatched by anyone. It was your passion of the dairy cow that made me the passionate student and dairy scientist that I am today. I know that times in the dairy industry are tough right now, but we never lose sight on why we do this. Thank you!

To Dave Winston: Whether you know it or not, **you** are the heart of the Dairy Science Department. It is your guidance and encouragement in the youth programs that help fuel the undergraduate program today. Thank you for allowing me to be part of the VT program. You made this Nittany Lion feel more at home.

To the Virginia Tech farm managers, staff, and veterinarians: Wow! You’ve had to deal with my shenanigans for a year and half. Not only am I surprised you didn’t kick me off that farm, I am eternally grateful for all of you supporting my research. It simply would not be possible without your help. Thank you!

To Nicole and Kellie: Thank you for keeping me sane. It was a challenge that I am sure of. I will miss you both dearly.

To Mark Ellett Jr., Katie McCauley, Courtney Henderson, and Anna Findlay – Thank you all so much for your help! It was a privilege and honor to work with you. I hope you gained something out these research projects – even if it was just money. Follow your dreams. The sky is the limit. Keep in touch – I know I will hear great things!

To the Dairy Science undergraduates and ****ADSA Quiz Bowl teams****: You made this experience for me such a great one – you have no idea! Serving as a TA was an absolute joy for me, and I enjoyed every minute of it. I was humbled to receive the Outstanding Graduate Teaching Assistant award. You inspired me. You made me a better person. You made me realize why I came back to graduate school. Thank you for giving this Nittany Lion a home. And... Drew Laffey... don't do anything I wouldn't do...

To my fellow graduate students, friends, mentors, and dairy producers: Thank you for what you do every day! We all have one central goal – providing the highest quality care possible to the beautiful bovine. I have learned so much from so many people – thank you for your time, your passion, your inspiration, your knowledge, and your friendship. I have no idea what I would be doing if it wasn't for your influence. Thank you!

And finally to Diane Glock-Cornman: This PhD would not have been possible without you. From 4-H and FFA, to coach, to teacher, to mentor, to AZ sister, to friend – you have always been the ray of sunshine and the encouragement that I needed to get to this level. It is amazing how a little over a decade ago, we stopped at Dr. Petersson-Wolfe's house on our way to the National Holstein Convention for quiz bowl. And now, I am writing a dissertation with Dr. Petersson-Wolfe as my advisor. You may not have physically been here at VT, but it was the knowledge, work ethic, skills, and compassion that you instilled in me that shined through during my PhD. Cheers – I dedicate this dissertation to you.

"The cow is the foster mother of the human race. From the time of the ancient Hindoo to this time have the thoughts of men turned to this kindly and beneficent creature as one of the chief sustaining forces of the human race" – W.D. Hoard

TABLE OF CONTENTS

ABSTRACT (academic).....	ii
ABSTRACT (public).....	iii
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS.....	vi
LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
CHAPTER 1: Literature Review.....	1
Introduction.....	1
Transition period.....	1
Inflammation during the transition period.....	3
Calving.....	6
Dystocia.....	8
Behavioral and physiological changes occurring around calving.....	11
Inflammatory cascade.....	15
NSAIDs.....	16
The use of NSAIDs in the periparturient dairy cow.....	17
Pre-weaning period.....	20
Group housing ad automatic calf feeders.....	20
Effect of dystocia and potential intervention strategies on calves.....	23
Research objectives.....	27
REFERENCES.....	28

Chapter 2. Technical Note: The use of an accelerometer for measuring step activity and lying behaviors in dairy calves.....	40
ABSTRACT.....	41
Technical note.....	42
REFERENCES.....	52
Chapter 3. Short Communication: Automated detection of behavioral changes from respiratory disease in pre-weaned calves.....	54
ABSTRACT.....	55
Short communication.....	56
REFERENCES.....	69
Chapter 4. Meloxicam administration either prior to or after parturition: Behavioral, physiological, and production responses.....	71
ABSTRACT.....	72
INTRODUCTION.....	74
MATERIALS AND METHODS.....	78
RESULTS.....	84
DISCUSSION.....	93
CONCLUSIONS.....	103
ACKNOWLEDGMENTS.....	104
REFERENCES.....	114
Chapter 5. Impact of meloxicam administration prior to calving to the dam on pre-weaned calf performance.....	122
ABSTRACT.....	123
Short communication.....	124
REFERENCES.....	142
Chapter 6. Research conclusions.....	144

LIST OF TABLES

Table 2.1	Steps, lying bouts, and lying time (min) from 5 calves in a 7-h period recorded either by video, AffTag II, or the HOBO Pendant G Data Logger, unfiltered and filtered.....	49
Table 2.2	Overall correlations between video and other recording techniques for step activity, lying bouts, and lying time for 5 calves during a 7-h period.....	50
Table 2.3	Correlations between recording techniques for lying bouts and lying time for 5 calves over a 10-d recording period (overall above diagonal, within calf below diagonal).....	51
Table 3.1	Definitions of the scoring system used to determine disease status adapted from the Calf Health Scorer app (University of Wisconsin, School of Veterinary Medicine).....	65
Table 3.2	<i>F</i> -value and probability level for the effect of day, health status, and the interaction of day and health status on step activity (no./d), lying bouts (no./d), and milk intake (mL/d)	66
Table 4.1	Mixed model (PROC GLIMMIX, SAS 9.4) of daily steps (no./d), lying bouts (no./d), and lying time (min/d) starting 2 d prior to calving until 7 d after calving with cows receiving meloxicam either prior to calving (n = 53), after calving (n = 59), or a negative control (n = 53).....	105
Table 4.2	Mixed model (PROC GLIMMIX, SAS 9.4) of daily milk yield and components (kg/d) averaged by week for the first 15 wk of lactation for 194 cows receiving meloxicam either prior to calving (n = 60), after calving (n = 69), or a negative control (n = 65).	106
Table 4.3	Clinical disease incidence by treatment group; data represented as percent of treatment group. Treatments included administration of meloxicam (1 mg/kg of BW by mouth) before calving (MEL-PRE , n = 60), after calving (MEL-POST , n = 69), and a negative control (CTL , n = 65).....	107
Table 5.1	Definitions of the scoring system used to determine disease status adapted from the Calf Health Scorer app (University of Wisconsin, School of Veterinary Medicine).....	136
Table 5.2	Mixed model (PROC GLIMMIX, SAS 9.4) of daily steps (no./d), lying bouts (no./d), and lying time (min/d) starting 2 d until 21 d of age from calves born from cows either receiving meloxicam prior to calving or a placebo.....	137

LIST OF FIGURES

Figure 1.1	Factors associated with inflammation in the periparturient dairy cow. Figure remade from Bradford et al., 2015. Cow photo credited to Cybil Fisher.....5
Figure 3.1	Mean (\pm SE) for daily steps and lying bouts of diseased and healthy calves at each day (with d 0 representing the day respiratory disease was first diagnosed). Differences between healthy and diseased calves: $*P \leq 0.05$, $\dagger P < 0.10$67
Figure 3.2	Mean (\pm SE) for milk intake of diseased and healthy calves at each day (with d 0 representing that day respiratory disease was first diagnosed). Differences between healthy and diseased calves: $*P \leq 0.05$68
Figure 4.1	Treatment regimens for control (CTL), meloxicam post-calving treatment (MEL-POST), and meloxicam pre-calving treatment (MEL-PRE). Meloxicam was dosed at 1 mg/kg of BW orally in a gel capsule. An empty gel capsule served as a placebo.....108
Figure 4.2	Least squares means (\pm SE) of the treatment by calving difficulty score interaction term, comparisons made within calving difficulty, for daily activity (steps/d). Data recorded for 10 d, beginning on d -2 and concluded on d 7. Treatments included administration of meloxicam (1 mg/kg of BW by mouth) before calving (MEL-PRE), after calving (MEL-POST), and a negative control (CTL). Sample sizes denoted within each bar. Differences between treatment groups: $*P \leq 0.05$109
Figure 4.3	Least squares means (\pm SE) of the treatment by parity by day interaction term (multiparous, A ; primiparous, B), comparisons made within day, for daily lying bouts (no./d). Data recorded for 10 d, beginning on d -2 and concluded on d 7. Day 0 was designated as the day of calving. Treatments included administration of meloxicam (1 mg/kg of BW by mouth) before calving (MEL-PRE ; primiparous, n = 17; multiparous, n = 36), after calving (MEL-POST ; primiparous, n = 21; multiparous, n = 38), and a negative control (CTL ; primiparous, n = 17; multiparous, n = 36). Dissimilar letters indicate differences between parity and treatment groups within each day: a,b; $P \leq 0.05$110
Figure 4.4	Least squares means (\pm SE) of the calving difficulty score by day interaction term (eutocia, n = 96; dystocia, n = 69), comparisons made within day, for daily lying bouts (A ; no./d) and lying bout duration (B ; min/bout). Data recorded for 10 d, beginning on d -2 and concluded on d 7. Day 0 was designated as the day of calving. Differences between calving difficulty groups: $*P \leq 0.05$, $\dagger P < 0.10$111
Figure 4.5	Least squares means (\pm SE) of the treatment by calving difficulty score interaction term, comparisons made within calving difficulty, for daily milk yield measured for the first 15 wk of lactation. Treatments included administration of meloxicam (1 mg/kg of BW by mouth) before calving (MEL-PRE), after calving (MEL-

POST), and a negative control (CTL). Sample sizes denoted within each bar.
 Differences between treatment groups: * $P \leq 0.05$, † $P < 0.10$112

Figure 4.6	Treatment least squares means (\pm SE) for daily milk fat (kg/d; A), protein (kg/d; B), and lactose (kg/d; C) measured for the first 15 wk of lactation. Treatments (n = 60 to 69 per treatment group) included administration of meloxicam (1 mg/kg of BW by mouth) before calving (MEL-PRE), after calving (MEL-POST), and a negative control (CTL). Differences between treatment groups: * $P \leq 0.05$113
Figure 5.1	Least squares means (\pm SE) for daily lying bouts from calves born difficultly and easily. Differences between dystocic and eutocic calves: * $P < 0.05$138
Figure 5.2	Least squares means (\pm SE) for daily steps and lying time starting 2 d of age until 21 d of age.....139
Figure 5.3	Least squares means (\pm SE) for daily lying time recorded from d 2 until 21 d of age. Treatment groups include calves born from dams that received meloxicam (MEL) or a placebo prior to calving (CTL). Calving difficulty was determined by the length of labor. Differences: * $P < 0.05$140
Figure 5.4	Least squares means (\pm SE) for daily health scores recorded for the first 21 d of age.....141

Chapter 1. Literature review

Introduction

Public interest in animal welfare continues to grow, making it increasingly important that the dairy industry evaluates management practices to further advance animal well-being. Historically, dairy cattle welfare has been conceptualized in a variety of methods from disease prevalence and milk yield (MY) to more subjective measures such as the freedom to express normal behaviors (Ventura et al., 2013). In 1965, the British government commissioned an investigation into the welfare of farm animals, which led to the development of the Brambell Committee. This committee recommended a set of five freedoms to access animal welfare (Brambell, 1965), which was later adopted by the Farm Animal Welfare Council. Brambell's report states that animal welfare is preserved if animals are free from (1) hunger and thirst, (2) discomfort, (3) pain, injury, and disease, (4) fear and stress, and were (5) free to express normal behaviors (Brambell, 1965). However, because cattle are stoic, identification of discomfort can be a challenging task (Weary et al., 2006). More recently, the use of technologies identifying changes in activity levels or feeding behaviors have proven capable of identifying discomfort and disease in cattle (Weary et al., 2009). The purpose of this research is to identify management techniques to improve welfare in periparturient cows, as well as to identify methods for early detection of disease in pre-weaned calves.

Transition period

The periparturient period, also known as the transition period, represents the 3 wk prior to and 3 wk after parturition, and is marked by dramatic endocrine, immune, and metabolic changes. Furthermore, fetal development in addition to the commencement of milk synthesis results in a pronounced increase in nutrient demand, subsequently leading to negative energy

balance. Due to these changes, the incidence of metabolic and infectious diseases, including hypocalcemia, retained placenta, metritis, mastitis, fatty liver syndrome, ketosis, and abomasal displacements are the highest during this period, and can be compounded by other diseases such as dystocia (Correa et al., 1993; Dematawewa and Berger, 1997; Drackley, 1999). This increase in disease incidence is multifactorial; however, immune dysfunction is widely accepted as one of the main causes of this quandary (Goff and Horst, 1997; Lewis, 1997).

Parturition is a stressful event that induces the production of cortisol (Aleri et al., 2016). Circulating cortisol reduces T-cell proliferation and development, and interferes with immunoglobulin function (Aleri et al., 2016). Cortisol downregulates adhesion molecule expression on the surface of neutrophils, thus reducing the efficiency of the immune response (Burton et al., 1995; Mallard et al., 2009). Neutrophil and lymphocyte activity is impaired during this time period, likely due to the increase in cortisol concentrations (Goff and Horst, 1997), as well as due to negative energy balance characterized by elevated non-esterified fatty acids (NEFA) concentrations (Hammon et al., 2006). The capacity of the liver to metabolize NEFAs is limited. Therefore, during negative energy balance, NEFAs and ketone bodies such as beta-hydroxybutyrate (BHBA) accumulate in the blood (Goff and Horst, 1997; Doepel et al., 2002). This accumulation has been associated with suppressing neutrophil function, leading to an increase in disease incidence (Suriyasathaporn et al., 2000; Hammon et al., 2006; Scalia et al., 2006). In a study examining the impact of NEFA concentrations and compositions, Contreras et al. (2012) concluded that NEFA profiles that mimic those of the transition period increased the production of pro-inflammatory eicosanoids via an increased expression of the cyclo-oxygenase-2 enzyme (COX-2). In another study, serum NEFAs collected from transition dairy cows

reduced the function and proliferation of peripheral blood mononuclear cells and decreased phagocytosis and oxidative burst capabilities of neutrophils in cell culture (Ster et al., 2012).

Hypocalcemia has been associated with elevated cortisol concentrations (Horst and Jorgensen, 1982), reducing the ability of peripheral blood mononuclear cells to recognize pathogens (Kimura et al., 2006), and decreasing the concentration and percentage of neutrophils undergoing phagocytosis and oxidative burst (Martinez et al., 2012; Martinez et al., 2014). Cows with subclinical hypocalcemia were at an increased risk for metritis, in addition to elevated NEFA (Martinez et al., 2012; Martinez et al., 2014) and BHBA concentrations (Martinez et al., 2012). Furthermore, hypocalcemia increases the likelihood of mastitis and retained placenta due to the loss of teat sphincter and uterine tone, respectively (Goff and Horst, 1997). Thus, when examining the above studies jointly, many metabolic disorders (ketosis and hypocalcemia) are interconnected with infectious disease due to immunosuppression (Aleri et al., 2016). In many ways, disease incidence during the periparturient period could be characterized as demonstrating the domino effect – one disease typically leads to another. Preventing the first domino from falling is key in improving animal welfare during the transition period.

Inflammation during the transition period

The periparturient period is characterized by an uncontrolled inflammatory response resulting in immune dysfunction, and an increase in disease incidence (Sordillo and Raphael, 2013). After calving, inflammation has been documented in both healthy and diseased cattle (Humblet et al., 2006; Bionaz et al., 2007; Huzzey et al., 2009; Graunard et al., 2012; Mullins et al., 2012; Qu et al., 2014). Past research has also demonstrated a large increase in pro-inflammatory eicosanoids immediately post-calving that decreased during the first week of lactation (Yuan et al., 2013). These data suggest that cattle experience at least some degree of

inflammation due to tissue damage associated with parturition, as well as due to onset of lactation. Uterine neutrophil populations, as well as the production of pro-inflammatory mediators such as IL-6 and IL-8, are much greater during the first week of lactation when compared to 42 Days In Milk (DIM; Yuan et al., 2015).

Haptoglobin, an acute phase protein, has been used widely as an indicator for inflammation. Elevated levels of haptoglobin during the first week after parturition have been discovered in healthy cows (Humblet et al., 2006; Bionaz et al., 2007; Graugnard et al., 2012; Qu et al., 2014), and are markedly higher in diseased cattle (Humblet et al., 2006; Qu et al., 2014). The odds of an elevated concentration of haptoglobin were 2.5 times greater in multiparous animals that experienced an assisted calving when compared to cows that did not require assistance (Pohl et al., 2015). Moreover, elevated levels of haptoglobin during the first 2 to 8 d of lactation have been associated with an increase in days open, indicating that a stronger acute phase response just after calving resulted in a diminishing of reproductive performance (Nightingale et al., 2015). These data concur with Dematawewa and Berger (1997) who demonstrated that dystocia impaired reproductive performance.

During lactation, nutrient demand outstrips feed intake, and consequently, dramatic changes in biological and metabolic processes occur, resulting in the breakdown of fat for energy. After parturition, total lipids in the liver has been positively correlated with acute phase proteins, haptoglobin and serum amyloid A (Ametaj et al., 2005). Inflammatory states have been documented in liver and adipose tissue during the first week of lactation (Loor et al., 2005; Sadri et al., 2010; Saremi et al., 2012; Gessner et al., 2013). Cows with reduced liver activity had elevated inflammatory states after calving, and these cows had lower blood calcium concentrations, more disease incidences, took longer to conceive, and produced less milk during

the first 4 wk of lactation when compared to cows with higher liver activity (Bertoni et al., 2008).

Furthermore, adipose tissue has been shown to produce pro-inflammatory cytokines such as Tumor Necrosis Factor ($TNF\alpha$), which can lead to low-grade inflammation in obese subjects (Hotamisligil et al., 1993); therefore, it may be possible that over-conditioned cows may experience low-grade inflammation. When other factors added in such as heat stress, social stress, diet changes, and contributing disease events, it can be collectively summarized that the above elements of the transition period set the stage for inflammation in the periparturient dairy cow (Figure 1.1, adapted from Bradford et al., 2015).

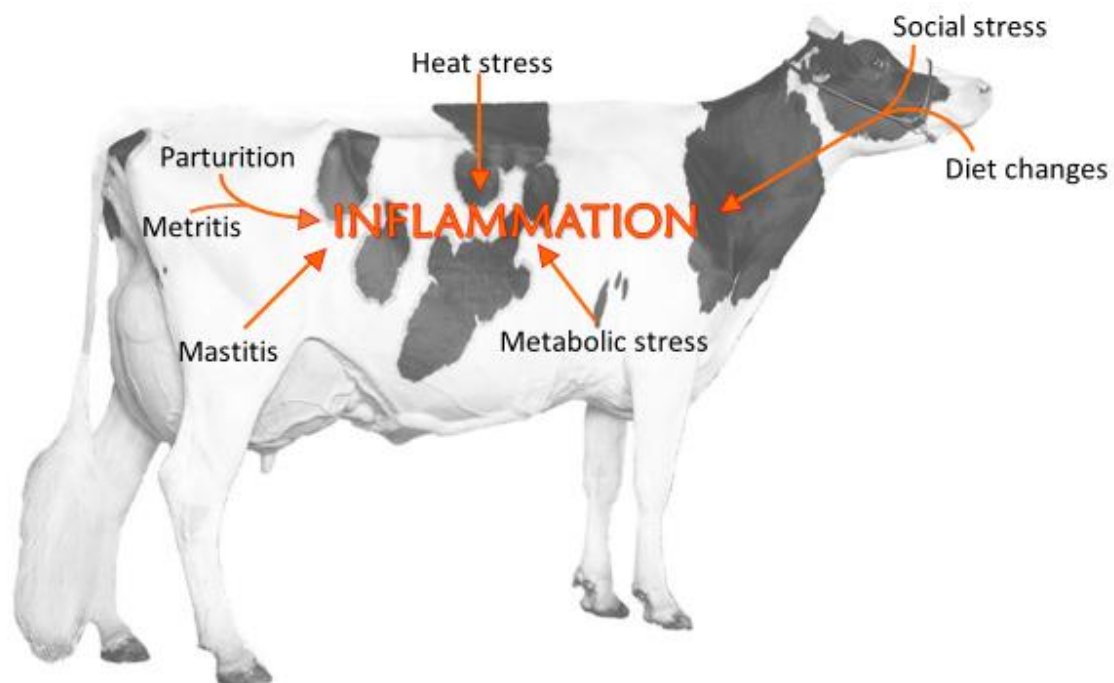


Figure 1.1 Factors associated with inflammation in the periparturient dairy cow. Figure adapted from Bradford et al., 2015. Cow photo credited to Cybil Fisher.

Inflammation requires a delicate balance. Too little inflammation insufficiently stimulates the immune system to clear a pathogen challenge; however, too much inflammation is associated with tissue damage, necrosis, and a loss of function (Serhan et al., 2008). Due to the excessive

amount of inflammation that occurs post-calving, an emerging hypothesis holds that the pro-inflammatory state may be contributing to immune dysfunction during the transition period. After calving, the inflammatory response is typically described as uncontrolled, and can result in a dysfunctional immune system (Sordillo and Raphael, 2013). For example, following LPS (lipopolysaccharide) challenge during early lactation, bovine peripheral blood mononuclear cells produced more TNF α , further enhancing the inflammatory response, as compared to LPS challenge during mid to late lactation (Sordillo et al., 1995). Increases in inflammatory cytokines, such as TNF α , have been associated with an increase in the severity of mastitis (Shuster et al., 1996). The implications of this heightened inflammatory state is not completely understood; however, inflammatory markers, such as haptoglobin, are markedly higher preceding clinical disease onset in cattle (Qu et al., 2014). Aside from milk fever (clinical hypocalcemia), clinical disease incidences during the periparturient period have remained unchanged in industry over the past few decades (Goff, 2006; USDA-APHIS, 2008; Bradford et al., 2015). Intervention strategies aimed at alleviating inflammation following parturition may be key in reducing disease incidence. Therefore, in this dissertation, the main focus is on mediation of inflammation around calving.

Calving

Typically, parturition is described in three separate stages. The first stage is characterized as the preparation of the birth canal for delivery of the fetus. During this stage, an onset of myometrial contractions allow for placement of the fetus for expulsion, and cervical dilation is initiated (Noakes et al., 2001b). Fetal cortisol induces the conversion of progesterone to estrogen through the enzymatic actions of 17 α -hydroxylase-C17,20-lyase (Lye, 1996; Schuler et al., 2006a; Schuler et al., 2006b). Consequently, as the cow approaches the onset of parturition,

progesterone declines, and estrogen increases (Smith et al., 1973; Schuler et al., 1994; Lye, 1996). The change in the progesterone to estrogen ratio plays a critical role in activating the myometrium and cervix for delivery of the fetus, as well as increasing the synthesis of prostaglandins (Lye, 1996). Prostaglandin F-2 α (PGF-2 α) is known to stimulate uterine contractions, and a large selective increase of PGF-2 α occurs immediately prior to parturition (Mitchell et al., 1976; Olson et al., 1984). Cortisol induces an up-regulation of COX-2 expression (Whittle et al., 2000b), occurring prior to luteolysis, suggesting that COX-2 increases PGF-2 α , which initiates parturition through lysing of the corpus luteum, causing the withdrawal of progesterone (Schuler et al., 2006b). In sheep, prostaglandin E2 (PGE2) progressively rises concurrently with cortisol concentrations during late gestation suggesting that PGE2 may act as a positive mediator, being part of a feedforward loop between the hypothalamic-pituitary-adrenal axis and intrauterine PGE2 production, and potentially promoting placental estradiol production (Whittle et al., 2000a, Whittle et al., 2001).

The second stage of parturition is marked by the appearance of abdominal contractions, amniotic sac presence, and expulsion of the fetus (Mainau and Manteca, 2011). Cervical widening caused by propulsion forces from the uterus allows for the fetus to pass into the birth canal (Taverne, 1992). The maternal birth canal distends, resulting in a large increase in oxytocin release, further amplifying uterine contractions (Noakes et al., 2001b). In many species, estrogen is involved with an increase of oxytocin receptors in the uterus, allowing for greater oxytocin sensitivity; however, in the bovine, an increase in oxytocin release, rather than elevated oxytocin binding capability, is the cause of the intensification of myometrial contractions (Taverne, 1992).

The final stage of parturition is the expulsion of the fetal membranes (Mainau and Manteca, 2011). Uterine contractions persist during this stage, but, decrease in intensity and

become less frequent. It should be noted that our understanding of the initiation of parturition in the bovine is still limited, and that much of the research performed in the past has been in the sheep model. A hypothetical model of the initiation of parturition in the bovine has been outlined in Shenavai et al. (2012) and readers are directed to that paper for further details. Additional studies performed in the bovine model may provide further insight in parturition in dairy cows, potentially providing additional clues into different techniques in preventing transition cow disorders.

Dystocia

Dystocia is defined as difficult or delayed birth, typically resulting in an assisted extraction of the calf (Mee, 2004). The incidence of dystocia can vary from herd to herd, however, literature suggests a range of 10 to 40 percent of calving events will be a difficult birth (Meyer et al., 2001; Lombard et al., 2007; USDA-APHIS, 2010). In a study examining dystocia trends from 1985 to 1996, Meyer et al. (2001) reviewed 666,341 calving records, and reported a dystocia incidence of 28.6 percent in primiparous and 10.7 percent in multiparous cows. More recently, dystocia incidence has been reported to be approximately 18.6 percent in primiparous and 10.8 percent in multiparous cows (USDA-APHIS, 2010).

Another study (Lombard et al., 2007) conducted on 7,380 calvings reported 51.2 percent of calves born from primiparous dams required assistance at birth, and when including multiparous animals, this study had an overall dystocia incidence of 37 percent. They also examined the effect of dystocia on calf morbidity and mortality. Heifer calves born difficultly were 20.7 times more likely to result in a stillborn calf when compared to eutocic calvings. Furthermore, severe dystocia-born heifer calves were 6.7 times more likely to die during the first 120 days of life when compared to eutocia-born calves, as the odds of digestive and respiratory

disorders were higher (1.3 and 1.7 odds ratio (OR), respectively), as well as stillbirth rate (20.7 OR; Lombard et al., 2007). In another study, dystocia increased the likelihood of retained placenta, displaced abomasum, and metritis in cows (Correa et al., 1993). Dematawewa and Berger (1997) found that dystocia resulted in a decrease in milk, fat, and protein yields, and an increase in days open, number of services, and mortality rates.

Dystocia is typically quantified in a scoring system using the amount of assistance as the determining factor for the degree of difficulty. A 1 to 5 scale (1 = no assistance, 2 = assistance by one person with no mechanical extraction needed, 3 = assistance by 2 or more people, 4 = mechanical extraction of the calf, and 5 = surgical procedure; Lombard et al., 2007) has been used in research, as well as a similar 5-point scale for genetic evaluations. Other research studies have used a simpler 3-point scale (1 = no assistance, 2 = easy assistance typically with only one person pulling the calf, and 3 = needed assistance with 2 or more people pulling; Meyer et al., 2001), and variations of the above scoring systems have been utilized (Newby et al., 2017). However, these scoring systems are subjective and therefore can be variable between observers. More recently, the amount of time between the appearance of the amniotic sac and the expulsion of the calf (Stage 2 of labor) has been examined between eutocic and dystocic calvings (Schuenemann et al., 2011). It has been generally accepted that normal bovine labor can vary from 30 min to 4 h, with an average of approximately 70 min (Noakes et al., 2001a). However, Schuenemann and coworkers (2011) found that the amount of time that elapsed during Stage 2 of labor is about twice as long in dystocic calvings when compared to eutocic calvings (86.7 vs. 45.2 min, respectively). The authors concluded that calving personnel should begin assisting cows 70 min after amniotic sac presence to help prevent potential stillbirth and injury to the dam (Schuenemann et al., 2011). Because of the subjectivity of scoring systems, it may be more

useful to quantify dystocia utilizing the length of Stage 2 labor rather than calving difficulty scores.

There are a number of reasons why dystocia occurs; however, the two most common causes of dystocia are fetal-maternal mismatch and mal-presentation of the fetus (Mainau and Manteca, 2011). Fetal-maternal mismatch or feto-pelvic disproportion is the most common cause of dystocia (> 50 percent of assisted births; Meijering et al., 1984), and is more common in primiparous cattle. Fetal gender, twinning, sire of the fetus, breed, nutrition, gestation length, weight of the dam, parity, and body condition of the dam are all factors that play a role in predisposing cattle to fetal-maternal mismatch (Mee, 2008). For every 1 kg increase in birth weight, there is a 13 percent increase in the odds of dystocia (Johanson and Berger, 2003). Therefore, strategies to reduce the number of calves with large calf birth weights, such as proper nutrition and the use of calving-ease sires, are sometimes employed on-farm to reduce dystocia incidence (Mainau and Manteca, 2011).

Fetal mal-presentation is the most common cause of dystocia in multiparous cows, representing 20 to 40 percent of dystocic cases (Meijering, 1984). Abnormal presentations of the fetus most commonly exhibited are posterior mal-presentation, foreleg mal-posture, breech presentation, or cranial mal-posture, in that order from most to least prevalent (Noakes et al., 2001a, Mee, 2008). Fetal mal-presentation is influenced heavily by twinning (Echternkamp and Gregory, 1999), as well as sex of the calf (male fetuses twice as likely than female fetuses), and fetal mortality (Holland et al., 1993, Mee, 2008, Mainau and Manteca, 2011). Other causes such as hypocalcemia (Curtis et al., 1983) and uterine torsion (Mee, 2008) represent a minority of dystocic calving events.

Behavioral and physiological changes occurring around calving

Much research has been completed on identifying behavioral and physiological changes occurring directly prior to calving in an effort to develop an alert system for calving events. Development of calving identification technologies has the capability to potentially reduce the negative consequences of dystocia. However, the timing of obstetrical assistance is key, as premature assistance (assistance within 70 min of presence of the amniotic sac) has been associated with more stress on the dam, vaginal lacerations, retained placenta, inhibition of maternal behaviors such as licking the calf, and higher stillbirth rates when compared to appropriately timed assistance (Kovacs et al., 2016a,b). Therefore, these technologies must be used appropriately. In fact, it may be more useful to identify the calving events that will require assistance, such as severe dystocia and malpresented calves, rather than identifying all calving events. To assess the accuracies of these tools, sensitivity (Se), the proportion of actual positives identified correctly, and specificity (Sp), the proportion of actual negatives identified correctly, are utilized.

Due to the dramatic endocrine changes occurring around parturition, some researchers have examined the ability of assays to identify the onset of calving events. As parturition approaches, circulating levels of estrone sulfate (E1S) and estradiol (E2) increase (Shah et al., 2006), as progesterone (P4) declines (Matsas et al., 1992; Streyll et al., 2011). For calving event identification within 24 h, the increase in E2 resulted in poor Se (Shah et al., 2006), and while the decrease in P4 resulted in acceptable Se (> 87 percent; Matsas et al., 1992), these methods are time consuming, expensive, and limited in application in the field (Saint-Dizier and Chastant-Maillard, 2015). Therefore, a large amount of research has been conducted examining other

clinical signs, physiological changes, and behavioral modifications that occur around the time of parturition.

Clinical signs of parturition, including teat filling and pelvic ligament relaxation, have been used with some success (Shah et al., 2006; Streyll et al., 2011). As parturition approached, pelvic ligament relaxation increased gradually, and then dramatically increased one day prior to calving (Shah et al., 2006). When the depth of the sacrosciatic ligament was ≥ 0.5 mm, with 0.94 accuracy in mature cows, calving occurred within 24 h (Shah et al., 2006). Increasing the cut-off from ≥ 5 mm to ≥ 8 mm led to higher Se, however, only about half of the cows exhibited this clinical sign.

Streyll and colleagues (2011) developed a parturition score based on clinical signs of calving: pelvic ligament relaxation and teat filling. Teat filling was scored on a 0 to 3 scale, with 0 representing a flaccid teat, and 3 representing a completely filled teat. The pelvic ligament relaxation score was scored on a 0 to 6 scale (0, 2, 4, and 6) to double the weight of the pelvic ligament relaxation in the total parturition score, and this variable was based on the softening and ability to palpate the ligament. The resulting combination of pelvic ligament relaxation and teat filling led to a 0.89 Se and 0.60 Sp, however, there was a great disparity between multiparous and primiparous animals (0.95 vs. 0.56 Se and 0.58 vs. 0.74 Sp for multiparous and primiparous, respectively). While identification of these clinical signs has merit in identifying calving, currently, none of these signs can be measured with automation, and therefore are very labor intensive. Development of automated technologies to identify calving events is warranted.

Vaginal and rectal temperature collected via data loggers have shown promise in calving identification. Rectal temperature decreased approximately 0.4°C (Burfeind et al., 2011), and vaginal temperature declined approximately 0.6°C when comparing the day of calving to 48 h

prior (Burfeind et al., 2011; Ouellet et al., 2016). Using a $\geq 0.1^{\circ}\text{C}$ decline within 24 h of calving, moderate Se and Sp for both rectal and vaginal temperature of approximately 0.70 to 0.80 were found, respectively (Burfeind et al., 2011; Ouellet et al., 2016). Similarly, reticuloruminal temperature has shown similar declines in temperature, with cows that will experience a dystocic calving displaying a drop in temperature 32 h prior to calving compared to 20 h prior to calving for eutocic calvings, suggesting the possibility of utilizing reticuloruminal temperature as an indicator of dystocia (Kovacs et al., 2017). Recent automated technologies utilizing vaginal temperature have become available to dairy producers as a calving identification tool (Vel'Phone, Medria, Châteaubourg, France). This device is inserted into the vaginal cavity of the cow and utilizes a drop in vaginal temperature to identify calving events to occur within 48 h. When the cow begins to calve, the thermometer is expelled during Stage 2 of labor, thus resulting in a drop in temperature once outside the body of the cow, and this drop in temperature is used to identify impending calving events.

Behavioral changes occurring around parturition have been heavily investigated. Behaviors such as lying time, lying and standing bouts, activity, tail raising, as well as feeding and drinking behaviors have been evaluated for their ability to identify calving. Data collected from tri-axial accelerometers have shown that cows were more active, spent less time lying, and exhibited more transitions from lying to standing on the day of calving when compared to the days preceding calving (Huzzey et al., 2005; Miedema et al., 2011a,b; Jensen, 2012). Cows that would experience a difficult calving exhibited more standing bouts (Proudfoot et al., 2009) and lying bouts (Yeiser, 2011) during the final day prior to calving or on the day of calving than cows that had eutocic calvings. Furthermore, recent studies have demonstrated that animals that experienced dystocia have a reduction in activity after calving (Barragan et al., 2017c). Lying

bouts and lying time were evaluated for accuracy in calving identification, however, the Se and Sp were disappointing (< 0.70 for Se and < 0.60 for Sp for both lying behaviors identifying calving within 24 h; Ouellet et al., 2016). Nevertheless, it appears that the change in lying behaviors and activity is isolated to the few hours directly prior to calving (Jensen, 2012; Ouellet et al., 2016), possibly hampering the development of a timely alert system. Potentially the increase in lying and standing bouts on the day of calving could be used as an indicator for animals that will experience a difficult calving (Proudfoot et al., 2009; Yeiser, 2011).

Dramatic changes in feeding behavior occur directly prior to calving. Feeding and drinking time, rumination time, water intake, and dry matter intake (DMI) have all shown alterations prior to parturition. Feeding time begins to decline 3 wk prior to calving (Bao and Giller, 1991), and this decline accelerates in the final 24 h before parturition (Proudfoot et al., 2009; Schirmann et al., 2013; Buchel and Sundrum, 2014), with a dramatic decrease during the final 2 h (Jensen, 2012). Proudfoot and colleagues (2009) found no difference in drinking behaviors around the time of calving; however, cows that would have a difficult calving consumed less water in the 24 h prior to calving when compared to cows with eutocic calvings. In contrast, Jensen (2012) found that cows approaching calving would spend less time drinking in the final 2 h prior to delivery. Dry matter intake tends to decline in the final 24 h prior to calving (Schirmann et al., 2013), and this decline is amplified in the final 6 h (Buchel and Sundrum, 2014). In agreement with water intake, Proudfoot and colleagues (2009) found that cows that would experience a difficult calving consumed less dry matter during the final 24 h prior to calving than cows that would experience a eutocic calving. Lastly, coinciding with feeding time and DMI, rumination time declined starting approximately 24 h prior to calving (Schirmann et al., 2013; Ouellet et al., 2016; Kovacs et al., 2017), and displayed a dramatic

decline within the final 6 h prior to delivery (Buchel and Sundrum, 2014; Pahl et al., 2014; Ouellet et al., 2016). Ouellet and coworkers (2016) tested the accuracies of rumination time for calving identification and found both Se and Sp values of approximately 0.5 for identification of calving within 24 h (using 3.6 min/6 h decline as a cut-off), and approximately 0.6 for calving identification within 6 h (using 12 min/6 h decline as a cut-off).

Finally, a combination of vaginal temperature, rumination time, lying bouts, and lying time were investigated for their accuracy in identifying calving events (Ouellet et al., 2016). When combining vaginal temperature, rumination time, lying bout, and lying time, a moderate Se and Sp of 0.77 was found for identification of calving within 24 h (Ouellet et al., 2016). For identification of calving within 6 h, a combination of vaginal temperature, rumination time, and lying bouts yielded the best results with both Se and Sp values of 0.71 (Ouellet et al., 2016). Unfortunately, the combination of multiple variables did not improve accuracies appreciably. Moreover, it may not be economically feasible, or justifiable, to utilize multiple technologies for the use of calving identification.

