Assessing and Mediating Pain in Dairy Cows
with Experimentally-Induced Clinical Mastitis

by

Colleen Fitzpatrick

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This thesis is an investigation of the objective assessment of pain through the use of pressure algometers and rumination tags and the effects of pain management therapy for experimentally-induced mastitis on behaviour and physiological measures in dairy cattle. Twenty-four lactating Holstein cows were enrolled in a lipopolysaccharide (LPS) endotoxin challenge study, where one mammary quarter was infused with 25 µg of *Escherichia coli* (*E. coli*) LPS endotoxin. Subsequently, a subcutaneous injection of either a non-steroidal anti-inflammatory drug (NSAID) (meloxicam; n=12) or placebo (n=12) was randomly allocated and administered using double-blind methods. Several behavioural, physiological and performance parameters were monitored throughout the study period. Beneficial effects of meloxicam administration on pain sensitivity, edema scores and dry matter intake were shown. For a subset of animals receiving placebo treatment, the algometer and rumination tags accurately detected changes in both pain sensitivity and rumination time after endotoxin challenge.
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CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

Despite the widespread implementation of mastitis control programs, clinical mastitis is a commonly occurring, and economically important, disease for the Canadian dairy industry (Olde Riekerink et al., 2008). Mastitis can be attributed to an annual economic loss of approximately $400 million for dairy producers (Canadian Bovine Mastitis Research Network (CBMRN), 2010). In the past 50 years, there has been a general decline of the incidence of clinical mastitis (Bradley, 2002). However, with an incidence rate of 23 cases per 100 cow years in Canadian herds (Olde Riekerink et al., 2008), a focus on research and extension on this issue is still greatly needed. Direct costs attributed to mastitis include milk production losses, treatment costs and potential long-term damage to the mammary gland as a result of inflammation (Fetrow et al., 2000). Indirect costs from mastitis can include somatic cell count (SCC) penalties and increased culling risk (Blowey and Edmondson, 2010). In Ontario, mastitis has been documented to be the third leading reason for culling in dairy herds (CanWest DHI, 2010). In addition, an association between clinical mastitis and reduced reproductive performance in lactating dairy cattle has been reported; cows with clinical mastitis prior to being confirmed pregnant showed increased days to first service, days open, and services per conception (Barker et al., 1998; Schrick et al., 2001). Furthermore, it is estimated that at least once a year, 50% of lactating dairy cows are infected in at least one mammary quarter, which results in many animals being at risk for subsequent illnesses as a consequence of these cases of mastitis (Hillerton and Berry, 2005).
With the state of our knowledge on mastitis and its effects, it is understandable that intensive research has been conducted, focusing on the clinical, physiological, immunological and molecular changes associated with mastitis. As such, our understanding of the biology and epidemiology of mastitis in dairy cattle has increased exponentially. Despite this increase in understanding, the effects of mastitis on cow behaviour and welfare remain largely unexplored (Leslie et al., 2010). A wide variety of tools and techniques are now available and validated for the assessment of animal behaviour and welfare. However the assessment of pain due to mastitis has not been adequately explored. Many researchers contend that animals suffering from mastitis have compromised welfare, and are in need of supportive pain management therapy (Leslie et al., 2010). Furthermore, some authors have asserted that appropriate analgesic treatment of clinical mastitis, to provide relief from suffering caused by pain, discomfort and distress, should be mandatory (Hillerton, 1998). Several non-steroidal anti-inflammatory drugs (NSAIDs) are readily available as supportive therapies for clinical mastitis, even though documented evidence of efficacy and regulatory approvals in this area is limited.

This review will focus on our general understanding of pain, valid methods of detecting pain in dairy cattle, and the state of our knowledge concerning the assessment, therapy and effects of mastitis on cow behaviour and welfare. Finally, the potential for increasing our knowledge in this area, through the incorporation of measures of cow behaviour and welfare into mastitis research, will be discussed.
1.2 DEFINING PAIN

Pain is a term generally associated with human experience. This term is relatively subjective in context, depending on the individual’s experience. The International Association for the Study of Pain defines pain as: “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” (IASP, 2011). This definition is broad and open to interpretation. Individuals can construe pain in many different ways, making it challenging to characterize. There are differences in pain responses between species, between individual animals, between different disease stages, and between acute versus chronic conditions. As such, defining pain is a controversial problem.

1.2.1 Perception of Pain in Dairy Cattle

Cases of clinical diseases, as seen with severely lame cows, or severe clinical cases of mastitis, are easy to characterize as being painful (Fitzpatrick et al., 1998). In such cases, the animal shows visible signs of pain and discomfort, including depressed appearance, decreased milk yield, weight loss, abnormal postures and decreased locomotion, to name a few (Huxley and Hudson, 2007). On the other hand, mild and moderate cases of clinical disease are not as easily characterized as being painful (Fitzpatrick et al., 1998). Unfortunately, the mild and moderate cases of disease occur at a substantially greater frequency. Thus, there is considerable potential for a large number of animals to experience pain to be overlooked. In most cases, our limited knowledge about how animals experience pain is due to our lack of understanding of the behavioural changes indicative of animal pain. As previously mentioned, the assessment and detection
of pain can be difficult, and is complicated by the stoic nature of dairy cattle (Fitzpatrick et al., 1998). This situation is exacerbated in that from an evolutionarily perspective, cattle are considered a prey species, and are predisposed to avoid showing pain and vulnerability, even when exposed to harmful stimuli (Dobromylskyj et al., 2000; Fitzpatrick et al., 1998). Although modern dairy production systems do not expose cattle to any forms of predation, the herd dynamic of a free-stall facility can still provide an opportunity for similar behavioural responses. In other words, the expression of pain and weakness could result in a more dominant animal causing restriction from access to feed and optimal housing areas (Huzzey et al., 2007; Lindgren, 2009). In these situations, pain can be hard to identify and characterize.

1.2.2 Producer Perception of Pain

In Norway, a pain assessment instrument (questionnaire) was developed for and completed by 149 dairy producers to evaluate their opinions about pain associated with various conditions in a dairy herd, using a visual analog scale (Kielland et al., 2010). These researchers found that a large proportion of the producers surveyed did agree that animals do experience physical pain (70%). It was also found that there was a wide range of pain scores allocated for the 21 conditions presented, ranging from 2.4 to 8.6 (on a 0 to 10-point scale). Severe, or serious, cases of mastitis received a score of 7.6, which correlated with conditions such as dystocia and distal limb fractures in calves. However, mastitis that was less severe (with only clots present) received a score of 5.7, which correlated with laminitis. It is evident that the severity of the disease can influence a producer’s opinion on how much pain the animal is experiencing.
1.2.3 Veterinary Perception of Pain

The attitudes and approaches of animal health professionals towards the recognition, prevention and alleviation of potential causes of reduced animal welfare has been explored to identify how animals are being treated in the dairy industry. For example, at a veterinary conference in Scotland, clinicians were administered a survey addressing the subject of dairy cattle welfare (Fitzpatrick et al., 2002). It was found that 68% of the respondents identified that it would be useful to have a scoring system for pain in cattle. In addition, these respondents also indicated that mastitis was not as painful as other clinical conditions or procedures, such as castration, caesarean section or lameness. It should be noted that the greatest variation amongst respondents was with respect to mastitis. It was concluded that the numerous causative agents involved, the range of environmental conditions and the varying levels of severity of infection that occur with cases of clinical mastitis may have been responsible for the wide range in response (Fitzpatrick et al., 2002).

A larger survey performed in the United Kingdom was used to assess the attitudes of cattle veterinarians towards the use of analgesics, and about pain in general (Huxley and Whay, 2006). The respondents were questioned about the severity of pain associated with a variety of cattle diseases, including mild clinical mastitis and severe endotoxic Escherichia coli (E. coli) mastitis. On a 10-point scale, respondents rated severe mastitis at a pain level of seven, comparable to a fracture or foot abscess. On the other hand, mild clinical mastitis was rated at a severity score of three, similar to hair loss from hock abrasion or a case of left displaced abomasum. There was also a significant difference
between male and female respondents. Women rated many of the clinical conditions to be significantly more painful than the rating assigned by men. Interestingly, even though the veterinarians who were surveyed recognized that there was pain associated with even mild cases of mastitis, the use of analgesics for this condition was not suggested. Yet, it is well documented that veterinarians have a responsibility to the producers and their animals to prevent both the pain and distress that result in altered behaviour and physiological changes from an animal’s normal state (Anderson and Muir, 2005).

Recently, a survey conducted by Thomsen et al. (2010) attempted to quantify the use of analgesics in cows and calves by bovine practitioners in Scandinavian countries. The results indicated that younger veterinarians that graduated in the 2000’s, in comparison to older graduates, were more likely to agree that recovery time is faster when analgesics are used. This result is understandable, considering the evolution of the teaching of pain management in veterinary medical education, especially over the last 10-15 years. Another important finding of this survey was the lack of difference between the attitudes of male and female veterinarians, which had been previously documented in the literature. Thomsen et al. (2010) speculated that this discrepancy could be due to an overall awareness of changes in national legislation that encompasses the use of anesthesia and analgesia for common husbandry practices, particularly in Scandinavia.

1.2.4 Public Perception of Pain

The increase in research on the behaviour and welfare of dairy cattle can be attributed to many reasons. One of the primary motivating factors may be public
perception. Various public media are focusing increased attention on the treatment, and overall welfare, of livestock animals. Scientists are encouraged to understand the behaviour of animals, and how to optimize management practices to decrease any stress and pain that they are experiencing (Fraser et al., 1997; von Keyserlingk et al., 2009).

There are three major questions that the public will ask when they are assessing the welfare of animals within a livestock industry (Fraser et al., 1997):

1. “Is the animal functioning well?”
2. “Is the animal feeling well?”
3. “Is the animal able to live according to its nature?”

In regard to clinical mastitis in dairy cattle, it is probable that the answer to all of these questions is “no”. As such, responses to these questions may affect the opinions of the general public that clinical mastitis, compromises the welfare of dairy cattle.

1.2.5  Research Focusing on Pain in Dairy Cattle

Recently, animal science research has placed increased emphasis on animal welfare. Specifically, quantifying and alleviating the effects of painful surgery, husbandry procedures and lameness have been the focus of most research involving pain in cattle. However, there is relatively little published literature aimed at determining the severity of pain linked with other specific cattle diseases, and at quantifying the importance of pain mitigation on animal welfare. An increasing focus has been placed on pain and distress exhibited due to management practices, and how it impacts the animal’s affective state (von Keyserlingk et al., 2009). These practices include dehorning and tail docking. As intervention procedures, humans performing these practices should easily identify when
the animal is in pain, and is suffering. In addition, recent emphasis has also been placed on the health and biological functioning of the animal, including acute diseases or injuries, such as lameness, transition diseases and dystocia (von Keyserlingk et al., 2009). In response, it is clear that these issues receive the most attention in prospective research on welfare and pain, as they are of importance to the industry.

1.3 PHYSIOLOGICAL AND BEHAVIOURAL CHARACTERISTICS ASSOCIATED WITH ILLNESS IN DAIRY CATTLE

Illness results in physiological and behavioural changes in dairy cattle. Physiological changes are useful in the diagnoses of illness. Producers and veterinarians can monitor deviations from normal physiological levels with considerable accuracy. However, the early detection of clinical disease by dairy producers may also be enhanced through the identification of behavioural changes. Therefore, the monitoring of both physiological and behavioural characteristics should be considered when monitoring disease.