Inflammatory cascade

Tissue injury or insult initiates the inflammatory cascade (Maroon et al., 2010). Damage to cell walls leads to the breakdown of phospholipids that are converted to arachidonic acid through the enzymatic action of phospholipase A2. Arachidonic acid is then transformed into prostaglandins and thromboxanes partially due to the action of cyclooxygenases (COX; Maroon et al., 2010). Two types of COX enzymes exist, COX-1 and COX-2. Cyclo-oxygenase-I is involved with gastric cytoprotection, renal water balance, and platelet aggregation (Morita, 2002), and consequently, COX-1 inhibitors are associated with an increased risk for adverse gastrointestinal and renal effects (Coetzee, 2013). The function of COX-2, however, is more

associated with inflammation (Morita, 2002). Therefore, much work has been done on developing drugs that selectively inhibit COX-2 to reduce inflammation, while preventing the consequences of blocking COX-1.

NSAIDs

Non-steroidal anti-inflammatory drugs (NSAID) are COX inhibitors. NSAIDs exist in three classes – nonselective (nonspecific), preferential, and selective. The NSAIDs that inhibit both COX-1 and COX-2 are referred to as nonselective COX inhibitors, whereas NSAIDs with partial selectivity for COX-2 are deemed as COX-2 preferential. Selective COX-2 NSAIDs only inhibit COX-2, however, this type of NSAID is only available in human medicine. In the United States, flunixin meglumine is the only FDA-approved NSAID for use in cattle (Coetzee, 2013). Flunixin meglumine is both a COX-1 and COX-2 inhibitor, and has greater anti-COX-1 activity than most other NSAIDs (Beretta et al., 2005). The elimination half-life of flunixin meglumine is 3 to 8 h (Anderson et al., 1990), and therefore requires daily dosing (Coetzee, 2013). A 4 d meat withhold and 36 h milk withhold after the final IV injection of flunixin meglumine has been established by the FDA (Coetzee, 2013).

Salicylic acid derivatives, such as acetylsalicylic acid (aspirin) and sodium salicylate, have been used in cattle for their anti-pyretic, analgesic, and anti-inflammatory effects; however, the use of these NSAIDs have not been approved by the FDA. The half-life of salicylates is only about 0.5 h in cattle (Gingerich et al., 1975; Kotschwar et al., 2009); however, when administered orally, the elimination half-life can be longer due to the rumen acting as a slow-release reservoir (Coetzee, 2013). Salicylates inhibit both COX isoforms, however, aspirin is more potent at inhibiting COX-1 than COX-2, and sodium salicylate is a weak inhibitor of both (Mitchell et al., 1993). Lastly, meloxicam has been approved for use in cattle in many European

countries as well as Canada. Meloxicam is a COX-2 preferential inhibitor in most species (Beretta et al., 2005), however, this affinity has not been proven in cattle. Meloxicam has an elimination half-life of 23 to 27 h in low producing and 17.5 h in high producing lactating cattle (EMA, 2007). In approved countries, a withdrawal period of 5 d for milk and 15 d for meat in European countries (EMA, 2007), and a 4 d milk withhold and a 20 d meat withhold in Canada have been established (Boehringer Ingelheim (Canada) Ltd., 2017).

The use of NSAIDs in the periparturient cow

A few studies have examined the effect of NSAIDs on the periparturient cow with a variety of results. The administration of flunixin meglumine around the time of calving has largely been unsuccessful (Shwartz et al., 2009, Newby et al., 2017). Shwartz and coworkers (2009) found that flunixin-treated cows consumed less feed over the first 5 wk of lactation, had a slight increase in rectal temperature, and produced similar milk yield (MY) when compared to controls. Likewise, Newby and colleagues (2017) found that flunixin-treated cows had an increased risk for retained placenta, elevated temperature, decreased MY, and a higher incidence of metritis when compared to controls. In fact, when flunixin meglumine was administered prior to calving, treated cows delivered a higher percentage of stillborn calves when compared to controls (26.5 percent vs. 5.3 percent; Newby et al., 2017).

Sodium salicylate and acetylsalicylic acid have been used in the periparturient cow with some success (Trevisi and Bertoni, 2008; Farney et al., 2013a,b; Grossi et al., 2013; Carpenter et al., 2016; Barragan et al., 2017a,b,c). Farney and colleagues (2013b) discovered that mature cows treated with sodium salicylate had higher whole lactation milk and milk fat yields when compared to controls; however, no difference was seen in the first or second parity groups. Furthermore, sodium salicylate treatment suppressed metabolic inflammation by reducing liver

mRNA TNF- α when compared to controls (Farney et al., 2013a). With that said, sodium salicylate-treated mature cows were also at a much higher risk for metritis when compared to controls (Farney et al., 2013b). Carpenter and coworkers (2016) found that sodium salicylate increased daily MY as well as 305-d milk and milk protein yields, with the majority of this increase noted during peak MY. Likewise, administration of acetylsalicylic acid after calving increased milk by 1 kg/d for the first 5 mo of lactation in organic herds (Barragan et al., 2017a), and Trevisi and Bertoni (2008) found that the administration of aspirin over the first 5 d of lactation resulted in a 13 percent increase in peak MY. Furthermore, aspirin-treated cows had a lower haptoglobin concentration 24 h after calving (Barragan et al., 2017b), and cows that calved easily and received aspirin were more active than eutocic control cows (Barragan et al., 2017c). However, when lysine acetylsalicylate (acetylsalicylic acid) was administered IM daily for 8 d prior to calving, treatment increased the incidence of retained placenta, as well as haptoglobin concentrations after calving (Grossi et al., 2013). It appears that the administration of salicylates after calving (12 h or later postpartum) increases MY, however, administration prior to calving may be problematic.

The effect of meloxicam on the periparturient cow has been mixed (Newby et al., 2013; Mainau et al., 2014; Carpenter et al., 2016). Meloxicam-treated dystocic cows exhibited an increase in feeding time and bunk visit frequency when compared to dystocic controls (Newby et al., 2013), in addition to meloxicam-treated primiparous eutocic animals had higher activity levels when compared to control eutocic heifers (Mainau et al., 2014). From these two studies, MY was similar for meloxicam-treated cows and controls (Newby et al., 2013; Mainau et al., 2014); however, it should be noted that these studies only monitored MY during the beginning of lactation before peak milk production (30 DIM or less). Additionally, both of these studies

administered meloxicam at 0.5 mg/kg of body weight subcutaneously. Carpenter and coworkers (2016) administered oral meloxicam at approximately 1 mg/kg of body weight. In this study, an increase in daily MY was seen in multiparous cows resulting in higher 305-d milk and milk protein yields, with the majority of this increase being attributed to a large increase in peak MY (Carpenter et al., 2016). Lastly, contrary to the effects of flunixin meglumine, meloxicam treatment did not increase the incidence of retained placenta (Newby et al., 2014).

Carprofen is another NSAID available to dairy producers in the European Union (Coetzee, 2013), however, the chief mode of action of this NSAID is unclear, and thought to be of something other than inhibition of COX in cattle (Delatour et al., 1996). In New Zealand, the administration of carprofen after calving did not elicit a milk response (Meier et al., 2014); however, these cows were managed in a rotational grazing system with a lower MY than of most other studies (Farney et al., 2013b; Carpenter et al., 2016). The authors argued that differences in metabolic stress in the cows between their study and past studies could be contributing to the lack of milk response from NSAID therapy. Another NSAID, ketoprofen, was administered following calving, and again 24 h later, had no impact on MY for the first individual milk recording of the lactation; however, ketoprofen-treated cows had a lower incidence of retained fetal membranes than controls (Richards et al., 2009). It should be noted that in Meier et al. (2014) as well as Richards et al. (2009) MY was only recorded in early lactation (6 wk or less); whereas, recent studies have reported that the effect of NSAID therapy may not become apparent until later in lactation (Carpenter et al., 2016).

It can be concluded from these studies that the administration of either salicylate derivatives or oral meloxicam after calving can increase peak MY. However, it is still unknown whether the administration of meloxicam prior to calving can mitigate the inflammatory response

that occurs during parturition, thus allowing for an easier calving. Inhibition of the inflammatory response during calving could improve welfare after calving by reducing disease incidences, and consequently resulting in higher MY. Finally, additional studies examining the effect of carprofen and ketoprofen on peak MY are needed.

Pre-weaning period

The pre-weaning period represents the period of time with the highest mortality rate of any time period during that animal's life (USDA-APHIS, 2010). Neonatal mortality has a wide range of causes from genetics, maternal illness, poor environmental conditions, failure of passive transfer, and disease events such as dystocia (Azzam et al., 1993; Uetake, 2013). Approximately 8 percent of calves are born dead or die within 48 h, and an additional 8 percent of calves die prior to weaning (USDA-APHIS, 2010). The majority of mortality (not including stillbirths) that occurs during the pre-weaning period is attributed to scours and then respiratory disease (USDA-APHIS, 2010); however, dystocic calvings increase the likelihood of the calf acquiring one of these diseases, in addition to dramatically increasing the odds of the calf being born dead when compared to eutocic calvings (Lombard et al., 2007). Therefore, much research has examined the effect of disease events, dystocia, and potential intervention strategies on calf performance.

Group housing and automatic calf feeders

The use of automatic calf feeders in pre-weaned calves is gaining popularity as dairy producers look to reduce labor costs and switch from individual-housing to group-housing (Kung et al., 1997). This technology requires a different style of calf management – the amount of time spent feeding calves will be greatly diminished; however, laborers will have to diligently monitor calves for disease. Automatic calf feeders as well as social housing benefit calves in many ways.

Automatic calf feeders allow calves to consume greater amounts of milk spread over more meals than traditional feeding systems. High milk intake diets have been associated with increased growth rates and these positive effects carry over into adulthood, as an increase in first lactational MY has been found (Soberon et al., 2012). Group- and pair-housing of pre-weaned calves increases in concentrate intake, and average daily gain, with these increases carrying through into the weaning period (Miller-Cushon and DeVries, 2016; Pempek et al., 2016). After weaning, Miller-Cushon and DeVries (2016) conducted a preference test to determine if housing type (individual housing versus pair housing) would impact eating habits, specifically examining if housing type influenced social and competitive feeding behaviors. A Y-maze was set-up, and a non-test calf was tethered on one of the sides of the maze. Then, a test calf was placed into the pen, and given the option to eat grain on either side of the Y-maze. Pair-housed calves spent more time eating with the non-test calf than individually-housed calves, suggesting that pair-housing during the pre-weaning period resulted in more social calves after weaning (Miller-Cushon and DeVries, 2016). Another preference test was performed, however, this time feed access was limited, specifically measuring competitive feeding behaviors. Again, a non-test calf was tethered to one side of the Y-maze, and a test calf was allowed to choose a side of the Y-maze to eat. Pair-housed calves were more likely to compete with the non-test calf for feed access than individually-housed calves, providing evidence that social housing during the pre-weaning period leads to more competitive feeding behaviors after weaning (Miller-Cushon and DeVries, 2016). Additional studies measuring this alteration in feeding behavior during adulthood is warranted, as group-housing during the pre-weaning period may have advantages that carry into adulthood and thus impact MY and other production measures. Pair-housed calves have also demonstrated an improved cognitive performance, as they were able to adapt to

environmental changes more quickly when compared to individually-housed calves (Gaillard et al., 2014).

While there are numerous benefits of group-housed calves on an automatic calf feeder, identifying diseased calves in large group sizes can be difficult when compared to individually-housed calves (Svensson et al., 2003). Mortality associated with respiratory disease in pre-weaned calves is 22.5 percent, and is approximately 56.5 percent for scours (USDA-APHIS, 2010). Therefore, the development of technologies to aid in the identification of these diseases is warranted.

Past research studies have examined feeding behavior around disease events utilizing an automatic calf feeder. Unrewarded visits are when a calf accesses the automatic calf feeder, however, at that time, no milk allowance is allotted to the calf. These unrewarded visits occur due to a calf consuming all of the allotted milk allowance recently, and therefore, the calf must wait until a milk allowance is provided. Unrewarded visits may be an indicator of appetite. Diseased calves had fewer unrewarded visits when compared to healthy calves; however, no difference was seen for drinking speed, milk consumption, and rewarded visits (Svensson and Jensen, 2007). In a field study, behavioral changes due to different disease events were identified (Knauer et al., 2017). Calves with scours drank milk more slowly beginning a few days before medical treatment and this reduction persisted until 10 d after medical treatment when compared to healthy controls; whereas, calves with respiratory disease drank milk more slowly on the day of medical treatment only (Knauer et al., 2017). Regardless of disease type, sick calves drank less milk and had fewer unrewarded visits than healthy controls (Knauer et al., 2017). Borderas and coworkers (2009) examined the effect of disease on feeding behaviors and the interaction of milk allowance with the effect of disease. Diseased calves on a high milk allowance visited the

feeder less, drank less milk, and yet spent more time at the feeder per visit than healthy calves (Borderas et al., 2009). In opposition, for calves on a low milk allowance, sick calves spent less time at the feeder per visit and no difference was noted for milk intake and total visits (Borderas et al., 2009). Therefore, when analyzing data from automatic calf feeders, it is important to take into account the effect of milk allowance. Algorithms to detect disease status will likely vary greatly between milk allowances, and potentially between different disease events.

Finally, another study using an experimentally induced pneumonia design examined the impact of *Mannheimia haemolytica* on step activity. Calves demonstrated a reduction in step activity 24 h post-inoculation when compared to negative controls (Hanzlicek et al., 2010). Step activity may prove to be a valuable behavior to detect disease incidence. Because behaviors may vary between diseases, it may be valuable to analyze the effect of each disease independently. Furthermore, because milk allowance directly impacts the effect of disease, utilizing additional tools, such as activity and lying behaviors, may be more useful in disease detection.

Effect of dystocia and potential intervention strategies on calves

The effect of dystocia on the newborn calf can be highly variable. The degree of trauma is dependent on a multitude of risk factors such as calf birth weight, calf gender, gestation length, calf presentation, genetics, breed, nutrition, climate, and maternal factors such as pelvic dimensions, parity, age, and body condition (Mee, 2008). Any one of these factors or a combination of factors can lead to a dystocic calving (Murray et al., 2016), and therefore, can lead to an increase in the odds of mortality (Meyer et al., 2001; Lombard et al., 2007) and morbidity (Lombard et al., 2007). Attempts to quantify the impact of calving on the calf has been developed utilizing a scoring system similar to the Apgar score used for human babies. Murray and colleagues (2015) developed a VIGOR score based on visual appearance, initiation of

movement, general responsiveness, oxygenation, and heart and respiratory rates. Based on the scoring system, calves born with assistance had reduced vigor when compared to calves born without assistance (Murray et al., 2015a; Murray et al., 2016).

Other physiological and behavioral measurements have been used to quantify the effect of dystocia on calves. Calves born with a hard pull took longer to obtain sternal recumbency (Murray et al., 2015b), make attempts to stand, achieve standing (Diesch et al., 2004; Barrier et al., 2012), and had a weaker suckling response than calves born unassisted (Murray et al., 2015b). Furthermore, increases in respiratory rate, lactate, and creatine kinase concentrations, and a decrease in blood pH were found in dystocic calves (Murray et al., 2015b), in addition to elevated cortisol concentrations, more days with health treatments, and reduced passive immune transfer when compared to eutocic calves (Barrier et al., 2013). Calves born when parturition was longer than 6 h had lower venous pH and higher L-lactate concentrations immediately after birth than calves born when parturition was shorter than 6 h (Bleul and Gotz, 2013). The above studies suggest calves born difficultly were subjected to respiratory acidosis due to extended duration of calving (Bleul and Gotz, 2013; Murray et al., 2015b). Elevated creatine kinase concentrations in dystocic calves have been associated with muscle damage likely due to a difficult extraction of the calf during parturition (Murray et al., 2015b), as well as an association with reduced colostrum antibody absorption (Boyd, 1989). The combined effects from the above studies demonstrate the negative consequences of dystocia on calf health around the time of birth.

Additional studies have been performed examining the effects of dystocia during the pre-weaning period as well as into adulthood. Lombard and colleagues (2007) found that calves born with severe difficulty were 1.7 and 1.3 times more likely to have a respiratory or digestive

disease event, respectively when compared to calves born from unassisted calvings. Excluding stillborn calves, heifer calves born with severe difficulty were 1.5 times more likely to experience a disease event as well as 1.5 times more likely to die than calves born from unassisted calvings during the first 120 d of life (Lombard et al., 2007). However, this effect is not limited to just the pre-pubertal period of a calf's life. Using a 1 to 3 calving difficulty scoring system (1 = unassisted, 2 = easy pull, and 3 = hard pull, mechanical extraction, or cesarean section), for every single increase in calving difficulty score, while holding all other variables constant, the resulting female calf produced 285 kg less milk in her first lactation when compared to calves born with no assistance (Heinrichs and Heinrichs, 2011). Similarly, a study in the United Kingdom found that veterinary-assisted calvings resulted in calves that produced 710 kg less milk in their first lactation when compared to calves born without assistance (Eaglen et al., 2011). Because of the above negative effects, intervention strategies have been investigated to determine if the consequences of dystocia can be reduced on the dairy calf.

Lombard and coworkers (2007) suggested that simple interventions during calving could reduce the severity of dystocia. However, research has been yet to be completed examining the impact of obstetrical assistance for dystocic calving events on dairy calves. Recently, research has examined the impact of early obstetrical assistance (assistance provided 15 min after first sight of the calf's hooves) in normal calving events. Early obstetrical assistance reduced the likelihood of stillborn calves and improved calf vigor when compared to late obstetrical assistance, and had no negative impact on average daily gain, health, or mortality (Villetaz Robichaud et al., 2017a,b). Furthermore, calves that were born quickly (< 1 h from the presence of feet until delivery) regardless of whether assistance was provided had improved vigor scores when compared to calves that had late assistance (> 1 h from the presence of feet until delivery).

Yet, even in normal calving events, calves born with assistance took longer to achieve sternal recumbency than calves born without assistance (Villettaz Robichaud et al., 2017a). Additional studies examining the impact of obstetrical assistance in dystocic calving events are warranted.

Previous studies have examined the effect of treatments on the newborn calf around the time of delivery to diminish the negative effects of dystocia on the newborn calf (Murray et al., 2015a, Murray et al., 2016). Murray and coworkers (2015) conducted a large field study (n = 842) where calves were randomly assigned to receive a subcutaneous injection of meloxicam with the target dose of 0.5 mg of meloxicam per kg of body weight. Calves that were assisted at birth and received meloxicam gained 1.1 kg more body weight during the first week of life when compared to calves assisted at birth that received a placebo (Murray et al., 2015a). The opposite effect was seen in calves born with observation but without assistance, as meloxicam-treated calves born easily grew 1.4 kg less than control calves during the first week of life (Murray et al., 2015). These data suggest that meloxicam administration had a negative effect on calves born with ease (Murray et al., 2015a). Nevertheless, meloxicam-treated calves had improved health during the first 6 wk of age (Murray et al., 2015a). In a similar study, Murray and colleagues (2016) utilized the previously mentioned VIGOR scoring system. Meloxicam-treated calves had an improved VIGOR score 6 h after treatment, a stronger suckling reflex, and consumed more milk from an automatic calf feeder than control calves (Murray et al., 2016). In conclusion, meloxicam treatment in dairy calves shows promise in alleviating some of the detrimental effects of dystocia; however, additional research examining the impact of meloxicam treatment in the dam prior to calving and the potential effects on the dairy calf performance warrant investigation.

Research objectives

The transition period is characterized by large endocrine, metabolic, and immune changes due to fetal development and milk production. These changes can subject the cow to a multitude of diseases, at a time that is already noted as having a heightened inflammatory state. The purpose of this dissertation is to contribute a better understanding of inflammatory mediation around the time of calving. The overall objective is to examine the effect of meloxicam around the time of calving on the welfare of both the dam and the calf.

The first objective was to evaluate the effect of meloxicam on the dam both before and after calving. Treatment groups include meloxicam treatment prior to calving, meloxicam treatment post-calving, and a negative control. The objective of this study was to evaluate the administration of meloxicam prior to calving on behavior, health, and production measures. Furthermore, we compared the effect of meloxicam administration prior to calving to post-calving meloxicam administration to determine if the time of administration yielded different responses. We hypothesized that the administration of meloxicam prior to calving would provide greater improvement of animal welfare and production measures than post-calving administration, and that both treatment groups would outperform the controls. Moreover, because dystocia exacerbates the heightened inflammatory state that already occurs around calving, we anticipated that NSAID-treated cows that experienced a difficult calving would display a larger response to treatment than NSAID-treated cows that calved easily.

The second objective was to evaluate the effect of meloxicam treatment of the dam prior to calving on the newborn calf. Because cows treated with meloxicam prior to calving may have an easier delivery, the welfare of the newborn calf could be positively impacted. We hypothesized that calves born from meloxicam-treated dams prior to calving would be more

active, consume more milk, have less disease events, and have improved average daily gains than calves born from non-treated dams.

The third objective was to measure feeding, activity, and lying behaviors in dairy calves around disease events. The pre-weaning age represents the time period with the highest mortality rate of any time during that animal's life. The use of automated technologies to monitor behavioral changes around these disease events may allow for easier detection. We hypothesized that diseased calves would be less active, spend more time lying, consume less milk from the automatic calf feeder, drink milk at a slower speed, and visit the feeder less than healthy calves.

REFERENCES

- Aleri, J. W., B. C. Hine, M. F. Pyman, P. D. Mansell, W. J. Wales, B. Mallard, and A. D. Fisher. 2016. Periparturient immunosuppression and strategies to improve dairy cow health during the periparturient period. *Res. Vet. Sci.* 108:8-17.
- Ametaj, B., B. Bradford, G. Bobe, R. Nafikov, Y. Lu, J. Young, and D. Beitz. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. *Can. J. Anim. Sci.* 85:165-175.
- Anderson, K. L., C. A. Neff-Davis, L. E. Davis, and V. D. Bass. 1990. Pharmacokinetics of flunixin meglumine in lactating cattle after single and multiple intramuscular and intravenous administrations. *Am. J. Vet. Res.* 51:1464-1467.
- Azzam, S. M., J. E. Kinder, M. K. Nielsen, L. A. Werth, K. E. Gregory, L. V. Cundiff, and R. M. Koch. 1993. Environmental effects on neonatal mortality of beef calves. *J. Anim. Sci.* 71:282-290.
- Bao, J. and P. Giller. 1991. Observations on the changes in behavioral activities of dairy-cows prior to and after parturition. *Ir. Vet. J.* 44:43-47.
- Barragan, A. A., L. M. Bauman, L. d. Costa, J. Velez, J. D. R. Gonzalez, G. M. Schuenemann, and S. Bas. 2017a. Effects of oral administration of acetylsalicylic acid after parturition on milk

yield and milk components in lactating dairy cows under certified organic management (abstract). *J. Dairy Sci.* 100, Suppl. 2:176-177.

Barragan, A. A., L. M. Bauman, J. Lakritz, J. F. Coetzee, J. Velez, J. D. R. Gonzalez, G. M. Schuenemann, and S. Bas. 2017b. Assessment of the effects of oral administration of acetylsalicylic acid on biomarkers of inflammation and stress in dairy cows after parturition (abstract). *J. Dairy Sci.* 100, Suppl. 2:137-138.

Barragan, A. A., L. M. Bauman, J. Velez, J. D. R. Gonzalez, G. M. Schuenemann, and S. Bas. 2017c. Effects of oral administration of acetylsalicylic acid after parturition on activity patterns, prevalence of diseases, mortality and culling rates in dairy cows (abstract). *J. Dairy Sci.* 100, Suppl. 2:17.

Barrier, A. C., M. J. Haskell, S. Birch, A. Bagnall, D. J. Bell, J. Dickinson, A. I. Macrae, and C. M. Dwyer. 2013. The impact of dystocia on dairy calf health, welfare, performance and survival. *The Veterinary Journal* 195:86-90.

Barrier, A. C., E. Ruelle, M. J. Haskell, and C. M. Dwyer. 2012. Effect of a difficult calving on the vigour of the calf, the onset of maternal behaviour, and some behavioural indicators of pain in the dam. *Prev. Vet. Med.* 103:248-256.

Beretta, C., G. Garavaglia, and M. Cavalli. 2005. COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin, carprofen and meloxicam: An in vitro analysis. *Pharmacol. Res.* 52:302-306.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. *J. Dairy Sci.* 91:3300-3310.

Bionaz, M., E. Trevisi, L. Calamari, F. Librandi, A. Ferrari, and G. Bertoni. 2007. Plasma Paraoxonase, Health, Inflammatory Conditions, and Liver Function in Transition Dairy Cows. *J. Dairy Sci.* 90:1740-1750.

Bleul, U. and E. Gotz. 2013. The effect of lactic acidosis on the generation and compensation of mixed respiratory-metabolic acidosis in neonatal calves. *Vet. Rec.* 172:528.

Boehringer Ingelheim (Canada) Ltd. 2017. Metacam - Product information. <http://www.metacamcattle.ca/productinformation> (accessed 15 February 2018).

Borderas, T. F., J. Rushen, M. A. G. von Keyserlingk, and A. M. B. de Passillé. 2009. Automated measurement of changes in feeding behavior of milk-fed calves associated with illness. *J. Dairy Sci.* 92:4549-4554.

Boyd, J. W. 1989. Relationships between acid-base balance, serum composition and colostrum absorption in newborn calves. *Br. Vet. J.* 145:249-256.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. *J. Dairy Sci.* 98:6631-6650.

- Brambell, R. 1965. Report of the Technical Committee to Enquire into the Welfare of Animals kept under Intensive Livestock Husbandry Systems. London: Her Majesty's Stationary Office.
- Buchel, S. and A. Sundrum. 2014. Short communication: Decrease in rumination time as an indicator of the onset of calving. *J. Dairy Sci.* 97:3120-3127.
- Burfeind, O., V. S. Suthar, R. Voigtsberger, S. Bonk, and W. Heuwieser. 2011. Validity of prepartum changes in vaginal and rectal temperature to predict calving in dairy cows. *J. Dairy Sci.* 94:5053-5061.
- Burton, J. L., M. Kehrli, S. Kapil, and R. L. Horst. 1995. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. *J. Leukoc. Biol.* 57:317-325.
- Carpenter, A. J., C. M. Ylloja, C. F. Vargas, L. K. Mamedova, L. G. Mendonca, J. F. Coetzee, L. C. Hollis, R. Gehring, and B. J. Bradford. 2016. Hot topic: Early postpartum treatment of commercial dairy cows with nonsteroidal antiinflammatory drugs increases whole-lactation milk yield. *J. Dairy Sci.* 99:672-679.
- Coetzee, J. F. 2013. A Review of Analgesic Compounds Used in Food Animals in the United States. *Vet. Clin. North Am. Food Anim. Pract.* 29:11-28.
- Contreras, G. A., W. Raphael, S. A. Mattmiller, J. Gandy, and L. M. Sordillo. 2012. Nonesterified fatty acids modify inflammatory response and eicosanoid biosynthesis in bovine endothelial cells. *J. Dairy Sci.* 95:5011-5023.
- Correa, M. T., H. Erb, and J. Scarlett. 1993. Path analysis for seven postpartum disorders of Holstein cows. *J. Dairy Sci.* 76:1305-1312.
- Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, P. A. Powers, M. C. Smith, M. E. White, R. B. Hillman, and E. J. Pearson. 1983. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. *J. Am. Vet. Med. Assoc.* 183:559-561.
- Delatour, P., R. Foot, A. Foster, D. Baggo, and P. Lees. 1996. Pharmacodynamics and chiralpharmacokinetics of carprofen in calves. *Br. Vet. J.* 152:183-198.
- Dematawewa, C. M. and P. J. Berger. 1997. Effect of dystocia on yield, fertility, and cow losses and an economic evaluation of dystocia scores for Holsteins. *J. Dairy Sci.* 80:754-761.
- Diesch, T. J., D. J. Mellor, K. J. Stafford, and R. N. Ward. 2004. The physiological and physical status of single calves at birth in a dairy herd in New Zealand. *N. Z. Vet. J.* 52:250-255.
- Doepel, L., H. Lapierre, and J. J. Kennelly. 2002. Peripartum performance and metabolism of dairy cows in response to prepartum energy and protein intake. *J. Dairy Sci.* 85:2315-2334.
- Drackley, J. K. 1999. Biology of Dairy Cows During the Transition Period: the Final Frontier? *J. Dairy Sci.* 82:2259-2273.

- Eaglen, S. A. E., M. P. Coffey, J. A. Woolliams, R. Mrode, and E. Wall. 2011. Phenotypic effects of calving ease on the subsequent fertility and milk production of dam and calf in UK Holstein-Friesian heifers. *J. Dairy Sci.* 94:5413-5423.
- Echternkamp, S. and K. Gregory. 1999. Effects of twinning on gestation length, retained placenta, and dystocia. *J. Anim. Sci.* 77:39-47.
- EMEA. 2007. Scientific discussion, Metacam CVMP/323/97. London, UK.
- Farney, J. K., L. K. Mamedova, J. F. Coetzee, B. KuKanich, L. M. Sordillo, S. K. Stoakes, J. E. Minton, L. C. Hollis, and B. J. Bradford. 2013a. Anti-inflammatory salicylate treatment alters the metabolic adaptations to lactation in dairy cattle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 305:R110-117.
- Farney, J. K., L. K. Mamedova, J. F. Coetzee, J. E. Minton, L. C. Hollis, and B. J. Bradford. 2013b. Sodium salicylate treatment in early lactation increases whole-lactation milk and milk fat yield in mature dairy cows. *J. Dairy Sci.* 96:7709-7718.
- Gaillard, C., R. K. Meagher, M. A. von Keyserlingk, and D. M. Weary. 2014. Social housing improves dairy calves' performance in two cognitive tests. *PLoS One* 9:e90205.
- Gessner, D. K., G. Schlegel, J. Keller, F. J. Schwarz, R. Ringseis, and K. Eder. 2013. Expression of target genes of nuclear factor E2-related factor 2 in the liver of dairy cows in the transition period and at different stages of lactation. *J. Dairy Sci.* 96:1038-1043.
- Gingerich, D. A., J. D. Baggot, and R. A. Yeary. 1975. Pharmacokinetics and dosage of aspirin in cattle. *J. Am. Vet. Med. Assoc.* 167:945-948.
- Goff, J. P. 2006. Major Advances in Our Understanding of Nutritional Influences on Bovine Health. *J. Dairy Sci.* 89:1292-1301.
- Goff, J. P. and R. L. Horst. 1997. Physiological Changes at Parturition and Their Relationship to Metabolic Disorders^{1,2}. *J. Dairy Sci.* 80:1260-1268.
- Graugnard, D. E., M. Bionaz, E. Trevisi, K. M. Moyes, J. L. Salak-Johnson, R. L. Wallace, J. K. Drackley, G. Bertoni, and J. J. Looor. 2012. Blood immunometabolic indices and polymorphonuclear neutrophil function in peripartum dairy cows are altered by level of dietary energy prepartum¹. *J. Dairy Sci.* 95:1749-1758.
- Grossi, P., G. Bertoni, F. P. Cappelli, and E. Trevisi. 2013. Effects of the precalving administration of omega-3 fatty acids alone or in combination with acetylsalicylic acid in periparturient dairy cows. *J. Anim. Sci.* 91:2657-2666.
- Hammon, D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff, and J. L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. *Vet. Immunol. Immunopathol.* 113:21-29.
- Hanzlicek, G. A., B. J. White, D. Mosier, D. G. Renter, and D. E. Anderson. 2010. Serial evaluation of physiologic, pathological, and behavioral changes related to disease progression of

experimentally induced *Mannheimia haemolytica* pneumonia in postweaned calves. *Am. J. Vet. Res.* 71:359-369.

Heinrichs, A. J. and B. S. Heinrichs. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. *J. Dairy Sci.* 94:336-341.

Holland, M., N. Speer, D. LeFever, R. Taylor, T. Field, and K. Odde. 1993. Factors contributing to dystocia due to fetal malpresentation in beef cattle. *Theriogenology* 39:899-908.

Horst, R. L. and N. A. Jorgensen. 1982. Elevated plasma cortisol during induced and spontaneous hypocalcemia in ruminants. *J. Dairy Sci.* 65:2332-2337.

Hotamisligil, G. S., N. S. Shargill, and B. M. Spiegelman. 1993. Adipose expression of tumor necrosis factor- α : direct role in obesity-linked insulin resistance. *Science* 259:87-91.

Humblet, M. F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, F. Rollin, and J. M. Godeau. 2006. Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. *Vet. Clin. Pathol.* 35:188-193.

Huzzey, J. M., T. F. Duffield, S. J. LeBlanc, D. M. Veira, D. M. Weary, and M. A. von Keyserlingk. 2009. Short communication: Haptoglobin as an early indicator of metritis. *J. Dairy Sci.* 92:621-625.

Huzzey, J. M., M. A. von Keyserlingk, and D. M. Weary. 2005. Changes in feeding, drinking, and standing behavior of dairy cows during the transition period. *J. Dairy Sci.* 88:2454-2461.

Jensen, M. B. 2012. Behaviour around the time of calving in dairy cows. *Appl. Anim. Behav. Sci.* 139:195-202.

Johanson, J. M. and P. J. Berger. 2003. Birth Weight as a Predictor of Calving Ease and Perinatal Mortality in Holstein Cattle. *J. Dairy Sci.* 86:3745-3755.

Kimura, K., T. A. Reinhardt, and J. P. Goff. 2006. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. *J. Dairy Sci.* 89:2588-2595.

Knauer, W. A., S. M. Godden, A. Dietrich, and R. E. James. 2017. The association between daily average feeding behaviors and morbidity in automatically fed group-housed preweaned dairy calves. *J. Dairy Sci.* 100:5642-5652.

Kotschwar, J. L., J. F. Coetzee, D. E. Anderson, R. Gehring, B. KuKanich, and M. D. Apley. 2009. Analgesic efficacy of sodium salicylate in an amphotericin B-induced bovine synovitis-arthritis model. *J. Dairy Sci.* 92:3731-3743.

Kovacs, L., F. L. Kezer, F. Ruff, and O. Szenci. 2016a. Timing of obstetrical assistance affects peripartal cardiac autonomic function and early maternal behavior of dairy cows. *Physiol. Behav.* 165:202-210.

Kovacs, L., F. L. Kezer, F. Ruff, and O. Szenci. 2017. Rumination time and reticuloruminal temperature as possible predictors of dystocia in dairy cows. *J. Dairy Sci.* 100:1568-1579.

- Kovacs, L., F. L. Kezer, and O. Szenci. 2016b. Effect of calving process on the outcomes of delivery and postpartum health of dairy cows with unassisted and assisted calvings. *J. Dairy Sci.* 99:7568-7573.
- Kung, L., Jr., S. Demarco, L. N. Siebenson, E. Joyner, G. F. Haenlein, and R. M. Morris. 1997. An evaluation of two management systems for rearing calves fed milk replacer. *J. Dairy Sci.* 80:2529-2533.
- Lewis, G. S. 1997. Uterine Health and Disorders. *J. Dairy Sci.* 80:984-994.
- Lombard, J. E., F. B. Garry, S. M. Tomlinson, and L. P. Garber. 2007. Impacts of dystocia on health and survival of dairy calves. *J. Dairy Sci.* 90:1751-1760.
- Loor, J. J., H. M. Dann, R. E. Everts, R. Oliveira, C. A. Green, N. A. J. Guretzky, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2005. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. *Physiol. Genomics* 23:217-226.
- Lye, S. J. 1996. Initiation of parturition. *Anim. Reprod. Sci.* 42:495-503.
- Mainau, E., A. Cuevas, J. L. Ruiz-de-la-Torre, E. Abbeloos, and X. Manteca. 2014. Effect of meloxicam administration after calving on milk production, acute phase proteins, and behavior in dairy cows. *Journal of Veterinary Behavior: Clinical Applications and Research* 9:357-363.
- Mainau, E. and X. Manteca. 2011. Pain and discomfort caused by parturition in cows and sows. *Appl. Anim. Behav. Sci.* 135:241-251.
- Mallard, B., B. McBride, M. Kehrl, and P. Coussens. 2009. Bovine immunophysiology and genetics: A review of the research and career of Jeanne L. Burton. *Vet. Immunol. Immunopathol.* 128:96-103.
- Maroon, J. C., J. W. Bost, and A. Maroon. 2010. Natural anti-inflammatory agents for pain relief. *Surg. Neurol. Int.* 1:80-89.
- Martinez, N., C. A. Risco, F. S. Lima, R. S. Bisinotto, L. F. Greco, E. S. Ribeiro, F. Maunsell, K. Galvao, and J. E. Santos. 2012. Evaluation of periparturient calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. *J. Dairy Sci.* 95:7158-7172.
- Martinez, N., L. D. Sinedino, R. S. Bisinotto, E. S. Ribeiro, G. C. Gomes, F. S. Lima, L. F. Greco, C. A. Risco, K. N. Galvao, D. Taylor-Rodriguez, J. P. Driver, W. W. Thatcher, and J. E. Santos. 2014. Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows. *J. Dairy Sci.* 97:874-887.
- Matsas, D., R. Nebel, and K. Pelzer. 1992. Evaluation of an on-farm blood progesterone test for predicting the day of parturition in cattle. *Theriogenology* 37:859-868.
- Mee, J. F. 2004. Managing the dairy cow at calving time. *Vet. Clin. North Am. Food Anim. Pract.* 20:521-546.

- Mee, J. F. 2008. Prevalence and risk factors for dystocia in dairy cattle: A review. *The Veterinary Journal* 176:93-101.
- Meier, S., N. V. Priest, C. R. Burke, J. K. Kay, S. McDougall, M. D. Mitchell, C. G. Walker, A. Heiser, J. J. Loor, and J. R. Roche. 2014. Treatment with a nonsteroidal antiinflammatory drug after calving did not improve milk production, health, or reproduction parameters in pasture-grazed dairy cows. *J. Dairy Sci.* 97:2932-2943.
- Meijering, A. 1984. Dystocia and stillbirth in cattle—A review of causes, relations and implications. *Livestock Production Science* 11:143-177.
- Meyer, C. L., P. J. Berger, K. J. Koehler, J. R. Thompson, and C. G. Sattler. 2001. Phenotypic Trends in Incidence of Stillbirth for Holsteins in the United States. *J. Dairy Sci.* 84:515-523.
- Miedema, H. M., M. S. Cockram, C. M. Dwyer, and A. I. Macrae. 2011a. Behavioural predictors of the start of normal and dystocic calving in dairy cows and heifers. *Appl. Anim. Behav. Sci.* 132:14-19.
- Miedema, H. M., M. S. Cockram, C. M. Dwyer, and A. I. Macrae. 2011b. Changes in the behaviour of dairy cows during the 24 h before normal calving compared with behaviour during late pregnancy. *Appl. Anim. Behav. Sci.* 131:8-14.
- Miller-Cushon, E. K. and T. J. DeVries. 2016. Effect of social housing on the development of feeding behavior and social feeding preferences of dairy calves. *J. Dairy Sci.* 99:1406-1417.
- Mitchell, J. A., P. Akarasereenont, C. Thiemermann, R. J. Flower, and J. R. Vane. 1993. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proceedings of the National Academy of Sciences* 90:11693-11697.
- Mitchell, M., A. Flint, and A. Turnbull. 1976. Stimulation of uterine activity by administration of prostaglandin F-2 α during parturition in sheep. *J. Reprod. Fertil.* 48:189-NP.
- Morita, I. 2002. Distinct functions of COX-1 and COX-2. *Prostaglandins Other Lipid Mediat.* 68–69:165-175.
- Mullins, C. R., L. K. Mamedova, M. J. Brouk, C. E. Moore, H. B. Green, K. L. Perfield, J. F. Smith, J. P. Harner, and B. J. Bradford. 2012. Effects of monensin on metabolic parameters, feeding behavior, and productivity of transition dairy cows. *J. Dairy Sci.* 95:1323-1336.
- Murray, C., T. Duffield, D. Haley, D. Pearl, D. Veira, S. Deelen, and K. Leslie. 2016. The Effect of Meloxicam NSAID Therapy on the Change in Vigor, Suckling Reflex, Blood Gas Measures, Milk Intake and Other Variables in Newborn Dairy Calves. *Journal of Veterinary Science & Animal Husbandry* 4:1-13.
- Murray, C., D. Haley, T. Duffield, D. Pearl, S. Deelen, and K. Leslie. 2015a. A field study to evaluate the effects of meloxicam NSAID therapy and calving assistance on newborn calf vigor, improvement of health and growth in pre-weaned Holstein calves. *The Bovine Practitioner* 49:1-12.

- Murray, C. F., D. M. Veira, A. L. Nadalin, D. M. Haines, M. L. Jackson, D. L. Pearl, and K. E. Leslie. 2015b. The effect of dystocia on physiological and behavioral characteristics related to vitality and passive transfer of immunoglobulins in newborn Holstein calves. *Canadian journal of veterinary research = Revue canadienne de recherche veterinaire* 79:109-119.
- Newby, N. C., K. E. Leslie, H. D. Dingwell, D. F. Kelton, D. M. Weary, L. Neuder, S. T. Millman, and T. F. Duffield. 2017. The effects of periparturient administration of flunixin meglumine on the health and production of dairy cattle. *J. Dairy Sci.* 100:582-587.
- Newby, N. C., D. L. Pearl, S. J. LeBlanc, K. E. Leslie, M. A. G. von Keyserlingk, and T. F. Duffield. 2013. Effects of meloxicam on milk production, behavior, and feed intake in dairy cows following assisted calving. *J. Dairy Sci.* 96:3682-3688.
- Newby, N. C., D. Renaud, R. Tremblay, and T. F. Duffield. 2014. Evaluation of the effects of treating dairy cows with meloxicam at calving on retained fetal membranes risk. *Can. Vet. J.* 55:1196-1199.
- Nightingale, C. R., M. D. Sellers, and M. A. Ballou. 2015. Elevated plasma haptoglobin concentrations following parturition are associated with elevated leukocyte responses and decreased subsequent reproductive efficiency in multiparous Holstein dairy cows. *Vet. Immunol. Immunopathol.* 164:16-23.
- Noakes, D., T. Parkinson, and G. England. 2001a. Dystocia and other disorders associated with parturition. Pages 229-244 in *Arthur's Veterinary Reproduction and Obstetrics*. WB Saunders, New York.
- Noakes, D., T. Parkinson, G. England, and G. Arthur. 2001b. Parturition and the care of parturient animals. *Arthur's Veterinary Reproduction and Obstetrics* 8:155-188.
- Olson, D. M., S. J. Lye, K. Skinner, and J. R. Challis. 1984. Early changes in prostaglandin concentrations in ovine maternal and fetal plasma, amniotic fluid and from dispersed cells of intrauterine tissues before the onset of ACTH-induced pre-term labour. *J. Reprod. Fertil.* 71:45-55.
- Ouellet, V., E. Vasseur, W. Heuwieser, O. Burfeind, X. Maldague, and E. Charbonneau. 2016. Evaluation of calving indicators measured by automated monitoring devices to predict the onset of calving in Holstein dairy cows. *J. Dairy Sci.* 99:1539-1548.
- Pahl, C., E. Hartung, A. Grothmann, K. Mahlkow-Nerge, and A. Haeussermann. 2014. Rumination activity of dairy cows in the 24 hours before and after calving. *J. Dairy Sci.* 97:6935-6941.
- Pempek, J. A., M. L. Eastridge, S. S. Swartzwelder, K. M. Daniels, and T. T. Yohe. 2016. Housing system may affect behavior and growth performance of Jersey heifer calves. *J. Dairy Sci.* 99:569-578.

- Pohl, A., O. Burfeind, and W. Heuwieser. 2015. The associations between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes, and metritis. *J. Dairy Sci.* 98:4544-4551.
- Proudfoot, K. L., J. M. Huzzey, and M. A. G. von Keyserlingk. 2009. The effect of dystocia on the dry matter intake and behavior of Holstein cows. *J. Dairy Sci.* 92:4937-4944.
- Qu, Y., A. N. Fadden, M. G. Traber, and G. Bobe. 2014. Potential risk indicators of retained placenta and other diseases in multiparous cows. *J. Dairy Sci.* 97:4151-4165.
- Richards, B. D., D. H. Black, R. M. Christley, M. D. Royal, R. F. Smith, and H. Dobson. 2009. Effects of the administration of ketoprofen at parturition on the milk yield and fertility of Holstein-Friesian cattle. *Vet. Rec.* 165:102-106.
- Sadri, H., R. M. Bruckmaier, H. R. Rahmani, G. R. Ghorbani, I. Morel, and H. A. Van Dorland. 2010. ORIGINAL ARTICLE: Gene expression of tumour necrosis factor and insulin signalling-related factors in subcutaneous adipose tissue during the dry period and in early lactation in dairy cows. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 94:e194-e202.
- Saint-Dizier, M. and S. Chastant-Maillard. 2015. Methods and on-farm devices to predict calving time in cattle. *Veterinary journal (London, England : 1997)* 205:349-356.
- Saremi, B., A. Al-Dawood, S. Winand, U. Muller, J. Pappritz, D. von Soosten, J. Rehage, S. Danicke, S. Haussler, M. Mielenz, and H. Sauerwein. 2012. Bovine haptoglobin as an adipokine: serum concentrations and tissue expression in dairy cows receiving a conjugated linoleic acids supplement throughout lactation. *Vet. Immunol. Immunopathol.* 146:201-211.
- Scalia, D., N. Lacetera, U. Bernabucci, K. Demeyere, L. Duchateau, and C. Burvenich. 2006. In vitro effects of nonesterified fatty acids on bovine neutrophils oxidative burst and viability. *J. Dairy Sci.* 89:147-154.
- Schirmann, K., N. Chapinal, D. M. Weary, L. Vickers, and M. A. G. von Keyserlingk. 2013. Short communication: Rumination and feeding behavior before and after calving in dairy cows. *J. Dairy Sci.* 96:7088-7092.
- Schuenemann, G. M., I. Nieto, S. Bas, K. N. Galvao, and J. Workman. 2011. Assessment of calving progress and reference times for obstetric intervention during dystocia in Holstein dairy cows. *J. Dairy Sci.* 94:5494-5501.
- Schuler, G., F. Hartung, and B. Hoffmann. 1994. Investigations on the use of C-21-steroids as precursors for placental oestrogen synthesis in the cow. *Exp. Clin. Endocrinol.* 102:169-174.
- Schuler, G., G. R. Ozalp, B. Hoffmann, N. Harada, P. Browne, and A. J. Conley. 2006a. Reciprocal expression of 17 α -hydroxylase-C17,20-lyase and aromatase cytochrome P450 during bovine trophoblast differentiation: a two-cell system drives placental oestrogen synthesis. *Reproduction (Cambridge, England)* 131:669-679.
- Schuler, G., U. Teichmann, M. P. Kowalewski, B. Hoffmann, E. Madore, M. A. Fortier, and K. Klisch. 2006b. Expression of Cyclooxygenase-II (COX-2) and 20 α -Hydroxysteroid

- Dehydrogenase (20 α -HSD)/Prostaglandin F-synthase (PGFS) in Bovine Placentomes: Implications for the Initiation of Parturition in Cattle. *Placenta* 27:1022-1029.
- Serhan, C. N., N. Chiang, and T. E. Van Dyke. 2008. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat. Rev. Immunol.* 8:349-361.
- Shah, K., T. Nakao, and H. Kubota. 2006. Plasma estrone sulphate (E 1 S) and estradiol-17 β (E 2 β) profiles during pregnancy and their relationship with the relaxation of sacrosciatic ligament, and prediction of calving time in Holstein-Friesian cattle. *Anim. Reprod. Sci.* 95:38-53.
- Shenavai, S., S. Preissing, B. Hoffmann, M. Dilly, C. Pfarrer, G. R. Ozalp, C. Caliskan, K. Seyrek-Intas, and G. Schuler. 2012. Investigations into the mechanisms controlling parturition in cattle. *Reproduction (Cambridge, England)* 144:279-292.
- Shuster, D. E., E. K. Lee, and M. E. Kehrli, Jr. 1996. Bacterial growth, inflammatory cytokine production, and neutrophil recruitment during coliform mastitis in cows within ten days after calving, compared with cows at midlactation. *Am. J. Vet. Res.* 57:1569-1575.
- Shwartz, G., K. L. Hill, M. J. VanBaale, and L. H. Baumgard. 2009. Effects of flunixin meglumine on pyrexia and bioenergetic variables in postparturient dairy cows. *J. Dairy Sci.* 92:1963-1970.
- Smith, V. G., L. A. Edgerton, H. D. Hafs, and E. M. Convey. 1973. Bovine Serum Estrogens, Progestins and Glucocorticoids during Late Pregnancy, Parturition and Early Lactation. *J. Anim. Sci.* 36:391-396.
- Soberon, F., E. Raffrenato, R. W. Everett, and M. E. Van Amburgh. 2012. Prewaning milk replacer intake and effects on long-term productivity of dairy calves. *J. Dairy Sci.* 95:783-793.
- Sordillo, L. M., G. M. Pighetti, and M. R. Davis. 1995. Enhanced production of bovine tumor necrosis factor-alpha during the periparturient period. *Vet. Immunol. Immunopathol.* 49:263-270.
- Sordillo, L. M. and W. Raphael. 2013. Significance of Metabolic Stress, Lipid Mobilization, and Inflammation on Transition Cow Disorders. *Vet. Clin. North Am. Food Anim. Pract.* 29:267-278.
- Ster, C., M. C. Loiselle, and P. Lacasse. 2012. Effect of postcalving serum nonesterified fatty acids concentration on the functionality of bovine immune cells. *J. Dairy Sci.* 95:708-717.
- Strey, D., C. Sauter-Louis, A. Braunert, D. Lange, F. Weber, and H. Zerbe. 2011. Establishment of a standard operating procedure for predicting the time of calving in cattle. *J. Vet. Sci.* 12:177-185.
- Suriyasathaporn, W., C. Heuer, E. N. Noordhuizen-Stassen, and Y. H. Schukken. 2000. Hyperketonemia and the impairment of udder defense: a review. *Vet. Res.* 31:397-412.
- Svensson, C. and M. B. Jensen. 2007. Short communication: Identification of diseased calves by use of data from automatic milk feeders. *J. Dairy Sci.* 90:994-997.

- Svensson, C., K. Lundborg, U. Emanuelson, and S. O. Olsson. 2003. Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. *Prev. Vet. Med.* 58:179-197.
- Taverne, M. A. M. 1992. Physiology of parturition. *Anim. Reprod. Sci.* 28:433-440.
- Trevisi, E. and G. Bertoni. 2008. Attenuation with acetylsalicylate treatments of inflammatory conditions in periparturient dairy cows. *Aspirin and Health Research Progress*. PI Quinn, ed. Nova Science Publishers 22-37.
- Uetake, K. 2013. Newborn calf welfare: a review focusing on mortality rates. *Animal science journal = Nihon chikusan Gakkaiho* 84:101-105.
- USDA-APHIS. 2008. Dairy 2007 Part II: Changes in the U.S. Dairy Cattle Industry, 1991–2007. USDA, Washington, DC.
- USDA-APHIS. 2010. Heifer calf health and management practices on U.S. dairy operations, 2007. USDA, Washington, DC.
- Ventura, B. A., M. A. G. von Keyserlingk, C. A. Schuppli, and D. M. Weary. 2013. Views on contentious practices in dairy farming: The case of early cow-calf separation. *J. Dairy Sci.* 96:6105-6116.
- Villettaz Robichaud, M., D. L. Pearl, S. M. Godden, S. J. LeBlanc, and D. B. Haley. 2017a. Systematic early obstetrical assistance at calving: I. Effects on dairy calf stillbirth, vigor, and passive immunity transfer. *J. Dairy Sci.* 100:691-702.
- Villettaz Robichaud, M., D. L. Pearl, S. M. Godden, J. Rushen, S. J. LeBlanc, and D. B. Haley. 2017b. Systematic early obstetrical assistance at calving: II. Effects on dairy heifer calf growth, health, and survival to weaning. *J. Dairy Sci.* 100:703-712.
- Weary, D. M., J. M. Huzzey, and M. A. von Keyserlingk. 2009. Board-invited review: Using behavior to predict and identify ill health in animals. *J. Anim. Sci.* 87:770-777.
- Weary, D. M., L. Niel, F. C. Flower, and D. Fraser. 2006. Identifying and preventing pain in animals. *Appl. Anim. Behav. Sci.* 100:64-76.
- Whittle, W., A. Holloway, S. Lye, W. Gibb, and J. Challis. 2000a. Prostaglandin Production at the Onset of Ovine Parturition Is Regulated by Both Estrogen-Independent and Estrogen-Dependent Pathways 1. *Endocrinology* 141:3783-3791.
- Whittle, W. L., A. C. Holloway, S. J. Lye, W. Gibb, and J. R. G. Challis. 2000b. Prostaglandin Production at the Onset of Ovine Parturition Is Regulated by Both Estrogen-Independent and Estrogen-Dependent Pathways*. *Endocrinology* 141:3783-3791.
- Whittle, W. L., F. A. Patel, N. Alfaidy, A. C. Holloway, M. Fraser, S. Gyomory, S. J. Lye, W. Gibb, and J. R. Challis. 2001. Glucocorticoid regulation of human and ovine parturition: the relationship between fetal hypothalamic-pituitary-adrenal axis activation and intrauterine prostaglandin production. *Biol. Reprod.* 64:1019-1032.

Yeiser, E. E. 2011. The use of activity measures in combination with physiological factors as indicators of disease in dairy cattle. Virginia Tech.

Yuan, K., J. K. Farney, L. K. Mamedova, L. M. Sordillo, and B. J. Bradford. 2013. TNFalpha altered inflammatory responses, impaired health and productivity, but did not affect glucose or lipid metabolism in early-lactation dairy cows. PLoS One 8:e80316.

Yuan, K., L. G. Mendonca, L. E. Hulbert, L. K. Mamedova, M. B. Muckey, Y. Shen, C. C. Elrod, and B. J. Bradford. 2015. Yeast product supplementation modulated humoral and mucosal immunity and uterine inflammatory signals in transition dairy cows. J. Dairy Sci. 98:3236-3246.

Evaluation of an accelerometer by Swartz. The goal of this study was to validate step activity, lying bouts, and lying time using an accelerometer in unweaned dairy calves. Through correlations and pairwise comparisons of the Afitag II accelerometer to a previously validated accelerometer, HOB0 Pendant G Data logger, and video, the accelerometer proved to be a valid management tool for lying behaviors and step activity.

Chapter 2. Technical Note: The use of an accelerometer for measuring step activity and lying behaviors in dairy calves

T. H. Swartz, M. L. McGilliard, and C. S. Petersson-Wolfe

Department of Dairy Science, Virginia Tech, Blacksburg, VA 24061

Christina S. Petersson-Wolfe

2120 Litton Reaves Hall, Virginia Tech

175 West Campus Drive

Blacksburg, VA 24061

Phone: 540-231-4767

Fax: 540-231-5014

cspw@vt.edu

ABSTRACT

Calf behaviors such as step activity, lying bouts, and lying time may be an indicator of calf health and welfare. To reduce time-consuming visual observations, the use of behavioral monitoring systems have been developed to capture these data. Previous studies have validated lying behaviors using an accelerometer (**HPG**; HOB0 Pendant G data logger, Onset Computer Corp., Bourne, MA) in calves. However, the HPG does not measure step activity. The objectives of this study were to (1) validate step activity, lying bouts, and lying time of Afitag II (**AT2**; Afitag II, Afimilk LTD., Kibbutz Afikim, Israel) to observations from video, and (2) to compare the behavioral data from AT2 to the HPG. Calves ($n = 5$) were group housed with an automatic calf feeder. Video cameras were installed at both sides of the pen, and observations were analyzed for 7-h/calf. AT2 and the HPG were both attached to the lateral side of the right rear leg of the calves, and data were recorded for 10 d. The full 10-d dataset was used to examine correlations for lying bouts and lying time between AT2 and the HPG. The HPG was set at a 60-s sampling interval and the output was analyzed both unfiltered as well as utilizing a 1-min event filter to remove potentially erroneous readings. AT2 recorded step activity, lying bouts, and lying time, and summarized these behaviors in 15-min periods. AT2 recorded lying time in 3-min intervals which was then automatically summarized in 15-min periods. The correlations of step activity, lying bouts, and lying time between video recordings and AT2 were 0.99. For the second objective, correlations between AT2 and the HPG were 0.99 for lying time and 0.93 for lying bouts. The 1-min event filter resulted in a 0.03 improvement in correlations for lying bouts between the HPG and AT2. The high correlation between video recordings and AT2 suggest that this device can be used to measure step activity, lying time, and lying bouts in unweaned dairy calves housed in groups.

Key words: validation, calf, lying behavior, activity

Technical Note

As herd size continues to increase in the dairy industry, many producers have turned to technology to assist with daily management tasks (Khanal et al., 2010). Tri-axial accelerometers have grown in popularity in part due to their usefulness in estrus detection (Dolecheck et al., 2015), but more recently research has shown value in disease detection (Alsaad et al., 2012; Fogsgaard et al., 2014; Itle et al., 2015). In dairy cattle, accelerometers have been used to detect behavioral changes around calving and disease events (Alsaad et al., 2012; Jensen, 2012; Fogsgaard et al., 2014; Itle et al., 2015). Early detection of these events can allow for timely intervention, potentially reducing the negative impact on the cow. In dry cows, a significant increase in standing times either during the week prior to calving or on the day of calving was indicative of clinical ketosis (Itle et al., 2015). In addition, cows close to calving showed an increase in step activity and lying bouts 6-h prior to calving and a decrease in lying time 24-h prior to calving, allowing for the development of calving alerts (Jensen, 2012). Furthermore, lameness in lactating dairy cattle housed in free stalls can be found with 76 percent accuracy utilizing deviations from a baseline for lying behaviors and step activity (Alsaad et al., 2012). Lastly, mastitic cows were more restless for 10-d after initial infection date, exhibiting an increased frequency in lying bouts and step activity, and decreased lying time (Fogsgaard et al., 2014).

Research in calves has shown promise in identifying disease through behavioral changes. When low doses of *Escherichia coli* endotoxin (0.025 µg or 0.05 µg of LPS/kg of BW) were injected IV, calves were less active while lying, and total lying time was unaffected (Borderas et al., 2008). In another study, calves inoculated with *Mannheimia haemolytica* to experimentally

induce pneumonia showed a significant decrease in step activity after inoculation (Hanzlicek et al., 2010). In studies examining social housing, calves were found to be more active when housed in pairs or groups, and also exhibited an increase in time spent standing (Jensen 1998; Chua et al., 2002). Lying behavior has also been shown to be affected by different housing systems, flooring types, and pen sizes in dairy calves (Webster et al., 1985; Bokkers and Koene, 2001; Hänninen et al., 2005; Færevik et al., 2008). These studies have demonstrated that behavioral measurements can provide quantitative evidence of calf welfare.

Lying behaviors and activity in dairy calves are typically collected via video or direct observation (Webster et al., 1985; Bokkers and Koene, 2001; Hänninen et al., 2005). However, these methods are time consuming. Lying behaviors have been validated in lactating dairy cattle and calves utilizing an accelerometer (**HPG**; HOBO Pendant G data logger, Onset Computer Corporation, Bourne, MA) (Ledgerwood et al., 2010; Bonk et al., 2013). However, the HPG does not measure step activity. Therefore, the objectives of this study were to (1) validate the use of an accelerometer (**AT2**; AfiTag II, Afimilk LTD., Kibbutz Afikim, Israel) in dairy calves by comparing step activity, lying bouts, and lying time to the gold standard of video recordings, and (2) to compare the behavioral data from AT2 to the HPG.

The current study was conducted during October 2015 at the Virginia Tech dairy farm. Five unweaned female calves (2 Jersey and 3 Holstein; age 44.6 ± 3.2 d) were housed in a group pen on a sawdust pack which contained a concrete feed alley with an automatic calf feeder and a feed bunk for grain. Calves had access to 12 L/d of a 22% CP, 20% CF milk replacer (Amplifier® Max, Land O'Lakes® Animal Milk Products Co., Shoreview, MN) via an automatic calf feeder (FA Förster-Technik GmbH, Engen, Germany), as well as access to a 22%

CP calf starter (Intensity 22% Textured Calf Starter Medicated, Southern States, Richmond, VA).

Video cameras (Canon HF M52, Canon USA, Inc., Melville, NY) with 32 GB of internal memory were installed at both sides of the pen. Video was recorded for a 7-h period during daylight hours for analysis. For each calf, the same 7-h period of video were analyzed using behavioral analysis software (The Observer XT, Version 12.0, Noldus Information Technology, Leesburg, VA). Video was analyzed continuously by one observer. Using correlations in Excel, intra-observer reliabilities ($n = 3$ calves for 2 h per calf) were found to be 1.00 for both lying time and lying bouts, and 0.93 for step activity. Step activity was defined as the right rear leg lifted off the floor while the calf was standing. A lying bout was defined as the transition from standing to lying, whereas lying time was defined as minutes per period spent lying.

Both AT2 and the HPG were attached to the lateral side of the right rear leg above the metacarpophalangeal joint. For 10-d, the HPG recorded the g -force and tilt of the x -, y -, and z -axes at 60-s intervals, and they were wrapped in gauze to provide cushioning and attached to the leg using Vet Wrap (Co-Flex, Andover Healthcare, Salisbury, MA). Output from the HPG was downloaded using graphing and analysis software (HOBOWare Lite, Onset Computer Corp., Bourne, MA), and then downloaded to spreadsheets. AT2 continuously recorded acceleration in the x -, y -, and z -axes and automatically transmitted these data as step activity and lying behaviors in 15-min intervals to herd management software (AfiAct II, Afimilk LTD., Kibbutz Afikim, Israel). AT2 data is cumulative; therefore, the output from AT2 was summarized to match the 24-h segments from the HPG, as well as the 7-h video recordings. AT2 recorded a lying bout when the calf spent a minimum of 3-min lying down. AT2 also recorded lying time to the nearest minute, and reported this behavior in 3-min intervals. Lying times not divisible by 3-

min had the remainder added to the next lying bout. The HPG recorded the degree of the y-tilt, which is used to determine standing or lying behaviors, with less than 60° indicating standing, and greater than or equal to 60° indicating lying. Using Excel, degrees of y-tilt were converted to either a lying or standing minute, and the change from a standing minute to a lying minute was used to denote a lying bout. Output was also edited to examine the effect of using a 1-min event filter to remove potentially erroneous readings of lying or standing events (Ledgerwood et al., 2010; Bonk et al., 2013). This filter converted behaviors of a 1-min duration back to the original behavior that preceded it. Previous studies in calves have shown an improvement in correlations between the HPG and direct observation for lying time and lying bouts when single lying and standing events were removed, justifying the use of a 1-min event filter (Bonk et al., 2013).

For the first objective, data from the video period were used to determine the accuracy of AT2 and the HPG (PROC REG, SAS) to identify video lying bouts and lying time, and AT2 to identify video steps. Because video was only 7-h, a mixed model containing device and calf was used to determine differences between the HPG, AT2, and video results for lying bouts and lying time, using a Dunnett test with video as the control (PROC GLIMMIX, SAS). A mixed model was also used to determine differences between AT2 and video for step activity. For the second objective, correlations for lying bouts and lying time between the HPG and AT2 were calculated, both overall (PROC CORR, SAS 9.4, SAS Institute Inc., Cary, NC) and within calf (MANOVA of PROC GLM, SAS). Daily lying bouts and lying time were analyzed for differences among AT2 and the HPG, unfiltered and filtered, using a mixed model of device, calf, device*calf, day, and device*day (PROC GLIMMIX, SAS).

Objective 1.

Seven hours of video were analyzed for each of the 5 calves to verify the HPG and AT2 readings, and behaviors are summarized in Table 2.1. AT2 was highly correlated with video recordings for step activity, and both accelerometers were highly correlated with video for lying time ($r = 0.99$, $P < 0.01$; Table 2.2). Lying bouts were also highly correlated between recording methods, with even larger correlations when the data from the HPG were filtered (AT2 with video, 0.99; AT2 with unfiltered and filtered HPG, 0.93 to 1.00; video with unfiltered and filtered HPG, 0.96 to 0.99).

Step activity from AT2 exceeded video by 118 ± 31 steps ($P = 0.02$). Occasionally during 15-min lying periods reported from AT2, a small number of steps was counted, suggesting that AT2 will sometimes record leg movement as a step while the calf is lying. This caused AT2 to measure more steps than counted on video.

The unfiltered output from the HPG reported 1.4 ± 0.5 more lying bouts per 7-h than recorded from video ($P = 0.03$), with no other comparisons significantly different. Since no difference was found between the filtered HPG and video, filtering the output improved the accuracy of identifying lying bouts, similar to Bonk et al., 2013. AT2 also showed no difference in lying bouts when compared to video analysis, providing evidence that AT2 accurately recorded the frequency of lying events. AT2 reported 5.0 ± 0.8 min less lying time in 7-h than the video ($P = 0.01$), but no difference was found between the HPG and video. Video is continuous, whereas AT2 records lying time in 3-min increments, and all behaviors are recorded cumulatively. For example, if a calf is lying for 5-min, and the calf stands, AT2 reports 1 lying bout of 3-min. Then, if the calf lies down again for 4-min, AT2 reports a cumulative lying time of 9-min, and 2 lying bouts. This difference in data collection may explain some of the differences noted for lying time.

Objective 2.

Table 2.3 shows the correlations between the HPG and AT2 for lying bouts and lying time as analyzed for a 10-d recording period. Correlations within calf have calf variation removed such that a single calf does not have undue influence on the correlation. Overall correlations as well as correlations within calf for lying time were 0.99 ($P < 0.01$), and were similar for filtered output from the HPG. For lying bouts, filtering the data from the HPG resulted in increased correlations both within calf and overall (0.93 to 0.96; 0.96 to 0.98, respectively).

During this 10-d period, the unfiltered HPG reported 982 ± 44 min/d of total lying time represented in 24.0 ± 2.4 lying bouts; filtered HPG reported 979 ± 44 min/d of total lying time represented in 19.4 ± 2.4 lying bouts; and AT2 reported 959 ± 44 min/d of total lying time represented in 20.0 ± 2.4 lying bouts (LSM \pm SE per calf). Lying time and lying bouts were similar to those found in previous studies with dairy calves (Chua et al., 2002; Hämmänen et al., 2005; Bonk et al., 2013).

Tukey pairwise comparisons were applied to the least squares means of the HPG, filtered and unfiltered, and AT2. Lying bouts recorded by the unfiltered HPG exceeded those of AT2 by 4.0 ± 0.5 bouts/d ($P < 0.01$) and exceeded those of the filtered HPG by 4.6 ± 0.5 bouts/d ($P < 0.01$). However, the 0.6 ± 0.5 difference in lying bouts between AT2 and the filtered HPG was not significant. By removing 1-min behavioral events from the HPG, filtering the output from the HPG resulted in closer agreement with AT2 for lying bouts as AT2 only records a lying bout if the duration is a minimum of 3-min.

The HPG, both unfiltered and filtered, and AT2 differed in lying time. Lying time from AT2 was 23 ± 0.7 min/d ($P < 0.01$) less than the unfiltered HPG, and 20 ± 0.7 min/d ($P < 0.01$) less than the filtered HPG. Additionally, the filtered HPG was 3.0 ± 0.7 min/d ($P = 0.02$) less than the unfiltered HPG. Differences between AT2 and the HPG may be due to AT2 continuously recording lying time, whereas the HPG is providing snapshots of behavior in 60-s intervals. The difference between the unfiltered and filtered HPG for lying time provides further evidence that a 1-min event filter improves accuracy by removing probable erroneous readings, as found in a previous study (Bonk et al., 2013).

The HPG was recently validated in unweaned calves (Bonk et al., 2013). However, this accelerometer does not measure step activity. The current study is the first to evaluate the use of AT2 attached to unweaned calves. AT2 was highly correlated with video analysis for step activity, lying bouts, and lying time. Moreover, AT2 was highly correlated to the HPG for both lying bouts and lying time. Applying a 1-min event filter to the HPG removed spurious readings and improved the accuracy of lying bouts. With high correlations, we declare AT2 as an acceptable tool for measuring step activity, lying time, and lying bouts in unweaned dairy calves. Automated behavioral measurements may have value in determining the effect of different housing and management systems on dairy calves. Technologies may have limitations on their ability to accurately identify behaviors; therefore, it is important to validate the results.

Table 2.1 Steps (steps per calf), lying bouts (bouts per calf), and lying time (min per calf) of 5 calves in a 7-h period recorded either by video, Afitag II, or the HOBOPendant G Data Logger, unfiltered and filtered.

	Video	AT2 ¹	HPG ² Unfiltered	HPG Filtered ³
Steps	662 ± 82	780 ± 82	N/A	N/A
Lying bouts	5.0 ± 0.9	4.8 ± 0.9	6.4 ± 0.9	4.8 ± 0.9
Lying time	264 ± 24	259 ± 24	265 ± 24	264 ± 24

¹AT2 = Afitag II

²HPG = HOBOPendant G Data Logger unfiltered

³Events ≤ 1-min from the HPG were converted back to the preceding behavior

Table 2.2 Overall correlations between video and other recording techniques for step activity, lying bouts, and lying time of 5 calves during a 7-h period.¹

	Video	AT2 ²	HPG ³ Unfiltered	HPG Filtered ⁴
----- Step Activity -----				
Video	-		N/A ⁵	N/A
AT2	0.99	-	N/A	N/A
----- Lying Bouts -----				
Video	-			
AT2	0.99	-		
HPG Unfiltered	0.96	0.93	-	
HPG Filtered	0.99	1.00	0.93	-
----- Lying Time -----				
Video	-			
AT2	0.99	-		
HPG Unfiltered	0.99	0.99	-	
HPG Filtered	0.99	0.99	0.99	-

¹All P < 0.01

²AT2 = AfTag II

³HPG = HOBO Pendant G Data Logger

⁴Events ≤ 1-min from the HPG were converted back to the preceding behavior

⁵N/A indicates correlations not available

Table 2.3 Correlations between recording techniques for lying bouts and lying time of 5 calves over a 10-d recording period (overall above diagonal, within calf below diagonal).^{1,2}

	AT2 ³	HPG ⁴ Unfiltered	HPG Filtered ⁵
----- Lying Bouts -----			
AT2	-	0.96	0.98
HPG Unfiltered	0.93	-	0.95
HPG Filtered	0.96	0.91	-
----- Lying Time -----			
AT2	-	0.99	0.99
HPG Unfiltered	0.99	-	0.99
HPG Filtered	0.99	0.99	-

¹All P < 0.01

²Within calf correlations after the removal of calf differences in mean bouts or time

³AT2 = AfTag II

⁴HPG = HOBO Pendant G Data Logger

⁵Events ≤ 1-min were converted back to the preceding behavior.

REFERENCES

- Alsaad, M., C. Römer, J. Kleinmanns, K. Hendriksen, S. Rose-Meierhöfer, L. Plümer, and W. Büscher. 2012. Electronic detection of lameness in dairy cows through measuring pedometric activity and lying behavior. *Appl. Anim. Behav. Sci.* 142:134-141.
- Bokkers, E. A. M., and P. Koene. 2001. Activity, oral behaviour and slaughter data as welfare indicators in veal calves: A comparison of three housing systems. *Appl. Anim. Behav. Sci.* 75:1-15.
- Bonk, S., O. Burnfeind, V. S. Suthar, and W. Heuwieser. 2013. Technical note: Evaluation of data loggers for measuring lying behavior in dairy calves. *J. Dairy Sci.* 96:3265-3271.
- Borderas, T. F., A. M. de Passillé and J. Rushen. 2008. Behavior of dairy calves after a low dose of bacterial endotoxin. *J. Anim. Sci.* 86:2920-2927.
- Chua, B., E. Coenen, J. van Delen, and D. M. Weary. 2002. Effects of Pair Versus Individual Housing on the Behavior and Performance of Dairy Calves. *J. Dairy Sci.* 85:360-364.
- Dolecheck, K. A., W. J. Silvia, G. Heersche Jr, Y. M. Chang, D. L. Ray, A. E. Stone, B. A. Wadsworth, and J. M. Bewley. 2015. Behavioral and physiological changes around estrus events identified using multiple automated monitoring technologies. *J. Dairy Sci.* 98:8723-8731.
- Færevik, G., K. Tjentland, S. Løvik, I. L. Anderson, and K. E. Bøe 2008. Resting pattern and social behaviour of dairy calves housed in pens with different sized lying areas. *Appl. Anim. Behav. Sci.* 114:54-64.
- Fogsgaard, K. K., T. W. Bennedsgarrd, and M. S. Herskin. 2015. Behavioral changes in freestall-housed dairy cows with naturally occurring clinical mastitis. *J. Dairy Sci.* 98:1730-1738.
- Hänninen, L., A. M. de Passillé, and J. Rushen. 2005. The effect of flooring type and social grouping on the rest and growth of dairy calves. *Appl. Anim. Behav. Sci.* 91:193-204.
- Hanzlicek, G. A., B. J. White, D. Mosier, D. G. Renter, and D. E. Anderson. 2010. Serial evaluation of physiologic, pathological, and behavioral changes related to disease progression of experimentally induced *Mannheimia haemolytica* pneumonia in postweaned calves. *Am. J. Vet. Res.* 71:359-369.
- Itle, A. J., J. M. Huzzey, D. M. Weary, and M. A. G. von Keyserlingk. 2015. Clinical ketosis and standing behavior in transition cows. *J. Dairy Sci.* 98:128-134.
- Khanal, A. R., J. Gillespie, and J. MacDonald. 2010. Adoption of technology, management practices, and production systems in US milk production. *J. Dairy Sci.* 93:6012-6022.
- Jensen, M. B. 2012. Behaviour around the time of calving in dairy cows. *Appl. Anim. Behav. Sci.* 139:195-202.
- Jensen, M. B., K. S. Vestergaard, and C. C. Krohn. 1998. Play behaviour in dairy calves kept in pens: the effect of social contact and space allowance. *Appl. Anim. Behav. Sci.* 56:97-108.

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

Webster, A. J. F., C. Saville, B. M. Church, A. Gnanasakthy, and R. Moss. 1985. The effect of different rearing systems on the development of calf behaviour. *Br. Vet. J.* 141:249-264.

Automated detection of respiratory disease in calves by Swartz. The goal of this study was to determine changes in step activity, lying behaviors, and feeding behaviors between calves with respiratory disease and healthy controls. When compared to healthy calves, step activity from diseased calves declined on the day prior to and on the day of respiratory disease diagnosis as well as after diagnosis. Lying bouts declined two days prior to diagnosis as well as after diagnosis. Milk intake was reduced on the day of diagnosis when compared to healthy controls.

Chapter 3. Short Communication: Automated detection of behavioral changes from respiratory disease in pre-weaned calves

T. H. Swartz, A. N. Findlay, and C. S. Petersson-Wolfe

Department of Dairy Science, Virginia Tech, Blacksburg, VA 24061

Christina S. Petersson-Wolfe

2120 Litton Reaves Hall, Virginia Tech

175 West Campus Drive

Blacksburg, VA 24061

Phone: 540-231-4767

Fax: 540-231-5014

cspw@vt.edu

ABSTRACT

Group-housing of calves can pose a challenge in identifying respiratory disease; therefore, there is a need to develop tools that can identify these disease events. In this experiment, pre-weaned calves (n = 30) were housed in groups with an automatic calf feeder, and were fitted with accelerometer. Step activity, lying behaviors, and feeding behaviors were recorded to determine the effect of respiratory disease. All calves were health scored twice daily, and calves with respiratory scores ≥ 5 were diagnosed with respiratory disease (n = 10). Each diseased calf was match paired with a healthy control based on the date of disease diagnosis, breed, and age. Control calves were determined to be healthy if they had respiratory scores ≤ 4 , as well as fecal, navel, and joint scores of a 0 or 1. Diseased calves were less active before, on the day of, and after respiratory disease diagnosis. Furthermore, diseased calves had reduced lying frequencies starting two days prior to diagnosis, as well as after diagnosis. Lastly, diseased calves consumed less milk on the day of diagnosis when compared to healthy controls. Step activity, lying bouts, and milk intake may prove to be a useful tool in identifying respiratory disease under practical farming, but this requires further research.