1.3.1 Physiological Predictors of Pain and Distress in Cattle

Inflammation and pain in animals are associated with neural, endocrine, hematological, immune, metabolic and behavioural changes that aim to restore homeostasis within the animal (Anderson and Muir, 2005). The immune system and the brain form a bi-directional communication network, whereby the immune system informs the brain about events occurring within the body. Through this communication network, initiation of a response by the immune system produces physiological, behavioural,
affective and cognitive changes that are jointly termed “sickness” or “sickness behaviour” (Maier and Watkins, 1998). Sickness behaviour is a sophisticated and highly conserved response to infection and inflammation. This sickness response is manifested by a number of distinct physiological and behavioural changes, including loss of appetite, adipsia, increased thermoregulatory behaviour, decreased social activity, somnolence, and changed grooming behaviours (Hart, 1988). In addition, sick animals may experience malaise, an affective state that involves negative feelings of depression, anhedonia, pain and lethargy (Millman, 2007). Sickness behaviour is not a maladaptive state. It is actually a highly adaptive response that acts together with the immune system to facilitate recuperation from both injury and illness (Aubert, 1999; Hart, 1988; Owen-Ashley et al., 2006).

An important aspect of an animal’s response to illness is fever (Hart, 1988). The physiological changes associated with inflammation can cause an increase in the body’s thermoregulatory set point, resulting in the animal developing a fever. This process may lead to the expression of the cardinal signs of inflammation, which include redness and heat, along with swelling and pain. The presence of these signs can be associated with depreciation of body function (Lees et al., 2004). An increase in core body temperature results in a less advantageous location for pathogen colonization. It has been suggested that to increase body temperature by 1°C, a 13% increase in metabolic activity is required (Hart, 1988). As such, fever is usually accompanied by other sickness behaviours that are less costly to an animal’s energy stores, which include reduced social activity, adipsia and anorexia.
1.3.2 Behavioural Predictors of Pain and Distress in Cattle

Over the years, dairy cattle have been expected to adapt to the increasing stressors of intensive production systems. Economic factors have forced the dairy industry towards increased milk production, while minimal attention has been given to the behaviours associated with inflammation and pain caused by important production diseases, such as mastitis.

There are obvious detrimental impacts of disease in dairy cattle, for both the animal and the producer. The greatest negative effect of disease is the influence it has on the animal. In the consideration of the basic principles of animal welfare, there are “Five Freedoms of Welfare” (Farm Animal Welfare Council (FAWC), 2009). Disease interferes with four of the five freedoms. The first two, “freedom from discomfort” and “freedom from pain, injury or disease”, are common effects of many bovine diseases. To avoid this situation, disease should be detected and assessed quickly, and therapy should be provided in a timely fashion, so the welfare of these animals is not jeopardized. The “freedom from fear and distress” can also be compromised with disease, particularly with cases of mastitis, from the stress induced due to illness. Finally, the fourth freedom, “freedom to express normal behaviour” can also be applicable with clinical mastitis, since the environment in commercial dairy facilities, especially with tie-stall facilities, restricts the opportunity for the animal to exhibit sickness behaviours, as compared to a cow’s natural environment. Given the dramatic effect that mastitis, and disease in general, has on cow welfare, it is important to identify and treat clinical mastitis cases as quickly and effectively as possible.
Cows experience many stressors throughout their life. It is clear that they may exhibit certain behaviours typical to times of distress. The behaviour of an animal is closely related to how it manages and survives in its environment. It is important to consider that disruptions in normal behavioural patterns could be indicative of a painful encounter or contact with another undesirable stimulus or event (O’Callaghan, 2002). For example, simple management decisions, such as pen changes, can cause stress due to unfamiliarity with the environment and the changes in social groupings (Hasegawa et al., 1997; Kondo and Hurnik, 1990; Schirmann et al., 2011). One study demonstrated the effect of pen movement on primiparous cows (Hasegawa et al., 1997). It was found that dominant animals showed few behavioural changes and no milk production losses two weeks after pen movement, whereas cows that were middle-ranked or subordinate in terms of dominance, exhibited a decrease in milk production of 3.8% and 5.5%, respectively. Similarly, other research has illustrated that in the first day after a cow is regrouped with other animals, she is involved in 9.6 agonistic interactions per hour, which is almost double the rate of agonistic behaviours of other cows in the same pen (Brakel and Leis, 1976). Agonistic behaviours have been classified as physical (bunting, fighting, pushing) or nonphysical (threatening and avoidance behaviours) interactions (Kondo and Hurnik, 1990). In addition, Schirmann et al. (2011) observed the behavioural effects of regrouping in new pens, and in existing pens. It was found that animals regrouped in new pens had a 9% decrease in DMI, while animals from both groups had decreased feeding rates by approximately 5.3 ± 2.1 g / min on the day of regrouping. Rumination times decreased on the day of regrouping for the animals that remained in their pens by 32.1 ± 11.0 min/d, and on the day after regrouping for the animals that were
moved to a new pen by $28.5 \pm 18.9$ min/d. Finally, animals that were regrouped to a new pen demonstrated an increased amount of lying bouts of $1.0 \pm 5.$ bouts/d on the day of regrouping. These results indicate that there is a substantial amount of stress that cows incur due to regrouping.

During times of sickness, animals spend less time eating and drinking and more time resting (Hart, 1988). Behavioural changes such as this likely cause shifts in the animal’s internal environment, such as changes in rumen microflora, and confer social benefits, since resting animals are less likely to be bullied by herdmates. In practical terms, these behavioural changes can aid in the identification of cows at risk of specific diseases. For instance, during the prepartum period, cows with lower dry matter intake (DMI) and reduced feeding times, as well as decreased water consumption, when compared to healthy cows, were significantly more likely to develop metritis after calving (Huzzey et al., 2007). These effects were demonstrated as early as two weeks prior to calving and persisted into the third week of lactation. Altered activity patterns and increased sleep often accompany sickness, possibly as a strategy to assist with healing, and to minimize pain (Molony and Kent, 1997). Extended periods of sleep, in conjunction with the animal’s immune response, can facilitate recovery with cases of infectious disease. Therefore, sleep is a beneficial behaviour during sickness to promote healing and recovery (Toth, 1995).

A number of husbandry practices with cattle have been found to cause tissue damage, inflammation and pain. Furthermore, veterinarians recognize that numerous
diseases of cattle, such as mastitis, metritis, digital dermatitis, pneumonia, and umbilical abscesses are painful conditions (Hewson et al., 2007; Huxley and Whay, 2006). Such organizations as the American Veterinary Medical Association (AVMA; Schaumburg, IL, US) have emphasized the importance of the use of pain therapies with procedures such as castration and dehorning, both prior to and after such procedures. The AVMA also promotes the use of NSAID supportive therapy for long lasting post-operative pain.

Recent research has focused on pain management practices, such as the use of analgesics for procedures in farm animals. It has been determined that the use of analgesics is relatively low (Anil et al., 2005). Reasons identified for this low rate of analgesic use include a lack of economically feasible, and safe to use, analgesics for farm animals. In addition, human food safety concerns and the challenge of recognizing pain in cattle are also important factors (Anil et al., 2005). Barrett (2004) reviewed the indications and rationale for the administration of NSAIDs to cattle. This author identified that there is a need for increased use of analgesia for a number of painful clinical situations and disease conditions of mature cattle. Also, it was reiterated in that review that NSAID administration for pain mitigation is important for ensuring the welfare of animals.

1.3.3 Physiological and Behavioural Indicators Associated with Mastitis

To effectively treat clinical mastitis, it is important to have reliable methods for the detection and classification of severity of infection. The implementation of a clinical evaluation system, which incorporates both local and systemic signs of disease has been
found to provide the most sensitive and precise classification system for clinical mastitis, with very few false positive results (Wenz et al., 2001; Wenz et al., 2006). However, clinical mastitis is still most often detected at milking, by direct observation of the milk and mammary gland. Yet, as farm sizes continue to increase and available labour continues to decrease, dairy producers will need to rely more heavily on automated systems, rather than visual detection. Less time is spent on the individual observation of each cow, and there is a greater risk of missing or misdiagnosing mild or moderate cases of clinical mastitis.

A recent study investigated the influences of feeding strategy on post-milking standing time in dairy cows, and whether this time relates to incidences of IMI (DeVries et al., 2010). The study demonstrated that feeding around milking time (between 30 min prior to 60 min after milking) resulted in the longest post-milking standing times. The shortest post-milking standing time was seen in cows that were fed >30 min prior to milking. It was identified that cows lying down for the first time at 40-60 min after milking had 1.4 times lower odds of acquiring a new environmental IMI than cows lying down less than 40 min after milking. However, as standing time after milking increased past 60 min, the odds of acquiring a new environmental IMI also increased. Cows lying between 60-90 min, 90-120 min and over 120 min after milking showed 3.2, 5.8, and 7.4 times greater odds of acquiring a new environmental IMI, respectively, compared to cows lying down within 40 min of milking (DeVries et al., 2010). These results suggest that feeding behaviour, and those management practices affecting that behaviour, may be important in the occurrence of adverse animal health events.
Mild and moderate cases of clinical mastitis cases have previously been studied, observing pain thresholds, altered stance, heart rate, respiratory rate and rectal temperature in affected cows, as compared with control cows (Fitzpatrick et al., 2000; Milne et al., 2003). It was found that animals with cases of moderate clinical mastitis had significantly greater heart rates, rectal temperatures and respiratory rates, when compared to cows with cases of mild clinical mastitis and normal cows. Cortisol levels and SCC were also significantly increased in cows with mastitis compared to normal cows (Fitzpatrick et al., 2000). Cows with both mild and moderate mastitis cases had significantly larger hock-to-hock distances compared to normal cows, thereby indicating an altered stance (Milne et al., 2003). These affected animals also exhibited an increased sensitivity to a mechanical pressure stimulus on the leg closest to the affected mammary quarter, suggesting a change in pain information processing as a result of inflammation.

In general, in the case of moderate or mild clinical mastitis, it is more difficult to determine whether ruminants experience pain and reduced welfare, as compared with severe cases of mastitis. Due to their stoic behaviour, it is also challenging to determine if NSAIDs would be beneficial to aid in recovery, and mitigate pain, during these less severe cases of mastitis (Milne, 2005).

As it is sometimes difficult to accurately identifying the initiation of illness in cases of naturally-occurring clinical mastitis, models which induce intramammary inflammation in dairy cattle are used. Recently, behavioural and physiological effects of lipopolysaccharide (LPS) endotoxin induced mastitis cases were examined in 20 lactating Holstein cows, randomly assigned to receive an intramammary infusion of either LPS
endotoxin or saline (Zimov et al., 2011). This endotoxin can have negative physiological effects on the cow, not only in the udder, but also throughout the whole body (Hogan and Smith, 2003). Cows receiving the LPS endotoxin had increased rectal temperatures, serum cortisol levels and peak milk SCC in the challenged quarter in the first 24 hours after infusion, as compared with saline-infused cows. In addition, endotoxin-infused cows spent reduced time eating, cud chewing and lying in their stalls compared with saline-infused cows. Furthermore, rumen contractions were reduced in endotoxin-infused cows at sample times, which corresponded with peak rectal temperatures. Results of this study suggest that endotoxin-induced mastitis affects both behavioural and physiological responses in lactating dairy cows (Zimov et al., 2011). These findings support the need for more investigation of behavioural and physiological responses in studies that utilize challenge models with mastitis-causing pathogens, as well as with naturally-occurring cases of clinical mastitis.