Key words: calf; respiratory disease; activity; feeding behavior

Short Communication

The use of automatic calf feeders in pre-weaned calves is gaining popularity as dairy producers look to reduce labor costs and improve calf welfare (Kung et al., 1997). Automatic calf feeders allow for calves to consume greater daily intakes of milk spread over more meals than traditional feeding systems. High milk intake diets have been positively correlated with increased growth rates and first lactation milk yield (Soberon et al., 2012). Group- and pair-housing of pre-weaned calves has demonstrated additional benefits including an increase in concentrate intake, and average daily gain, with these increases culminating in the weaning period (Miller-Cushon and DeVries, 2016; Pempek et al., 2016). Furthermore, pair-housed calves have improved social abilities, were less fearful (Duve et al., 2012), and adapted to environmental changes more quickly when compared to individually-housed calves (Gaillard et al., 2014), providing evidence that individual-housing impairs social as well as cognitive performance. While there are numerous benefits of group-housed calves on an automatic calf feeder, large group sizes are associated with increased risk for respiratory disease and difficulties in identifying diseased calves when compared to individually-housed calves (Svensson et al., 2003; Svensson and Jensen, 2007). Mortality associated with respiratory disease in pre-weaned calves is 22.5% (USDA-APHIS, 2010). Therefore, the development of technologies to aid in the identification of this disease is warranted.

Several methods of respiratory disease detection in calves currently exist, from health examinations (McGuirk, 2008), to approach tests with either a human or a novel object (Cramer and Stanton, 2015), and through automated technologies such as noninvasive infrared thermography (Schaefer et al., 2012). While these methods have merit, daily examinations may be time consuming, and could require additional labor. Furthermore, noninvasive infrared

thermography may not be economically feasible for all farms. Therefore, alternative methods of respiratory disease detection may be needed. Some farms may already be utilizing an automatic calf feeder to reduce labor costs, thus, maximizing the use of the data from these feeders for respiratory disease detection may provide additional value. Other farms may already be utilizing accelerometers in adult cattle for heat detection (Dolecheck et al., 2015) and disease detection (Rutten et al., 2013); however, these accelerometers may also prove valuable for disease detection in calves (Trenel et al., 2009).

Past research studies have examined feeding behavior around disease events in pre-weaned calves. Svensson and Jensen (2007) found restrictively-fed diseased calves have less unrewarded visits when compared to healthy restrictively-fed calves; however, no difference was seen for drinking speed, milk consumption, and rewarded visits. Another study found that diseased calves on a high milk allowance demonstrated fewer total visits, reduced milk intake, and an increase in visit duration when compared to healthy calves; conversely, when examining calves on a low milk allowance, sick calves demonstrated a reduction in visit duration and no difference was noted for milk intake and total visits (Borderas et al., 2009). Another study, using an experimentally induced pneumonia design, showed a reduction in step activity 24 h post-inoculation of *Mannheimia haemolytica* when compared to negative controls (Hanzlicek et al., 2010).

Previous studies have examined the effect of multiple diseases on feeding behavior (Svensson and Jensen, 2007; Borderas et al., 2009). Because behaviors may differ between diseases, it could be useful to examine the effect of each disease separately. Furthermore, because milk allowances will vary from farm-to-farm, utilizing additional tools may have value in detecting disease in pre-weaned calves to complement feeding behavior. Therefore, the aim of

this study was to assess the changes in feeding behavior as well as step activity and lying behaviors in pre-weaned dairy calves before and during the onset of respiratory disease. We hypothesized that diseased calves would be less active and would, therefore, spend more time lying around the time of respiratory disease onset than healthy calves. Furthermore, we hypothesized that diseased calves would consume less milk at a slower drinking speed, and have less total visits around the time of respiratory disease onset when compared to healthy calves.

Animals, Housing, and Feeding

This study was conducted from March 2016 through August 2016 at the Virginia Tech dairy in Blacksburg, VA, in accordance with guidelines set by the Institutional Animal Care and Use Committee (16-002).

Female Holstein and Jersey calves (n = 30; 19 Holstein and 11 Jersey) were enrolled in this trial at birth, moved into individual hutches, ear tagged, and fed 4 L of colostrum after birth. Calves remained in individual hutches for the first 6 d of life. While in hutches, calves were fed 2 L of 40.6°C water mixed with 300 grams of milk replacer (Cow's Match®, Warm Front® PB, Land O'Lakes Animal Milk Products Co., Shoreview, MN) twice daily.

When the calf was 7 d of age, it was moved into group-housing with an automatic calf feeder (FA Förster-Technik GmbH, Engen, Germany). The pen had a 6.3 by 6.0 m bedded pack, and a 4.3 by 6.0 m concrete alley which was maintained by the farm staff. To reflect what occurs on most dairy farms, group size was dynamic, as calves entered group housing 7 d after birth, and left at weaning, 55 d of age. The automatic calf feeder gradually increased milk allowances from 6 L to a maximum of 12 L of milk per day; maximum milk allowance was reached when calves were 16 d of age. Weaning began at 46 d of age until 55 d of age when calves were

weaned and removed from the pen. Calves were allowed ad libitum access to water and calf starter (Intensity 22% Textured Calf Starter Medicated, Southern States, Richmond, VA). Water was provided through an automatic waterer (WaterMatic 150, Ritchie Industries Inc., Conrad, IA), and grain was provided in a metal feed bunk. Grain refusals were discarded daily, and new grain was provided to ensure ad libitum access. No other feedstuff was provided.

Experimental measurements

Accelerometers previously validated on calves (AfiTag II, AfiMilk LTD., Kibbutz Afikim, Israel) were attached to the calves' right rear legs at birth to collect step activity and lying behaviors (Swartz et al., 2016). These variables were collected wirelessly every 15 min and transmitted to a computer (AfiAct II, Afimilk LTD., Kibbutz Afikim, Israel). Data was then summarized into daily steps (no./d), lying time (min/d), and lying bouts (no./d) using Excel spreadsheets (Microsoft Corp., Redmond, WA). From 7 d through 55 d of age, the automatic calf feeder recorded milk intake (mL/d), drinking speed (mL/min), as well as rewarded and unrewarded visits (no./d) for each calf. The automatic calf feeder continuously records and calculates feeding behavior data in spreadsheets utilizing a commercial software program marketed with the feeder (Institute Software, FA Förster-Technik GmbH, Engen, Germany).

All calves were health scored twice daily (0800 and 1600 h) using the Calf Health Scorer App (University of Wisconsin, School of Veterinary Medicine) until weaning. This app closely follows the criteria of the calf health scoring chart previously developed by Dr. Sheila McGuirk (McGuirk, 2008). A description of this scoring system is provided in Table 3.1. Calves were scored on eye discharge, ear disposition, nasal discharge, coughing, and rectal temperature for respiratory scoring, with scores of 0 representing normal, and 3 being severely abnormal. Respiratory disease was indicated when the sum of these five variables were ≥ 5 , provided that at

least two of the five variables were ≥ 2 (McGuirk, 2008). Calves diagnosed with respiratory disease were treated with a single subcutaneous injection of an antibiotic, tulathromycin, using label recommendations (Draxxin®, Zoetis Inc., Kalamazoo, MI). Fecal consistency scores as well as joint and navel scores were performed to ensure that calves diagnosed with respiratory disease were only affected by respiratory disease.

Statistical analysis

For each diseased calf, a healthy calf on the same date, of the same breed, and similar age (age: Mean \pm SD; healthy calves: 29.1 ± 6.4 d; diseased calves: 33.1 ± 8.8 d) was identified as the matched pair. Day 0 was assigned as the day when respiratory disease was first diagnosed with a respiratory score of ≥ 5 . Healthy controls were defined as calves with fecal, joint, and navel scores of a 1 or 0, and a total respiratory score of ≤ 4 . Furthermore, a healthy control calf was required to maintain the above health scores for 7 d on both sides of d 0 (d -7 to d 7) of its matched diseased calf. A healthy calf was eligible to serve as a control more than once, however, no control calf was used more than twice. A diseased calf was defined as a calf with a respiratory score ≥ 5 , with fecal, joint, and navel scores of a 1 or 0. Diseased calves were required to maintain fecal, joint, and navel scores of a 1 or 0 for 7 d on both sides of d 0 (d -7 to d 7). This was done to ensure that that diseased calves were only affected by respiratory disease, and no other disease events. Of the 30 calves, 14 calves were diagnosed with respiratory disease. Four of these calves were excluded either due to the absence of an appropriate control calf ($n = 3$) or the disease event occurring outside of the maximum milk allowance period ($n = 1$). Thus, only data from respiratory disease events occurring during maximum milk allowance periods were included in the analyses.

The residuals were analyzed for outliers and normality. No outliers were identified, and no data were missing. Therefore, for each dependent variable, there was a total of 140 observations divided over 7 d and 10 pairs of calves. A repeated-measures mixed model (PROC GLIMMIX, SAS) was used with health status, day (repeated measure), and the interaction of day and health status as fixed components, and the matched pair of calves and the interaction of pair and health status as random components. All variables were modeled with the autoregressive covariance structure, and the Kenward-Roger procedure was used for degrees of freedom approximation (Kenward and Roger, 1997). Table 3.2 provides F -values, probability levels, and degrees of freedom of fixed effects. When the interaction term of day and health status was $P < 0.50$, the effect of health status was compared at each day using the SLICE option in SAS. Significance was declared when $P \leq 0.05$. Daily steps (no./d) and lying bouts (no./d) for diseased and healthy calves are shown in Figure 3.1. Daily milk intake (mL/d) for diseased and healthy calves are shown in Figure 3.2.

Activity monitoring

Diseased calves took fewer steps than healthy controls on d -1 (healthy vs. diseased: $2,617 \pm 184.9$ vs. $2,091.8 \pm 184.9$ steps/d; $F_{1,70} = 6.34$, $P = 0.01$) and on d 0 (healthy vs. diseased: $2,478.4 \pm 184.9$ vs. $1,740 \pm 184.9$ steps/d; $F_{1,70} = 12.54$, $P < 0.01$). After medical treatment, the difference between healthy and diseased calves was diminished, with diseased calves being less active on d 1, (healthy vs. diseased: $2,384.7 \pm 184.9$ vs. $1,918 \pm 184.9$ steps/d; $F_{1,70} = 5.01$, $P = 0.03$), d 2 (healthy vs. diseased: $2,568.6 \pm 184.9$ vs. $2,129.2 \pm 184.9$ steps/d; $F_{1,70} = 4.44$, $P = 0.04$), and d 3 (healthy vs. diseased: $2,789.9 \pm 184.9$ vs. $2,286.3 \pm 184.9$ steps/d; $F_{1,70} = 5.83$, $P = 0.02$).

No difference in lying time was observed. However, diseased calves had a reduction in lying frequency on d -2, (healthy vs. diseased: 26.9 ± 1.95 vs. 22.6 ± 1.95 lying bouts/d; $F_{1,69} = 4.04$, $P = 0.05$), d -1 (healthy vs. diseased: 27.4 ± 1.95 vs. 23.0 ± 1.95 lying bouts/d; $F_{1,69} = 4.23$, $P = 0.04$), d 1 (healthy vs. diseased: 24.1 ± 1.95 vs. 17.2 ± 1.95 lying bouts/d; $F_{1,69} = 10.39$, $P < 0.01$), and d 3 (healthy vs. diseased: 27.9 ± 1.95 vs. 22.7 ± 1.95 lying bouts/d; $F_{1,69} = 5.90$, $P = 0.02$) when compared to healthy controls.

Feeding behavior

No difference in milk intake was observed on d -1 (healthy vs. diseased: $8,647.2 \pm 580.2$ vs. $7,793.2 \pm 580.2$ mL/d; $F_{1,96} = 1.08$; $P = 0.30$); however, a significant difference was observed on d 0 (healthy vs. diseased: $8,863.9 \pm 580.2$ vs. $7,261.7 \pm 580.2$ mL/d; $F_{1,96} = 3.81$, $P = 0.05$) with diseased calves consuming approximately 1.6 L less milk when compared to healthy controls. No difference was found for total visits, rewarded visits, and drinking speed, or in any feeding behavior after medical treatment (after d 0).

Calves with respiratory disease were less active, had fewer lying bouts, and consumed less milk around the time of diagnosis as compared to healthy calves. These differences were most apparent on the day prior to and the day of diagnosis. A previous study found a reduction in steps during the 24 h period after experimentally inducing pneumonia (Hanzlicek et al., 2010). In the current study, step activity declines one day prior to clinical signs of respiratory disease, and on the day of diagnosis in naturally occurring respiratory disease events, with a smaller difference still noted post-treatment. Additionally, lying bouts began to decrease two days prior to diagnosis, as well as after diagnosis; however, no difference was seen on the day of diagnosis. In the present trial, calves diagnosed with respiratory disease were treated with tulathromycin (Draxxin®, Zoetis Inc., Kalamazoo, MI) on the day of diagnosis. In a safety study, calves treated

with tulathromycin displayed transient pain behaviors such as head shaking and pawing at the ground following administration (Zoetis, 2014). In a past research study examining pain management following castration of calves, an increase in lying bouts was associated with pain (Olson et al., 2016). In the present study, a lack of difference in lying bouts between diseased calves and controls on d 0 was observed. This could be due to transient pain from tulathromycin administration that caused an increase in lying frequency in diseased calves.

Previous research examined the effect of disease on feeding behavior (Svensson and Jensen, 2007; Borderas et al., 2009). Borderas et al. (2009) found that diseased calves on a high milk allowance (12 L/d or ad libitum) consumed less milk in fewer visits, whereas Svensson and Jensen (2007) found a reduction in unrewarded visits, but no difference in milk intake in calves fed restrictively (5.6 to 8.5 L/d). In the present study, only respiratory disease events that occurred during the high milk allowance period were utilized. Our findings were in agreement with Borderas et al. (2009), as diseased calves on a high milk allowance in the present study consumed less milk on the day of diagnosis when compared to healthy controls.

However, in contrast to previous studies, no difference was found in total visits or rewarded visits. Previous studies clinically examined calves for disease during the first 3 to 4 weeks of age (Svensson and Jensen, 2007; Borderas et al., 2009). In this study, calves were examined until weaning (d 55), with the average respiratory disease event occurring at 33.1 ± 8.8 d (age: Mean \pm SD). It is possible that the effect of disease on visit frequency is limited to the first few weeks of age, or potentially to diseases other than respiratory events. The lack of difference found in feed intake after medical treatment suggests that the administration of tulathromycin restores appetite back to normal levels.

In diseased animals, behaviors that are associated with health benefits, such as play and exploratory behavior (Jensen et al., 1998; Cramer and Stanton, 2015; Cramer et al., 2016), are typically the first to decline, as sick animals divert resources from less critical functions to those that are essential for life (Weary et al., 2009). Play and exploratory behavior were not specifically measured in the current study; however, it is likely that the reduction in step activity is a result of diseased calves having less motivation to express play and/or exploratory behaviors (de Passillé et al., 2010). It should also be noted that feeding behaviors did not display any differences prior to d 0. Furthermore, milk intake returned to normal levels more quickly after medical treatment (d 0) than activity. Because activity displays differences prior to d 0 and after d 0, it appears that sickness behavior is more accentuated in activity than in feed intake. This may be due to the critical role of feeding behaviors in life functions, whereas activity is a behavior that is less essential for life, and therefore, more apt to decline when an animal is diseased.

In conclusion, calves with respiratory disease were less active, had reduced lying frequencies, and consumed less milk than healthy controls before or during the day of diagnosis. After treatment, milk intake of diseased calves returned to normal levels; however, a difference was still noted in activity and lying bouts. Future research is needed to test the ability of these technologies to identify disease prospectively.

Acknowledgements

The authors would like to recognize H. Schramm for her veterinary medicine knowledge, M. McGilliard for his statistical expertise, C. Henderson for her calf management assistance, and the farm staff at the Virginia Tech dairy facility for their support.

Table 3.1 Definitions of the scoring system used to determine disease status adapted from the Calf Health Scorer app (University of Wisconsin, School of Veterinary Medicine).

	0	1	2	3
Respiratory scoring				
Eye discharge	Normal serous discharge	Mild or small amount of discharge	Moderate amount of discharge on both eyes	Heavy discharge
Ear disposition	Normal	Ear flick or head shake	One ear with a slight droop	Both ears droop and/or severe head tilt
Nasal discharge	Normal, clear discharge	Unilateral slightly cloudy discharge	Bilateral cloudy discharge	Heavy bilateral purulent discharge
Coughing	No coughing	Single induced cough	Induced multiple coughs or occasional spontaneous cough	Repeated spontaneous coughs
Rectal Temperature (°C)	37.8 to 38.2	38.3 to 38.8	38.9 to 39.4	Greater than 39.4
Fecal scoring				
Fecal consistency	Normal	Pasty, semi-formed	Loose, but remains on top of the bedding	Watery, sifts through the bedding
Other scoring				
Navel scoring	Normal	Slightly enlarged, but not warm or painful	Slightly enlarged, with some pain or moisture	Enlarged, painful, and warm, with a foul odor
Joint scoring	Normal	Slight swelling, no pain or warmth	Swelling with pain, some lameness	Swelling with severe pain, heat, and lameness

Table 3.2 *F*-value and probability level for the effect of day, health status, and the interaction of day and health status on step activity (no./d), lying bouts (no./d), and milk intake (mL/d).

Source	Num df ¹	Steps ²		Lying bouts ²		Milk intake ³	
		<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
Health status	1	15.36	< 0.01	9.20	< 0.01	0.11	0.74
Day	6	2.20	0.05	5.41	< 0.01	1.77	0.11
Day * Health status	6	0.94	0.47	3.49	< 0.01	1.15	0.34

¹For random components, num df = 9, 9, and 108 for pair, pair * health status, and the residual, respectively.

²Den df = 20, 97, and 97 for health status, day, and day * health status, respectively for both steps and lying bouts. Den df approximated using the Kenward-Roger method.

³Den df = 30, 103, and 103 for health status, day, and day * health status, respectively for milk intake. Den df approximated using the Kenward-Roger method.

Figure 3.1 Mean (\pm SE) for daily steps and lying bouts of diseased and healthy calves at each day (with d 0 representing the day respiratory disease was first diagnosed). Differences between healthy and diseased calves: * $P \leq 0.05$, † $P < 0.10$.

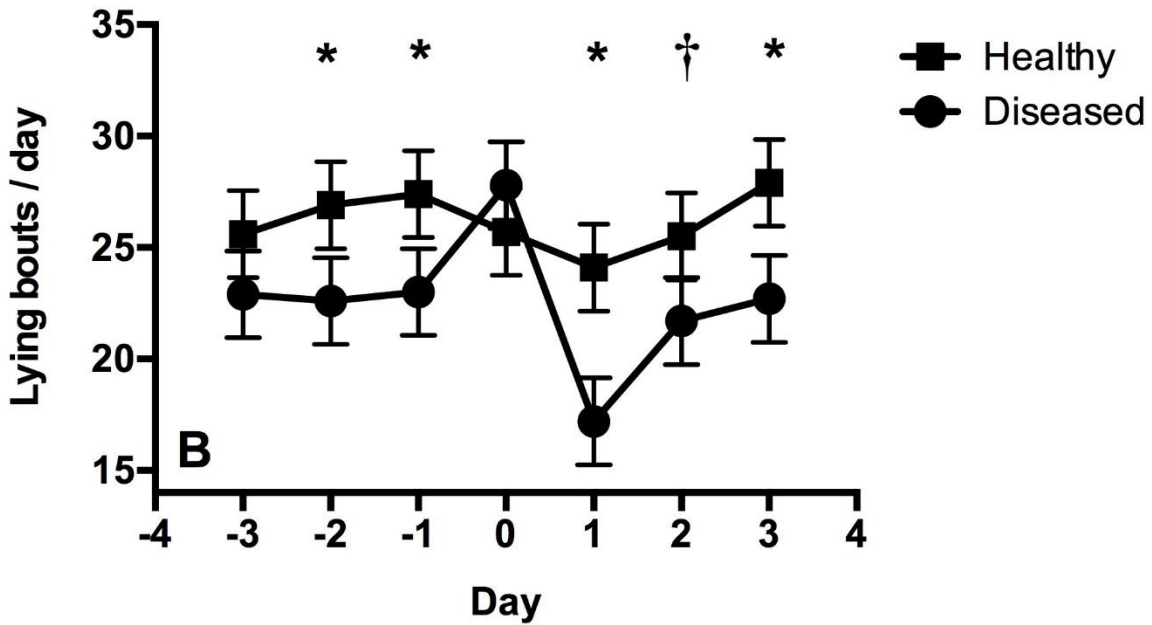
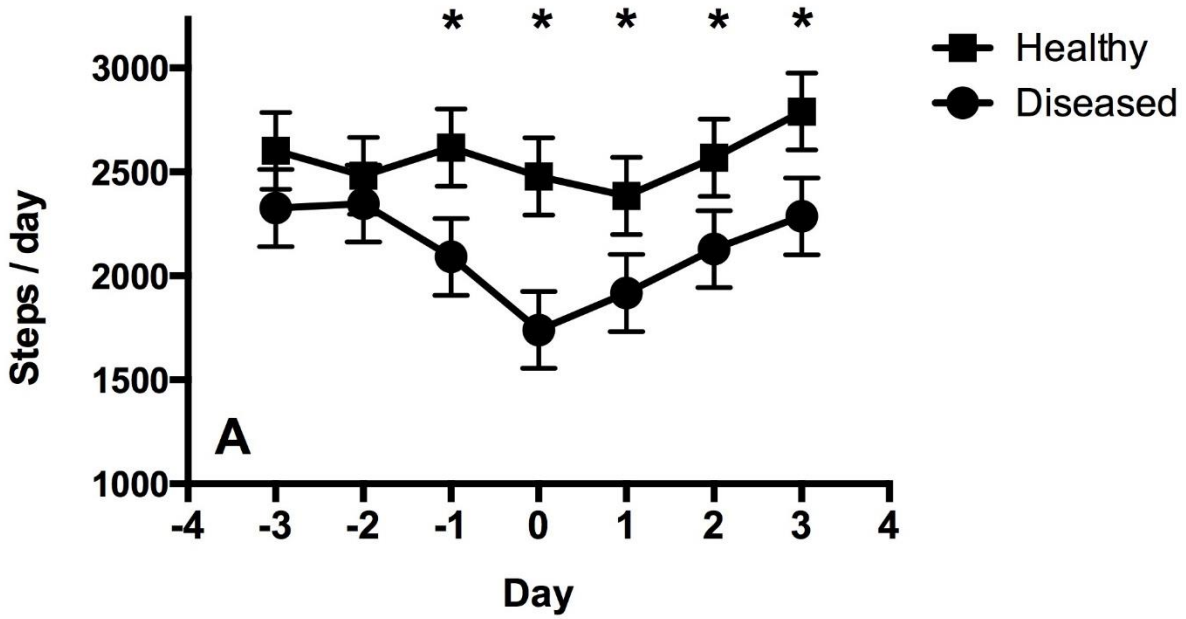
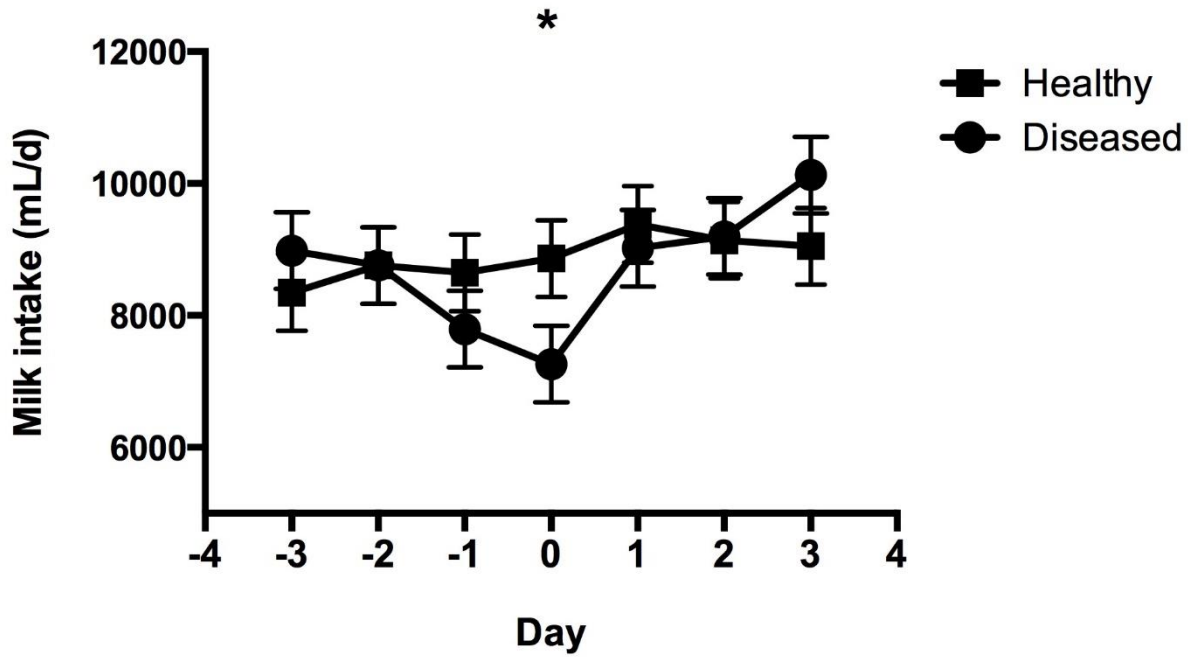


Figure 3.2 Mean (\pm SE) for milk intake of diseased and healthy calves at each day (with d 0 representing the day respiratory disease was first diagnosed). Differences between healthy and diseased calves: $*P \leq 0.05$.



REFERENCES

- Borderas, T. F., J. Rushen, M. A. G. von Keyserlingk, and A. M. B. de Passillé. 2009. Automated measurement of changes in feeding behavior of milk-fed calves associated with illness. *J. Dairy Sci.* 92:4549-4554.
- Cramer, M. C., T. L. Olivett, and A. L. Stanton. 2016. Associations of behavior-based measurements and clinical disease in preweaned, group-housed dairy calves. *J. Dairy Sci.* 99:7434-7443.
- Cramer, M. C. and A. L. Stanton. 2015. Associations between health status and the probability of approaching a novel object or stationary human in preweaned group-housed dairy calves. *J. Dairy Sci.* 98:7298-7308.
- de Passillé, A. M., M. B. Jensen, N. Chapinal, and J. Rushen. 2010. Technical note: Use of accelerometers to describe gait patterns in dairy calves. *J. Dairy Sci.* 93:3287-3293.
- Dolecheck, K. A., W. J. Silvia, G. Heersche Jr, Y. M. Chang, D. L. Ray, A. E. Stone, B. A. Wadsworth, and J. M. Bewley. 2015. Behavioral and physiological changes around estrus events identified using multiple automated monitoring technologies. *J. Dairy Sci.* 98:8723-8731.
- Duve, L. R., D. M. Weary, U. Halekoh, and M. B. Jensen. 2012. The effects of social contact and milk allowance on responses to handling, play, and social behavior in young dairy calves. *J. Dairy Sci.* 95:6571-6581.
- Gaillard, C., R. K. Meagher, M. A. von Keyserlingk, and D. M. Weary. 2014. Social housing improves dairy calves' performance in two cognitive tests. *PLoS One* 9:e90205.
- Hanzlicek, G. A., B. J. White, D. Mosier, D. G. Renter, and D. E. Anderson. 2010. Serial evaluation of physiologic, pathological, and behavioral changes related to disease progression of experimentally induced *Mannheimia haemolytica* pneumonia in postweaned calves. *Am. J. Vet. Res.* 71:359-369.
- Jensen, M. B., K. S. Vestergaard, and C. C. Krohn. 1998. Play behaviour in dairy calves kept in pens: the effect of social contact and space allowance. *Appl. Anim. Behav. Sci.* 56:97-108.
- Kenward, M. G. and J. H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 983-997.
- Kung, L., Jr., S. Demarco, L. N. Siebenson, E. Joyner, G. F. Haenlein, and R. M. Morris. 1997. An evaluation of two management systems for rearing calves fed milk replacer. *J. Dairy Sci.* 80:2529-2533.
- McGuirk, S. M. 2008. Disease management of dairy calves and heifers. *Vet. Clin. North Am. Food Anim. Pract.* 24:139-153.

- Miller-Cushon, E. K. and T. J. DeVries. 2016. Effect of social housing on the development of feeding behavior and social feeding preferences of dairy calves. *J. Dairy Sci.* 99:1406-1417.
- Olson, M. E., B. Ralston, L. Burwash, H. Matheson-Bird, and N. D. Allan. 2016. Efficacy of oral meloxicam suspension for prevention of pain and inflammation following band and surgical castration in calves. *BMC Vet. Res.* 12:102.
- Pempek, J. A., M. L. Eastridge, S. S. Swartzwelder, K. M. Daniels, and T. T. Yohe. 2016. Housing system may affect behavior and growth performance of Jersey heifer calves. *J. Dairy Sci.* 99:569-578.
- Rutten, C. J., A. G. J. Velthuis, W. Steeneveld, and H. Hogeveen. 2013. Invited review: Sensors to support health management on dairy farms. *J. Dairy Sci.* 96:1928-1952.
- Schaefer, A. L., N. J. Cook, C. Bench, J. B. Chabot, J. Colyn, T. Liu, E. K. Okine, M. Stewart, and J. R. Webster. 2012. The non-invasive and automated detection of bovine respiratory disease onset in receiver calves using infrared thermography. *Res. Vet. Sci.* 93:928-935.
- Soberon, F., E. Raffrenato, R. W. Everett, and M. E. Van Amburgh. 2012. Prewaning milk replacer intake and effects on long-term productivity of dairy calves. *J. Dairy Sci.* 95:783-793.
- Svensson, C. and M. B. Jensen. 2007. Short communication: Identification of diseased calves by use of data from automatic milk feeders. *J. Dairy Sci.* 90:994-997.
- Svensson, C., K. Lundborg, U. Emanuelson, and S. O. Olsson. 2003. Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. *Prev. Vet. Med.* 58:179-197.
- Swartz, T. H., M. L. McGilliard, and C. S. Petersson-Wolfe. 2016. Technical note: The use of an accelerometer for measuring step activity and lying behaviors in dairy calves. *J. Dairy Sci.* 99:9109-9113.
- Trenel, P., M. B. Jensen, E. L. Decker, and F. Skjoth. 2009. Technical note: Quantifying and characterizing behavior in dairy calves using the IceTag automatic recording device. *J. Dairy Sci.* 92:3397-3401.
- USDA-APHIS. 2007. Heifer calf health and management practices on U.S. dairy operations, 1991-2007. National Animal Health Monitoring System, Animal and Plant Health Inspection Service and Veterinary Services, Fort Collins, CO.
- Weary, D. M., J. M. Huzzey, and M. A. von Keyserlingk. 2009. Board-invited review: Using behavior to predict and identify ill health in animals. *J. Anim. Sci.* 87:770-777.
- Zoetis. 2014. Tulathromycin injection package insert - Draxxin.
<https://www.zoetisus.com/products/pages/draxxindairy/documents/draxxin-marketing-pi.pdf>
(accessed 21 April 2017).

Meloxicam administration to dairy cows around the time of calving by Swartz. The goal of this study was to determine if meloxicam administration around the time of calving would affect animal activity and milk yield in transition dairy cows. Meloxicam administration before calving increased milk yield after an easy calving, when compared to administration of meloxicam after calving and negative controls. Furthermore, pre-calving meloxicam administration increased milk components regardless of calving difficulty. Lastly, dystocia increased lying bouts on the day of calving and increased the duration of lying bouts after calving. Activity after a difficult calving was reduced when compared to cows that calved easily.

Chapter 4. Meloxicam administration either prior to or after parturition: Behavioral, physiological, and production responses

T. H. Swartz,* H. H. Schramm,† J. M. Bewley,‡ C. M. Wood,# K. E. Leslie,§ and C. S. Petersson-Wolfe*

*Department of Dairy Science, Virginia Tech, Blacksburg, VA 24061

†VA-MD College of Veterinary Medicine, Blacksburg, VA 24061

‡CowFocused Housing, Bardstown, KY 40004

#Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA 24061

§Department of Population Medicine, University of Guelph, Guelph, ON N1G 2W1

Christina Petersson-Wolfe

2120 Litton Reaves Hall, Virginia Tech

175 West Campus Drive

Blacksburg, VA 24061

Phone: (540) 231-4767

milk@vt.edu

ABSTRACT

Periparturient dairy cows have been described as having an uncontrolled inflammatory response that contributes to a dysfunctional immune system. This phenomenon is a major contributor to the increase in disease incidence that occurs during the transition period. Previous research has shown that NSAID administration after calving can increase milk yield. To our knowledge, this is the first study that administered meloxicam prior to calving. Meloxicam was dosed at 1 mg/kg of BW, and an empty gel capsule served as a placebo; both of which were administered orally with a balling gun. Dairy cows were randomly assigned to one of three treatment groups: (1) meloxicam administration prior to calving, with a placebo administered after calving (**MEL-PRE**, n = 60), (2) placebo administered prior to calving, and meloxicam administered after calving (**MEL-POST**, n = 69), and (3) a placebo administered prior to calving and after calving (**CTL**, n = 65). In order to identify imminent calving events, a vaginal thermometer was inserted approximately 2 wk prior to the expected calving date, and a drop in temperature was used to identify cows close to calving. Calving events were monitored via video cameras. The amount of time that elapsed between the appearance of the amniotic sac at the vulva until delivery of the calf was used to determine calving difficulty score. Cows that calved in ≤ 70 min were designated as eutocic calving events, and cows that took longer than 70 min were designated as dystocia. Milk yield and components were measured for the first 15 wk of lactation. The effects of treatment, breed, parity, calving difficulty, and, when applicable, a repeated measure, along with interaction terms were analyzed in mixed models. Eutocic MEL-PRE animals produced 6.8 kg/d more milk than eutocic CTL. Regardless of calving difficulty, MEL-PRE animals produced more milk fat, protein, and lactose (kg/d) than the CTL. Cows that received meloxicam after calving, regardless of calving difficulty, produced more milk fat kg/d than CTL. Meloxicam administration around the time of calving increased milk yield and

components. Additional research is needed to determine appropriate treatments for dystocic calvings.

Key words: periparturient dairy cow, nonsteroidal anti-inflammatory drug, meloxicam, behavior

INTRODUCTION

Parturition is necessary for dairy production. Yet, it is a risky period, where the incidence of disease, injury, and mortality are high. This increase in disease incidence has been associated with an uncontrolled inflammatory response (Sordillo and Raphael, 2013). After calving, inflammation has been documented in both healthy and diseased cattle (Humblet et al., 2006; Bionaz et al., 2007; Huzzey et al., 2009; Graugnard et al., 2012; Mullins et al., 2012; Qu et al., 2014). These data suggest that cattle experience at least some degree of inflammation due to tissue damage associated with parturition, as well as the immense metabolic demand associated with the onset of lactation. Dystocia, defined as delayed or difficult calving, is associated with a heightened inflammatory response. The odds of an elevated concentration of haptoglobin, an inflammatory marker, were 2.5 times greater in multiparous animals that experienced an assisted calving compared to those who did not require assistance (Pohl et al., 2015). Furthermore, dystocia increases the odds of retained placenta, displaced abomasum, and metritis (Correa et al., 1993), resulting in a decrease in milk, fat, and protein yields, as well as an increase in days open, number of services, and mortality rates (Dematawewa and Berger, 1997). Dystocia reduced 305-d lactation milk yield in both primiparous and multiparous animals, and decreased peak milk yield by approximately 2 kg/d in multiparous cows (Atashi et al., 2012). The incidence of dystocia can vary from herd to herd; however, in North America, literature suggests 10 to 40 percent of calvings will experience a difficult birth (Meyer et al., 2001; Lombard et al., 2007; USDA-APHIS, 2010).

In addition to inflammation associated with the process of parturition, the postpartum period is often characterized by a low-grade chronic inflammatory state (Bradford et al., 2015). This chronic inflammation associated with metabolic disorders is typically referred to as

metabolic inflammation (Hotamisligil, 2006). During early lactation, nutrient demand outstrips feed intake, and consequently, dramatic changes in biological and metabolic processes occur, resulting in the breakdown of fat for energy. After parturition, total lipids in the liver have been positively correlated with acute phase proteins, haptoglobin and serum amyloid A (Ametaj et al., 2005). Inflammatory states have been documented in liver and adipose tissue during the first week of lactation (Loor et al., 2005; Sadri et al., 2010; Saremi et al., 2012; Gessner et al., 2013). Cows with reduced liver activity had elevated inflammatory states after calving, and these cows had lower blood calcium concentrations, greater incidence of disease, took longer to conceive, and produced less milk during the first 4 wk of lactation when compared to cows with higher liver activity (Bertoni et al., 2008).

As nutrient demand exceeds intake, non-esterified fatty acids (NEFA) begin to increase in blood during the final few weeks before calving, and this surge has been associated with an escalation of disease incidence in postpartum dairy cows (LeBlanc et al., 2005; Ospina et al., 2010). To combat this problem, administration of monensin, an ionophore, 3 wk prior to calving decreased β -hydroxybutyrate (BHBA) and NEFA concentrations, and this effect carried over into lactation by reducing the prevalence of subclinical ketosis (Duffield et al., 2003). In a study examining the impact of NEFA concentrations and compositions, the authors concluded that NEFA profiles that mimic those of the transition period increased the production of pro-inflammatory eicosanoids via an increased expression of the cyclo-oxygenase-II enzyme (COX-2; Contreras et al., 2012). In another study, serum NEFAs collected from transition dairy cows reduced the function and proliferation of peripheral blood mononuclear cells and decreased phagocytosis and oxidative burst capabilities of neutrophils in cell culture (Ster et al., 2012). Therefore, when examining the above studies jointly, alleviation of metabolic stress, and thus

metabolic inflammation, pre-calving may have merit in reducing both metabolic and infectious disease incidence post-calving.

Considering the above elements in addition to ones not mentioned (heat stress, social stress, to name a few), it can be collectively summarized that the transition period sets the stage for inflammation in the periparturient dairy cow (Bradford et al., 2015). The implications of this heightened inflammatory state are not completely understood; however, inflammatory markers, such as haptoglobin, are markedly higher preceding clinical disease onset in cattle (Qu et al., 2014). Aside from milk fever, clinical disease incidences during the periparturient period have remained unchanged in the dairy industry over the past 20 to 30 years (Goff, 2006; USDA-APHIS, 2008; Bradford et al., 2015). Studies investigating mitigation of the inflammatory response around parturition could potentially lead to better results.

One potential solution to this problem is the administration of a nonsteroidal anti-inflammatory drug (NSAID) to reduce inflammation in the periparturient dairy cow. Nonsteroidal anti-inflammatory drugs are cyclo-oxygenase (COX) inhibitors, which can be utilized to mediate prostaglandin production associated with inflammation. Two isoforms of COX enzymes exist, COX-1 and COX-2. Cyclo-oxygenase-I is involved with gastric cytoprotection, renal water balance, and platelet aggregation (Morita, 2002), and consequently, COX-1 inhibitors are associated with an increased risk for adverse gastrointestinal and renal effects (Coetzee, 2013). The function of COX-2, however, is more associated with inflammation (Morita, 2002). Therefore, much work has been focused on the development of drugs that selectively inhibit COX-2 to reduce inflammation, while preventing the consequences of blocking COX-1. In the United States, flunixin meglumine is the only FDA-approved NSAID for use in cattle (Coetzee, 2013). Flunixin meglumine is both a COX-1 and COX-2 inhibitor. It has

greater anti-COX-1 activity than most other NSAIDs (Beretta et al., 2005), and has an elimination half-life of 3 to 8 h (Anderson et al., 1990). Unfortunately, the administration of flunixin meglumine around the time of calving has been unsuccessful in reducing disease incidence (Shwartz et al., 2009; Newby et al., 2017). In fact, flunixin meglumine administration increased the risk for retained placenta, elevated temperature, decreased milk yield, and a higher incidence of metritis when compared to controls (Newby et al., 2017). It is possible that the mode of action of flunixin meglumine may be contributing to this disappointment. Another NSAID, meloxicam, has been approved for use in cattle in many European countries as well as Canada. Meloxicam is a COX-2 preferential inhibitor in most species (Beretta et al., 2005); however, this affinity has not been proven in cattle. Meloxicam has an elimination half-life of 23 to 27 h in low producing and 17.5 h in high producing lactating cattle (EMEA, 2007), and this long half-life potentially makes a single dose more effective. A withdrawal period of 5 d for milk and 15 d for meat in the EU (EMEA, 2007), and a 4 d milk withhold and a 20 d meat withhold in Canada have been established (Boehringer Ingelheim (Canada) Ltd., 2017).

Past research has examined the effect of meloxicam administration in periparturient dairy cows with variable results (Newby et al., 2013; Mainau et al., 2014; Carpenter et al., 2016). However, these studies administered meloxicam after calving only. Dystocia has been identified as a risk factor for clinical ketosis (Duffield et al., 2009). This finding suggests that the inflammation from a difficult calving may impact metabolic stress, and therefore, exacerbate metabolic inflammation. Recognizing that metabolic stress induces inflammation via COX-2 (Contreras et al., 2012), meloxicam, a COX-2 preferential inhibitor, may be a more efficacious treatment. Therefore, if the processes of parturition are the underpinning of the heightened inflammatory state, administration of an NSAID directly prior to calving to alleviate

inflammation may have merit. Recent automated technologies have become available which are moderately accurate in identifying imminent calving events, thus allowing for the possibility of a treatment to be administered before parturition. Therefore, the objective of this study was to evaluate the administration of meloxicam prior to calving on behavior, health, and production measures. Furthermore, we compared the effect of meloxicam administration prior to calving to post-calving meloxicam administration to determine if the time of administration yielded different responses. We hypothesized that the administration of meloxicam prior to calving would provide greater improvement of animal welfare and production measures than post-calving administration, and that both treatment groups would outperform the controls. Moreover, because dystocia exacerbates the heightened inflammatory state that already occurs around calving, we anticipated that NSAID-treated cows that experienced a difficult calving would display a larger response to treatment than NSAID-treated cows that calved easily.

MATERIALS AND METHODS

This study was conducted at the Virginia Tech Dairy Research Complex in Blacksburg, VA with enrollment occurring from August 2016 through August 2017. All experimental procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee (14-265).

Design and treatments

Approximately 3 wk prior to the expected calving date, dry cows and pregnant heifers were moved to group housing in a compost bedded pack barn with access to a close-up dry cow Total Mixed Ration (TMR). Just prior to arrival in the close-up dry cow pen, animals were weighed using an automated scale (AfiWeigh, Afimilk LTD., Kibbutz Afikim, Israel), and this

weight was used to determine the appropriate meloxicam dosage for administration around calving (1 mg/kg of BW, administered by mouth; Meloxicam tablets, USP(15 mg), Cipla LTD., Kurkumbh, India). Meloxicam tablets were placed into gel capsules, and an empty gel capsule was utilized as a placebo; both were administered with a balling gun. To administer boluses, cows were restrained using a headlock at the feed bunk. Cows were randomly assigned to one of three treatment groups and the regimens were as follows (Figure 1.): (1) meloxicam administration no more than 48 h prior to calving and no less than 6 h prior to calving, and a placebo administered within 12 h post-calving (**MEL-PRE**), (2) placebo administration no more than 48 h prior to calving and no less than 6 h prior to calving, and meloxicam administered within 12 h post-calving (**MEL-POST**), and (3) placebo administration no more than 48 h prior to calving and no less than 6 h prior to calving, and a placebo administered within 12 h post-calving (**CTL**). The pre-calving treatment window was based upon a previous pharmacokinetic study, where meloxicam concentration peaked approximately 11 h after oral administration (Malreddy et al., 2013). Dry cows (n = 237) had a vaginal thermometer (Vel'Phone, Medria, Châteaubourg, France) inserted approximately 14 d prior to their expected calving date. This device was used to detect the drop in vaginal temperature that occurs within 48 h prior to calving (Burfeind et al., 2011; Saint-Dizier and Chastant-Maillard, 2015; Ouellet et al., 2016).

Dystocia scoring

Dry cows were continuously monitored 24-h/d using three video cameras (Axis P1353-E, Axis Communications AB, Lund, Sweden). These cameras were equipped with Lightfinder technology and as a result required only minimal lighting to capture calvings during night hours. The camera feeds were transmitted via an Ethernet cord to a computer and were recorded in 3-h segments using video recording software (Media Recorder, Noldus Information Technology Inc.,

Leesburg, VA). The video was used for the purpose of calving difficulty scoring. Dystocia is typically quantified in a scoring system using the amount of assistance as the determining factor for the degree of difficulty; however, these scoring systems can be subjective and therefore variable between observers. More recently, the amount of time between the presence of the amniotic sac at the vulva and the expulsion of the calf (Stage 2 of labor) has been examined between eutocic and dystocic calvings (Schuenemann et al., 2011). The authors concluded that a calving event with Stage 2 labor > 70 min was indicative of a calving that should be assisted, and this threshold has been utilized in previous research (Kovacs et al., 2016a; Kovacs et al., 2016b; Villettaz Robichaud et al., 2017a; Villettaz Robichaud et al., 2017b). Therefore, in the present study, dystocia was defined when Stage 2 of labor was > 70 min, and eutocia was defined as \leq 70 min. Apart from two cows, eutocic calving events were not provided with assistance. In those two calving events, the farm managers deemed that the calf needed to be extracted due to an emergency such as malpresentation of the fetus. These two cows remained as part of the study, and were deemed as eutocic calving events, as both calving events were less than 70 min in length. The determination of which animals required assistance during calving was left to the discretion of the farm managers and staff, and was performed as part of routine farm management. Farm managers and staff were masked to treatments during the calving to prevent bias towards assisting calvings in one treatment group over another.

Behavior

Upon entry into the close-up dry cow pen, animals were fitted with an accelerometer (AfiTag II, AfiMilk LTD., Kibbutz Afikim, Israel) which measured steps, lying time, and lying bouts. These behaviors were collected wirelessly every 30 min and transmitted to a computer (AfiAct II, Afimilk LTD., Kibbutz Afikim, Israel). Data were summarized into daily steps

(no./d), lying time (min/d), and lying bouts (no./d) using the SUM option in PROC MEANS (SAS ver. 9.4, SAS Institute Inc., Cary, NC). Lying bout duration was calculated by dividing the lying time by the number of lying bouts on that day. Data recording from the accelerometer began 14 d before the expected calving date and concluded at 7 DIM. Data from day -3, the day prior to treatment regimen, was used as a covariate to account for individual differences in behaviors.

Health

Rectal temperatures for the first 7 DIM were taken at approximately 0900 h using a digital rectal thermometer (SharpTemp V, Cotran Corp., Portsmouth, RI). Clinical disease events were diagnosed by university veterinarians, who were masked to treatments, for the first 30 DIM. Health events were recorded in farm management software (PC Dart, Dairy Records Management Systems, Raleigh, NC). These diseases included retained fetal membranes, milk fever, clinical mastitis, clinical ketosis, metritis, and left displaced abomasum. All clinical disease events were defined following Kelton et al. (1998). Stillbirth was defined as a calf that was born dead. A single blood sample was taken 3 d after calving for beta-hydroxybutyrate (BHBA) testing. A previously validated handheld meter (Nova Vet Meter, Nova Biomedical, Waltham, MA) was adjusted using the 1.25 calibration slope according to the user manual for the purpose of testing ketone levels in bovine blood (Bach et al., 2016). A quality control test was done using a control solution (Nova Max Plus Glu/Ket Control Solution – Mid, Nova Biomedical, Waltham, MA) to ensure accuracy of the meter. A minimum of 0.1 mL blood sample was collected by coccygeal venipuncture with a 1-mL syringe and 20-gauge needle. A test strip was inserted into the handheld meter and the blood was placed on the test strip. Within

a few seconds, the handheld meter provided a numerical BHBA value. Lastly, Body Condition Score (BCS) was recorded on the day of calving, using a 1 to 5 scale (Ferguson et al., 1994).

Milk yield and milk components

On the day of calving, cows were moved into an adjacent compost bedded pack pen where they remained through the first 7 DIM. After which, cows were moved into a deep sand-bedded freestall barn for the remainder of the trial. Cows were fed a lactating cow TMR and were milked twice daily. Milk was weighed through an automated milk meter and milk components were analyzed via near-infrared spectroscopy (NIR) at each milking for the first 15 wk of lactation (AfiMilk MPC Milk Meter and AfiLab Milk Analyzer, Afimilk LTD., Kibbutz Afikim, Israel). This NIR system has been previously validated (Kaniyamattam and De Vries, 2014). Although a milk withhold of 5 d should be sufficient (Malreddy et al., 2013), milk was discarded for the first 7 DIM, after it passed through the meter and the NIR system, to ensure no residues from meloxicam treatment would enter the food supply.

Statistical analysis

A mixed model (PROC GLIMMIX, SAS ver. 9.4, SAS Institute Inc., Cary, NC) was used to analyze the data. Fixed effects included treatment (MEL-PRE, MEL-POST, or CTL), breed (Holstein or Jersey), parity (primiparous or multiparous), and calving difficulty score (eutocia or dystocia). A repeated measure (week of lactation in milk responses or day in other models) was included when applicable. Cow was modeled as a random effect. Repeated measures over time within cow were modeled using the autoregressive error structure and the Kenward-Rogers procedure was used for degrees of freedom approximation (Kenward and Roger, 1997). All two- and three-way interaction terms of the fixed effects were introduced into the model. For milk

data, the Predicted Transmitting Ability (PTA) for the variable of interest (PTA Milk, PTA Fat, or PTA Protein) was included as a covariate in the model to account for genetic differences contributing to variation. Because a genetic parameter for lactose yield does not exist, the PTA for milk yield was used for the lactose model instead. Body condition score was tested as a covariate in milk responses, in addition to the BHBA model. Additionally, for milk data, interaction terms of treatment with the covariates (PTA milk, PTA fat, PTA protein, or BCS) were tested. For behavioral measurements (steps, lying time, lying bouts, and lying bout duration), data from d -3 (the day prior to treatment regimen) for the variable of interest was used as a covariate to account for individual differences in those behaviors. Backwards elimination was used to eliminate non-significant terms from highest to least significant until all variables were significant ($P \leq 0.05$); however, the main effect of treatment was forced into the model regardless of significance. Main effects were retained in the model if $P \leq 0.05$ or if the main effect was involved in a significant interaction term. Two-way interaction terms were retained if $P \leq 0.05$ or if the interaction term was involved in a significant three-way interaction term. For interaction terms involving treatment, simple effects tests were investigated using the SLICEDIFF option and Tukey adjusted P -values were declared significant at $P \leq 0.05$.

Calving length was analyzed as a continuous response variable to determine if pre-calving meloxicam administration prolonged parturition. This model included fixed effects of treatment (MEL-PRE, MEL-POST, CTL), breed (Holstein or Jersey), parity (primiparous or multiparous), and calving assistance (assisted or not assisted). For all models, the residuals were checked for normality and outliers. Calving length was not normally distributed, and therefore a logarithmic transformation was used. All other data were normally distributed, and all assumptions of the model were met.

For health events, Fisher's exact test was used (PROC FREQ, SAS ver. 9.4, SAS Institute Inc., Cary, NC). Significance was declared at $P \leq 0.05$.

RESULTS

Because vaginal temperature has a moderate sensitivity for identifying imminent calving events, some cows did not receive treatment in the pre-calving treatment window and were therefore excluded from analysis. Furthermore, cows that calved with twins or calved via cesarean section were also excluded. Therefore, the final sample size ($n = 194$; 87 multiparous Holstein, 48 primiparous Holstein, 41 multiparous Jersey, and 18 primiparous Jersey) included only cows that calved vaginally with single births (MEL-PRE, $n = 60$; MEL-POST, $n = 69$; CTL, $n = 65$).

Provided below are descriptive characteristics of the calving events. Using the 70 min threshold for calving length, 81 cows (42%) were designated as having dystocic calvings, similar to dystocia incidence found by Lombard et al., 2007, and 113 cows (58%) were designated as having eutocic calvings. Gender of the fetus was split approximately evenly within each treatment group (MEL-PRE, 32 female, 28 male; MEL-POST, 35 female, 34 male; CTL, 32 female, 33 male). Of the 81 cows that experienced a difficult calving, 41 of those calvings resulted in male calves, and 40 yielded female calves. Of the 113 cows that had an easy calving, 54 of those calvings resulted in male calves, and 59 of those calvings yielded female calves. Calf birthweights were approximately the same between treatment groups (Mean \pm SD; MEL-PRE, 36.5 ± 8.0 ; MEL-POST, 37.9 ± 7.7 ; CTL, 34.9 ± 7.6 kg). Calf birthweight was 35.8 ± 7.5 and 37.4 ± 8.3 kg for eutocic and dystocic calving events, respectively. For Holstein cattle, the genetic parameter, Sire Calving Ease (SCE), for the sire of the fetuses were similar between treatment groups (Mean \pm SD; MEL-PRE, 7.2 ± 0.8 ; MEL-POST, 7.0 ± 0.8 ; CTL, 7.0 ± 0.9).

Sire Calving Ease was 7.0 ± 0.9 and 7.0 ± 0.7 for eutocic and dystocic calving events, respectively. Sire Calving Ease data are not available for the Jersey breed. The number of cows that received assistance during calving was similar across treatment groups and within industry norms (USDA-APHIS, 2010; n = number of assisted calvings, percent of treatment group; MEL-PRE, n = 14, 23%; MEL-POST, n = 17, 25%; CTL, n = 11; 17%). Gestation length was similar across treatments (MEL-PRE, 278 ± 5 ; MEL-POST, 277 ± 5 ; CTL, 277 ± 5 d). For multiparous cows, the length of the dry period was similar between treatment groups (Mean \pm SD; MEL-PRE, 68 ± 20 ; MEL-POST, 66 ± 15 ; CTL, 70 ± 23 d). Parities were distributed similarly across treatment groups, with approximately 33 to 35 percent first lactation, 20 to 25 percent second lactation, and 42 to 45 percent 3+ lactation cows represented within each treatment group (All parities: Mean \pm SD; MEL-PRE, 2.4 ± 1.2 ; MEL-POST, 2.2 ± 1.1 ; CTL 2.4 ± 1.5). For just multiparous animals, parities were distributed similarly (Mean \pm SD; MEL-PRE, 3.0 ± 0.9 ; MEL-POST, 2.9 ± 0.8 ; CTL 3.2 ± 1.3). Body weight was measured approximately 21 d before expected calving date, and this weight was similar across treatment groups (Mean \pm SD; Holstein: MEL-PRE, 694 ± 97 ; MEL-POST, 711 ± 108 ; CTL, 677 ± 109 ; Jersey: MEL-PRE, 521 ± 53 ; MEL-POST, 540 ± 90 ; CTL, 540 ± 90 kg). Body Condition Score at calving was similar across treatment groups (Mean \pm SD; MEL-PRE 3.3 ± 0.4 , MEL-POST 3.3 ± 0.4 , CTL 3.4 ± 0.4).

Behavioral data

Significant main effects and interactions for activity, lying bouts, lying time, and lying bout duration are provided in Table 4.1. Day -3 was used as a covariate to account for individual differences in behavior; however, some cows did not have d -3 data due to either missing data, accelerometer malfunction, or technical problems with the wireless data collection system.

Therefore, the final sample size for the behavioral data included 165 cows (MEL-PRE, n = 53; MEL-POST, n = 59; CTL n = 53). No effect of treatment or calving difficulty score was identified for lying time. Treatment LSM for lying time were 578 ± 10 , 586 ± 10 , and 560 ± 10 min/d for MEL-PRE, MEL-POST, and CTL, respectively. The breed by parity interaction was significant ($P < 0.01$). Least squares means were primiparous Holstein 594 ± 11 , multiparous Holstein 590 ± 8 , primiparous Jersey 511 ± 18 , and multiparous Jersey 602 ± 11 min/d.

For steps, the treatment by calving difficulty score interaction term was significant ($P = 0.03$), and this term was sliced by calving difficulty score to determine the simple effects of treatment within calving difficulty (Figure 4.2). No treatment effect was found in the eutocic calving animals. However, dystocic MEL-PRE animals were significantly less active than dystocic CTL (dystocic MEL-PRE $2,555 \pm 78$ vs. dystocic CTL $2,913 \pm 97$ steps/d, $P < 0.01$). Moreover, dystocic MEL-POST animals were less active than dystocic CTL (dystocic MEL-POST $2,480 \pm 91$ vs. dystocic CTL $2,913 \pm 97$ steps/d, $P < 0.01$). No difference was noted between the meloxicam treatment groups that had a difficult calving ($P = 0.79$).

Continuing with steps, the three-way interaction of treatment, breed, and day was significant ($P = 0.05$). This three-way interaction term was sliced by the two-way interaction term, breed by day, to determine the simple effect of treatment within breed and day. No differences were found between treatment groups within the Holstein breed; however, treatment effect was noted in the Jersey breed. Jersey animals that received meloxicam prior to calving were significantly less active on d -1 (MEL-PRE Jersey $2,503 \pm 162$ vs. CTL Jersey $3,069 \pm 166$ steps/d, $P = 0.03$), d 1 (MEL-PRE Jersey $3,335 \pm 159$ vs. CTL Jersey $4,033 \pm 166$ steps/d, $P < 0.01$), and d 2 (MEL-PRE Jersey $2,868 \pm 160$ vs. CTL Jersey $3,436 \pm 166$ steps/d, $P = 0.02$) when compared to CTL Jersey. Furthermore, MEL-PRE Jersey were less active than MEL-POST

Jersey on d 2 (MEL-PRE Jersey $2,868 \pm 160$ vs. MEL-POST Jersey $3,509 \pm 202$ steps/d, $P = 0.03$). Jersey cattle who received meloxicam after calving were less active than CTL Jersey on d 6 (MEL-POST Jersey $2,402 \pm 194$ vs. CTL Jersey $3,051 \pm 169$ steps/d, $P = 0.02$) and d 7 (MEL-POST Jersey $2,368 \pm 194$ vs. CTL Jersey $3,235 \pm 166$ steps/d, $P < 0.01$).

In the steps model, the three-way interaction term of calving difficulty score, breed, and day was significant ($P = 0.05$). This interaction term was sliced by the two-way interaction term, breed by day, to determine the simple effects of calving difficulty within breed and day. Dystocic Holstein animals demonstrated a reduction in activity on d 1 (dystocic Holstein $2,600 \pm 95$ vs. eutocic Holstein $3,010 \pm 95$ steps/d, $P < 0.01$), d 4 (dystocic Holstein $2,278 \pm 96$ vs. eutocic Holstein $2,546 \pm 94$ steps/d, $P = 0.05$), and d 5 (dystocic Holstein $2,222 \pm 97$ vs. eutocic Holstein $2,504 \pm 94$ steps/d, $P = 0.04$), when compared to eutocic Holstein calvings. The effect of dystocia on Jersey cattle was a bit less pronounced, as dystocic Jersey animals displayed a reduction in activity only on d 0 (dystocic Jersey $2,993 \pm 171$ vs. eutocic Jersey $3,627 \pm 140$ steps/d, $P < 0.01$) and d 2 (dystocic Jersey $3,006 \pm 166$ vs. eutocic Jersey $3,535 \pm 145$ steps/d, $P = 0.02$) when compared to eutocic Jersey.

For lying bouts, the three-way interaction term of treatment, parity, and day was significant (Figure 4.3; $P < 0.01$). Simple effects of the interaction term were investigated to determine the effect of treatment on lying bouts within each day. No effect of treatment was found between treatment groups of multiparous animals. However, primiparous MEL-PRE animals had more lying bouts on d 0 than primiparous CTL (primiparous MEL-PRE 23.0 ± 1.1 vs. primiparous CTL 17.9 ± 1.1 lying bouts/d, $P < 0.01$) and primiparous MEL-POST (primiparous MEL-PRE 23.0 ± 1.1 vs. primiparous MEL-POST 16.3 ± 0.9 lying bouts/d, $P < 0.01$). Moreover, on d 0, primiparous MEL-PRE animals had a similar number of lying bouts as

multiparous cows (all comparisons of primiparous MEL-PRE to multiparous MEL-PRE, MEL-POST, and CTL, $P \geq 0.89$). Furthermore, primiparous CTL had fewer lying bouts on the day of calving than that of all treatment groups of multiparous animals (primiparous CTL 17.9 ± 1.1 vs. 21.8 ± 0.7 multiparous CTL, $P = 0.02$; primiparous CTL 17.9 ± 1.1 vs. 21.6 ± 0.7 multiparous MEL-POST, $P = 0.04$; primiparous CTL 17.9 ± 1.1 vs. 22.9 ± 0.7 multiparous MEL-PRE, all comparisons $P < 0.01$). Similarly, primiparous MEL-POST animals had fewer lying bouts on the day of calving when compared to all treatment groups of multiparous animals (primiparous MEL-POST 16.3 ± 0.9 vs. 21.8 ± 0.7 multiparous CTL; primiparous MEL-POST 16.3 ± 0.9 vs. 21.6 ± 0.7 multiparous MEL-POST; primiparous MEL-POST 16.3 ± 0.9 vs. 22.9 ± 0.7 lying bouts multiparous MEL-PRE, all comparisons $P < 0.01$). A parity effect was identified on the day prior to calving, as primiparous MEL-POST animals showed more lying bouts than multiparous MEL-POST (primiparous MEL-POST 16.0 ± 0.9 vs. multiparous MEL-POST lying bouts 12.5 ± 0.7 , $P = 0.03$).

For lying bouts, the calving difficulty score by day interaction term was significant (Figure 4.4a; $P < 0.01$). Animals that experienced dystocia had a higher frequency of lying on d 0 than eutocic calving animals (dystocia 23.0 ± 0.5 vs. eutocia 18.2 ± 0.5 lying bouts/d, $P < 0.01$). After calving, animals that had a difficult calving displayed a reduction in lying bouts on d 2 (dystocia 10.2 ± 0.5 vs. eutocia 11.9 ± 0.5 lying bouts/d, $P = 0.02$) when compared to eutocia animals.

For lying bout duration, the calving difficulty score by day interaction term was significant ($P = 0.04$), and simple effects of calving difficulty were investigated within day (Figure 4.4b). Animals that experienced dystocia spent more time lying per bout on d 1 (dystocia 57 ± 2 vs. eutocia 48 ± 2 min/bout, $P < 0.01$), d 2 (dystocia 60 ± 2 vs. eutocia 49 ± 2 min/bout, P

< 0.01), d 4 (dystocia 61 ± 2 vs. eutocia 53 ± 2 min/bout, $P = 0.01$), and d 5 (dystocia 59 ± 2 vs. eutocia 52 ± 2 min/bout, $P = 0.03$). Treatment was not different ($P = 0.84$); LSM include MEL-PRE 51 ± 1 , MEL-POST 52 ± 1 , and CTL 51 ± 1 min/bout.

Health events, rectal temperature, calving length and BHBA

No treatment effect was identified in any of the health events data (Table 4.3). Total clinical disease events by treatment group include: MEL-PRE, $n = 6$; MEL-POST, $n = 18$; and CTL, $n = 14$. No treatment effect was identified in the number of cows with multiple disease events (Table 4.3; MEL-PRE, $n = 0$; MEL-POST, $n = 2$; and CTL, $n = 3$). For the cows with multiple disease events, 1 MEL-POST cow had four clinical disease events, 1 CTL cow had three clinical disease events, 1 MEL-POST had two clinical disease events, and 2 CTL cows had two clinical disease events. No treatment effect was identified in the number of stillborn calves ($P = 0.09$; Table 4.3). One calf was born deformed, and required euthanasia; therefore, this calf was excluded from the stillbirth analysis.

Calving length was not different between treatment groups (Logarithmic LSM \pm SE; MEL-PRE 1.94 ± 0.04 , MEL-POST 1.89 ± 0.04 , CTL 1.87 ± 0.04 $P = 0.32$). Holsteins took longer to calve than Jerseys (Logarithmic LSM \pm SE; Holstein 1.95 ± 0.03 vs. Jersey 1.85 ± 0.04 , $P = 0.02$). Primiparous animals took longer to calve than multiparous (Logarithmic LSM \pm SE; primiparous 1.99 ± 0.03 vs. multiparous 1.81 ± 0.03 , $P < 0.01$). Animals that received assistance at calving had a longer calving than cows that calved unassisted (Logarithmic LSM \pm SE; assisted 2.11 ± 0.04 vs. unassisted 1.69 ± 0.02 , $P < 0.01$).

The final model for rectal temperature included treatment ($P = 0.59$), calving difficulty score ($P < 0.01$), breed ($P < 0.01$), parity ($P = 0.24$), breed*calving difficulty score ($P = 0.09$),

day ($P < 0.01$), calving difficulty score*day ($P = 0.59$), parity*day ($P = 0.02$), breed*day ($P = 0.03$), and breed*calving difficulty score*day ($P = 0.03$). The three-way interaction term of breed, calving difficulty score, and day was sliced by the two-way interaction term, breed by day, to investigate the simple effects of calving difficulty within breed and day. Dystocic Holstein had a higher rectal temperature on d 3 (dystocic Holstein 38.9 ± 0.05 vs. eutocic Holstein $38.7 \pm 0.05^\circ\text{C}$, $P < 0.01$) than eutocic Holstein. Jersey cattle that had a difficult calving displayed increased rectal temperature on d 3 (dystocic Jersey 38.6 ± 0.09 vs. eutocic Jersey $38.4 \pm 0.07^\circ\text{C}$, $P = 0.05$), d 4 (dystocic Jersey 38.7 ± 0.09 vs. eutocic Jersey $38.5 \pm 0.07^\circ\text{C}$, $P = 0.04$), and d 6 (dystocic Jersey 38.7 ± 0.09 vs. eutocic Jersey $38.5 \pm 0.07^\circ\text{C}$, $P = 0.01$) after calving than compared to eutocic Jersey animals. Investigation of the parity by day interaction term determined that primiparous animals had a higher rectal temperature on d 2 (primiparous 38.6 ± 0.05 vs. multiparous $38.4 \pm 0.04^\circ\text{C}$, $P < 0.01$) when compared to multiparous animals. Treatment was not different ($P = 0.59$); treatment LSM include MEL-PRE 38.6 ± 0.03 , MEL-POST 38.6 ± 0.03 , and CTL $38.6 \pm 0.03^\circ\text{C}$.

The final model for β -hydroxybutrate (BHBA) included treatment ($P = 0.27$), body condition score at calving ($\beta = 0.06$, $P = 0.01$), breed ($P = 0.67$), parity ($P = 0.48$), and breed*parity ($P = 0.02$). All pairwise comparisons of the breed by parity interaction term were investigated. Multiparous Holstein had greater BHBA concentrations than primiparous Holstein (0.88 ± 0.03 vs. 0.72 ± 0.05 mmol/L, respectively; $P = 0.02$). Parity was not significant for Jersey (multiparous, 0.73 ± 0.05 mmol/L and primiparous, 0.82 ± 0.07 mmol/L). Additionally, treatment did not significantly affect BHBA concentration (MEL-PRE 0.78 ± 0.04 mmol/L, MEL-POST 0.75 ± 0.04 mmol/L, and CTL 0.84 ± 0.04 mmol/L; $P = 0.27$).

Milk yield and components

Significant main effects and interactions are listed in Table 4.2. The genetic parameters for milk yield (MY) and milk components were significant in their respective models (daily MY: PTA milk, $\beta = 0.0027$, $P < 0.01$; daily fat yield: PTA fat, $\beta = 0.0027$, $P < 0.01$; daily protein yield: PTA protein, $\beta = 0.0033$, $P < 0.01$; daily lactose yield: PTA milk, $\beta = 0.0001$, $P < 0.01$). Breed and parity, as well as the interaction between both terms were significant ($P \leq 0.02$) in all MY and milk components models. Least squares means for MY and components are listed: MY: multiparous Holstein, 52.9 ± 0.6 ; multiparous Jersey, 37.6 ± 0.9 ; primiparous Holstein, 36.5 ± 0.8 ; and primiparous Jersey, 28.6 ± 1.3 kg/d; For daily milk fat: multiparous Holstein, 2.09 ± 0.02 ; multiparous Jersey, 1.59 ± 0.04 ; primiparous Holstein, 1.40 ± 0.03 ; and primiparous Jersey, 1.26 ± 0.05 kg/d; For daily milk protein: multiparous Holstein, 1.54 ± 0.02 ; multiparous Jersey, 1.19 ± 0.03 ; primiparous Holstein, 1.07 ± 0.02 ; and primiparous Jersey: 0.91 ± 0.04 kg/d; For daily milk lactose: multiparous Holstein, 2.07 ± 0.03 ; multiparous Jersey, 1.49 ± 0.05 ; primiparous Holstein, 1.42 ± 0.04 ; and primiparous Jersey, 1.07 ± 0.07 kg/d.

Milk yield and components were measured for the first 15 wk of lactation, and the effect of time (week) was found to be significant ($P < 0.01$), as the yield of milk and milk components gradually increased as the cow proceeded towards peak lactation. In general, the effects of genetics, breed, parity, and time on milk and milk component yields were as expected, and were accounted for in each milk data model.

For daily MY, the treatment by calving difficulty score interaction term was significant ($P = 0.02$), and was sliced by calving difficulty score to determine the effect of treatment within calving difficulty (Figure 4.5). Eutocic MEL-PRE animals produced more milk than eutocic CTL (eutocic MEL-PRE 43.0 ± 1.0 vs. eutocic CTL 36.2 ± 0.8 kg/d, $P < 0.01$), as well as producing more milk than eutocic MEL-POST (eutocic MEL-PRE 43.0 ± 1.0 vs. eutocic MEL-POST 38.7

± 0.9 kg/d, $P < 0.01$). Eutocic MEL-POST was not statistically different from eutocic CTL for MY, albeit these cows produced approximately 2.5 kg/d more milk (eutocic MEL-POST 38.7 ± 0.9 kg/d vs. eutocic CTL 36.2 ± 0.8 kg/d, $P = 0.07$). No differences were found between treatment groups that experienced a difficult calving. Treatment LSM for dystocic calvings include dystocic MEL-PRE 39.7 ± 0.9 , dystocic MEL-POST 37.9 ± 1.0 , and dystocic CTL 38.1 ± 1.1 kg/d.

Treatment effects for milk components are illustrated in Figure 4.6. For daily milk fat yield, treatment was significant ($P < 0.01$), as MEL-PRE produced more fat than CTL (1.66 ± 0.03 vs. 1.50 ± 0.03 kg/d, respectively; $P < 0.01$). Furthermore, MEL-POST animals produced more fat than CTL as well (1.60 ± 0.03 vs. 1.50 ± 0.03 kg/d, respectively; $P = 0.02$). No difference was noted between the meloxicam treatment groups ($P = 0.30$).

For daily milk protein yield, treatment was significant ($P < 0.01$), as MEL-PRE produced more protein than CTL (1.23 ± 0.02 vs. 1.12 ± 0.02 kg/d, respectively; $P < 0.01$). However, no difference was found between MEL-POST animals and the CTL (1.18 ± 0.02 vs. 1.12 ± 0.02 kg/d, respectively; $P = 0.11$), as well as no difference was noted between the meloxicam treatment groups ($P = 0.17$).

For daily milk lactose yield, treatment was significant ($P < 0.01$). Similarly to milk protein yield, MEL-PRE animals produced more lactose than CTL (1.59 ± 0.04 vs. 1.43 ± 0.04 kg/d, respectively; $P < 0.01$). No difference was found between MEL-POST animals and the CTL (1.52 ± 0.04 vs. 1.43 ± 0.04 kg/d, respectively; $P = 0.23$), as well as no difference was noted between the meloxicam treatment groups ($P = 0.27$).

DISCUSSION

Our study is the first study to examine the impact of meloxicam administration prior to calving. Past research has examined the impact of NSAID therapy on behavior around the time of calving. Mainau et al. (2014) found that meloxicam administration post-calving in primiparous animals that experienced an easy calving resulted in an increase in activity for the first 2 d post-calving when compared to eutocic primiparous controls. Likewise, aspirin administration to cows that had an easy calving increased activity post-calving when compared to eutocic controls (Barragan et al., 2017c). In the present study, no treatment effect was identified on activity in eutocic calving events. In beef cows, meloxicam administration after caesarean section increased lying time, but had no effect on activity when compared to cows with no analgesia after surgery (Barrier et al., 2014). Meloxicam-treated animals that experienced a difficult calving in the present study were significantly less active than dystocic controls. We anticipated that meloxicam treatment would increase activity post-calving regardless of calving difficulty; however, our results oppose our original hypothesis. We speculate that the decline in activity in meloxicam-treated animals that had difficult calvings is due to alterations in feeding behaviors. In a past study, dystocia decreased dry matter intake and feeding time on the day prior to calving (Proudfoot et al., 2009). Furthermore, cows with reduced rumination time during the first few days of lactation had higher concentrations of haptoglobin, suggesting severe inflammation in periparturient dairy cows reduces rumination time (Calamari et al., 2014). Newby et al. (2013) found that meloxicam-treated cows following an assisted calving visited the feed bunk more often and spent more time feeding than control dystocic cows. From these studies, it appears that mitigation of inflammation via meloxicam administration can recover feeding behaviors following a difficult calving. Past research has demonstrated an

inverse relationship with rumination time and activity (Abeni and Galli, 2017). During periods of heat stress, cows spent less time ruminating and were more active than when not heat stressed (Abeni and Galli, 2017). We hypothesize that meloxicam-treated animals that had a difficult calving spent more time ruminating, which in turn, reduced activity levels. In human studies, women who had chronic insomnia after childbirth reported higher levels of bodily pain (Sivertsen et al., 2017). Furthermore, NSAID therapies administered to women within 24 h of childbirth helped alleviate this pain and reduced the need for additional treatment (Hedayati et al., 2003). It is possible that in the present study administration of meloxicam to animals that had dystocic calvings reduced activity by alleviating inflammation allowing the cow to rest more easily, similarly as Barrier et al. (2014). This, in turn, could enhance sleep and rumination time after a challenging calving. However, arguing against these theories, our study also showed that dystocia increased the duration of lying bouts and decreased activity levels in the present study, and this decrease in activity after a difficult calving has been identified in a past study (Barragan et al., 2017c). It may be that the quantity of steps or duration of lying bouts are not an adequate measure of animal welfare, whereas the quality of the time spent resting may be a better indicator (Klefot et al., 2016). Nevertheless, these theories are merely speculation, and additional studies investigating feeding and sleep behaviors in periparturient cows may reveal interesting findings.