These studies have successfully shown that both severe and moderate clinical mastitis alter normal behaviours, and causes systemic physiological changes that are indicative of pain. It is generally concluded that there is a need for analgesia in these clinical situations, and that NSAID therapy could provide useful anti-inflammatory and anti-pyretic activity for these cattle, along with an analgesic effect, that would result in substantially improved animal welfare.
1.4 OBJECTIVE ASSESSMENT OF ILLNESS, DISTRESS AND PAIN IN DAIRY CATTLE

Chronic pain, as observed in cases of mastitis, can be detrimental to the health of an animal, and is often regarded as a pathological process (Muir and Woolf, 2001; Watkins and Maier, 2005). Recent research has focused on behavioural observations and assessing physiologic parameters to objectively define if an animal is experiencing pain or distress (Rutherford, 2002). Although research-based equipment is an ideal method for assessing discomfort as precise indicators of pain, some of the more traditional measurements can be applicable on-farm. For example, the most basic indicators of illness or pain are decreased DMI, water consumption and milk production (Weary et al., 2006). Since cows generally have relatively consistent intakes and production, a decline in either measure may be a good indicator of a problem. Similarly, prolonged changes in milk quality can also be an indirect indicator of inflammation and potential pain. An elevated SCC can be associated with poor udder health and inflammation, which can subsequently indicate pain (Harmon, 2001). Another milk component that can be objectively measured is L-Lactate Dehydrogenase (LDH), which is an enzyme in the milk which increases during mastitis (Chagunda et al., 2006). A bio-sensor to detect this component has become commercially available, and can be used as an early indicator of clinical mastitis (Hogeveen et al., 2010). In terms of physiological parameters, acute phase proteins, such as serum amyloid A and haptoglobin have also been shown to be good indicators of infection, stress, inflammation and pain (Grönlund et al., 2005). All of these parameters have been quite successful in assessing the pain associated with mastitis. However, one of the most effective, but frequently unused, methods of assessing discomfort is observing the overall behaviour of the animal (Anil et al., 2005; Blowey
and Edmondson, 2010). Behavioural responses related to pain and discomfort may include changes in activity, gait, mental state, vocalization and posture. Some of these behaviours are reflexive, whereas others are manifested to decrease the occurrence of tissue damage, reduce the recurrence of tissue damage, and promote overall recovery (Molony and Kent, 1997).

Pedometry systems are available for activity monitoring in the dairy industry, and have been used for the detection of lameness, oestrus and other conditions. There are many commercial pedometry systems currently available. Systems with monitors attached to the leg, rather than the neck or body, produce the most accurate representation of lying behaviour (Ledgerwood et al., 2010). A field study in Israel revealed that 92% of cows that developed clinical lameness had a decrease in pedometric activity of at least 15% (Mazrier et al., 2006). Conversely, oestrus causes an increase in physical activity, and can increase the pedometric activity of free-stall housed cows four-fold (Kiddy, 1977). Evidence is accumulating that cow activity increases significantly in the period immediately prior to calving. It has been suggested that this increased restlessness may be a result of discomfort or distress (Huzzey et al., 2005; von Keyserlingk and Weary, 2007). Huzzey et al. (2005) observed that in the 3 d before calving for dairy cows housed indoors, the number of standing bouts increased by 80%. With the development of automated recording of activity data and the development of algorithms for interpretation, there is considerable potential for pedometric measurements to be a beneficial on-farm tool for the early detection of clinical mastitis, and in turn, help to mitigate potential pain in cattle.
There have been some new research developments that show considerable potential for assessment of pain in dairy cows. These automated measures are precise and easily identify subtle changes in cow behaviour to detect pain. For example, electronic data loggers that measure the orientation of the animal (recumbent or upright) can be used to monitor lying and standing behaviour. Specific measurements include time spent lying, time spent standing, the frequency of lying bouts and lying laterality that the cow exhibits (Ledgerwood et al., 2010). Significant changes in these behaviours, such as decreased lying times, and increased lying bouts, can be an indicator of discomfort in the animal.

In other recent research, specialized weighing platforms have been used to identify lameness in dairy cattle. This weight scale system has the ability to calculate the weight distribution on each hoof, allowing the identification of discomfort in dairy cows (Chapinal et al., 2011). Similarly, research conducted by Pastell et al. (2010) looked at weight distribution in cattle and its ability to detect lameness and other hoof care issues. This measure was proven to be sensitive for the detection of lameness in cows, and particularly those suffering from sole ulcers. However, this method was not useful in detecting cases of mild lameness. Combined with the use of other tools, this technology has the potential of being a useful indicator of discomfort.

The pressure algometer is another technology that has been shown to accurately measure pain. The use of this instrument involves exerting and measuring pressure on an affected body region. Interpretation of changes in sensitivity to this pressure can be used
to determine the pain threshold, which is defined as “the minimum intensity of a stimulus that is perceived as painful” (International Association for the Study of Pain (IASP), 2011). The algometer has been used successfully in dairy cattle research, specifically after the procedure of dehorning (Heinrich et al., 2010), and in cases of lameness caused by integument lesions (Dyer et al., 2007; Liu et al., 2009).

Changes in nociceptive thresholds have been observed in situations of acute stress (Rodgers and Randall, 1988). These thresholds can be measured using a laser-based method to detect thermal nociception (Herskin et al., 2003; Veissier et al., 2000). Nociceptive pain is defined as “pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors” (International Association for the Study of Pain (IASP), 2011). In this method, a laser beam is focused on the animal’s lower limb. A change of behaviour in response to the laser, such as kicking or tail flicking, can be used to identify discomfort. Research suggests that the behavioural responses elicited in response to laser stimulation are both valid and reliable as an indication of nociceptive responses in the cow (Herskin et al., 2003).

A decrease in rumination has been shown to be a good indicator of discomfort in cows with cases of mastitis (Siivonen et al., 2011). It is possible that monitoring rumination may provide a reliable measure of systemic discomfort in cases of clinical disease. It has been shown that when dairy calves were intravenously infused with LPS endotoxin, during peak fever response, they had a rumination time of 6.42 ± 3.69 min, while animals treated with saline had a rumination time of 24.57 ± 6.64 min (Borderas et
Similarly, Bristow and Holmes (2007) also reported that a reduction in rumination time was associated with increased cortisol levels in cattle. Although visual detection of rumination has proven to be a good method to monitor rumination patterns, as with any subjective measurement, it can sometimes be tough to detect changes solely from visual observations, which necessitates an automated device to measure rumination. A recent technologically advanced tool has been developed, validated and become commercially available for dairy farm use. This new tool, rumination HR-Tags (SCR Engineers Ltd., Netanya, IL), is an enhanced version of an activity monitoring tag for the measurement of rumination, and can be used for monitoring and detecting changes in the daily rumination of dairy cattle (Schirmann et al., 2009). It is evident that there are many instruments that can be used by both scientists and producers to successfully evaluate the pain associated with disease, such as with clinical mastitis.

1.5 THE USE OF NSAIDS IN CATTLE

Treatment of inflammation relies on relieving the pain and other systemic effects that commonly accompany inflammation, and slowing any further tissue damage. NSAIDs are commonly used in animals to reduce inflammation (anti-inflammatory), reduce pain (analgesic), reduce pain sensitivity (anti-hyperalgesic), and decrease overall body temperature (anti-pyretic). These drugs act by inhibiting cyclooxygenase enzymes (COX-1 and COX-2), which in turn prevents prostaglandin synthesis (Figure 1.1).
Figure 1.1. The mechanism of action of NSAIDs (MedScape® [WebMD LLC; New York, NY, US])

Around the world, commercially available NSAIDs are approved for anti-inflammatory and anti-pyretic indications. The actual intended pharmacological effect of NSAID administration has not been documented, meaning that the frequency of use of NSAIDs in cattle with an intention to mitigate pain is not well understood. One survey performed by the Colorado Veterinary Medical Association (CVMA; Denver, CO, US) determined that approximately 50% of veterinarians use NSAIDs for pain management following surgery (Wagner and Hellyer, 2000). A Canada-wide survey was conducted to describe the use of analgesics in cattle. Of the 309 veterinarians that reported treating acute toxic mastitis cases, 93% of them provided analgesia in the form of ketoprofen or flunixin meglumine as supportive therapy (Hewson et al., 2007). This Canadian study did not attempt to determine the distribution of analgesic use in moderate versus severe clinical mastitis cases. It is noteworthy that of the 309 veterinarians questioned, 300 of them had graduated prior to 2001. Of those respondents, 27% had never participated in a continuing education program for pain management in animals, yet, a large majority of
the veterinarians that graduated prior to 2001 thought that their knowledge of pain management in dairy cattle was adequate. These veterinarians were asked to rank common dairy management procedures such as, dehorning, displaced abomasum surgery, castration, toxic mastitis, etc., with respect to the amount of pain endured by the animal. None of the procedures were considered to be painless. It was therefore concluded that there is a need for continuing education opportunities for veterinarians with respect to pain identification and analgesic use in food animal species (Hewson et al., 2007).

A recent Canadian study evaluated the efficacy of a single dose of an NSAID at the onset of a naturally-occurring case of neonatal calf diarrhea complex, in conjunction with oral rehydration therapy, and antibiotic treatment (Todd et al., 2010). It was found that NSAIDs successfully reduced the behaviours associated with sickness and pain, and significantly improved calf welfare. Calves treated with NSAID were 5.3 times more likely to consume their entire milk allowance than untreated calves during sickness. In addition, at the end of the study period, calves treated with NSAID consumed 0.15 kg / d more of their starter ration, drank 1.1 L / d more water, gained 4.3 kg more body weight and exhibited less pain-related behaviour, as compared to calves that received placebo treatment (Todd et al., 2010). Similarly, when investigating NSAID use for cases of induced diarrhea in calves, treatment with flunixin meglumine decreased the fecal output (Roussel, Jr. et al., 1988) and improved the clinical status (Barnett et al., 2003) of these animals. Bednarek et al. (2003) observed the use of NSAIDs in addition to antimicrobial therapy for calves with bronchopneumonia, and found that there was an improvement in recovery rates with these treated animals.
With many diseases of dairy cattle, the largest cost to the producer is decreased milk production, which is largely due to decreased feed intake (Bareille et al., 2003). It has been documented that with mastitis, reduced milk production can contribute to approximately 70% of the total cost of the disease (Blosser, 1979). There may be benefit to the use of NSAIDs for management of inflammation and alleviation of pain. Research concerning the use of NSAIDs to manage pain in lame cows has been widely studied. By administering ketoprofen to lame cows, the resulting hyperalgesia was regulated following treatment; showing promising potential to reduce hyperalgesic effects (Whay et al., 2005). These studies suggest that incorporating NSAID therapy into treatment protocols for a variety of clinical problems should improve the welfare of diseased animals and correspondingly, decrease the economic losses to food animal producers.