Previous research studies have identified that an increase in lying bouts (Yeiser, 2011) and standing bouts (Proudfoot et al., 2009) on the day of calving as a promising indicator for animals that will experience a difficult calving. In the present study, cows that had a difficult calving had five more lying bouts on the day of calving than cows that experienced an easy calving. In turn, cows that had dystocia had a reduction of approximately 1 to 2 bouts/d for the

first few days after calving, and spent approximately 10 more min lying per bout. These data suggest that the process of a difficult labor increases restlessness during calving, as was seen in a previous study (Barrier et al., 2012a), and that the inflammation associated with dystocia after calving decreases the motivation to rise from a lying state. Alarming, in first lactation animals, heifers that received meloxicam before calving had an increase in lying bouts on the day of calving when compared to the control, similar to animals experiencing a dystocic calving. However, pre-calving meloxicam heifers did not display a reduction in lying bouts in the day following calving, as animals that experienced a difficult calving did. We are unsure as to why meloxicam administration before calving increased lying bouts on the day of calving in heifers, and why this effect was limited to first lactation animals and not apparent in multiparous cows. It is interesting that the number of lying bouts on the day of calving in the primiparous pre-calving meloxicam group is more similar to that of the multiparous animals. As multiparous animals typically deliver calves more easily, these data could suggest that the administration of meloxicam before calving to primiparous heifers allowed for an easier calving, or a calving more similar to that of a multiparous cow.

Lastly, our study failed to demonstrate any treatment effect on health outcomes. Animals that experienced dystocia had higher rectal temperature than eutocic animals, likely due to an enhanced inflammatory response and tissue damage associated with a longer calving. However, it should be noted that the increase in rectal temperature after dystocic calving events was small (~0.2°C increase) and short-lived, and therefore, potentially not biologically meaningful. Furthermore, meloxicam treated cows had similar BHBA concentrations as controls; previous meloxicam studies also failed to detect treatment effect (Newby et al., 2013; Carpenter et al.,

2016). Additional large studies examining the impact of NSAID therapy around the time of calving on disease outcomes are still needed.

In the present study, administration of meloxicam increased MY and milk components. This effect was accentuated if the administration was before calving, and in cows that calved easily. In fact, eutocic animals that received meloxicam before calving produced 6.8 kg/d more milk for the first 15 wk of lactation when compared to eutocic controls. While this treatment effect appears large, it is not without precedent. In multiparous animals, past research studies have demonstrated that meloxicam administration after calving increases daily MY by 4 kg/d (Carpenter et al., 2016), and sodium salicylate increased daily MY by approximately 8 kg/d (Farney et al., 2013). Other meloxicam studies failed to identify a milk response to treatment; however, these studies only measured MY during the first 30 d after calving (Mainau et al., 2014), or just in assisted calving events (Newby et al., 2013). Past research has identified that NSAID treatment effect on MY is accentuated during peak lactation (Trevisi and Bertoni, 2008; Carpenter et al., 2016). Therefore, in the present study, MY was measured for the first 15 wk of lactation. Additionally, it should be noted that in the present study, meloxicam was dosed at 1 mg/kg of BW by mouth, similar to Carpenter and coworkers (2016); whereas, other studies administered meloxicam at 0.5 mg/kg of BW subcutaneously (Newby et al., 2013; Mainau et al., 2014). This could also explain why our treatment effect is more similar to that of Carpenter and colleagues (2016) than of that in the other studies.

Non-steroidal anti-inflammatory drug administration around the time of calving has yielded highly variable results on milk production, with some studies demonstrating an increase as much as 8 kg/d in multiparous cows, and other studies indicating a decline in production. We argue that this variable response is due in part to the duration of milk production measurement,

as well as the differences in the mode of action of each NSAID and potentially the interaction between the mode of action and the timing of administration. Sodium salicylate and acetylsalicylic acid have been used in the periparturient cow with some success (Trevisi and Bertoni, 2008; Farney et al., 2013; Carpenter et al., 2016; Barragan et al., 2017a). Salicylates inhibit both COX isoforms, however, acetylsalicylic acid is more potent at inhibiting COX-1 than COX-2, and sodium salicylate is a weak inhibitor of both (Mitchell et al., 1993). Furthermore, both of these NSAIDs inhibit the activation of the inflammatory mediator, Nuclear Factor kappa B (NF- κ B; Kopp and Ghosh, 1994, Pierce et al., 1996). Farney and colleagues (2013) reported that mature cows treated with sodium salicylate produced nearly 2,500 kg more milk and 130 kg more milk fat in a 305-d lactation when compared to controls; however, no difference was seen in the first or second parity groups. Carpenter and coworkers (2016) found that sodium salicylate increased daily MY by 3.5 kg/d as well as 305-d milk and milk protein yields, with the majority of this increase noted during peak MY. Likewise, administration of acetylsalicylic acid after calving increased milk by 1 kg/d for the first 5 mo of lactation in organic herds (Barragan et al., 2017a), and another aspirin study demonstrated a 13 percent increase in peak MY (Trevisi and Bertoni, 2008).

However, other NSAID studies have not identified a positive milk response to treatment. Flunixin meglumine, a non-specific COX inhibitor that has greater anti-COX-1 activity than most other NSAIDs (Beretta et al., 2005), has largely been unsuccessful when administered around the time of calving (Shwartz et al., 2009; Newby et al., 2017). Shwartz and coworkers (2009) found that flunixin-treated cows produced 3.5 kg/d less milk during the first week of lactation when compared to controls. Likewise, Newby and colleagues (2017) found that flunixin-treated cows produced 1.6 kg/d less milk for the first 2 wk of lactation when compared

to controls. Furthermore, flunixin-treated cows consumed less feed over the first 5 wk of lactation (Shwartz et al., 2009), had an increased risk for retained placenta, elevated temperature, and a higher incidence of metritis when compared to controls (Newby et al., 2017).

Carprofen is another NSAID available to dairy producers in the European Union (Coetzee, 2013), however, the chief mode of action of this NSAID is unclear, and thought to be of something other than inhibition of COX in cattle (Delatour et al., 1996). In New Zealand, the administration of carprofen after calving did not elicit a milk response (Meier et al., 2014); however, these cows were managed in a rotational grazing system with a lower MY than of that in the current study as well as other studies (Farney et al., 2013; Carpenter et al., 2016). The authors argued that differences in metabolic stress in the cows between these studies and Meier et al. (2014) could be contributing to the lack of milk response from NSAID therapy. Furthermore, Meier and coworkers (2014) recorded MY for the first 6 wk after calving, whereas, recent studies have reported that the effect of NSAID therapy may not become apparent until later in lactation (Carpenter et al., 2016).

Considering the aforementioned studies collectively, we speculate that measuring MY through peak production is key to identifying a response to NSAID treatment. Furthermore, we hypothesize that the mode of action of each NSAID is contributing to differences noted between studies. Inhibition of COX-1 after calving, via flunixin meglumine, reduced MY and has blatant negative effects on health (Newby et al., 2017); whereas, inhibition of COX-2, via meloxicam, appears to improve MY in the present study as well as another study (Carpenter et al., 2016) and has no apparent negative impacts (Newby et al., 2013; Mainau et al., 2014; Newby et al., 2014; Carpenter et al., 2016). It should be noted that in both flunixin meglumine studies the NSAID was administered within a few hours after calving. It is possible that inhibition of COX-1 via

flunixin administration this soon after calving led to the increase in reproductive disorders such as retained placenta and metritis. Potentially, delaying the administration of flunixin until the second day after calving may reduce the incidence of reproductive disorders and improve treatment response. Efficacy of salicylates is a bit more perplexing; however, the inhibition of NF- κ B may be contributing to its success. Additional studies examining the mechanism behind the ascent in peak MY due to NSAID therapy may provide clarity.

The increase in peak MY due to NSAID administration has been perplexing, especially considering that NSAID treatment was weeks, if not months, in advance of treatment effect. Maybe even more puzzling is that this increase in MY in the present study was further enhanced if meloxicam was administered prior to calving. It is well-known that MY can be enhanced through either an increase in the activity of the mammary epithelial cell or through alterations in mammary epithelial cell number – e.g., either an increase in cell proliferation or a decrease in involution/apoptosis. Past research has demonstrated that inflammatory signaling impairs milk secretion in mice (Connelly et al., 2010). The mechanism for this outcome was an increase in the activation of NF- κ B, a transcription factor associated with inflammation (Connelly et al., 2010). In that experiment, increased activation of NF- κ B amplified the rate of apoptosis in the mammary epithelium, contributing to the decline in milk secretion (Connelly et al., 2010). Furthermore, in mice, reduced activity of NF- κ B, via knockout of IKK β (an upstream regulator of NF- κ B), resulted in a delay in involution and a reduction of apoptotic cells in the mammary gland (Baxter et al., 2006). In the bovine, a similar effect with milk secretion was assessed. During the first 4 wk of lactation, cows in the highest quartile for inflammatory markers produced 20 percent less milk than cows in the lowest quartile (Bertoni et al., 2008). Furthermore, cows treated with acetylsalicylic acid after calving had a lower haptoglobin

concentration 24 h after calving (Barragan et al., 2017b), and these cows produced approximately 1 kg/d more milk for the first 5 mo of lactation (Barragan et al., 2017a). We speculate that the administration of meloxicam in the present study impaired inflammatory signaling which led to an increase in MY, and that this effect was further accentuated when meloxicam was administered prior to calving. Furthermore, we hypothesize that the administration of meloxicam prior to calving blunted the inflammatory cascade that occurs during parturition. Whereas, when meloxicam was administered after calving, the inflammatory cascade was already sufficiently initiated by parturition, and therefore a belated treatment would have a diminished effect. Additional studies examining inhibition of the inflammatory cascade around the time of calving are warranted.

We hypothesized that administration of meloxicam around the time of calving would be more efficacious in dystocic rather than eutocic calving events. We speculated that cows with a difficult calving would be more responsive to treatment due to a heightened inflammatory state that occurs due to dystocic calving events. We anticipated this effect because past research studies have demonstrated that administration of an NSAID after a disease challenge improved performance and well-being. For instance, in an experimentally induced *E. coli* mastitis study, flunixin meglumine administration after challenge increased MY when compared to challenged counterparts (Yeiser et al., 2012). This effect on MY, however, was not found in another mastitis study that utilized meloxicam in cases of mild clinical mastitis (McDougall et al., 2009). However, McDougall et al. (2009) as well as another mastitis study (McDougall et al., 2016) did find that meloxicam administration after a mild mastitis disease event reduced the risk of culling, reduced somatic cell count (McDougall et al., 2009), improved bacteriological cure rates, and increased reproductive performance (McDougall et al., 2016). Moreover, in a lameness study,

flunixin meglumine administration reduced weight shifting, alleviating lameness-associated pain when compared to saline-treated controls (Wagner et al., 2017). In reproductive diseases, administration of carprofen improved pregnancy rates among cows with subclinical endometritis (Priest et al., 2013), and similar improvements were seen via flunixin meglumine administration by reducing days to first service and accelerating involution in cows with metritis (Amiridis et al., 2001).

However, our results for MY demonstrate the opposite response as to what we were expecting – as treatment effect was more efficacious in eutocic calving events rather than dystocia. The reason for this diverging effect may be that the dose of meloxicam administered to animals who experienced dystocia was insufficient, and that those cows may require larger doses or administration of the drug more than once, as only a single dose was administered in the present study. Our study confirms the findings of Newby and coworkers (2013), where meloxicam treatment did not elicit a milk response in dystocic calving events. In Mee’s review on dystocia (Mee, 2008), he eloquently paraphrases McClintock (2004): *“There is no such thing as an easy calving... just varying degrees of difficulty... from the dam’s perspective”*. In fact, an inflammatory response has been documented in seemingly healthy cattle after calving (Humblet et al., 2006; Bionaz et al., 2007; Graugnard et al., 2012; Mullins et al., 2012), suggesting that nearly all cattle experience inflammation due to parturition. Therefore, it appears that our hypothesis was incorrect, and that an exacerbated inflammatory state from dystocia would actually make it more difficult to detect treatment effect. Additional studies examining treatments for dystocic calving events are warranted.

This is the first study to our knowledge that establishes a beneficial effect of NSAID treatment prior to calving. Past research administered flunixin meglumine directly prior to

calving and demonstrated a large increase in the incidence of stillborn calves (Newby et al., 2017). In the present study, administering meloxicam prior to calving had no effect on the incidence of stillborn calves. It is perplexing as to why flunixin meglumine administered prior to calving would increase stillbirth rates, but meloxicam would not. Furthermore, when administering flunixin meglumine directly after calving, treatment resulted in an increase in reproductive diseases such as retained placenta and metritis (Newby et al., 2017), whereas this effect is not noted in the present study or other meloxicam studies (Newby et al., 2013; Mainau et al., 2014; Carpenter et al., 2016). However, additional larger meloxicam studies are needed to confirm the effect of meloxicam on clinical health outcomes.

In another study, lysine acetylsalicylate (acetylsalicylic acid) was administered IM daily for 8 d prior to calving, and treatment increased the incidence of retained placenta, as well as haptoglobin concentrations after calving (Grossi et al., 2013); however, it should be noted that this study had a small sample size (n = 8 to 9 per treatment group) and treatment administration prior to calving was much longer than in the present study. It is possible that the difference in the mode of action of the aforementioned drugs is contributing to this outcome. Flunixin meglumine has greater anti-COX-1 activity than most other NSAIDs (Beretta et al., 2005) and acetylsalicylic acid irreversibly binds to COX-1 (Vane and Botting, 2003), whereas meloxicam is a COX-2 preferential inhibitor (Beretta et al., 2005). However, to further complicate this conundrum, reproductive physiologists have argued that COX-2 is a critical enzyme for parturition (Shenavai et al., 2012). During initiation of parturition, cortisol induces an up-regulation of COX-2 expression (Whittle et al., 2000), occurring prior to luteolysis, suggesting that COX-2 increases PGF-2 α , which initiates parturition through lysing of the corpus luteum, causing the withdrawal of progesterone (Schuler et al., 2006). Therefore, inhibition of COX-2 via meloxicam

administration prior to calving could, in theory, cause dystocia. It is possible that in the present study meloxicam was not administered in a large enough dose or was not administered early enough in the initiation of parturition phase to elicit this effect. However, we would also note that meloxicam administration after calving has not been associated with a higher incidence of reproductive diseases such as retained placenta and metritis, as flunixin meglumine has. It seems more likely that the mode of action of the NSAID is playing a larger role than previously thought. Additional studies detailing the initiation of parturition and the role of the COX enzymes in the bovine may provide clarity. Furthermore, pharmacodynamic studies examining the mechanism of meloxicam are desired to confirm that meloxicam behaves similarly in the bovine as it does in other species.

CONCLUSIONS

Our study confirms that the administration of meloxicam around the time of calving increases MY and components. To the best of our knowledge, this is the first study to demonstrate that meloxicam administration is more efficacious in increasing MY when administered prior to calving, and to cows that had an easy calving. Furthermore, our study confirms that difficult calving events increase lying bouts on the day of calving and decreases activity thereafter. We believe this is the first study to demonstrate that dystocia decreases lying bouts and increases lying bout duration in the days following calving. The authors would like to acknowledge a major limitation in the widespread application of these conclusions to the dairy industry. In this study, 237 cows were enrolled; however, only 194 cows were used for analysis. A large portion of this was due to cows not receiving the pre-calving treatment within the specified timeframes, because the accuracies of calving identification technologies are still moderate at best. While improving the accuracy of calving identification is beyond the scope of

the current project, the application of the administration of meloxicam prior to calving may be problematic in industry until the accuracy of these technologies have been improved. Recent advances in identifying the onset of calving utilizing either ruminating, lying, or activity behaviors (Borchers et al., 2017; Mahmoud et al., 2017) create optimism in making this treatment regimen a possibility in the dairy industry. Additional research studies examining intervention strategies for dystocic calving events are still needed.

ACKNOWLEDGMENTS

The authors would like to recognize M. Ellett Jr. and K. McCauley for their assistance in data collection, M. McGilliard and R. White for their statistical expertise, and the farm staff at the Virginia Tech dairy facility for their support.

Table 4.1 Mixed model (PROC GLIMMIX, SAS 9.4) of daily steps (no./d), lying bouts (no./d), and lying time (min/d) starting 2 d prior to calving until 7 d after calving with cows receiving meloxicam prior to calving (n = 53), after calving (n = 59), or a negative control (n = 53).

Fixed effects	Steps	Lying bouts	Lying time	Lying bout duration
	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value	<i>P</i> -value
Covariate steps (d -3) ¹	<0.01	NA ²	NA	NA
Covariate lying bouts (d -3) ¹	NA	<0.01	NA	NA
Covariate lying time (d -3) ¹	NA	NA	<0.01	NA
Covariate lying bout duration (d -3) ¹	NA	NA	NA	<0.01
Treatment	<0.01	0.60	0.12	0.84
Breed	<0.01	0.02	<0.01	0.01
Parity	<0.01	0.24	<0.01	<0.01
Calving difficulty	<0.01	0.71	-. ³	<0.01
Breed*parity	<0.01	-	<0.01	-
Calving difficulty*breed	0.60	-	-	-
Treatment*breed	<0.01	-	-	-
Treatment*parity	-	0.93	-	-
Treatment*calving difficulty	0.03	-	-	-
Day	<0.01	<0.01	<0.01	<0.01
Treatment*day	0.53	<0.01	-	-
Breed*day	<0.01	-	0.02	-
Parity*day	0.82	<0.01	<0.01	<0.01
Calving difficulty*day	0.07	<0.01	-	0.04
Breed*parity*day	0.03	-	-	-
Calving difficulty*breed*day	0.05	-	-	-
Treatment*breed*day	0.05	-	-	-
Treatment*parity*day	-	<0.01	-	-

¹Behavioral data from d -3, the day before treatment regimen began, was used as a covariate to account for individual differences in behavior. Steps from d -3 was only included in the steps model, just as lying bouts from d -3 was only included in the lying bouts model, lying time from d -3 was only included in the lying time model, and lying bout duration from d -3 was only included in the lying bout duration model.

²NA = not applicable.

³ - Dashed line represents a term that was not significant for that specific model ($P > 0.05$) and therefore was removed during backwards elimination. Terms that were not significant for any of the behavioral models were removed during backwards elimination and are not included in the table.

Table 4.2 Mixed model (PROC GLIMMIX, SAS 9.4) of daily milk yield and components (kg/d) averaged by week for the first 15 wk of lactation for 194 cows receiving meloxicam prior to calving (n = 60), after calving (n = 69), or a negative control (n = 65).

Fixed effects	Daily milk yield <i>P</i> -value	Daily fat yield <i>P</i> -value	Daily protein yield <i>P</i> -value	Daily lactose yield <i>P</i> -value
PTA milk ¹	<0.01	NA ²	NA	<0.01
PTA fat ¹	NA	<0.01	NA	NA
PTA protein ¹	NA	NA	<0.01	NA
Treatment	<0.01	<0.01	<0.01	<0.01
Breed	<0.01	<0.01	<0.01	<0.01
Parity	<0.01	<0.01	<0.01	<0.01
Calving difficulty	0.36	- ³	-	-
Breed*parity	<0.01	<0.01	<0.01	0.02
Treatment*calving difficulty	0.02	-	-	-
Week	<0.01	<0.01	<0.01	<0.01
Breed*week	<0.01	<0.01	-	<0.01
Parity*week	<0.01	<0.01	<0.01	<0.01

¹The genetic parameter for milk yield (PTA milk) was only included in the daily milk yield and lactose models. The genetic parameter for fat yield (PTA fat) was only included in the daily fat yield model. The genetic parameter for protein yield (PTA protein) was only included in the daily protein model.

²NA = not applicable.

³ - Dashed line represents a term that was not significant for that specific model ($P > 0.05$) and therefore was removed during backwards elimination. Terms that were not significant for any of the milk yield and components models were removed during backwards elimination and are not included in the table.

Table 4.3 Clinical disease incidence by treatment group; data represented as percent of treatment group. Treatments included administration of meloxicam (1 mg/kg of BW by mouth) before calving (**MEL-PRE**, n = 60), after calving (**MEL-POST**, n = 69), and a negative control (**CTL**, n = 65).

Disease	MEL-PRE	MEL-POST	CTL	<i>P</i> -value
Ketosis	3.3	7.3	4.6	0.66
Milk fever	1.7	2.9	3.0	1.00
Retained placenta	3.3	8.7	6.2	0.49
Displaced abomasum	0.0	1.5	3.1	0.65
Metritis	1.7	4.4	0.0	0.27
Mastitis	0.0	1.5	4.6	0.21
Stillbirth ¹	3.3	13.2	4.6	0.09
Multiple ²	0.0	2.9	4.6	0.33

¹Stillbirth was defined as a calf that was born dead.

²Percent of cows within each treatment group that had more than one clinical disease event during the first 30 DIM.

Figure 4.1 Treatment regimens for control (**CTL**), meloxicam post-calving treatment (**MEL-POST**), and meloxicam pre-calving treatment (**MEL-PRE**). Meloxicam was dosed at 1 mg/kg of BW orally in a gel capsule. An empty gel capsule served as a placebo.

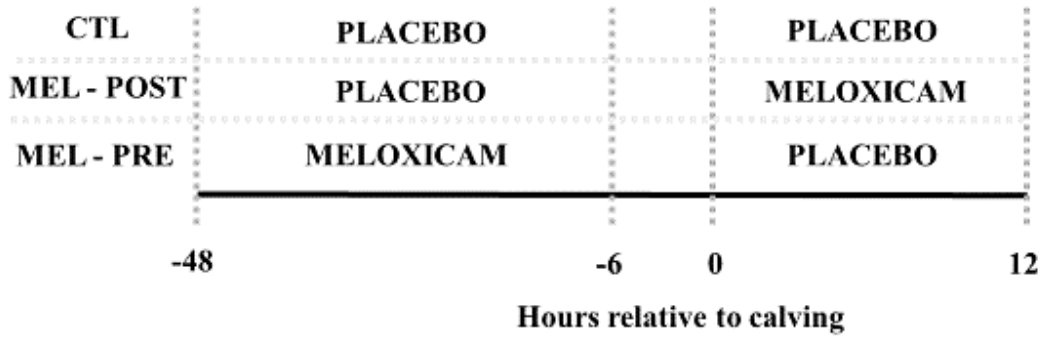


Figure 4.2 Least squares means (\pm SE) of the treatment by calving difficulty score interaction term, comparisons made within calving difficulty, for daily activity (steps/d). Data recorded for 10 d, beginning on d -2 and concluded on d 7. Treatments included administration of meloxicam (1 mg/kg of BW by mouth) before calving (**MEL-PRE**), after calving (**MEL-POST**), and a negative control (**CTL**). Sample sizes denoted within each bar. Differences between treatment groups: * $P \leq 0.05$.

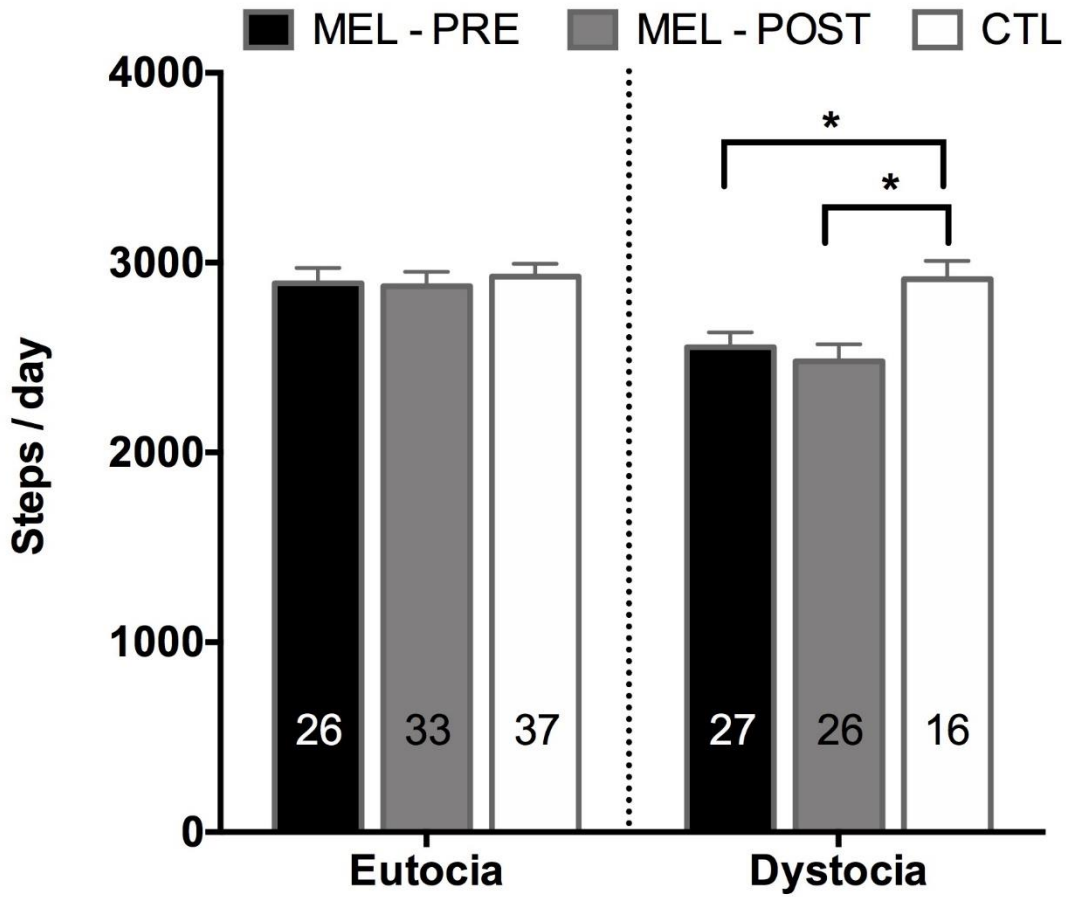


Figure 4.3 Least squares means (\pm SE) of the treatment by parity by day interaction term (multiparous, **A**; primiparous, **B**), comparisons made within day, for daily lying bouts (no./d). Data recorded for 10 d, beginning on d -2 and concluded on d 7. Day 0 was designated as the day of calving. Treatments included administration of meloxicam (1 mg/kg of BW by mouth) before calving (**MEL-PRE**; primiparous, n = 17; multiparous, n = 36), after calving (**MEL-POST**; primiparous, n = 21; multiparous, n = 38), and a negative control (**CTL**; primiparous, n = 17; multiparous, n = 36). Dissimilar letters indicate differences between parity and treatment groups within each day: a,b; $P \leq 0.05$.

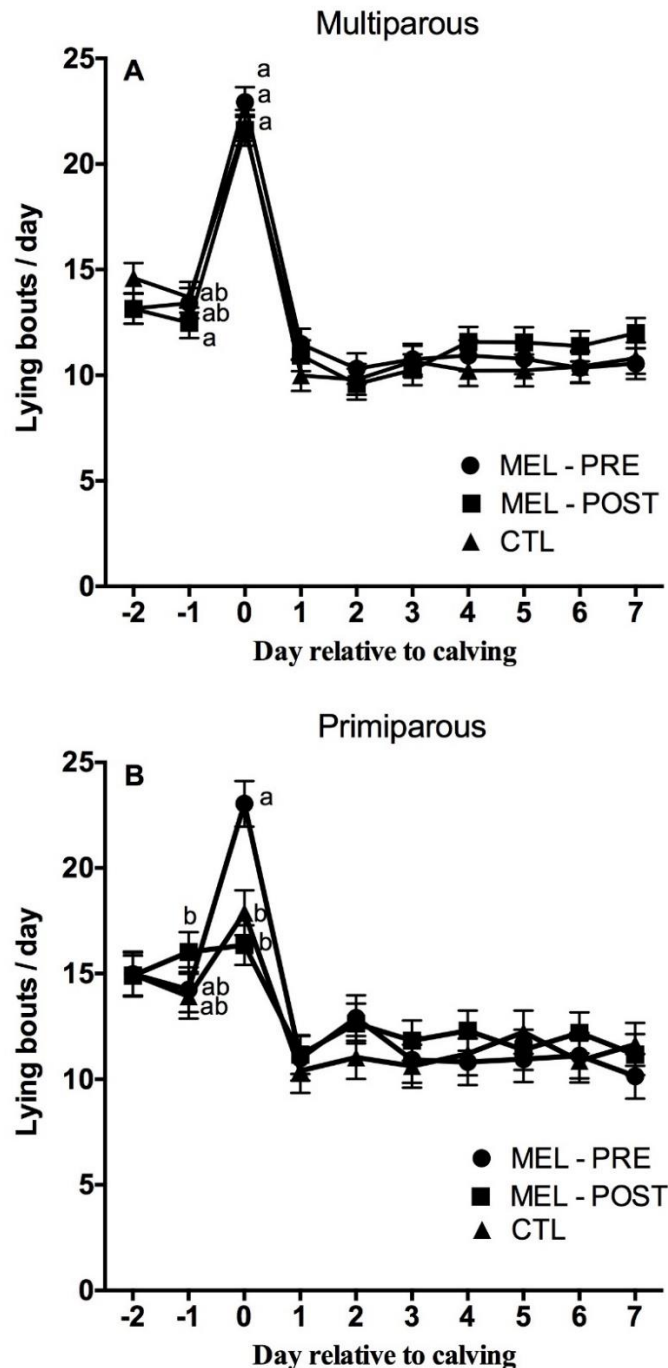


Figure 4.4 Least squares means (\pm SE) of the calving difficulty score by day interaction term (eutocia, $n = 96$; dystocia, $n = 69$), comparisons made within day, for daily lying bouts (**A**; no./d) and lying bout duration (**B**; min/bout). Data recorded for 10 d, beginning on d -2 and concluded on d 7. Day 0 was designated as the day of calving. Differences between calving difficulty groups: * $P \leq 0.05$, † $P < 0.10$.

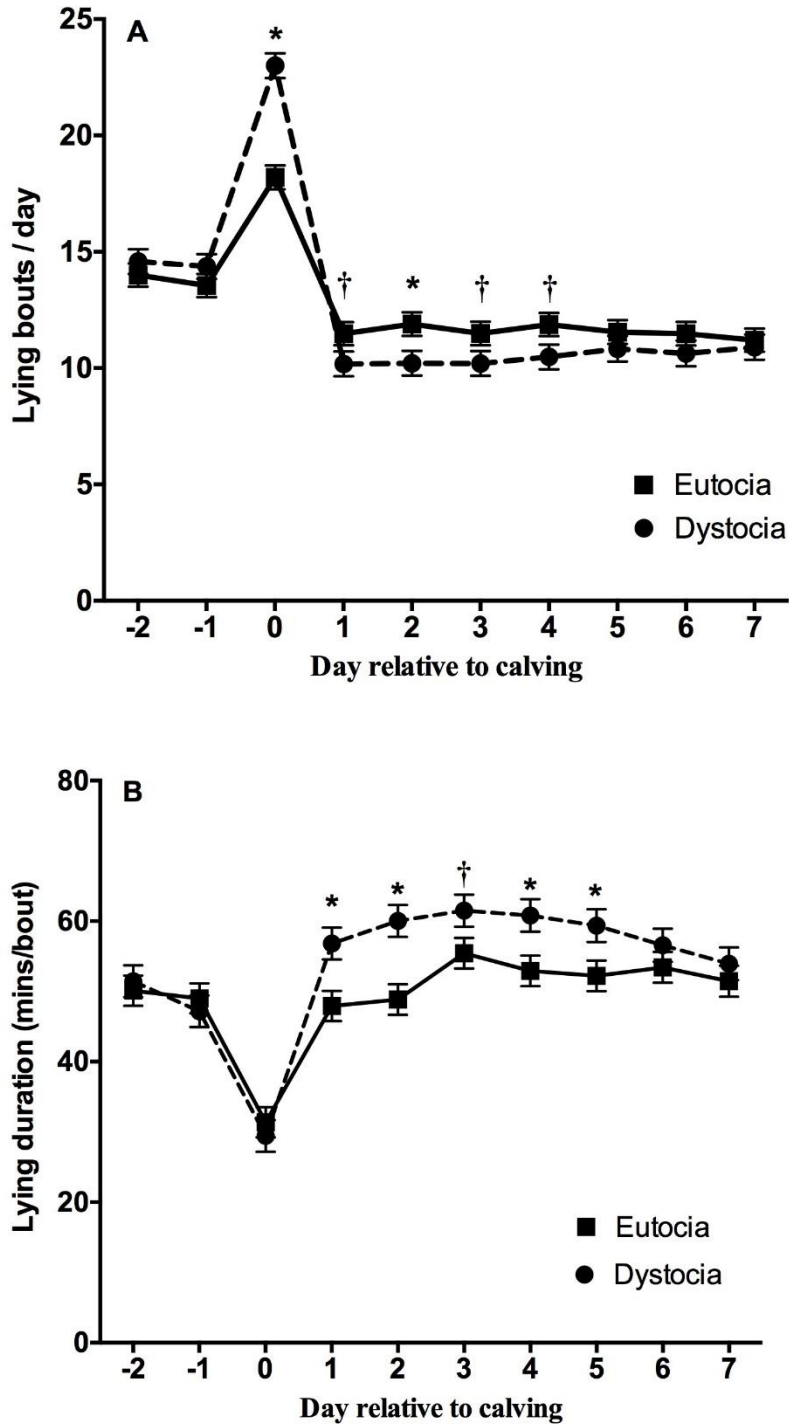


Figure 4.5 Least squares means (\pm SE) of the treatment by calving difficulty score interaction term, comparisons made within calving difficulty, for daily milk yield measured for the first 15 wk of lactation. Treatments included administration of meloxicam (1 mg/kg of BW by mouth) before calving (**MEL-PRE**), after calving (**MEL-POST**), and a negative control (**CTL**). Sample sizes denoted within each bar. Differences between treatment groups: * $P \leq 0.05$, † $P < 0.10$.

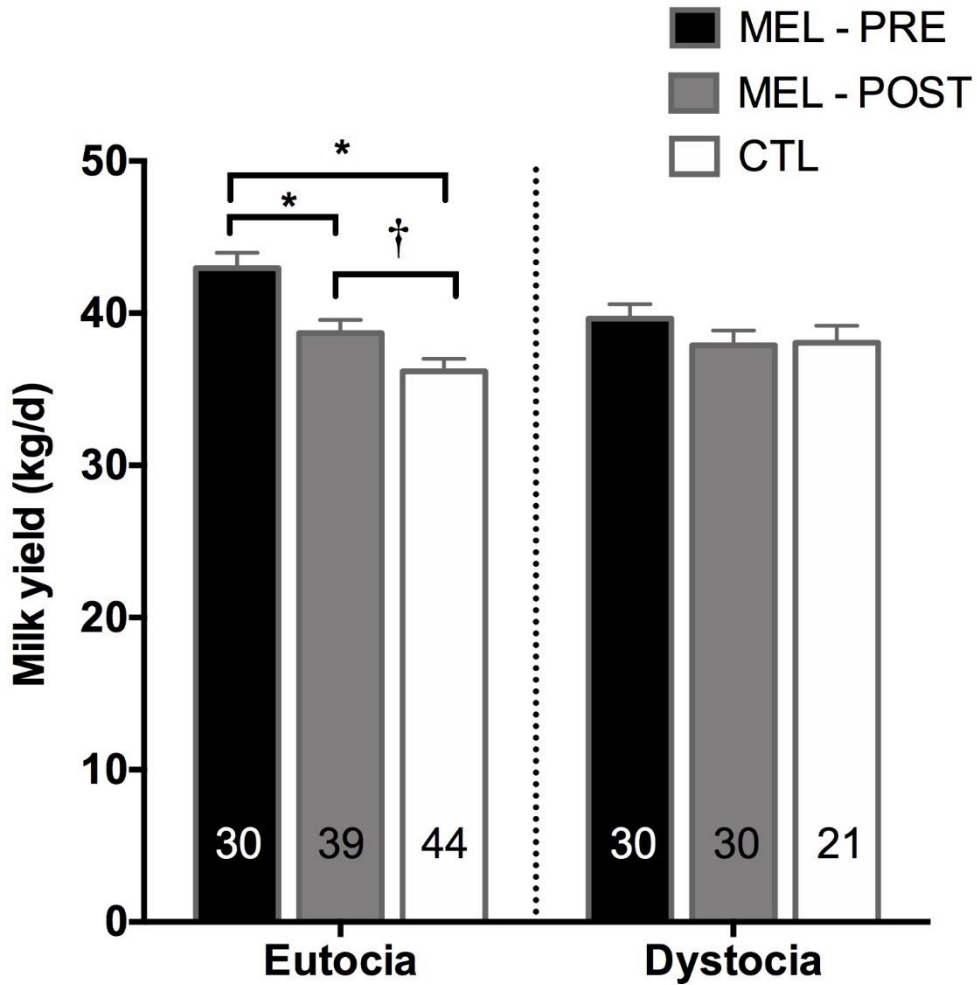
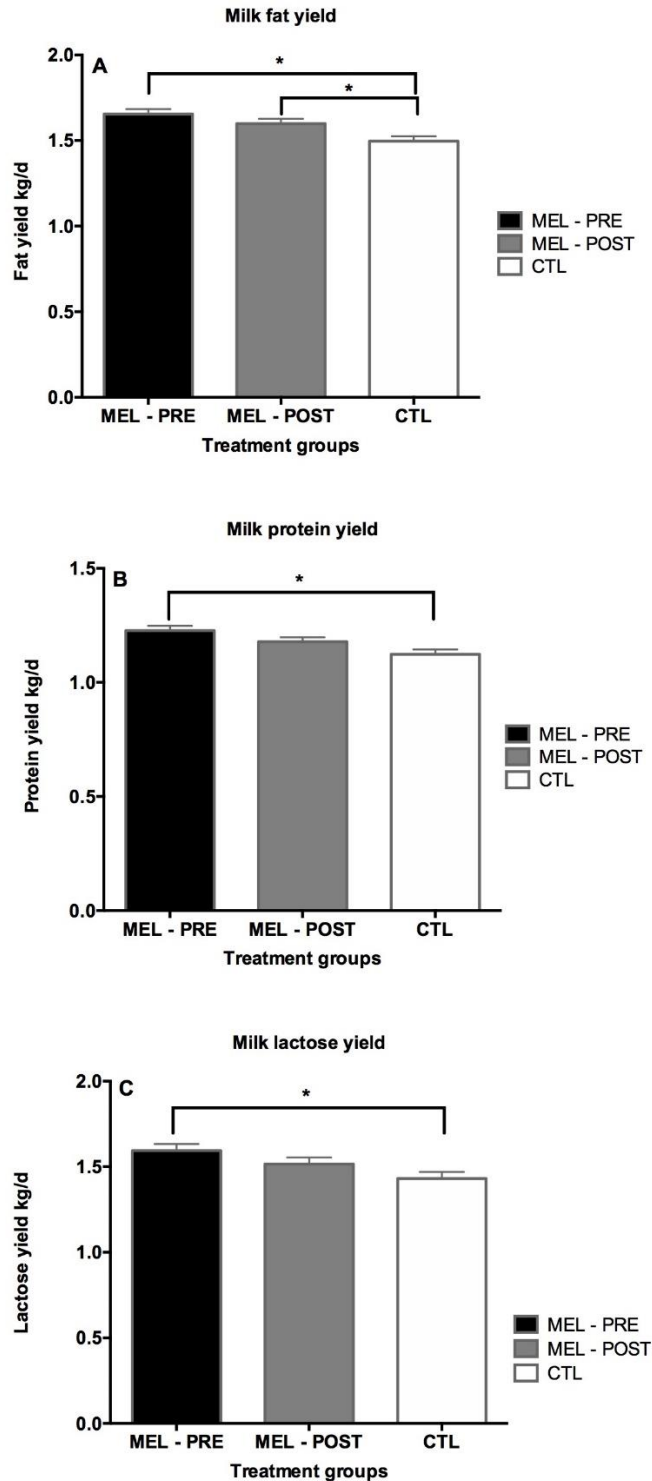


Figure 4.6 Treatment least squares means (\pm SE) for daily milk fat (kg/d; **A**), protein (kg/d; **B**), and lactose (kg/d; **C**) measured for the first 15 wk of lactation. Treatments ($n = 60$ to 69 per treatment group) included administration of meloxicam (1 mg/kg of BW by mouth) before calving (**MEL-PRE**), after calving (**MEL-POST**), and a negative control (**CTL**). Differences between treatment groups: $*P \leq 0.05$.