1.6 USE OF NSAIDS WITH CASES OF MASTITIS

Treatment options for animals with severe clinical mastitis most often involves veterinary intervention. Survey research has shown that both dairy producers and veterinarians generally agree that severe cases of mastitis can cause the animal significant pain and distress (Milne, 2005). As such, it is common practice to provide the severely mastitic cow with NSAID therapy, in addition to antibiotics. This combination will hopefully decrease the signs of pain and discomfort associated with clinical mastitis, while effectively treating the cow to clear the infection.
1.6.1 NSAID Therapy with Endotoxin-Induced Mastitis

The use of NSAIDs has been shown to decrease rectal temperatures, decrease signs of inflammation, maintain rumen motility, and reduce heart rates in cows challenged with LPS to mimic early coliform mastitis, as compared with their unaffected counterparts (Anderson et al., 1986; Wagner and Apley, 2004). Decreased heart rate could be interpreted as a result of a decrease in animal distress or alleviation of pain by the NSAID. There was also an observed reduction in fever of over 1.6 °C in treated animals at 6 h after endotoxin challenge (Anderson et al., 1986). As previously stated, fever is a strategy used by animals to combat infection. As such, it is unknown whether the reduction of fever is actually advantageous for animals with an early case of clinical mastitis. There is generally a lack of published literature supporting the beneficial or detrimental effects of reducing fever in these cases.

1.6.2 NSAID Therapy with Experimental-Challenge Mastitis

Anderson and Muir (2005) reviewed numerous articles concerning the use of NSAIDs in dairy cattle, which clearly demonstrated an improved response to treatment in affected animals after a variety of veterinary procedures. These animals also returned to a normal physiological state more quickly when an NSAID was administered prior to specific procedures, such as castration and dehorning. When cows were infused with *E. coli* and given a NSAID prior to the development of clinical signs of infection, it was found that two NSAIDs almost entirely blocked the febrile response and delayed the decrease in rumen activity of affected animals (Lohuis et al., 1989a). Other studies with experimentally-induced coliform mastitis have also shown improved recovery in these treated animals (Vangroenweghe et al., 2005). Oral and intravenous NSAIDs provided
equal systemic responses (Odensvik and Magnusson, 1996). In a similar experiment, it was found that NSAIDs decreased mammary inflammation and rectal temperature, but did not prevent milk production losses or appetite reduction (Morkoç et al., 1993).

1.6.3 NSAID Therapy with Naturally-Occurring Mastitis

The effect of NSAIDs on naturally-occurring clinical mastitis is not well documented in the literature. As it is challenging to perform research on naturally-occurring infections, most of the published literature reports on results obtained from experimentally-induced infections. However, it may be inappropriate to directly compare cases of clinical mastitis resulting from LPS endotoxin infusion or experimental-challenge with live organism to naturally-occurring mastitis. Nonetheless, there are many similarities between clinical signs for natural infections and experimentally-induced infections. Both induced and naturally-occurring infections result in increases in milk SCC, body temperature, concentrations of TNF-α, mammary gland swelling, and a decrease in milk production (Van Oostveldt et al., 2002). Dascanio et al. (1995) documented the administration of antibiotics and one IV NSAID treatment at the time of first physical examination after the detection of naturally-occurring clinical mastitis. Overall, these researchers found no difference in body temperature, milk production or need for additional treatment between the treatment groups, when monitoring animal responses every 24 hours. However, when treating cows with severe clinical mastitis using an NSAID, the treated animals recovered more quickly than the control animals, and reduced rectal temperatures were observed (Dascanio et al., 1995). Similarly, in a study in Israel, it was found that giving ketoprofen intramuscularly for 5 d allowed
affected cows to return to 75% of their daily milk production recorded prior to their mastitis infection (Shpigel et al., 1994).

Fitzpatrick et al. (1998) studied whether or not cows with clinical mastitis suffered pain over time, and if treatment with a NSAID would help with pain alleviation. Cows with mild or moderate mastitis were given an NSAID, either by intramammary or intravenous route of administration. Pain thresholds were determined using a mechanical device that exerted pressure to the hind limb of each cow. Cows with mild and moderate cases of clinical mastitis showed a heightened responsiveness to pain that persisted for days or weeks after onset. The cows with mild clinical mastitis exhibited reduced sensitivity to pain when treated with a NSAID intravenously. A beneficial effect of the relief of pain was documented (Fitzpatrick et al., 1998). However, similar results were not found with the moderate cases of clinical mastitis, which may have been attributed to the dosage of NSAID being too low. In addition, the observed pain relief by the NSAID in that study was short-lived, and it was recommended that repeated doses of intravenous NSAID might allow for more long-term pain relief (Fitzpatrick et al., 1999; Fitzpatrick et al., 1998).

In another study, 100 dairy cows with both mild and moderate naturally-occurring cases of mastitis were assessed for pain (Milne et al., 2004). It was found that the respiratory rate, rectal temperature and heart rate were all significantly increased in cases of moderate mastitis, when compared to mild clinical mastitis cases. Animals were administered the NSAID, meloxicam, in either a single or a three-dose regimen. Pain
threshold levels were then measured. Animals treated with NSAID returned to their normal threshold levels for these outcome variables significantly faster than untreated animals. The effect was similar whether an animal received one or three doses of meloxicam. It was concluded that by promoting recovery of moderate or mild mastitis by alleviating pain associated with a case of mastitis, cattle welfare will be improved. Other studies in which cows have been treated with meloxicam have reported the alleviation of pain and discomfort associated with mastitis, by reducing heart and respiratory rates and pain responses in lactating dairy cows (Banting et al., 2000). In addition, meloxicam has been shown to be effective as a pain management therapy as a single intravenous dose administration in conjunction with antibiotics (Friton et al., 2002).

In a recent study in New Zealand, treatment of mild and moderate clinical mastitis with a combination of meloxicam and a parenteral antibiotic (penethamate hydriodide) was evaluated for its effect on SCC, milk yield losses, clinical outcomes, and culling rates as compared with antibiotic therapy alone (McDougall et al., 2009). Cows were treated with 5 g of penethamate hydriodide daily for 3 d after the clinical detection of mastitis. Half of these cows were also treated with 250 mg of meloxicam and the other half were treated with a placebo vehicle (control group). It was found that there was no difference between treatment groups in the number of cows that were defined as treatment failures (i.e., re-treated within 24 days of initial treatment, died, or the treated gland stopped producing milk). There was also no difference in milk yield for the cows treated with meloxicam compared with the control cows. However, SCC was lower in the meloxicam-treated group compared with the control group after treatment (550 ± 48 vs. 711 ± 62...
...respectively) and fewer meloxicam-treated cows were removed from the herds (39/237 (16.4%) vs. 67/237 (28.2%), respectively). It was concluded that treating cows with a combination of meloxicam and penethamate resulted in a lower SCC and a reduced risk of removal from the herd (culling) as compared with the penethamate treatment alone (McDougall et al., 2009).

The use of NSAIDs for the treatment of mastitis has been most commonly prescribed for cases of severe endotoxic mastitis, and has not been widely adopted as a standard treatment for cases of mild and moderate clinical mastitis. For such cases, treatment decisions most often do not directly involve veterinarians. Usually, the therapy of these cases at the time of their detection is up to the discretion of the dairy producer or farm manager. Farm personnel often follow a treatment protocol that is designed by both farm staff and the herd health advisory team. It is desirable to create a set of standard operating procedures as a treatment protocol for all cases of clinical mastitis, such as found with the Canadian Quality Milk Program (Dairy Farmers of Canada (DFC), 2010), and to consult with a veterinarian about how to carry these plans out efficiently. As such, there may be an opportunity for greater use of NSAID therapy in mild and moderate clinical mastitis cases.

1.7 CONCLUSION

It is clear that clinical mastitis has severe detrimental effects on the animal and negative economic impacts on dairy producers. However, pain associated with clinical mastitis is generally not measured or treated, unlike some other disease conditions in the...
dairy industry. Attention to behavioural and physiological indicators should be used to monitor animal health, as mild and moderate clinical mastitis are more challenging to detect on-farm, and may therefore be overlooked by the producer. However, it is important to note that new technologies may allow dairy producers to identify clinical mastitis in its very early stages or even before clinical changes occur. Furthermore, automated measures of activity, such as step counts and lying time show promise as a predictor of clinical problems. These new technologies have the potential for improving the screening methods for pre-clinical mastitis, and act as early indicators for the onset of a clinical mastitis event. With this opportunity for the early detection of infection, there is a potential for early intervention with NSAID therapy, which may allow for maximum efficacy from its use, as appropriate therapy of all clinical mastitis cases should be a major goal of the dairy industry. Change in the perception of welfare in farm animals and an increase in our knowledge base surrounding this issue will enhance the creation and implementation of appropriate therapy programs for conditions such as clinical mastitis in dairy cattle.

1.8 RESEARCH OBJECTIVES

The first objective of this dissertation was to investigate the use of both the pressure algometer and rumination tags as tools to help to objectively detect pain and systemic discomfort. It was hypothesized that both technologies will be able to accurately detect changes in response to pain sensitivity and rumination in cases of intramammary inflammation after an experimentally-induced endotoxin challenge.
The second objective of this dissertation was to evaluate the efficacy of pain management medication on the physiological and behavioural impacts associated with pain in dairy cattle during experimentally-induced mastitis following LPS infusion. It was hypothesized that the use of an NSAID (meloxicam) would improve the physiological state of the cow after endotoxin challenge, and reduce the negative behavioural effects of experimentally-induced mastitis. Furthermore, it was thought that the use of meloxicam would mitigate the pain associated with clinical mastitis.
CHAPTER 2: THE OBJECTIVE ASSESSMENT OF PAIN AND SYSTEMIC DISCOMFORT IN DAIRY CATTLE WITH CLINICAL MASTITIS THROUGH THE USE OF PRESSURE ALGOMETERS AND RUMINATION TAGS

2.1 INTRODUCTION

Mastitis continues to be a prevalent and economically detrimental disease in dairy cattle. The negative effects of mastitis, including discomfort and pain, are probably underestimated. In a study by Kielland et al. (2010), it was found that on a 10-point scale, dairy producers ranked only severe cases of mastitis as extremely painful in relation to other conditions. Milder cases of mastitis were ranked much lower for the intensity of pain. Dairy cattle health and welfare scientists have suggested that cows can experience significant pain in even mild cases of mastitis, which can decrease welfare (Leslie et al., 2010).

Pain and discomfort can be assessed in experimental settings and using on-farm measures such as decreased DMI, water consumption and milk production (Weary et al., 2006). Observing the behaviour of the animal can identify abnormalities that could be an indicator of distress and pain due to mastitis (Blowey and Edmondson, 2010). However, for more objective indicators of pain, validated research-based equipment and methods are needed. Recently, new developments have shown promise for the assessment of pain in dairy cows. These measures are quantitative and may be able to identify subtle changes in behaviour that are indicative of a pain response. One of these novel technologies is the pressure algometer. This instrument is used to quantify the pressure exerted on an affected area of the body. The pressure at which the animal responds (moves “away” from the pressure) is interpreted as the pain threshold response of the animal. The
algometer has been used successfully in studies evaluating pain associated with dehorning and lameness (Dyer et al., 2007; Heinrich et al., 2010). However, to the knowledge of the authors, there has been no previous work conducted to quantify pain during cases of clinical mastitis using an algometer. Another technology that has recently been validated and marketed to commercial dairy farms is an automated rumination and activity monitoring system (Schirmann et al., 2009; Burfeind et al., 2011). Specifically, this tool monitors rumination and regurgitation sounds that are detected by a small microphone in the tag. The output data from this system provide information on rumination time, chewing rate, and the interval between bolus regurgitation (Schirmann et al., 2009). There is some evidence to suggest that a decrease in rumination time could be used as an indicator of systemic discomfort in mastitis (Siivonen et al., 2011). The objective of this study was to evaluate the use of algometers and automated rumination monitoring devices to objectively assess changes in response to pain and systemic discomfort in dairy cattle during experimentally-induced clinical mastitis.

2.2 MATERIALS AND METHODS

2.2.1 Animals, Housing and Management

Twenty-one dairy cows (9 primiparous and 12 multiparous) from the University of Guelph Research Centres (Guelph, ON, CA) were enrolled in this study from August 2010 through March 2011. Only animals with no clinical signs of illness were selected. Sample size was determined based on a recently published experimentally-induced clinical mastitis trial (Zimov et al., 2011). All study animals were between 30 and 100 days in milk (DIM). All animals were housed in a tie-stall facility at the Ponsonby Dairy
Research Centre (Ponsonby, ON, CA). Cows were fed a total-mixed ration (TMR) daily at 0900, 1300 and 1500 h, and were milked daily at 0530 and 1600 h. The handling and care of all animals was conducted in accordance with the Canadian Council on Animal Care regulations and standards (2009), and the study was completed within the guideline of an approved Animal Utilization Protocol (AUP#10R050) from the University of Guelph Animal Care Committee.

2.2.2 Intramammary Infusion of *E. coli* LPS Endotoxin

Animals were inoculated by intramammary infusion with either a 25 µg (n = 18) or 100 µg (n = 3) dose of *E. coli* 0111:B4 LPS endotoxin (Sigma-Alderich Co., St. Louis, MO, US). The animals that received 25 µg were enrolled at the beginning of the trial, and those that were infused with 100 µg of endotoxin were enrolled at the end of the trial, after the study protocol was altered. The endotoxin was obtained in a 1 mg dosage, and was re-suspended in sterile phosphate buffer saline to form aliquots of either 25µg/10 mL or 100µg/10 mL solution. This 10 mL dosage was administered into the mammary quarter of all 21 animals (1 quarter per cow). All quarters selected for endotoxin challenge were dipped with an iodine-based teat disinfectant, and cleaned with cotton swabs soaked in 70% ethanol prior to infusion. The infusion was conducted using a sterile 12 mL syringe, fitted with a sterile teat cannula (Jorgensen Laboratories Inc., Loveland, CO, US). All inoculated quarters were again dipped with an iodine-based teat disinfectant immediately after the infusion. Endotoxin infusions were administered on the day of challenge (d 0) between 0700 and 0800 h. The infused, or “challenged” quarters, were selected based on their SCC status. Three days prior to the endotoxin challenge,
quarters of all animals were tested using milk samples that were collected aseptically, and processed through a DeLaval Cell counter DCC (DeLaval International AB, Tumba, SE). Only quarters with a SCC of $\leq 200,000$ cells/mL were selected to challenge with endotoxin, with preference given to the right hind quarter. This SCC value is an industry standard to determine the health status of the udder, but it is a relatively conservative estimate, as it does apply to composite, not quarter, samples. Each cow demonstrated systemic signs of experimentally-induced clinical mastitis, regardless of endotoxin dosage.

2.2.3 Pain Sensitivity

Pain sensitivity readings were measured at 6 time periods over the course of the trial, always by the same operator to minimize variability of the pressure readings between operators. These time periods include 3 d prior to and on the day of endotoxin inoculation, when measurements were taken directly before the inoculation (0 h), as well as 3 h, 6 h, 12 h and 24 h after the time of inoculation. Due to differences in the sampling time intervals between the data collected in 2010 and 2011, because of changes in the study protocol, only the 15 animals (7 primiparous and 8 multiparous) enrolled in 2010 were selected for the pain sensitivity analysis.

Pain sensitivity was measured with a pressure algometer (Force Ten FDX 50, Wagner Instruments, Greenwich, CT, US). This algometer was equipped with a curved pressure pad for easier application on the contour of the quarter. This tool measured the amount of pounds of force (lbf) that could be applied to both the quarter that was
inoculated (challenged quarter) and the ipsilateral quarter that was not inoculated (control quarter). When the cow was standing square on all 4 legs, even pressure was applied perpendicularly to the quarter, approximately 15 cm ventral to the udder attachments, until the operator of the algometer could not press any further, or the animal had an adverse reaction to the pressure (i.e. tail swishing, kicking, shifting weight). The maximum reading at the time of removal of the algometer indicated how much pressure could be applied at each specific time period. Pressure was applied first to the control quarter, followed directly by the challenged quarter. This was done to minimize the chance of increased sensitivity readings in the control quarter if the animal was experiencing pain in her inoculated quarter. Pain sensitivity of the unaffected control quarter was also analyzed to determine if it followed the same trend as the challenged quarter. The SOP for this procedure is found in Appendix 1.

2.2.4 Rumination

Automated rumination monitoring devices (HR-Tags, SCR Engineers Ltd., Netanya, IL), as validated by Schirmann et al. (2009), were used to monitor changes in rumination throughout the study. The tags continuously recorded the time spent ruminating in 2 h intervals by monitoring rumination and regurgitation sounds that are detected by a small microphone in the tag. These tags were attached to a collar, and placed on the upper left side of the cow’s neck, to ensure the most accurate rumination readings. The tags were applied 3 d prior to 3 d following the challenge. The raw rumination data were collected twice daily using a tag reader, and saved with the rumination software that operates with the tags, and the output data provide information.
on rumination time, chewing rate, and the interval between bolus regurgitation (Schirmann et al., 2009).

### 2.2.5 Statistical Analysis

All data were imported or entered into a Microsoft Office Excel spreadsheet (Microsoft Office Excel 2008 for Mac, Microsoft Corporation, Redmond, WA, US). From here, these spreadsheets in .xsl or .csv format were all imported into the statistical software, SAS (SAS 9.2. Software, SAS Institute, Inc., Cary, NC, US). The pain sensitivity readings were imported directly into SAS. Data that were obtained through technological means (i.e. rumination tags) had multiple spreadsheets that had to be consolidated first before importing into SAS. Rumination was analyzed both by day, and by period (2 h intervals). This was done to identify overall changes on a daily basis, and specific changes in diurnal patterns in relation to time of endotoxin challenge. Experimental days were adjusted to start at 0800 h, which is the time when all animals had been challenged with endotoxin. Daily rumination time was calculated by summing 12 2-h periods within each day, and discarding incomplete days. After the removal of these incomplete days, daily rumination data were available from 2 d prior to 2 d after the day of endotoxin challenge.

Initial descriptive analysis was done using the univariate procedure in SAS. Outliers were identified by visual assessment of the data distribution, as defined using this procedure. Extreme outliers were defined as observations that lay more than 3 times the interquartile range from the first or third quartile. These observations were further
investigated and deletion of these points were considered. Data were summarized by cow and the appropriate experimental time periods, as previously described, using the summary procedure in SAS. Summarized data were exported to Excel, where PivotTables were used for exploratory analysis of the data prior to the inferential analysis. Based on these analyses, it was determined, both visually and statistically, that measures occurring prior to the endotoxin challenge showed no difference. As such, a baseline value for each outcome measure was calculated for each cow, averaging the measures taken before the day of challenge. For pain sensitivity, the baseline was averaged from measurements taken at 3 d and directly before the endotoxin challenge (0 h), and for rumination, the 2 days before the endotoxin challenge were averaged for a baseline reading.

Data were analyzed using mixed models with repeated measures (PROC MIXED), including cow as the experimental unit, enrollment group as a random effect, and time as a repeated measure over cow. Fixed effects that were considered in the model included: parity, DIM at enrollment and time. Two-way interactions between fixed effects were tested if biologically plausible. The covariance structure with the lowest Aikake’s Information Criterion was selected. Contrast statements were used to test differences between baseline readings and subsequent time points. Residuals were examined after each model to verify normality and homogeneity of variances as well as to detect possible outliers and influential points. The level of significance used for all statistical analyses was $P \leq 0.05$. 
2.3 RESULTS

2.3.1 Pain Sensitivity

There was an effect of time on the pressure applied to the challenged quarter ($P = 0.01$; Table 2.1). Specifically, there was a decrease at 3 h ($2.19 \pm 1.04$ lbf; $P = 0.05$), 6 h ($2.33 \pm 0.94$ lbf; $P = 0.02$) and 24 h ($3.21 \pm 0.94$ lbf; $P = 0.004$) after endotoxin challenge, as compared with baseline values. There was also an overall parity effect on the pressure applied to the quarter, as $2.7 \pm 0.7$ lbf ($P = 0.002$) more pressure could be placed on the quarters of multiparous cows as compared to primiparous cows. Days in milk did not have an effect on pressure.

The pressure applied to the control quarter did not change over time (Table 2.2). As with the challenged quarter, there was an overall effect of parity on the pressure applied to the quarter, as $2.6 \pm 0.5$ lbf ($P = 0.0002$) more pressure could be placed on the quarters of multiparous cows as compared to primiparous cows, which is consistent with the challenged quarter. Time and DIM did not have an effect on pressure.

2.3.2 Rumination

One cow was removed from rumination analysis by day, as she was identified as an extreme outlier, with excessively low rumination values. This was most likely due to a recording error from the rumination tags. There were no changes in the daily rumination time after the endotoxin challenge. There was an overall effect of parity on rumination, as multiparous cows ruminated $103.7 \pm 15.0$ min ($P < 0.001$) more than primiparous cows. Days in milk did not have an effect on rumination.
When analyzing rumination by 2-h periods, there was a period by day interaction ($P < 0.001$; Figure 2.1) between the diurnal patterns of the baseline data and d 0, after controlling for parity ($P = 0.01$). Specifically, cows experienced a decrease in rumination time during the first 8 h following the endotoxin challenge, and later experienced an increase in rumination time, as compared to the baseline data. Days in milk did not have an effect on rumination.

2.4 DISCUSSION

The present study was conducted to evaluate the use of algometers and automated rumination monitoring devices in dairy cattle experiencing experimentally-induced clinical mastitis. The pressure algometer is used to quantify the pressure placed on an affected area of the body, which is interpreted as the pain threshold response of the animal (Heinrich et al., 2010). Rumination monitoring devices have been validated as an objective way to detect changes of rumination time in dairy cattle (Schirmann et al., 2009), which may be beneficial in detecting decreases in rumination, which have been associated with discomfort due to mastitis (Siivonen et al., 2011). It was hypothesized that both the algometer and rumination monitoring device would objectively assess changes in response to pain and discomfort in dairy cattle with experimentally-induced mastitis.

To the knowledge of the authors, there has been no previous work conducted using the pressure algometer as a method to quantify pain during cases of clinical mastitis. However, this tool has been used in previous studies with dairy cattle (Dyer et
al., 2007; Liu et al., 2009), sheep (Stubsjøen et al., 2010) and horses (Haussler et al., 2007; Haussler et al., 2008; De Heus et al., 2010), and it has proven to be a useful method of identifying pain. Much of the work done using the algometer for dairy cattle has been with lameness studies. Wu et al (2011) found that sound cattle were able to withstand 4.5 kg of force (kgf) of pressure in the soft interdigital tissue of the hoof, and those animals that were not able to reach this threshold were considered to have some form of lameness.

The algometer readings were always conducted first on the control quarter, followed directly by the challenged quarter. This was done to try and minimize the chance of increased sensitivity readings in the control quarter, if the animal was experiencing pain in her challenged quarter. Although this method decreased the probability of pain sensitivity in the control quarter being inaccurately high, there was also a risk of the animal being desensitized to the pressure placed on her challenged quarter, because she was already exposed to the algometer on her control quarter. However, since this was such an acute inflammation, it was hypothesized that the animals would not be desensitized in the challenge quarter, and the pain sensitivity readings would be accurate.