REFERENCES

- Abeni, F. and A. Galli. 2017. Monitoring cow activity and rumination time for an early detection of heat stress in dairy cow. *Int. J. Biometeorol.* 61:417-425.
- Ametaj, B., B. Bradford, G. Bobe, R. Nafikov, Y. Lu, J. Young, and D. Beitz. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. *Can. J. Anim. Sci.* 85:165-175.
- Amiridis, G. S., L. Leontides, E. Tassos, P. Kostoulas, and G. C. Fthenakis. 2001. Flunixin meglumine accelerates uterine involution and shortens the calving-to-first-oestrus interval in cows with puerperal metritis. *J. Vet. Pharmacol. Ther.* 24:365-367.
- Anderson, K. L., C. A. Neff-Davis, L. E. Davis, and V. D. Bass. 1990. Pharmacokinetics of flunixin meglumine in lactating cattle after single and multiple intramuscular and intravenous administrations. *Am. J. Vet. Res.* 51:1464-1467.
- Atashi, H., A. R. Abdolmohammadi, A. Asaadi, A. Akhlaghi, M. Dadpasand, and Y. J. Ahangari. 2012. Using an incomplete gamma function to quantify the effect of dystocia on the lactation performance of Holstein dairy cows in Iran. *J. Dairy Sci.* 95:2718-2722.
- Bach, K. D., W. Heuwieser, and J. A. A. McArt. 2016. Technical note: Comparison of 4 electronic handheld meters for diagnosing hyperketonemia in dairy cows. *J. Dairy Sci.* 99:9136-9142.
- Barragan, A. A., L. M. Bauman, L. d. Costa, J. Velez, J. D. R. Gonzalez, G. M. Schuenemann, and S. Bas. 2017a. Effects of oral administration of acetylsalicylic acid after parturition on milk yield and milk components in lactating dairy cows under certified organic management (abstract). *J. Dairy Sci.* 100, Suppl. 2:176-177.
- Barragan, A. A., L. M. Bauman, J. Lakritz, J. F. Coetzee, J. Velez, J. D. R. Gonzalez, G. M. Schuenemann, and S. Bas. 2017b. Assessment of the effects of oral administration of acetylsalicylic acid on biomarkers of inflammation and stress in dairy cows after parturition (abstract). *J. Dairy Sci.* 100, Suppl. 2:137-138.
- Barragan, A. A., L. M. Bauman, J. Velez, J. D. R. Gonzalez, G. M. Schuenemann, and S. Bas. 2017c. Effects of oral administration of acetylsalicylic acid after parturition on activity patterns, prevalence of diseases, mortality and culling rates in dairy cows (abstract). *J. Dairy Sci.* 100, Suppl. 2:17.
- Barrier, A. C., T. M. Coombs, C. M. Dwyer, M. J. Haskell, and L. Goby. 2014. Administration of a NSAID (meloxicam) affects lying behaviour after caesarean section in beef cows. *Appl. Anim. Behav. Sci.* 155:28-33.
- Barrier, A. C., M. J. Haskell, A. I. Macrae, and C. M. Dwyer. 2012. Parturition progress and behaviours in dairy cows with calving difficulty. *Appl. Anim. Behav. Sci.* 139:209-217.

Baxter, F. O., P. J. Came, K. Abell, B. Kedjouar, M. Huth, K. Rajewsky, M. Pasparakis, and C. J. Watson. 2006. IKKbeta/2 induces TWEAK and apoptosis in mammary epithelial cells. *Development* 133:3485-3494.

Beretta, C., G. Garavaglia, and M. Cavalli. 2005. COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin, carprofen and meloxicam: An in vitro analysis. *Pharmacol. Res.* 52:302-306.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. *J. Dairy Sci.* 91:3300-3310.

Bionaz, M., E. Trevisi, L. Calamari, F. Librandi, A. Ferrari, and G. Bertoni. 2007. Plasma Paraoxonase, Health, Inflammatory Conditions, and Liver Function in Transition Dairy Cows. *J. Dairy Sci.* 90:1740-1750.

Boehringer Ingelheim (Canada) Ltd. 2017. Metacam - Product information. <http://www.metacamcattle.ca/productinformation> (accessed 15 February 2018).

Borchers, M. R., Y. M. Chang, K. L. Proudfoot, B. A. Wadsworth, A. E. Stone, and J. M. Bewley. 2017. Machine-learning-based calving prediction from activity, lying, and ruminating behaviors in dairy cattle. *J. Dairy Sci.* 100:5664-5674.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. *J. Dairy Sci.* 98:6631-6650.

Burfeind, O., V. S. Suthar, R. Voigtsberger, S. Bonk, and W. Heuwieser. 2011. Validity of prepartum changes in vaginal and rectal temperature to predict calving in dairy cows. *J. Dairy Sci.* 94:5053-5061.

Calamari, L., N. Soriani, G. Panella, F. Petrera, A. Minuti, and E. Trevisi. 2014. Rumination time around calving: An early signal to detect cows at greater risk of disease. *J. Dairy Sci.* 97:3635-3647.

Carpenter, A. J., C. M. Ylloja, C. F. Vargas, L. K. Mamedova, L. G. Mendonca, J. F. Coetzee, L. C. Hollis, R. Gehring, and B. J. Bradford. 2016. Hot topic: Early postpartum treatment of commercial dairy cows with nonsteroidal antiinflammatory drugs increases whole-lactation milk yield. *J. Dairy Sci.* 99:672-679.

Coetzee, J. F. 2013. A Review of Analgesic Compounds Used in Food Animals in the United States. *Vet. Clin. North Am. Food Anim. Pract.* 29:11-28.

Connelly, L., W. Barham, R. Pigg, L. Saint-Jean, T. Sherrill, D. S. Cheng, L. A. Chodosh, T. S. Blackwell, and F. E. Yull. 2010. Activation of nuclear factor kappa B in mammary epithelium promotes milk loss during mammary development and infection. *J. Cell. Physiol.* 222:73-81.

Contreras, G. A., W. Raphael, S. A. Mattmiller, J. Gandy, and L. M. Sordillo. 2012. Nonesterified fatty acids modify inflammatory response and eicosanoid biosynthesis in bovine endothelial cells. *J. Dairy Sci.* 95:5011-5023.

Correa, M. T., H. Erb, and J. Scarlett. 1993. Path analysis for seven postpartum disorders of Holstein cows. *J. Dairy Sci.* 76:1305-1312.

Delatour, P., R. Foot, A. Foster, D. Baggo, and P. Lees. 1996. Pharmacodynamics and chiralpharmacokinetics of carprofen in calves. *Br. Vet. J.* 152:183-198.

Dematawewa, C. M. and P. J. Berger. 1997. Effect of dystocia on yield, fertility, and cow losses and an economic evaluation of dystocia scores for Holsteins. *J. Dairy Sci.* 80:754-761.

Duffield, T. F., S. LeBlanc, R. Bagg, K. Leslie, J. Ten Hag, and P. Dick. 2003. Effect of a Monensin Controlled Release Capsule on Metabolic Parameters in Transition Dairy Cows. *J. Dairy Sci.* 86:1171-1176.

Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. *J. Dairy Sci.* 92:571-580.

EMEA. 2007. Scientific discussion, Metacam CVMP/323/97. London, UK.

Farney, J. K., L. K. Mamedova, J. F. Coetzee, J. E. Minton, L. C. Hollis, and B. J. Bradford. 2013. Sodium salicylate treatment in early lactation increases whole-lactation milk and milk fat yield in mature dairy cows. *J. Dairy Sci.* 96:7709-7718.

Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci.* 77:2695-2703.

Gessner, D. K., G. Schlegel, J. Keller, F. J. Schwarz, R. Ringseis, and K. Eder. 2013. Expression of target genes of nuclear factor E2-related factor 2 in the liver of dairy cows in the transition period and at different stages of lactation. *J. Dairy Sci.* 96:1038-1043.

Goff, J. P. 2006. Major Advances in Our Understanding of Nutritional Influences on Bovine Health. *J. Dairy Sci.* 89:1292-1301.

Graugnard, D. E., M. Bionaz, E. Trevisi, K. M. Moyes, J. L. Salak-Johnson, R. L. Wallace, J. K. Drackley, G. Bertoni, and J. J. Looor. 2012. Blood immunometabolic indices and polymorphonuclear neutrophil function in peripartum dairy cows are altered by level of dietary energy prepartum. *J. Dairy Sci.* 95:1749-1758.

- Grossi, P., G. Bertoni, F. P. Cappelli, and E. Trevisi. 2013. Effects of the precalving administration of omega-3 fatty acids alone or in combination with acetylsalicylic acid in periparturient dairy cows. *J. Anim. Sci.* 91:2657-2666.
- Hedayati, H., J. Parsons, and C. A. Crowther. 2003. Rectal analgesia for pain from perineal trauma following childbirth. *The Cochrane Library*.
- Hotamisligil, G. S. 2006. Inflammation and metabolic disorders. *Nature* 444:860-867.
- Humblet, M. F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, F. Rollin, and J. M. Godeau. 2006. Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. *Vet. Clin. Pathol.* 35:188-193.
- Huzzey, J. M., T. F. Duffield, S. J. LeBlanc, D. M. Veira, D. M. Weary, and M. A. von Keyserlingk. 2009. Short communication: Haptoglobin as an early indicator of metritis. *J. Dairy Sci.* 92:621-625.
- Kaniyamattam, K. and A. De Vries. 2014. Agreement between milk fat, protein, and lactose observations collected from the Dairy Herd Improvement Association (DHIA) and a real-time milk analyzer. *J. Dairy Sci.* 97:2896-2908.
- Kelton, D. F., K. D. Lissemore, and R. E. Martin. 1998. Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. *J. Dairy Sci.* 81:2502-2509.
- Kenward, M. G. and J. H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 983-997.
- Klefot, J. M., J. L. Murphy, K. D. Donohue, B. F. O'Hara, M. E. Lhamon, and J. M. Bewley. 2016. Development of a noninvasive system for monitoring dairy cattle sleep. *J. Dairy Sci.* 99:8477-8485.
- Kopp, E. and S. Ghosh. 1994. Inhibition of NF-kappa B by sodium salicylate and aspirin. *Science* 265:956-959.
- Kovacs, L., F. L. Kezer, F. Ruff, and O. Szenci. 2016a. Timing of obstetrical assistance affects periparturient cardiac autonomic function and early maternal behavior of dairy cows. *Physiol. Behav.* 165:202-210.
- Kovacs, L., F. L. Kezer, and O. Szenci. 2016b. Effect of calving process on the outcomes of delivery and postpartum health of dairy cows with unassisted and assisted calvings. *J. Dairy Sci.* 99:7568-7573.
- LeBlanc, S. J., K. E. Leslie, and T. F. Duffield. 2005. Metabolic Predictors of Displaced Abomasum in Dairy Cattle. *J. Dairy Sci.* 88:159-170.

- Lombard, J. E., F. B. Garry, S. M. Tomlinson, and L. P. Garber. 2007. Impacts of dystocia on health and survival of dairy calves. *J. Dairy Sci.* 90:1751-1760.
- Loor, J. J., H. M. Dann, R. E. Everts, R. Oliveira, C. A. Green, N. A. J. Guretzky, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2005. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. *Physiol. Genomics* 23:217-226.
- Mahmoud, F., B. Christopher, A. Maher, H. Jürg, S. Alexander, S. Adrian, and H. Gaby. 2017. Prediction of calving time in dairy cattle. *Anim. Reprod. Sci.* 187:37-46.
- Mainau, E., A. Cuevas, J. L. Ruiz-de-la-Torre, E. Abbeloos, and X. Manteca. 2014. Effect of meloxicam administration after calving on milk production, acute phase proteins, and behavior in dairy cows. *Journal of Veterinary Behavior: Clinical Applications and Research* 9:357-363.
- Malreddy, P. R., J. F. Coetzee, B. Kukanich, and R. Gehring. 2013. Pharmacokinetics and milk secretion of gabapentin and meloxicam co-administered orally in Holstein-Friesian cows. *J. Vet. Pharmacol. Ther.* 36:14-20.
- McClintock, S. E. J. 2004. A genetic evaluation of dystocia in Australian Holstein-Friesian cattle. University of Melbourne, Institute of Land and Food Resources.
- McDougall, S., E. Abbeloos, S. Piepers, A. S. Rao, S. Astiz, T. van Werven, J. Statham, and N. Perez-Villalobos. 2016. Addition of meloxicam to the treatment of clinical mastitis improves subsequent reproductive performance. *J. Dairy Sci.* 99:2026-2042.
- McDougall, S., M. A. Bryan, and R. M. Tiddy. 2009. Effect of treatment with the nonsteroidal antiinflammatory meloxicam on milk production, somatic cell count, probability of re-treatment, and culling of dairy cows with mild clinical mastitis. *J. Dairy Sci.* 92:4421-4431.
- Mee, J. F. 2008. Prevalence and risk factors for dystocia in dairy cattle: A review. *The Veterinary Journal* 176:93-101.
- Meier, S., N. V. Priest, C. R. Burke, J. K. Kay, S. McDougall, M. D. Mitchell, C. G. Walker, A. Heiser, J. J. Loor, and J. R. Roche. 2014. Treatment with a nonsteroidal antiinflammatory drug after calving did not improve milk production, health, or reproduction parameters in pasture-grazed dairy cows. *J. Dairy Sci.* 97:2932-2943.
- Meyer, C. L., P. J. Berger, K. J. Koehler, J. R. Thompson, and C. G. Sattler. 2001. Phenotypic Trends in Incidence of Stillbirth for Holsteins in the United States¹. *J. Dairy Sci.* 84:515-523.
- Mitchell, J. A., P. Akarasereenont, C. Thiemermann, R. J. Flower, and J. R. Vane. 1993. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proceedings of the National Academy of Sciences* 90:11693-11697.

- Morita, I. 2002. Distinct functions of COX-1 and COX-2. *Prostaglandins Other Lipid Mediat.* 68–69:165-175.
- Mullins, C. R., L. K. Mamedova, M. J. Brouk, C. E. Moore, H. B. Green, K. L. Perfield, J. F. Smith, J. P. Harner, and B. J. Bradford. 2012. Effects of monensin on metabolic parameters, feeding behavior, and productivity of transition dairy cows. *J. Dairy Sci.* 95:1323-1336.
- Newby, N. C., K. E. Leslie, H. D. Dingwell, D. F. Kelton, D. M. Weary, L. Neuder, S. T. Millman, and T. F. Duffield. 2017. The effects of periparturient administration of flunixin meglumine on the health and production of dairy cattle. *J. Dairy Sci.* 100:582-587.
- Newby, N. C., D. L. Pearl, S. J. LeBlanc, K. E. Leslie, M. A. G. von Keyserlingk, and T. F. Duffield. 2013. Effects of meloxicam on milk production, behavior, and feed intake in dairy cows following assisted calving. *J. Dairy Sci.* 96:3682-3688.
- Newby, N. C., D. Renaud, R. Tremblay, and T. F. Duffield. 2014. Evaluation of the effects of treating dairy cows with meloxicam at calving on retained fetal membranes risk. *Can. Vet. J.* 55:1196-1199.
- Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Evaluation of nonesterified fatty acids and β -hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. *J. Dairy Sci.* 93:546-554.
- Ouellet, V., E. Vasseur, W. Heuwieser, O. Burfeind, X. Maldague, and E. Charbonneau. 2016. Evaluation of calving indicators measured by automated monitoring devices to predict the onset of calving in Holstein dairy cows. *J. Dairy Sci.* 99:1539-1548.
- Pierce, J. W., M. A. Read, H. Ding, F. W. Luscinskas, and T. Collins. 1996. Salicylates inhibit I kappa B-alpha phosphorylation, endothelial-leukocyte adhesion molecule expression, and neutrophil transmigration. *J. Immunol.* 156:3961-3969.
- Pohl, A., O. Burfeind, and W. Heuwieser. 2015. The associations between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes, and metritis. *J. Dairy Sci.* 98:4544-4551.
- Priest, N. V., S. McDougall, C. R. Burke, J. R. Roche, M. Mitchell, K. L. McLeod, S. L. Greenwood, and S. Meier. 2013. The responsiveness of subclinical endometritis to a nonsteroidal antiinflammatory drug in pasture-grazed dairy cows. *J. Dairy Sci.* 96:4323-4332.
- Proudfoot, K. L., J. M. Huzzey, and M. A. G. von Keyserlingk. 2009. The effect of dystocia on the dry matter intake and behavior of Holstein cows. *J. Dairy Sci.* 92:4937-4944.
- Qu, Y., A. N. Fadden, M. G. Traber, and G. Bobe. 2014. Potential risk indicators of retained placenta and other diseases in multiparous cows. *J. Dairy Sci.* 97:4151-4165.

- Sadri, H., R. M. Bruckmaier, H. R. Rahmani, G. R. Ghorbani, I. Morel, and H. A. Van Dorland. 2010. ORIGINAL ARTICLE: Gene expression of tumour necrosis factor and insulin signalling-related factors in subcutaneous adipose tissue during the dry period and in early lactation in dairy cows. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 94:e194-e202.
- Saint-Dizier, M. and S. Chastant-Maillard. 2015. Methods and on-farm devices to predict calving time in cattle. *Veterinary journal (London, England : 1997)* 205:349-356.
- Saremi, B., A. Al-Dawood, S. Winand, U. Muller, J. Pappritz, D. von Soosten, J. Rehage, S. Danicke, S. Haussler, M. Mielenz, and H. Sauerwein. 2012. Bovine haptoglobin as an adipokine: serum concentrations and tissue expression in dairy cows receiving a conjugated linoleic acids supplement throughout lactation. *Vet. Immunol. Immunopathol.* 146:201-211.
- Schuenemann, G. M., I. Nieto, S. Bas, K. N. Galvao, and J. Workman. 2011. Assessment of calving progress and reference times for obstetric intervention during dystocia in Holstein dairy cows. *J. Dairy Sci.* 94:5494-5501.
- Schuler, G., U. Teichmann, M. P. Kowalewski, B. Hoffmann, E. Madore, M. A. Fortier, and K. Klisch. 2006. Expression of Cyclooxygenase-II (COX-2) and 20 α -Hydroxysteroid Dehydrogenase (20 α -HSD)/Prostaglandin F-synthase (PGFS) in Bovine Placentomes: Implications for the Initiation of Parturition in Cattle. *Placenta* 27:1022-1029.
- Shenavai, S., S. Preissing, B. Hoffmann, M. Dilly, C. Pfarrer, G. R. Ozalp, C. Caliskan, K. Seyrek-Intas, and G. Schuler. 2012. Investigations into the mechanisms controlling parturition in cattle. *Reproduction (Cambridge, England)* 144:279-292.
- Shwartz, G., K. L. Hill, M. J. VanBaale, and L. H. Baumgard. 2009. Effects of flunixin meglumine on pyrexia and bioenergetic variables in postparturient dairy cows. *J. Dairy Sci.* 92:1963-1970.
- Sivertsen, B., K. J. Petrie, J. C. Skogen, M. Hysing, and M. Eberhard-Gran. 2017. Insomnia before and after childbirth: The risk of developing postpartum pain-A longitudinal population-based study. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 210:348-354.
- Sordillo, L. M. and W. Raphael. 2013. Significance of Metabolic Stress, Lipid Mobilization, and Inflammation on Transition Cow Disorders. *Vet. Clin. North Am. Food Anim. Pract.* 29:267-278.
- Ster, C., M. C. Loiselle, and P. Lacasse. 2012. Effect of postcalving serum nonesterified fatty acids concentration on the functionality of bovine immune cells. *J. Dairy Sci.* 95:708-717.
- Trevisi, E. and G. Bertoni. 2008. Attenuation with acetylsalicylate treatments of inflammatory conditions in periparturient dairy cows. *Aspirin and Health Research Progress*. PI Quinn, ed. Nova Science Publishers 22-37.

USDA-APHIS. 2008. Dairy 2007 Part II: Changes in the U.S. Dairy Cattle Industry, 1991–2007. USDA, Washington, DC.

USDA-APHIS. 2010. Heifer calf health and management practices on U.S. dairy operations, 2007. USDA, Washington, DC.

Vane, J. R. and R. M. Botting. 2003. The mechanism of action of aspirin. *Thromb. Res.* 110:255-258.

Villettaz Robichaud, M., D. L. Pearl, S. M. Godden, S. J. LeBlanc, and D. B. Haley. 2017a. Systematic early obstetrical assistance at calving: I. Effects on dairy calf stillbirth, vigor, and passive immunity transfer. *J. Dairy Sci.* 100:691-702.

Villettaz Robichaud, M., D. L. Pearl, S. M. Godden, J. Rushen, S. J. LeBlanc, and D. B. Haley. 2017b. Systematic early obstetrical assistance at calving: II. Effects on dairy heifer calf growth, health, and survival to weaning. *J. Dairy Sci.* 100:703-712.

Wagner, S. A., J. M. Young, J. K. Tena, and B. H. Manning. 2017. Short communication: Behavioral evaluation of the analgesic effect of flunixin meglumine in lame dairy cows. *J. Dairy Sci.* 100:6562-6566.

Whittle, W. L., A. C. Holloway, S. J. Lye, W. Gibb, and J. R. G. Challis. 2000. Prostaglandin Production at the Onset of Ovine Parturition Is Regulated by Both Estrogen-Independent and Estrogen-Dependent Pathways*. *Endocrinology* 141:3783-3791.

Yeiser, E. E. 2011. The use of activity measures in combination with physiological factors as indicators of disease in dairy cattle. Virginia Tech.

Yeiser, E. E., K. E. Leslie, M. L. McGilliard, and C. S. Petersson-Wolfe. 2012. The effects of experimentally induced *Escherichia coli* mastitis and flunixin meglumine administration on activity measures, feed intake, and milk parameters. *J. Dairy Sci.* 95:4939-4949.

Effect of meloxicam administration to the dam prior to calving on pre-weaned calf

behavior by Swartz. The goal of this study was to determine if meloxicam administration to the dam prior to calving would impact newborn calf behavior. Calves born difficultly from dams that received meloxicam before calving spent more time lying when compared to eutocic calves born from dams that received meloxicam before calving, as well as when compared to eutocic and dystocic calves born from control dams. Calves born difficultly displayed less lying bouts for the first few days after birth when compared to calves born easily.

Chapter 5. Impact of meloxicam administration to the dam prior to calving on pre-weaned calf performance

T. H. Swartz,* H. H. Schramm,† J. M. Bewley,‡ C. M. Wood,# K. E. Leslie,§ and C. S. Petersson-Wolfe*

*Department of Dairy Science, Virginia Tech, Blacksburg, VA 24061

†VA-MD College of Veterinary Medicine, Blacksburg, VA 24061

‡CowFocused Housing, Bardstown, KY 40004

#Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA 24061

§Department of Population Medicine, University of Guelph, Guelph, ON N1G 2W1

Christina Petersson-Wolfe

2120 Litton Reaves Hall, Virginia Tech

175 West Campus Drive

Blacksburg, VA 24061

Phone: (540) 231-4767

milk@vt.edu

ABSTRACT

Dystocia has severe implications on calf performance and health, as well as long-term negative effects on future milk production. Furthermore, previous research identified that administration of flunixin meglumine to the dam prior to calving increased the number of stillborn calves. In the present study, we examined the impact of pre-calving meloxicam administration to the dam on newborn calf behavior and health for the first 21 d of life. Dystocia was defined by the length of labor. A calf born from a dam when the length of stage 2 of labor was > 70 min was considered a dystocic calving event, and a calving ≤ 70 min was considered eutocic. An accelerometer was attached to calves' right hind legs after birth. This accelerometer monitored activity (steps/d), lying time (min/d), and lying bouts (bouts/d). Furthermore, calves were health scored daily. Calves born difficultly had fewer lying bouts on d 2, 3, and 4 of age when compared to calves born easily. Moreover, calves born difficultly from dams that received meloxicam before calving spent more time lying when compared to eutocic calves born from dams that received meloxicam before calving, as well as when compared to eutocic and dystocic calves born from control dams. Moreover, no effect on health due to treatment or calving difficulty was identified. To the best of our knowledge, this is the first study to quantify activity measures in calves that transitioned from individual hutches to group housing. We identified a large spike in activity and a decline in lying time on the day (d 5) calves were moved from individual housing to group housing. Additional studies are needed to examine intervention strategies for calves born difficultly. Lastly, in general, calves born from meloxicam-treated dams appear to have similar behaviors and health as calves born from dams that did not receive meloxicam prior to calving; however, additional research is needed to confirm this effect.

Key words: calf; dystocia; activity; meloxicam

Short Communication

The effect of dystocia on the newborn calf can be highly variable, but often has severe implications for newborn calf health and performance, in addition to long-term effects on future milk production. Lombard and colleagues (2007) found that calves born with severe difficulty were 1.7 and 1.3 times more likely to have a respiratory or digestive disease event, respectively, when compared to calves born unassisted. During the first 4 months of life, heifer calves born with severe difficulty were 1.5 times more likely to experience a disease event as well as 1.5 times more likely to die when compared to calves born unassisted (Lombard et al., 2007). However, this effect is not limited to just the pre-pubertal period of a calf's life. Using a calving difficulty scoring system (1 = unassisted, 2 = easy pull, and 3 = hard pull, mechanical extraction, or cesarean section), for each increase in calving difficulty score, while holding all other variables constant, the resulting female calf produced 285 kg less milk in her first lactation when compared to calves born with no assistance (Heinrichs and Heinrichs, 2011). Similarly, a study in the United Kingdom found that veterinary-assisted calvings resulted in calves that produced 710 kg less milk in their first lactation when compared to calves born without assistance (Eaglen et al., 2011).

Physiological and behavioral measurements have been used to quantify the effect of dystocia on calves. Calves born with a hard pull took longer to obtain sternal recumbency (Murray et al., 2015b), make attempts to stand, achieve standing (Diesch et al., 2004; Barrier et al., 2012b), and had a weaker suckling response than calves born unassisted (Murray et al., 2015b). Attempts to quantify the impact of calving on the calf have been developed utilizing a scoring system similar to the Apgar score used for human babies. Murray and colleagues (2015a)

developed a VIGOR score based on visual appearance, initiation of movement, general responsiveness, oxygenation, and heart and respiratory rates. Based on the scoring system, calves born with assistance had reduced vigor when compared to calves born without assistance (Murray et al., 2015a; Murray et al., 2016). Because of the above negative effects, intervention strategies have been investigated to determine if the consequences of dystocia on the dairy calf can be reduced.

Previous studies have examined the effect of a non-steroidal anti-inflammatory drug (NSAID) administered to the newborn calf around the time of delivery (Murray et al., 2015a; Murray et al., 2016). Murray and coworkers (2015a) conducted a large field study where calves were randomly assigned to receive a subcutaneous injection of meloxicam with a target dose of 0.5 mg of meloxicam per kg of BW. Calves that were assisted at birth and received meloxicam gained 1.1 kg more BW during the first week of life when compared to calves assisted at birth that received a placebo (Murray et al., 2015a). Conversely, the opposite effect was seen in calves born with observation but without assistance, as meloxicam-treated calves grew at a slower rate than control calves that were born with observation but without assistance (Murray et al., 2015a). Nevertheless, meloxicam-treated calves had improved health during the first 6 weeks of age when compared to controls (Murray et al., 2015a). Meloxicam-treated calves had an improved vigor 6 h after treatment, a stronger suckling reflex, and consumed more milk from an automatic calf feeder than control calves (Murray et al., 2016). Overall, meloxicam treatment in dairy calves shows promise in alleviating some of the detrimental effects of dystocia. However, past research has demonstrated that when a NSAID (flunixin meglumine) is administered to the dam just prior to calving, the number of stillborn calves was increased 5-fold when compared to

controls (Newby et al., 2017). Therefore, additional research examining the impact of other NSAID therapies utilized prior to calving on calf performance warrant investigation.

Flunixin meglumine is both a COX-1 and COX-2 inhibitor, and has greater anti-COX-1 activity than most other NSAIDs (Beretta et al., 2005). Another NSAID, meloxicam, is a COX-2 preferential inhibitor in most species (Beretta et al., 2005); however, this affinity has not been proven in cattle. In a companion paper from our research group, we administered meloxicam before calving and were unable to find treatment effect on the number of stillborn calves (Swartz et al., In Review). It is possible that the mode of action is contributing to the different responses seen from our study and the flunixin meglumine study (Newby et al., 2017). Nevertheless, it has yet to be seen whether or not meloxicam administration to the dam prior to calving could impact newborn calf performance. Therefore, the objective of the present study was to investigate the impact of meloxicam administration to the dam prior to calving on newborn calf performance and health. Moreover, we investigated the impact of a difficult calving on calf behavior.

Utilizing accelerometers, we quantified activity and lying behaviors over the course of the first 3 wk of the calf's life. We hypothesized that meloxicam administration to the dam prior to calving would allow for the dam to deliver the calf more easily, and therefore, an improvement in calf performance would be identified. We hypothesized that calves born from meloxicam-treated dams would be more active, have more lying bouts, and spend less time lying when compared to calves born from untreated dams. Furthermore, we hypothesized that calves born difficultly would be less active, have fewer lying bouts, and spend more time lying when compared to calves born easily. Because scours and respiratory disease events are the leading cause of pre-weaned calf death (USDA-APHIS, 2010), reducing the impact of dystocia on newborn calves may have merit in reducing the incidence of other diseases. Therefore, we

hypothesized that calves born easily and from dams that received meloxicam prior to calving would be healthier than calves born difficultly or from dams that did not receive meloxicam prior to calving. This project was conducted alongside a periparturient cow study examining the impact of meloxicam administration (Swartz et al., In Review).

Animals, Housing, and Feeding

This study was conducted from March 2017 through August 2017 at the Virginia Tech dairy in Blacksburg, VA, in accordance with guidelines set by the Institutional Animal Care and Use Committee (16-002).

Holstein and Jersey calves (n = 105) were enrolled at birth, moved into individual hutches, and ear tagged. Holstein calves were fed 4 L of colostrum, and Jersey calves were fed 3 L of colostrum after birth. If a calf did not suckle colostrum from a nipple, an esophageal feeder was utilized. Calves remained in individual hutches for the first 4 d of life. While in hutches, calves were fed 2 L of 40.6°C water mixed with 300 grams of milk replacer (Cow's Match®, Warm Front® PB, Land O'Lakes Animal Milk Products Co., Shoreview, MN) twice daily.

When each calf was 5 d of age, they were moved into group-housing with an automatic calf feeder (FA Förster-Technik GmbH, Engen, Germany) at approximately 0800 h. The pen had a 6.3 by 6.0 m bedded pack, and a 4.3 by 6.0 m concrete alley which was maintained by the farm staff. Calves remained in the pen for the remainder of the trial, which concluded when each calf was 21 d old. The 40FIT feeding program was utilized on the automatic calf feeder which provided calves with controlled ad libitum access to milk during the trial. The automatic calf feeder provided an allotment of 2 L of milk per 2 h time period. Therefore, if a calf consumed all of the 2 L allotment, the calf would not be provided any additional milk for the next 2 h, after

which, the calf regained access to milk. This was done to ensure that calves did not gorge themselves during a single meal. In group housing, calves were allowed ad libitum access to water and calf starter (Intensity 22% Textured Calf Starter Medicated, Southern States, Richmond, VA). Water was provided through an automatic waterer (WaterMatic 150, Ritchie Industries Inc., Conrad, IA), and grain was provided in a metal feed bunk. No other feedstuff was provided.

Experimental design

Cow treatment protocol has been previously described in a companion paper (Swartz et al., In Review). In short, dams were randomly assigned to either receive meloxicam (**MEL**; 1 mg/kg of BW, administered by mouth; Meloxicam tablets, USP(15 mg), Cipla LTD., Kurkumbh, India) or a placebo (**CTL**; empty gel capsule) prior to calving. Dams were weighed approximately 3 wk prior to calving and this weight was used to determine the appropriate dose (1 mg/kg of BW). Pre-calving treatment was administered no more than 48 h prior to calving, and no less than 6 h prior to calving. This treatment window was based off of a previous pharmacokinetic study, where meloxicam reached peak concentration 11 h after oral administration (Malreddy et al., 2013).

Dystocia definition

The definition of calving difficulty has been previously described (Swartz et al., In Review). Briefly, close-up dry cows were continuously monitored 24-h/d using three video cameras (Axis P1353-E, Axis Communications AB, Lund, Sweden). The camera feeds were transmitted via an Ethernet cord to a computer and were stored in 3-h segments using video recording software (Media Recorder, Noldus Information Technology Inc., Leesburg, VA). The

amount of time between the presence of the amniotic sac at the vulva and the expulsion of the calf was used to objectively distinguish between eutocic and dystocic calvings. Utilizing thresholds from a previous study (Schuenemann et al., 2011), a calving event with Stage 2 labor > 70 min was indicative of a difficult calving (**DYS**), and a eutocic calving was defined as calving that was ≤ 70 min (**EUT**).

Serum total protein

Approximately 5 ml of blood was collected by a single jugular venipuncture from calves 1 to 7 d of age. After blood collection, samples were immediately centrifuged at 2000 rpm for 20 min at 15°C to separate serum. An optical serum refractometer (Clinical refractometer 300005, Sper Scientific, Scottsdale, AZ) calibrated with distilled water prior to each session, and was used to estimate serum total proteins.

Experimental measurements

Previously validated accelerometers (AfiTag II, AfiMilk LTD., Kibbutz Afikim, Israel) were attached to the calves' right rear legs at birth to collect activity and lying behaviors (Swartz et al., 2016). These variables were collected wirelessly every 30 min and transmitted to a computer (AfiAct II, Afimilk LTD., Kibbutz Afikim, Israel). Data was then summarized into daily steps (no./d), lying time (min/d), and lying bouts (no./d).

Calves were weighed at birth, again on d 5 (the day the calf was moved to group housing and placed onto an autfeeder), and again at the end of the trial (21 d). Furthermore, milk intake was recorded during the entire trial. From d 1 through d 4 when calves were fed 4 L of milk by hand, any refusals were recorded using a graduated cylinder. From 5 d through 21 d of age, the automatic calf feeder recorded milk intake (mL/d) and drinking speed (mL/min). The automatic

calf feeder continuously records and calculates feeding behavior data in spreadsheets utilizing a commercial software program marketed with the feeder (Institute Software, FA Förster-Technik GmbH, Engen, Germany).

Health Scoring

All calves were health scored daily (1600 h) using the Calf Health Scorer App (University of Wisconsin, School of Veterinary Medicine). This app closely follows the criteria of the calf health scoring chart previously developed by Dr. Sheila McGuirk (McGuirk, 2008). A description of this scoring system is provided in Table 5.1. Calves were scored on eye discharge, ear disposition, nasal discharge, coughing, and temperature for respiratory scoring, with scores of 0 representing normal, and 3 being severely abnormal. Respiratory disease was indicated when the sum of these five variables were ≥ 5 , provided that at least two of the five variables were ≥ 2 (McGuirk, 2008). Calves diagnosed with respiratory disease were treated with an injectable antibiotic using label recommendations (either Nuflor, Merck Animal Health, Madison, NJ or Draxxin®, Zoetis Inc., Kalamazoo, MI). Fecal consistency as well as joint and navel scores were performed as well. Calves with a fecal score of 2 or greater were provided with electrolytes and an injectable antibiotic (either Nuflor, Merck Animal Health, Madison, NJ or Excenel, Zoetis Inc., Kalamazoo, MI). If a calf became severely dehydrated, fluids were administered IV. Calves with a joint or navel score of a 2 or greater were treated with an injectable antibiotic (Nuflor, Merck Animal Health, Madison, NJ). All treatments were administered with advisement from a veterinarian.