Unlike the study by Wu et al. (2011) that used specific threshold points to determine if a cow was experiencing pain, the current study relies on baseline values of a cow’s pain sensitivity to determine if she is feeling discomfort after the endotoxin challenge. Like humans, cows have a varying degree of pain sensitivity, and they respond to this pain in very different ways (Underwood, 2002). Because of this variation, a
general threshold point to determine whether or not the animal is experiencing discomfort is not always an objective or practical way to assess pain. This is especially true with cases of mastitis, as the size and shape of a cow’s udder can play a key role in how much pressure can actually be applied on the inflamed area. It is well known that primiparous animals have smaller, and more underdeveloped udders than their multiparous counterparts (Klaas et al., 2004). Because of this lack of tissue, in general, less pressure can be applied to these primiparous cows, which may be construed as these animals having a greater pain sensitivity, as described in the current study. However, in reality, they could be experiencing just as much, or more discomfort than multiparous animals.

Although there is variation between individual animals, it was observed that animals experienced the greatest amount of pain at approximately 3 to 6 h after the endotoxin challenge. This peak in pain sensitivity is consistent with physiological parameters in animals that were administered intramammary *E. coli* endotoxin in previous challenge studies. Rectal temperatures (Wagner and Apley, 2004; Banting et al., 2008; Zimov et al., 2011), respiration rate (Banting et al., 2008), thromboxane B₂ (Banting et al., 2000; Banting et al., 2008), mastitis scores (Banting et al., 2008; Zimov et al., 2011), mammary surface temperature (Anderson and Hunt, 1989), size of udder (Banting et al., 2008) and signs of pain after palpating the udder (Banting et al., 2008) have been shown to have their peak increase at 6 h post endotoxin infusion. Further, white blood cell counts (Lohuis et al., 1989a), time spent feeding and chewing cud (Zimov et al., 2011), rumen motility (Banting et al., 2000; Wagner and Apley, 2004), and lying time (Zimov et al., 2011) have been shown to have their nadir at 6 h after endotoxin
infusion. Because of the consistency of these parameters to have their greatest detrimental impact at 6 h after infusion, it can be inferred that the animal is experiencing the most physiological and behavioural effects of the endotoxin at that time point, which as previously stated, is coherent with the pain sensitivity findings from the current study. It can be concluded from these findings that the algometer can detect increases in pain sensitivity in dairy cattle with cases of experimentally-induced endotoxin mastitis.

The rumination monitoring tags that were used in the current study have previously been validated for use in both dairy heifers and cows (Schirmann et al., 2009; Burfeind et al., 2011). These tags monitor rumination and regurgitation sounds that are detected by a small microphone in the tag; the output data provide information on rumination time, chewing rate, and the interval between bolus regurgitation (Schirmann et al., 2009).

Rumination patterns in dairy cattle have been used as an indicator of behaviour and overall welfare in both research and on-farm. A decrease in normal rumination patterns can be indicative of stress (Anderson and Muir, 2005; Bristow and Holmes, 2007), disease (Collier et al., 1982) and pain (Anderson and Muir, 2005) in dairy cattle. Although there was no effect of daily rumination time in the current study, there were significant differences in diurnal patterns between the baseline data, and the day of endotoxin challenge, which can be indicative of systemic discomfort. Some studies have found that rumination decreases as a result of both naturally occurring and experimentally induced cases of mastitis. Siivonen et al (2011), who observed the effects of
experimentally-induced endotoxin mastitis, found that rumination decreased 4-8 h after the infusion of endotoxin, as compared with the previous day. This is generally consistent with the current study, however, in the current study, there were decreases in the first 8 h after endotoxin challenge. This variation between time periods could be due to both the lack of accuracy of visual observations, as well as the small sample size of 6 animals in the previously mentioned study. In another endotoxin challenge study it was also reported that a decrease in cud chewing occurs at 3-9 h after endotoxin infusion, which follows both the previously stated and current study (Zimov et al., 2011). Fogsgaard et al. (In Press) recently observed the effects of experimentally-induced E. coli mastitis on ruminating time and found that at 12-20 h after endotoxin challenge there was a decrease in overall rumination time, which differs from the current study. However, since the previous study used an actual E. coli isolate, the systemic effects of the infection would commence much later than if the animals were inoculated with E. coli LPS endotoxin, as in the current study (Borderas et al., 2008). From what is known about decreases in rumination patterns due to sickness, and the effects of rumination due to experimentally-induced mastitis, it can be concluded from the results in the current study that the rumination tag is an accurate tool to detect changes in rumination due to endotoxin-induced mastitis.

2.5 CONCLUSIONS

In conclusion, both the pressure algometer and the rumination tag have shown to provide accurate and quantitative assessments of changes in response to pain associated with cases of experimentally-induced mastitis. The algometer measured pain sensitivity
values that were consistent with physiological parameters from other studies associated with experimentally-induced cases of mastitis. The rumination tags identified decreases in rumination after endotoxin challenge, which is comparable with previous challenge trials, under visual observation. In summary, both of these monitoring tools provide an objective assessment of the behaviour of dairy cattle with cases of experimentally-induced mastitis.
Table 2.1. Least square mean (±SE) pressure (lbf) in challenged quarters of 15 Holstein dairy cows before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.

<table>
<thead>
<tr>
<th>Time Interval Relative to Endotoxin Challenge (h)</th>
<th>Pressure (lbf)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>13.09 ± 1.26</td>
<td>-------</td>
</tr>
<tr>
<td>3</td>
<td>10.90 ± 1.61</td>
<td>0.05</td>
</tr>
<tr>
<td>6</td>
<td>10.76 ± 1.54</td>
<td>0.02</td>
</tr>
<tr>
<td>12</td>
<td>11.51 ± 1.55</td>
<td>0.12</td>
</tr>
<tr>
<td>24</td>
<td>9.88 ± 1.54</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Table 2.2. Least square mean (±SE) pressure (lbf) in control quarters of 15 Holstein dairy cows before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.

<table>
<thead>
<tr>
<th>Time Interval Relative to Endotoxin Challenge (h)</th>
<th>Pressure (lbf)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>14.03± 1.11</td>
<td>-------</td>
</tr>
<tr>
<td>3</td>
<td>13.68 ± 1.11</td>
<td>0.69</td>
</tr>
<tr>
<td>6</td>
<td>13.89 ± 1.11</td>
<td>0.88</td>
</tr>
<tr>
<td>12</td>
<td>13.18 ± 1.11</td>
<td>0.35</td>
</tr>
<tr>
<td>24</td>
<td>12.45 ± 1.11</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Figure 2.1. Least square means (±SE) rumination time (min/2 h) of 21 Holstein dairy cows before (baseline, average of 2 d prior to inoculation; ○) and on the day of (●) intramammary inoculation of *E. coli* lipopolysaccharide. Experimental days were adjusted to start at 0800 h, time when all cows were inoculated.

● denotes feeding time and ◀ denotes milking time.

Significant differences between experimental day are denoted by symbols (** = $P \leq 0.01$; *** = $P \leq 0.001$).
CHAPTER 3: EFFECTS OF PAIN MANAGEMENT THERAPY FOR EXPERIMENTALLY-INDUCED MASTITIS ON BEHAVIOUR AND PHYSIOLOGICAL MEASURES IN DAIRY CATTLE

3.1 INTRODUCTION

Clinical mastitis results in many detrimental impacts, for both the animal and the producer. A case of clinical mastitis can cause a significant decrease in milk production and overall productivity throughout lactation. These effects can have economic implications if a large proportion of a herd is affected (Hogeveen, 2005). Although the economic impacts receive a great deal of attention, perhaps the greatest negative effect of clinical mastitis is on animal welfare. Clinical mastitis can cause extreme pain and swelling of the mammary gland, as well as severe systemic effects in the animal (Fitzpatrick et al., 1998). Therefore, in addition to antibiotic therapy for the bacterial infection, supportive therapy to mitigate the pain, discomfort, and inflammation may be of critical importance.

Non-steroidal anti-inflammatory drugs are widely used in dairy cattle as pain management therapy for a variety of procedures and diseases, including clinical mastitis (Fajt et al., 2011). In Canada, 2 NSAIDs are currently approved and available for pain management therapy in lactating dairy cattle. These products are flunixin meglumine and ketoprofen. Both of these NSAIDs predominately inhibit the prostaglandin synthesis of COX-1, an enzyme that plays a housekeeping role throughout the animal’s body (Hawkey, 2001). By this action, these NSAIDs are able to regulate and maintain different physiological effects, such as gastrointestinal and renal functions, as well as maintain a homeostatic state in the body. Flunixin meglumine (Banamine 50 mg/mL Sterile Solution
Injectable, Merck Animal Health, Boxmeer, NL) and ketoprofen (Anafen Injection 100 mg/mL, Merial Canada, Inc., Baie D’Urfé, QC, CA) have active half-lives of 3.1 to 8.1 and 2 h, respectively.

In addition to NSAIDs that predominately inhibit prostaglandin synthesis of COX-1 enzymes, there are also NSAIDs that selectively target COX-2 enzymes (Hawkey, 2001). These enzymes act specifically on sites of inflammation. Furthermore, their actions are enhanced when there are increased levels of inflammatory stimuli, growth factors and cytokines (Hawkey, 2001). In Canada and elsewhere, the NSAID, meloxicam (Metacam 20mg/mL solution of injection, Boehringer Ingelheim GmbH, Ingelheim am Rhein, DE), has been approved for use in calves during dehorning (Heinrich et al., 2010), as well as an aid in the therapy of cases of neonatal calf diarrhea complex (Todd et al., 2010). However, there are currently no NSAIDs that target COX-2 enzymes approved for use in lactating dairy cattle in Canada. Such NSAIDs are approved in Europe and other regions of the world. In lactating cattle, meloxicam has a half-life of 17.5 h. In the EU specifically, meloxicam has been approved for use in lactating dairy cattle with clinical cases of mastitis, as administration of this drug has been shown to decrease inflammatory and clinical condition, improve milk yield and restore pain-threshold responses in affected cows (Friton et al., 2002; Milne et al., 2004; Friton and Banting, 2005). Meloxicam has also been evaluated with mild and moderate cases of clinical mastitis in New Zealand (McDougall et al., 2009), where the use of meloxicam reduced SCC following a clinical case of mastitis, as well as the subsequent risk of culling in treated animals. Because of the specific mechanisms of meloxicam, and its
extended half-life, there is considerable potential for using this NSAID as a pain management therapy in cases of clinical mastitis. However, to determine the true efficacy of meloxicam, a variety of objective parameters should be measured, specifically pain sensitivity and behavioural indicators of pain, as these are not frequently examined, but important to consider during cases of clinical mastitis. The objective of this study is to evaluate the efficacy of the pain management therapy on activity, pain sensitivity and physiological indicators of inflammation in dairy cattle with experimentally-induced clinical mastitis.

3.2 MATERIALS AND METHODS

3.2.1 Animals, Housing and Management

Twenty-four dairy cows from the University of Guelph Research Centres (Guelph, ON, CA) were enrolled in this study in August 2010. Only animals with no clinical signs of illness were selected. Sample size was determined based on a recently published experimentally-induced clinical mastitis trial (Zimov et al., 2011). All study animals were between 10 and 100 DIM and the study group was blocked into primiparous (n = 12) and multiparous (n = 12) groups. All animals were housed in a tie-stall facility at the Ponsonby Dairy Research Centre (Ponsonby, ON, CA). Cows were fed a total-mixed ration (TMR) daily at 0900, 1300 and 1500 h, and were milked daily at 0530 and 1600 h. The handling and care of all animals was conducted in accordance with the Canadian Council on Animal Care regulations and standards (2009), and the study was completed within the guideline of an approved Animal Utilization Protocol (AUP#10R050) from the University of Guelph Animal Care Committee.
3.2.2 Intramammary Infusion of *E. coli* LPS Endotoxin

Animals were inoculated by intramammary infusion with a 25 µg dose of *E. coli* 0111:B4 LPS endotoxin (Sigma-Alderich Co., St. Louis, MO, US). The endotoxin was obtained in a 1 mg dosage, and was re-suspended in sterile phosphate buffer saline to form aliquots of 25 µg/10 mL solution. This 10 mL dosage was administered into the mammary quarter of all 24 animals (1 quarter per cow). All quarters that were selected for endotoxin challenge were dipped with an iodine-based teat disinfectant, and further cleaned with cotton swabs soaked in 70% ethanol prior to infusion. The infusion was conducted using a sterile 12 mL syringe, fitted with a sterile teat cannula (Jorgensen Laboratories Inc., Loveland, CO, US). All inoculated quarters were again dipped with an iodine-based teat disinfectant immediately after the infusion. Endotoxin infusions were administered on the day of challenge (d 0), between 0700 and 0800 h. The infused, or “challenged” quarters, were selected based on their SCC status. Three days prior the day of endotoxin challenge, quarters of all animals were tested using milk samples that were collected aseptically, and processed through a DeLaval Cell counter DCC (DeLaval International AB, Tumba, SE). Only quarters with a SCC of ≤200,000 cells/mL were selected to challenge with endotoxin, with preference given to the right hind quarter. This SCC value is an industry standard to determine the health status of the udder, but it is a relatively conservative estimate, as it does apply to composite, not quarter, samples. Each cow demonstrated systemic signs of experimentally-induced clinical mastitis, regardless of endotoxin dosage.
3.2.3 Experimental Treatments

Animals were randomly assigned with the use of a random number generator (Random.org, Mads Haahr, 2011) to either meloxicam or placebo treatment within their parity blocks, where half of the cows received meloxicam (n = 6) (Metacam 20mg/mL solution of injection, Boehringer Ingelheim GmbH, Ingelheim am Rhein, DE) and the other half received an equivalent volume of placebo (n = 6) for each parity group. Negative control animals (animals that were not inoculated with LPS and received a placebo injection) were not included in this trial, since with previously published studies, there did not appear to be any adverse behavioural effects with any of the procedures preformed (Zimov et al., 2011). The experimental solutions were provided in individual sterile glass vials, which were labeled with a unique trial identification number. The placebo solution was the product vehicle, with only the active meloxicam ingredient removed. As such, both treatment solutions were identical in colour and consistency. All research technicians and researchers were blind to the treatment identity. The specific identifier code was provided only after the trial was completed, including data entry, data validation and preliminary descriptive analyses. From recent herd measurements, the average weights of 10 primiparous cows and 10 multiparous cows from the Ponsonby Dairy Research Centre were obtained. The average weight from each group was used to calculate treatment dosages for primiparous and multiparous animals for the current study. At a rate of 2.5 mL/100 kg, and primiparous animals received dosages of 15 mL, while multiparous animals received dosages of 17 mL. These injections were performed subcutaneously, and given immediately after *E. coli* endotoxin challenge.
3.2.4 Sampling Time Periods

For most of the experimental parameters included in this study, there were specific time periods that were identified to take each measurement. These time periods started 3 days prior to the endotoxin infusion. On d 0, measurements were taken directly before the inoculation and experimental treatment (0 h), as well as at 3 h, 6 h, 12 h and 24 h after the time of infusion. This gave a total of 6 time periods over the course of the trial.

3.2.5 Clinical Parameters

Several clinical parameters were measured following the endotoxin challenge, as a means to monitor the physiological changes of the animals due to the systemic effects of clinical mastitis. These parameters included clinical score of the milk, udder edema score, rectal and core body temperature, SCC and acute phase protein (haptoglobin and serum amyloid A [SAA]) concentrations in blood.

A clinical score modified from Wenz et al. (2006) and Zimov et al. (2011) was used to assess the appearance of the milk and changes in the conformation of the udder (Table 3.1). Clinical status was determined at the 6 time-points, as described above.

Edema of the udder was also assessed at the 6 time periods. Edema was assessed on both the challenged quarter, and the control quarter on a 5-point scale (Table 3.2), as described by Nestor, Jr. et al. (1988).
Temperatures were monitored rectally using a digital thermometer. These measurements were taken not only for research purposes, but to monitor the cow, and ensure that she was not experiencing adverse effects from the endotoxin challenge. Temperatures were taken at the 6 specific time periods, as well as at 2 d post inoculation. This additional reading was done to ensure that the animals had returned back to their homeostatic states.

Continual temperature monitoring was also conducted from 3 d prior to 3 d following inoculation using a temperature data logger (Vemco Minilog 8, Vemco Division of AMIRIX Systems Inc., Halifax, NS, CA), which monitored the animal’s temperature in 1-min intervals. This logger was attached to a modified controlled internal drug release device (CIDR 1380, Pfizer Animal Health, Madison, NJ, US) and inserted vaginally into the cow. The full standard operating procedure for this logger, which is modified from the original SOP used by Vickers et al. (2010), is found in Appendix 2. Data were extracted from the loggers after they were removed from the animal, and organized in a spreadsheet for statistical analysis.

Milk samples were aseptically collected throughout the experimental trial to determine SCC. Samples were taken in accordance with the National Mastitis Council protocols (National Mastitis Council, Verona, WI, US). Samples were taken at the 6 sample time periods, as indicated above. Samples were frozen at -20°C, and were submitted to the Atlantic Veterinary College (Charlottetown, PEI, CA). Samples were then thawed, and ran through a Fossomatic Cell Counter (Foss Instruments, Hillerod,
DK) for determination of SCC. The SCC values were converted to somatic cell scores (SCS) for statistical analysis using the following equation:

\[
SCS = \log^2 (\frac{SCC}{100,000}) + 3
\]

Blood samples were taken at the 6 specified time points, as indicated above. For the analyses of acute phase proteins, approximately 10 mL of blood was obtained by coccygeal venipuncture at each sampling time. The samples were processed through a centrifuge at 3000 revolutions per min (RPM) for 12 min, frozen at -20ºC and sent to the Animal Health Laboratory (Guelph, ON, CA) to be analyzed for both serum amyloid A (SAA) and haptoglobin.

### 3.2.6 Pain Sensitivity

Pain sensitivity was measured with a pressure algometer (Force Ten FDX 50, Wagner Instruments, Greenwich, CT, US). This algometer was equipped with a curved pressure pad for easier application on the contour of the quarter. This tool measured the amount of pounds of force (lbf) that could be applied to both the quarter that was inoculated (challenged quarter) and the ipsilateral quarter that was not inoculated (control quarter), and was always conducted by the same operator to minimize variability of the pressure readings between operators. When the cow was standing square on all 4 legs, even pressure was applied perpendicularly to the quarter, approximately 15 cm ventral to the udder attachments, until the operator of the algometer could not press any further, or the animal had an adverse reaction to the pressure (i.e. tail swishing, kicking, shifting...
weight). The maximum reading at the time of removal of the algometer indicated how much pressure could be applied at each specific time period. Pressure was applied first to the control quarter, followed directly by the challenged quarter. This was done to minimize the chance of increased sensitivity readings in the control quarter if the animal was experiencing pain in her inoculated quarter. Pain sensitivity of the unaffected control quarter was analyzed to determine if it followed the same trend as the challenged quarter. Pain sensitivity readings were taken at the 6 specific time periods, as previously indicated. The SOP for this procedure is found in Appendix 1.

3.2.7 Rumination

Automated rumination monitoring devices (HR-Tags, SCR Engineers Ltd., Netanya, IL), as validated by Schirmann et al. (2009), were used to monitor changes in rumination throughout the study. The tags continuously recorded the time spent ruminating in 2 h intervals by monitoring rumination and regurgitation sounds that are detected by a small microphone in the tag. These tags were attached to a collar, and placed on the upper left side of the cow’s neck, to ensure the most accurate rumination readings. The tags were applied 3 d prior to 3 d following the challenge. The raw rumination data were collected twice daily using a tag reader, and saved with the rumination software that operates with the tags, and the output data provide information on rumination time, chewing rate, and the interval between bolus regurgitation (Schirmann et al., 2009).
3.2.8 Lying Behaviour

Electronic data loggers (HOBO Pendant G Logger Data Logger, Onset Computer Corporation, Pocasset, MA, US) were used to monitor both standing and lying behaviour. These loggers were programmed to record the position of the cow (both standing or lying, and the side they were lying on) in 1-min intervals. These recordings were used to calculate different lying behaviour parameters, including the frequency of lying bouts and their duration, daily lying time and time spent lying on each side. This device has previously been validated as an accurate method to monitor activity in dairy cattle (Ledgerwood et al., 2010). These data loggers were attached on the animals 3 d prior to 3 d following the challenge. Loggers were placed in the mid metatarsal region on the lateral aspect of the right hind leg. After the trial period, loggers were removed, and data, including g force readings on the x, y and z-axis, were extracted into the HOBOware Pro software (HOBOware Pro Version 3.1.0, Onset Computer Corporation, Pocasset, MA, US) and converted into degrees of tilt for statistical analysis (Ito, 2009).

3.2.9 Dry Matter Intake

Dry matter intake for each individual cow was measured daily by subtracting the amount of total mixed ration (TMR) orts from the amount of TMR delivered the previous day. These intakes were taken 3 d prior to 3 d following endotoxin challenge. At 3 time periods throughout the trial (d -3, d 1 and d 4), samples were collected from the TMR weigh-backs, and frozen. At a later date, these samples were thawed, and dried (60°C for 48 h) to determine their moisture content. These 3 values were averaged, and applied to the daily weigh-backs to determine their DM content.
3.2.10 Milk Production

Daily milk yield was recorded twice daily, from 3 d prior to 3 d following the day of endotoxin challenge. The data were captured by the on-farm dairy management software (DairyCOMP 305, Valley Agricultural Software, Tulare, CA, US), and later exported into a spreadsheet for statistical analysis.

3.2.11 Statistical Analysis

All data were imported or entered into a Microsoft Office Excel spreadsheet (Microsoft Office Excel 2008 for Mac, Microsoft Corporation, Redmond, WA, US). From here, these spreadsheets in .xsl or .csv format were all imported into the statistical software, SAS (SAS 9.2. Software, SAS Institute, Inc., Cary, NC, US). All data that were entered manually were imported directly into SAS. Data that were obtained through technological means (i.e. temperature data loggers, rumination tags and electronic data loggers) had multiple spreadsheets that had to be consolidated first before importing into SAS. These parameters obtained through technological means were summarized both by day (removing any incomplete days), and by 2-h periods for rumination, and by hour for core body temperature and lying behaviour. This was done to identify overall changes on a daily basis, and specific changes in diurnal patterns in relation to time of endotoxin challenge. After the removal of incomplete days, data from all technological parameters were available from 2 d prior to 2 d after the day of endotoxin challenge. Experimental days were adjusted to start at 0800 h, which is the time when all animals had been challenged with endotoxin.
Initial descriptive analysis was done using the univariate procedure in SAS. Outliers were identified by visual assessment of the data distribution, as defined using this procedure. Extreme outliers were defined as observations that lay more than 3 times the interquartile range from the first or third quartile. These observations were further investigated and deletion of these points were considered. Data were summarized by cow and the appropriate experimental time period using the summary procedure in SAS. Summarized data were exported to Excel, where PivotTables were used for exploratory analysis of the data prior to the inferential analysis. Based on these analyses, it was determined, both visually and statistically, that measures occurring prior to the endotoxin challenge showed no difference. As such, a baseline value for each outcome measure was calculated for each cow, averaging the measures taken before the day of challenge. For pain sensitivity, the baseline was averaged from measurements taken at 3 d and directly before the endotoxin challenge (0 h), and for rumination, the 2 days before the endotoxin challenge were averaged for a baseline reading.