Statistical analysis

Of the 105 calves enrolled, 11 calves were born from dams that received meloxicam prior to calving and calved difficultly. These 11 calves were matched to 11 calves from dams who received meloxicam prior to calving that calved easily, as well as matched to 11 calves from dams that did not receive meloxicam prior to calving and calved difficultly, and matched to 11 calves from dams that did not receive meloxicam and calved easily. Therefore, 44 calves were included in the analysis (11 MEL DYS, 11 MEL EUT, 11 CTL DYS, and 11 CTL EUT; 28 Holstein and 16 Jersey). To control for colostrum management, as well as other factors, calves were matched by birth weight, breed, and serum total protein values. Therefore, calf birthweights were similar (Mean \pm SD; MEL DYS, 35.5 ± 7.9 ; MEL EUT, 35.8 ± 6.4 ; CTL DYS, 35.0 ± 9.2 , and CTL EUT, 35.6 ± 8.1 kg), as were serum total protein values (Mean \pm SD; MEL DYS, 5.8 ± 0.6 ; MEL EUT, 5.9 ± 0.6 ; CTL DYS, 6.0 ± 0.6 , and CTL EUT, 6.0 ± 0.6 g/dl).

The residuals were analyzed for outliers and normality. Data met the assumptions of the model. A repeated-measures mixed model (PROC GLIMMIX, SAS ver. 9.4, SAS Institute Inc., Cary, NC) was used with treatment (MEL or CTL), calving difficulty score (eutocia or dystocia), day (repeated measure), and all two- and three-way interaction terms, with calf modeled as a random component. The first order autoregressive covariance structure and the Kenward-Roger procedure for degrees of freedom approximation was utilized. Day 1, or the day just after the calf was born, was removed from the behavioral analyses due to missing or incomplete data. When the interaction term was $P \leq 0.05$, simple effects were investigated using the SLICE option in SAS; otherwise, the interaction term was removed from the model. The final model for each response variable is provided in Table 5.2. Significance was declared when $P \leq 0.05$.

No effect of treatment or calving difficulty was found in the milk intake (treatment: MEL 5397 ± 185 vs. CTL 5283 ± 184 , calving difficulty: dystocic 5588 ± 184 vs. eutocic 5092 ± 185

mL/d, both $P > 0.05$). No effect of treatment or calving difficulty was noted on drinking speed (treatment: MEL 504 ± 22 vs. CTL 500 ± 22 , calving difficulty: dystocic 487 ± 22 vs. eutocic 518 ± 22 mL/min, both $P > 0.05$).

Average daily gain was calculated from birth to 5 d of age, as well as from 5 d of age to 21 d, and finally ADG was calculated from birth to 21 d of age. No effect of treatment or calving difficulty was noted on ADG during any time period. For example, for treatment ($P = 0.63$), ADG from birth to 21 d of age was 0.44 ± 0.05 kg/d and 0.40 ± 0.05 kg/d for MEL and CTL, respectively; for calving difficulty ($P = 0.20$), 0.47 ± 0.05 and 0.37 ± 0.05 kg/d for dystocic and eutocic calves, respectively.

Daily lying bouts (no./d) are displayed for eutocic and dystocic calves in Figure 5.1. No effect of treatment was identified (MEL 18.4 ± 0.63 vs. CTL 17.7 ± 0.62 bouts/d, $P = 0.43$). A significant calving difficulty by day interaction was found ($P = 0.05$). Calves born difficultly had less lying bouts on d 2 (eutocic 26.1 ± 1.3 vs. dystocic 19.7 ± 1.4 bouts, $P < 0.01$), d 3 (eutocic 28.2 ± 1.3 vs. dystocic 20.5 ± 1.4 bouts, $P < 0.01$), and d 4 (eutocic 27.6 ± 1.3 vs. dystocic 19.9 ± 1.3 bouts, $P < 0.01$) when compared to calves born easily.

Daily steps (no./d) and lying time are shown in Figure 5.2. For steps, no effect of treatment was identified (MEL $1,531 \pm 60$ vs. CTL $1,588 \pm 60$ steps/d, $P = 0.50$) or for calving difficulty (eutocic 1609 ± 60 vs dystocic 1510 ± 60 steps/d, $P = 0.24$). Figure 5.3 represents the treatment by calving difficulty interaction for lying time. Calves born difficultly from dams that received meloxicam prior to calving spent more time lying than calves born easily in either treatment group or control calves born difficultly (MEL DYS $1,109 \pm 9$ vs. MEL EUT $1,063 \pm 9$, $P < 0.01$; MEL DYS $1,109 \pm 9$ vs. CTL EUT $1,068 \pm 9$, $P < 0.01$; MEL DYS $1,109 \pm 9$ vs. CTL DYS $1,066 \pm 9$ mins/d, $P < 0.01$).

Respiratory scores, fecal, joint, and navel scores were summed to form a total health score value. No effect of treatment (CTL 3.2 ± 0.08 vs. MEL 3.4 ± 0.08 total health score/d, $P = 0.31$) or calving difficulty (EUT 3.3 ± 0.08 vs DYS 3.3 ± 0.08 total health score/d, $P = 0.66$) was identified. A significant effect of time ($P < 0.01$) was identified; LSM are provided in Figure 5.4.

A past study demonstrated that administration of flunixin meglumine to the dam prior to calving increased the number of stillborn calves. In a companion paper from our research group, we were unable to identify an effect of meloxicam treatment to the dam prior to calving on the number of stillborn calves. To further ensure that meloxicam treatment administered prior to calving had no impact on calf health, the present study was conducted examining health scores, activity, and lying behaviors. We were unable to identify any treatment effect on calf health. Calves born difficultly from dams that received meloxicam prior to calving spent approximately 40 to 50 more minutes lying per day than calves born easily from meloxicam-treated dams, or when compared to control calves. Similarly, calves born difficultly had fewer lying bouts than calves born easily. In past studies, calves that experienced dystocia took longer to obtain sternal recumbency (Murray et al., 2015b), make attempts to stand, and achieve standing (Diesch et al., 2004; Barrier et al., 2012b). Furthermore, calves born difficultly had elevated concentrations of creatine kinase and this effect has been associated with muscle damage likely due to a difficult extraction of the calf during parturition (Murray et al., 2015b). Collectively, the reduction in lying bouts and the increase in lying time by calves born difficultly from the present study is in agreement with past studies, and is likely due to muscle damage associated with a difficult, delayed birth. The difference in lying time between dystocic calves born from meloxicam-treated dams when compared to dystocic control calves is a bit perplexing. In human medicine, PGE2 administered exogenously induces cervical ripening, thus allowing for an easier delivery of the

newborn (Keirse, 1993). Therefore, if meloxicam administration prior to calving inhibits PGE2 synthesis, this could, in theory, cause dystocia. It is possible that a more rigid cervix during labor could increase muscle damage in the newborn calf, and therefore increased the lying time in calves born from meloxicam-treated dams. Yet, this effect is not noted in the calves born from meloxicam-treated dams who calved easily. Additional studies are needed to confirm if pre-calving administration of meloxicam to the dam does affect lying behaviors in calves.

This is the first study to our knowledge to quantify activity and lying behaviors in pre-weaned calves that transition from individual housing to group housing. In the present study, calves were moved from individual hutches to group housing on d 5, and this coincided with a large spike in activity and a decrease in lying time. This effect is most likely due to socialization, adjustment to a larger pen, and play behavior. In a recent paper from our research group, we identified that calves inflicted with respiratory disease were less active, and this reduction in activity began 1 d prior to clinical onset (Swartz et al., 2017). In the present study, activity levels dramatically spiked upon entry into the pen, and gradually declined for the first few days afterwards. When taking the activity data in context with the health scores, the decline in activity reached a trough during the middle of second week of a calf's life, which coincided with the greatest health scores (most clinical signs of disease). As health scores gradually declined from the second to third week of the calf's life, activity levels steadily increased. In lying time, a similar effect was noted, as lying time peaked at d 10, near the peak of health scores. Afterwards, lying time gradually declined with age, which corresponded with a decline health scores. While these behavioral alterations coinciding with health scores seem promising for disease detection, additional research teasing out the effect of disease from confounding effects of socialization due to housing changes will be challenging. In the previously mentioned respiratory disease study

(Swartz et al., 2017), we controlled for the effect of age (and thus, socialization) by pair matching diseased calves to healthy control calves of a similar age. Furthermore, in that study, all disease events occurred well past the adjustment period, as the average diseased calf was approximately 1 mo of age. However, in the present study, we identified some potential challenges in continuing with disease detection research utilizing these behaviors. Typically, in industry, deviations in behaviors such as activity are used to identify disease events. These deviations are calculated within the subject (cow or calf), utilizing behavioral data from the preceding days to generate individual baselines. Therefore, if a decline in activity is occurring due to adjustment to a new social environment, this decline in activity could be identified as a disease event when, in fact, it may be due to an adjustment to group housing. Therefore, a large number of false alerts would be generated. Studies examining the effect of disease on activity and lying behaviors with a consistent housing type throughout the trial are needed.

In conclusion, calves born from a dystocic calving had fewer lying bouts for the first few days after birth when compared to calves born easily. Furthermore, calves born difficultly from dams that received meloxicam prior to calving spent more time lying than calves born easily from dams that received meloxicam prior to calving, as well as spent more time lying when compared to calves born from control dams. No effect of treatment or calving difficulty was identified on health scores. Additional research is needed to confirm that meloxicam treatment to the dam prior to calving has no negative impact on newborn calf performance. Furthermore, additional studies examining potential intervention strategies aimed at alleviating the negative consequences of dystocia on newborn calves are needed. Lastly, activity measures provide promise in identifying disease events; however, controlling for the confounding effect of socialization due to housing changes are needed.

Table 5.1 Definitions of the scoring system used to determine disease status adapted from the Calf Health Scorer app (University of Wisconsin, School of Veterinary Medicine).

	0	1	2	3
Respiratory scoring				
Eye discharge	Normal serous discharge	Mild or small amount of discharge	Moderate amount of discharge on both eyes	Heavy discharge
Ear disposition	Normal	Ear flick or head shake	One ear with a slight droop	Both ears droop and/or severe head tilt
Nasal discharge	Normal, clear discharge	Unilateral slightly cloudy discharge	Bilateral cloudy discharge	Heavy bilateral purulent discharge
Coughing	No coughing	Single induced cough	Induced multiple coughs or occasional spontaneous cough	Repeated spontaneous coughs
Rectal Temperature (°C)	37.8 to 38.2	38.3 to 38.8	38.9 to 39.4	Greater than 39.4
Fecal scoring				
Fecal consistency	Normal	Pasty, semi-formed	Loose, but remains on top of the bedding	Watery, sifts through the bedding
Other scoring				
Navel scoring	Normal	Slightly enlarged, but not warm or painful	Slightly enlarged, with some pain or moisture	Enlarged, painful, and warm, with a foul odor
Joint scoring	Normal	Slight swelling, no pain or warmth	Swelling with pain, some lameness	Swelling with severe pain, heat, and lameness

Table 5.2 Mixed model (PROC GLIMMIX, SAS 9.4) of daily steps (no./d), lying bouts (no./d), and lying time (min/d) starting at 2 d of age until 21 d of age of calves born from cows receiving meloxicam prior to calving or a placebo.

Source	Steps		Lying bouts		Lying time	
	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
Treatment	0.46	0.50	0.64	0.43	4.98	0.03
Calving difficulty	1.40	0.24	9.24	< 0.01	6.53	0.01
Treatment*Calving difficulty	– ¹	–	–	–	7.86	< 0.01
Day	24.84	< 0.01	4.30	< 0.01	6.22	< 0.01
Calving difficulty*Day	–	–	1.59	0.05	–	–

¹- Dashed line represents a term that was not significant for that specific model and therefore was removed. Terms that were not significant for any of the behavioral models were removed and are not included in the table.

Figure 5.1 Least squares means (\pm SE) for daily lying bouts from calves born difficultly and easily. Differences between dystocic and eutocic calves: * $P < 0.05$.

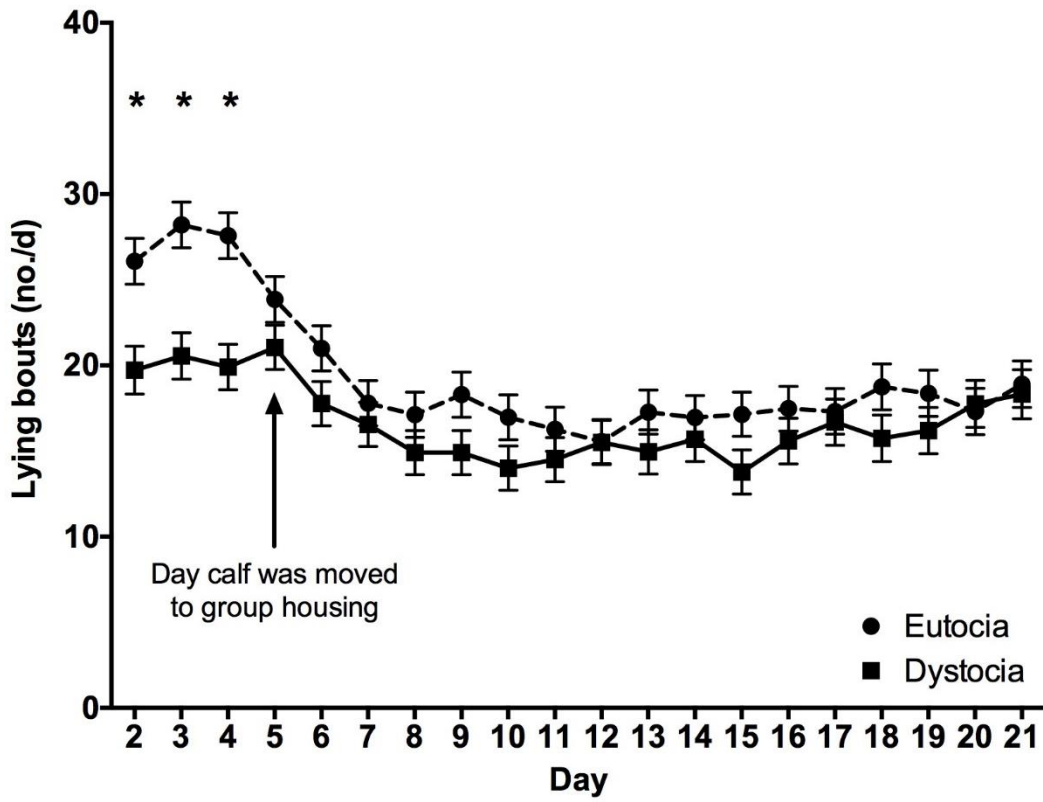


Figure 5.2 Least squares means (\pm SE) for daily steps and lying time starting 2 d of age until 21 d of age.

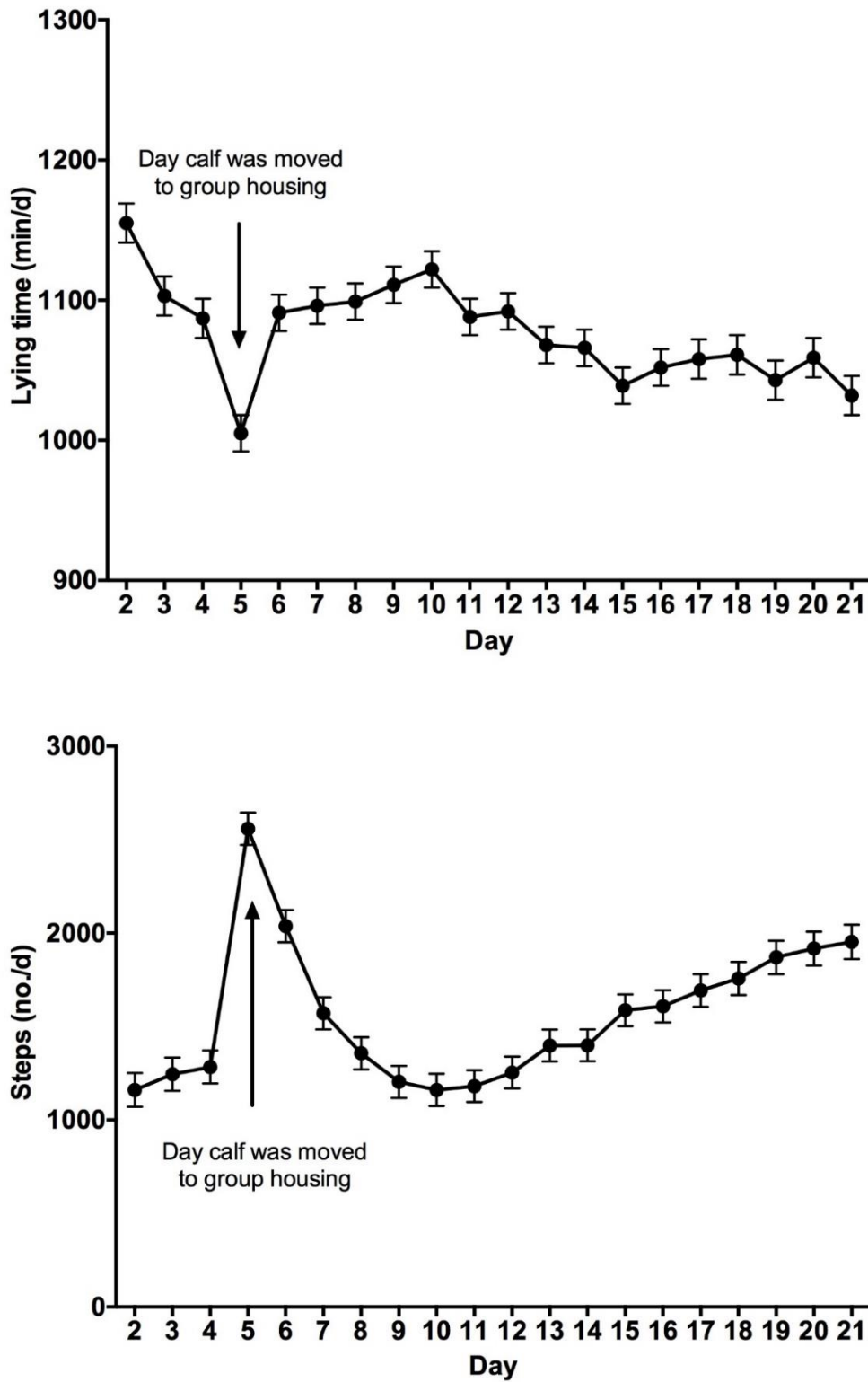


Figure 5.3 Least squares means (\pm SE) for daily lying time recorded from d 2 until 21 d of age. Treatment groups include calves born from dams that received meloxicam (MEL) or a placebo prior to calving (CTL). Calving difficulty was determined by the length of labor. Differences: * P < 0.05.

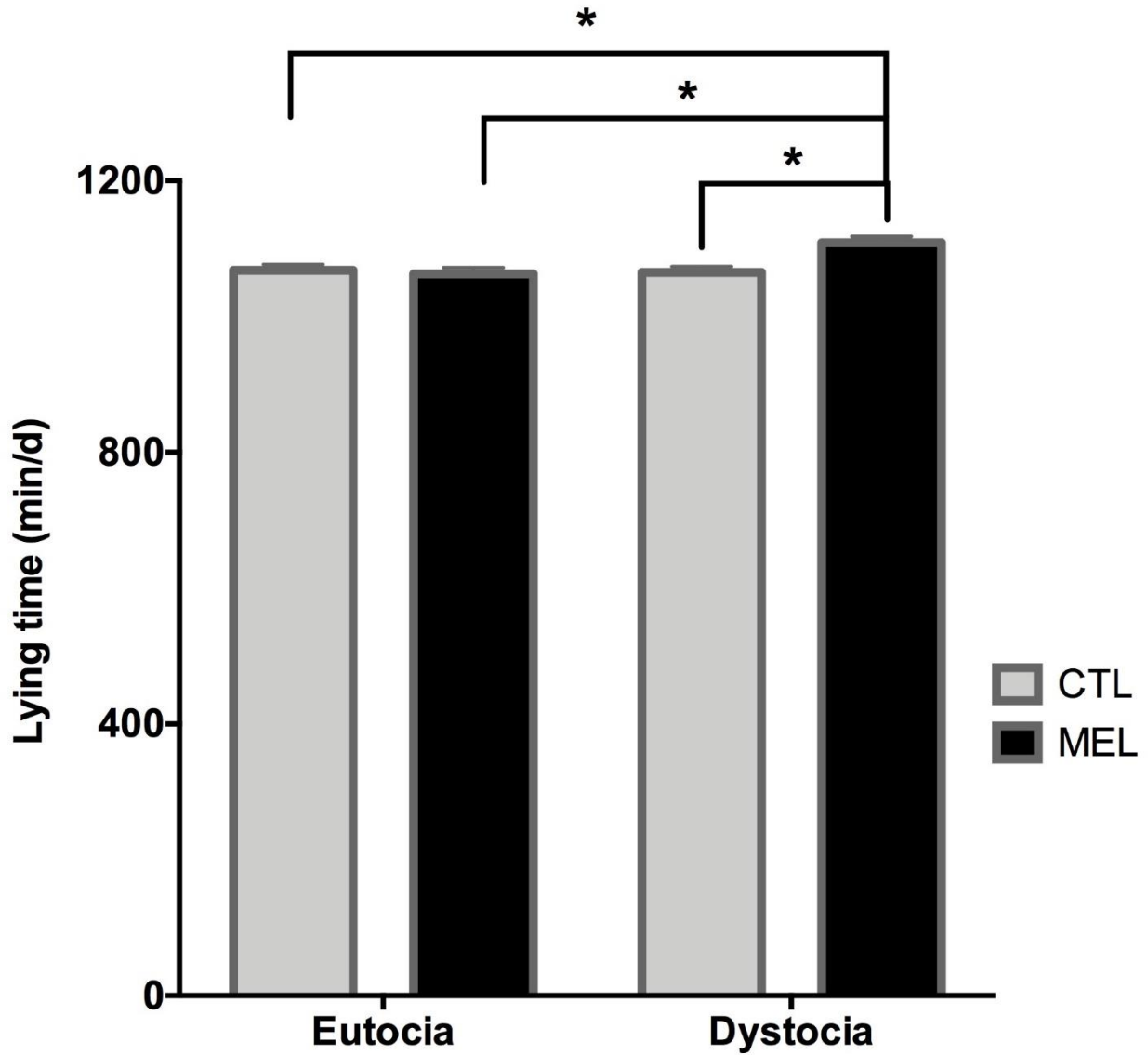
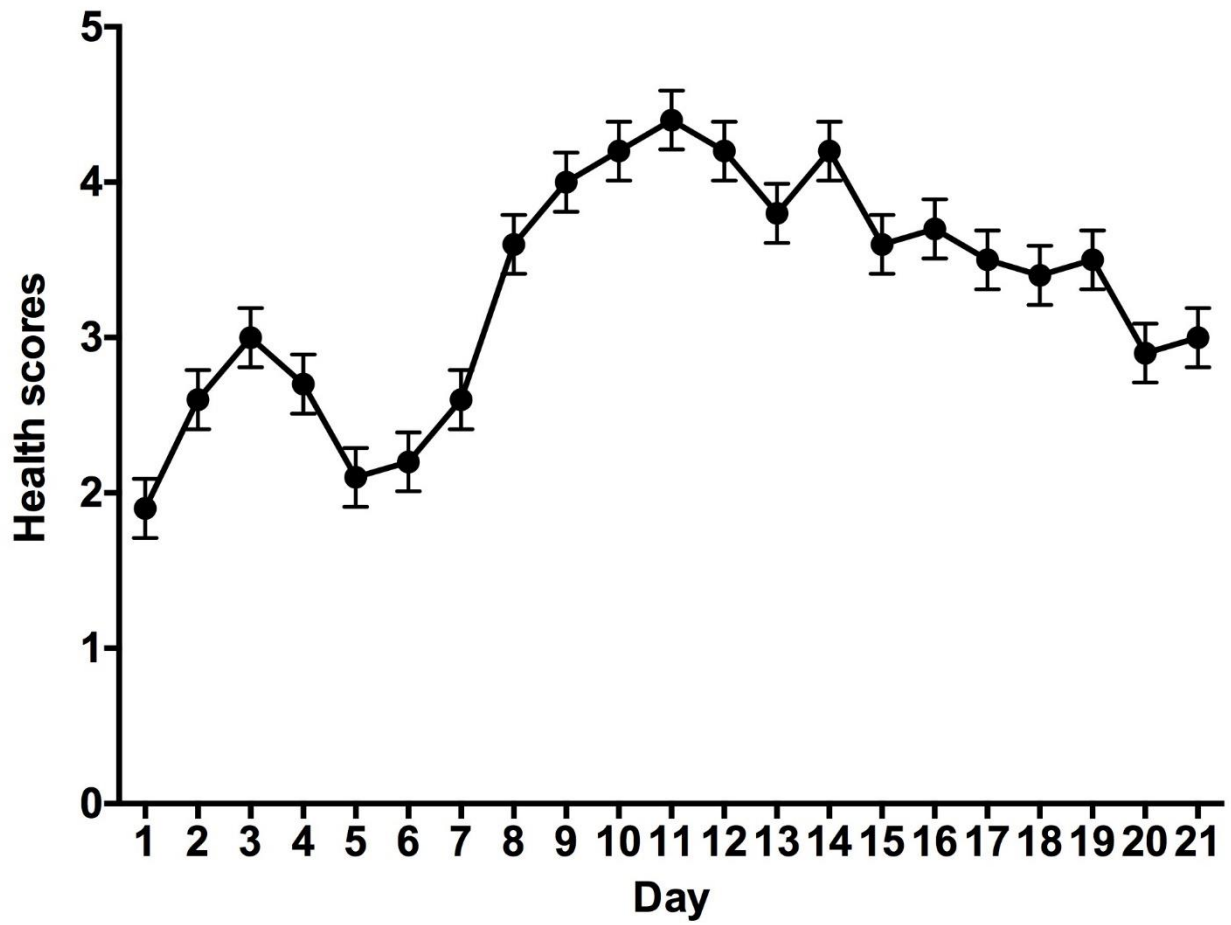


Figure 5.4 Least squares means (\pm SE) for daily health scores recorded for the first 21 d of age.



REFERENCES

- Barrier, A. C., E. Ruelle, M. J. Haskell, and C. M. Dwyer. 2012. Effect of a difficult calving on the vigour of the calf, the onset of maternal behaviour, and some behavioural indicators of pain in the dam. *Prev. Vet. Med.* 103:248-256.
- Beretta, C., G. Garavaglia, and M. Cavalli. 2005. COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin, carprofen and meloxicam: An in vitro analysis. *Pharmacol. Res.* 52:302-306.
- Diesch, T. J., D. J. Mellor, K. J. Stafford, and R. N. Ward. 2004. The physiological and physical status of single calves at birth in a dairy herd in New Zealand. *N. Z. Vet. J.* 52:250-255.
- Eaglen, S. A. E., M. P. Coffey, J. A. Woolliams, R. Mrode, and E. Wall. 2011. Phenotypic effects of calving ease on the subsequent fertility and milk production of dam and calf in UK Holstein-Friesian heifers. *J. Dairy Sci.* 94:5413-5423.
- Heinrichs, A. J. and B. S. Heinrichs. 2011. A prospective study of calf factors affecting first-lactation and lifetime milk production and age of cows when removed from the herd. *J. Dairy Sci.* 94:336-341.
- Keirse, M. J. 1993. Prostaglandins in preinduction cervical ripening. Meta-analysis of worldwide clinical experience. *The Journal of reproductive medicine* 38:89-100.
- Lombard, J. E., F. B. Garry, S. M. Tomlinson, and L. P. Garber. 2007. Impacts of dystocia on health and survival of dairy calves. *J. Dairy Sci.* 90:1751-1760.
- Malreddy, P. R., J. F. Coetzee, B. Kukanich, and R. Gehring. 2013. Pharmacokinetics and milk secretion of gabapentin and meloxicam co-administered orally in Holstein-Friesian cows. *J. Vet. Pharmacol. Ther.* 36:14-20.
- McGuirk, S. M. 2008. Disease management of dairy calves and heifers. *Vet. Clin. North Am. Food Anim. Pract.* 24:139-153.
- Murray, C., T. Duffield, D. Haley, D. Pearl, D. Veira, S. Deelen, and K. Leslie. 2016. The Effect of Meloxicam NSAID Therapy on the Change in Vigor, Suckling Reflex, Blood Gas Measures, Milk Intake and Other Variables in Newborn Dairy Calves. *Journal of Veterinary Science & Animal Husbandry* 4:1-13.
- Murray, C., D. Haley, T. Duffield, D. Pearl, S. Deelen, and K. Leslie. 2015a. A field study to evaluate the effects of meloxicam NSAID therapy and calving assistance on newborn calf vigor, improvement of health and growth in pre-weaned Holstein calves. *The Bovine Practitioner* 49:1-12.
- Murray, C. F., D. M. Veira, A. L. Nadalin, D. M. Haines, M. L. Jackson, D. L. Pearl, and K. E. Leslie. 2015b. The effect of dystocia on physiological and behavioral characteristics related to

vitality and passive transfer of immunoglobulins in newborn Holstein calves. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire 79:109-119.

Newby, N. C., K. E. Leslie, H. D. Dingwell, D. F. Kelton, D. M. Weary, L. Neuder, S. T. Millman, and T. F. Duffield. 2017. The effects of periparturient administration of flunixin meglumine on the health and production of dairy cattle. *J. Dairy Sci.* 100:582-587.

Schuenemann, G. M., I. Nieto, S. Bas, K. N. Galvao, and J. Workman. 2011. Assessment of calving progress and reference times for obstetric intervention during dystocia in Holstein dairy cows. *J. Dairy Sci.* 94:5494-5501.

Swartz, T. H., A. N. Findlay, and C. S. Petersson-Wolfe. 2017. Short communication: Automated detection of behavioral changes from respiratory disease in pre-weaned calves. *J. Dairy Sci.* 100:9273-9278.

Swartz, T. H., M. L. McGilliard, and C. S. Petersson-Wolfe. 2016. Technical note: The use of an accelerometer for measuring step activity and lying behaviors in dairy calves. *J. Dairy Sci.* 99:9109-9113.

Swartz, T. H., H. H. Schramm, J. M. Bewley, C. M. Wood, K. E. Leslie, and C. S. Petersson-Wolfe. In Review. Meloxicam administration either prior to or after parturition: Behavioral, physiological, and production responses. *J. Dairy Sci.* 101:(In Review).

USDA-APHIS. 2010. Heifer calf health and management practices on U.S. dairy operations, 2007. USDA, Washington, DC.

Chapter 6. Research conclusions

In our study, meloxicam administration prior to calving increased MY by 6.8 kg/d in cows that calved easily when compared to eutocic CTL. While we hypothesized a positive effect on MY from pre-calving meloxicam administration, this increase in MY was larger than anticipated. In fact, this finding opposes the results of past pre-calving NSAID administration studies, which led to an increase in disease incidences or stillborn calves. Pharmacology studies are needed to determine the reason for this diverging effect – why meloxicam, and why not flunixin meglumine or acetylsalicylic acid? Inhibition of the synthesis of specific eicosanoids could be part of the answer. For instance, flunixin meglumine could inhibit the synthesis of PGF-2 α , which potentially could be leading to the increase in reproductive diseases, as PGF-2 α is associated with myometrial activation. Whereas, inhibition of PGF-2 α from meloxicam administration may be reduced when compared to flunixin, thereby providing the mechanism for why meloxicam administration has no effect on reproductive disease incidence. Pharmacology studies could provide insight into the mechanism of action behind each NSAID; however, this author is more interested in reducing disease incidence in periparturient dairy cows. Because of the large MY response noted from pre-calving administration of meloxicam in eutocic animals, we now hypothesis that meloxicam administration prior to calving mediated the uncontrolled inflammatory response that occurs around the time of calving, and this effect resulted in healthier cattle. While our study did not demonstrate an effect on any health outcomes, we would contend that a much larger sample size is needed to adequately test the effect of NSAID administration around the time of calving on disease events.

The first project to be conducted would be a large scale commercial herd study where clinical disease outcomes are the primary variables of interest. To adequately test the effect of

meloxicam administration prior to calving on these variables, a large sample size of approximately 1,000 cattle or more would be necessary – largely dependent upon the clinical disease incidence within the herd utilized. Herd veterinarians and trained farm managers would be blinded to treatment groups and diagnose all clinical disease events. These disease events would be defined using previous literature characterizations. Treatments would be administered by research staff – with meloxicam administered by mouth directly prior to calving at a dose of 1 mg/kg of BW. All descriptive statistics, such as calving assistance and calf gender, would be recorded; preferably, only Holstein cattle would be enrolled. Daily MY would be recorded for the entirety of a 305-d lactation.

A subset of the animals from the above project would be utilized for an immunology study. The large scale commercial herd study would answer the research question of – does meloxicam administration prior to calving reduce clinical disease incidences? However, if meloxicam does reduce clinical disease incidences, an immunological-based study answering the “why” behind this phenomenon would provide additional insight. In this study, we would measure pro- and anti-inflammatory eicosanoids, cytokines such as Tumor Necrosis Factor α , immune cell profiling, neutrophil phagocytosis and oxidative burst, cortisol, metabolites (NEFA, BHBA, and glucose), cytobrush technique (quantification of uterine neutrophil population), and haptoglobin. As reactive oxygen species can oxygenate fatty acids to synthesize eicosanoids, another potential outcome variable would be oxidative stress. Intensive sampling would occur over the first 3 wk after calving, with additional sample points at 30, 60, and at 75 DIM during peak production. Interpretation of immunological data can be challenging, as an increase in one variable may or may not be a positive or negative effect. However, by tying the results from the immunological study back to a large scale clinical disease study, interpretation of the data can be

made more conclusively. For instance, if meloxicam reduced clinical disease incidence in a large scale study, and in the immunological study treatment reduced the concentration of a pro-inflammatory eicosanoid, such as PGE₂, this would provide evidence that the mechanism of action to the reduction in clinical disease incidence is due to a reduction in PGE₂ synthesis.

Our study failed to identify a response to treatment for MY in animals that experienced dystocia. A logical next step would be to test the number of treatment administrations of meloxicam (n = 70 per treatment group). In this study, there would be three treatment groups: 1) meloxicam administered prior to calving, and a second dose administered 24 h after calving (both dosed at 1 mg/kg of BW), 2) meloxicam administered prior to calving (1 mg/kg of BW), and a placebo administered 24 h after calving, and 3) a negative control receiving a placebo both before and after calving. Dystocia would be defined similarly to the present study, utilizing the length of labor as the determinant. Milk yield and components, cortisol, inflammatory markers, metabolites, activity, lying behaviors, rumination time, and disease outcomes would be measured during the transition period, with MY and components measurements being assessed until the end of the 305-d lactation.

Periparturient flunixin meglumine studies have failed to identify a positive treatment effect. In fact, most flunixin meglumine studies have decreased milk yield, increased disease incidences, and when administered prior to calving, increased the incidence of stillborn calves. However, it should be noted that in all of these studies flunixin meglumine was administered either prior to calving or within a few hours after calving. Sodium salicylate and acetylsalicylic acid have both yielded positive MY responses when administered 12 or more hours after calving. Because salicylate derivatives are thought to have a relatively similar mode of action as flunixin meglumine, it is likely that delaying flunixin meglumine therapy to 12 or more hours after

calving could result in an increase in MY. This author would prefer to delay treatment to 24 h post-calving, and block cows on the study by retained placenta status and calving difficulty. The purpose of delaying treatment to 24 h post-calving would be to identify cows that retained fetal membranes. Treatments would include administration of flunixin meglumine dosed at 2.2 mg/kg of BW for 3 consecutive daily IV injections starting 24 h post-calving, and a negative control receiving daily doses of a placebo. Milk yield and components would be measured for 305-d, in addition to disease outcomes, inflammatory markers, metabolites, activity, lying behaviors, and rumination time.

Finally, we identified a decline in activity as a promising indicator for detecting calves inflicted with respiratory disease. However, we also found that activity spikes upon entry into group housing from individual hutches, followed by a gradual decline over the next few days. This phenomenon potentially creates a problem in identifying disease events occurring in calves directly after relocation to group housing, as changes in behavior due to disease could be confounded by changes in behavior due to relocation of the calf to group housing. A future study conducted utilizing a consistent housing type would be more appropriate to identify the effect of disease on activity and lying behaviors in pre-weaned calves. Therefore, in the next project, calves would be housed in group housing starting at birth for the first 3 wk of life. Outcome variables would include activity, lying time, lying bouts, lying duration, and milk intake. Calves would be fed 6 L of milk/d, and any refusals would be recorded. Calves would be health scored twice daily for the entire 3 wk observation period. A retrospective analysis would be conducted pair-matching diseased calves with healthy controls based on age, gender, breed, and calf birthweight.

In conclusion, whether it is periparturient dairy cows or pre-weaned dairy calves, research is needed to reduce disease incidence in the dairy industry. Animal welfare is paramount to improving the productivity and sustainability of dairy farms. Research continues to make advances in disease detection, prevention, and control; however, clinical disease incidence remains mostly unchanged in industry. Not only is it vital for research to identify methods to reduce clinical disease incidence in the dairy industry, but more importantly, to apply these solutions in a practical manner to the dairy industry.