Data were analyzed using mixed models with repeated measures (PROC MIXED), including cow as the experimental unit, enrollment group as a random effect, and time as a repeated measure over cow. Fixed effects that were considered in the model included: parity, DIM at enrollment, experimental treatment and time. Two-way interactions between fixed effects were tested if biologically plausible. The covariance structure with the lowest Aikake’s Information Criterion was selected. Contrast statements were used to test differences between baseline readings and subsequent time points, and between treatments at specific time points. Residuals were examined after
each model to verify normality and homogeneity of variances as well as to detect possible outliers and influential points. The level of significance used for all statistical analyses was $P \leq 0.05$.

### 3.3 RESULTS

#### 3.3.1 Clinical Parameters

There was no effect of treatment (Figure 3.1) or parity on the clinical score of milk. However, after controlling for DIM, there was an effect of time on the clinical score of milk throughout the trial ($P < 0.001$). There was an increase in clinical scores between the baseline values, and both 6 h ($1.9 \pm 0.3; P < 0.001$) and 12 h ($1.3 \pm 0.3; P < 0.001$) after endotoxin challenge.

When edema scores were analyzed, there was a trend for the interaction between treatment and time ($P = 0.06$; Figure 3.2; Table 3.3). Because of this level of significance, and from exploratory analysis, this relationship was further investigated. It was found that there were significant differences of edema scores between treatments. Specifically, the placebo group had an increased edema score as compared to the meloxicam group at 3 h ($0.5 \pm 0.2; P = 0.04$), 6 h ($0.7 \pm 0.3; P = 0.05$) and 24 h ($0.6 \pm 0.2; P = 0.01$) after endotoxin challenge. At these time intervals, it was found that there was an increase in edema score in control cows as compared with meloxicam cows. There was also a within treatment effect for both treatments. At 3 h, 6 h, 12 h and 24 h, there were increases in edema score as compared with baseline values for both treatment groups ($P < 0.05$). Parity or DIM did not have an effect on edema score.
There was no effect of treatment (Figure 3.3), parity or DIM on rectal temperatures, however there was an effect of time ($P < 0.001$). To be specific, there were increases in rectal temperature at time points 3 h ($0.4 \pm 0.1^\circ C$), 6 h ($2.0 \pm 0.1^\circ C$) and 12 h ($0.8 \pm 0.1^\circ C$) after endotoxin challenge, as compared to the baseline temperature values.

Two temperature loggers were lost during the experimental study, therefore 22 cows were available for statistical analyses. Temperature was analyzed on both a daily and hourly basis. For day analysis, when controlling for treatment, there was an effect of time on temperature ($P < 0.001$; Figure 3.4). Specifically, there was an increase of $0.2 \pm 0.1^\circ C$ ($P = 0.04$) in temperature on d 0 as compared to the baseline data. Parity or DIM did not have an effect. From exploratory analysis, on an hourly basis, it was apparent that there was little difference between treatments before and after d 0, therefore, only d 0 was included in this analysis. It was found that there was an interaction between hour and treatment ($P = 0.03$; Figure 3.5) on d 0. Parity or DIM did not have an effect on cow temperature.

There were no effects of treatment (Figure 3.6) parity or DIM on the SCS of the milk, however, there was a significant effect of time ($P < 0.001$). There were increases of SCS at 6 h ($2.1 \pm 0.4$; $P < 0.001$), 12 h ($3.7 \pm 0.6$; $P < 0.001$), and 24 h ($6.5 \pm 0.3$; $P < 0.001$) after endotoxin challenge, as compared with the baseline values.

For both blood parameters, 1 cow was removed from analyses because she had increased levels of both haptoglobin and SAA throughout the entire trial, including
baseline values, which may have indicated an underlying infection. This left 23 cows for analyses. In addition, 2 cows were removed from both haptoglobin and SAA analyses and 1 additional cow was removed from haptoglobin analysis because they had more than one sample with a protein level being excessively higher than the reference level. This left data from 20 and 21 cows for analysis, respectively. With haptoglobin levels, there was a trend for the interaction between treatment and time \((P = 0.10; \text{Figure } 3.7)\). There was a difference of haptoglobin levels between the baseline value, and 24 h \((0.21 \text{ g/L; } P < 0.001)\) after endotoxin challenge. Haptoglobin was not affected by parity or DIM. SAA levels were also not effected by treatment (Figure 3.8) or DIM, however, there was both a within and between group interaction between parity and time \((P = 0.02)\). For primiparous cows, there was an increase between baseline readings and 12 h \((0.3 \pm 0.1 \text{ mg/L; } P = 0.03)\) and 24 h \((0.7 \pm 0.1 \text{ mg/L; } P < 0.001)\) after endotoxin challenge. For multiparous cows, there was an increase between baseline readings and 12 h \((0.5 \pm 0.1 \text{ mg/L; } P = 0.0003)\) and 24 h \((1.0 \pm 0.1 \text{ mg/L; } P = 0.001)\) after endotoxin challenge. There were also differences between parity groups at baseline \((0.6 \pm 0.2 \text{ mg/L; } P = 0.007)\), 3 h \((0.7 \pm 0.2 \text{ mg/L; } P = 0.0002)\) and 6 h \((0.7 \pm 0.2 \text{ mg/L; } P = 0.001)\) after endotoxin challenge, where primiparous cows had increased SAA levels.

### 3.3.2 Pain Sensitivity

There was an interaction involving the pressure applied to the challenged quarter within and between treatment and time \((P = 0.02; \text{Figure } 3.9; \text{Table } 3.4)\). When comparing baseline to the subsequent time points, no differences were found within the treatment group. Within the placebo group, there were decreases at 3 h \((2.95 \pm 1.11 \text{ lbf; } P = 0.01)\), 6 h \((2.66 \pm 1.05 \text{ lbf; } P = 0.02)\) and 24 h \((2.96 \pm 1.03 \text{ lbf; } P = 0.007)\) after
endotoxin challenge, as compared with baseline values. Between treatments, there were differences at 3 h (3.61 ± 1.63 lbf; \( P = 0.04 \)) and 6 h (4.38 ± 1.49 lbf; \( P = 0.008 \)) after the endotoxin challenge, where more pressure could be placed on the challenged quarters of those animals that received meloxicam than the animals that received placebo. Parity or DIM did not have an effect on pressure.

The results were not comparable to the challenged quarter, in that treatment did not have an effect on the pressure applied to the quarter (Figure 3.10), nor did DIM. There was an effect of time (\( P = 0.01 \)) on the amount of pressure that could be put on the control quarter. There was a difference at 12 h (-1.57 ± 0.70 lbf; \( P = 0.03 \)) and 24 h (-1.78 ± 0.54 lbf; \( P = 0.002 \)) after endotoxin challenge as compared to the baseline values. There was also a parity effect, as it was observed that more pressure could be placed on the control quarters of multiparous cows (1.69 ± 0.50 lbf; \( P = 0.002 \)) throughout the study, as compared with primiparous cows.

**3.3.3 Rumination**

Two cows were removed from the rumination analyses as they were both identified as extreme outliers, with excessively low rumination values. This was most likely due to a recording error from the rumination tags. Rumination, as calculated by day, was not affected by treatment (Figure 3.11), DIM or time. Rumination was however affected by parity, as multiparous cows ruminated 140.2 ± 42.7 min (\( P = 0.004 \)) more throughout the trial than their primiparous counterparts. When analyzing rumination by 2-h periods, neither treatment (Figure 3.12) nor DIM had an effect. However, when
controlling for parity, there was a period by day interaction for rumination \((P < 0.001)\). Specifically, there were decreases in rumination directly after endotoxin challenge on d 0 as compared with the baseline data.

### 3.3.4 Lying Behaviour

One logger was damaged during the experimental study, therefore 23 cows were available for statistical analyses. For analyses by day, for all 4 parameters (lying time, lying time on the inoculated quarter, number of bouts and average bout duration), treatment did not have an effect (Figures 3.13 – 3.16). In fact, for lying time, lying time on the inoculated quarter and average bout duration, there were no effects of time, parity or DIM. For lying bouts, there was an effect of day \((P = 0.01)\), specifically, there were differences between baseline values, and d 1 \((1.9 \pm 0.8 \text{ bouts}; P = 0.03)\). Parity or DIM did not have an effect on lying bouts. When lying time was analyzed by hour, there were not differences in the diurnal pattern between treatments (Figure 3.17 and Figure 3.18) or between baseline and subsequent days.

### 3.3.5 Dry Matter Intake

One cow was removed from DMI analysis as she was identified as an extreme outlier, with excessively low DMI. This cow was diagnosed following the trial with a displaced abomasum, which may explain her sudden drop in DMI. When controlling for parity, there was a trend for a day effect \((P = 0.06)\), such that there was a decrease of 1.0 \(\pm 0.4 \text{ kg of DMI} (P = 0.006)\) on d 0 as compared to baseline (Figure 3.19; Table 3.5). However, this difference was driven only by the placebo animals. When d 0 was
compared to the baseline within each treatment, it was found that the placebo group had a decrease of $1.6 \pm 0.5$ kg of DMI ($P = 0.004$), while cows that received meloxicam did not show a significant decrease of DMI ($0.5 \pm 0.5$ kg; $P = 0.34$). Dry matter intake did not have an effect on DMI.

3.3.6 Milk Production

Due to a recording error with DairyCOMP 305®, there was not sufficient data to evaluate milk production, therefore no statistical analysis was completed.

3.4 DISCUSSION

The present study was conducted to evaluate the efficacy of meloxicam as a supportive NSAID therapy for cows who are experiencing cases of severe clinical mastitis. Previous research in cases of naturally-occurring mild and moderate clinical mastitis concluded that the administration of meloxicam after diagnosis of mastitis improved SCC levels in affected cows, and reduced the risk of culling from the herd (McDougall et al., 2009). This NSAID therapy has also been shown to decrease inflammatory and clinical condition, improve milk yield and restore pain-threshold responses in cows with both naturally-occurring and experimentally-induced mastitis (Friton et al., 2002; Milne et al., 2004; Friton and Banting, 2005). From these findings, it was hypothesized that the administration of this drug would mitigate the adverse effects of an experimentally-induced case of mastitis, and improve physiological, behavioural and performance responses in the cow. Many objective parameters, specifically regarding
pain sensitivity and behavioural responses of the cow, were used to determine the efficacy of this drug with cases of clinical mastitis.

Clinical parameters are markers of health, and give researchers insight into what physiological changes are happening in the body when an animal is in a state of distress. This is especially true with mastitis-related studies, as visual observation and laboratory analyses of both blood and milk can help monitor the progression of infection, and determine whether supportive therapies can help improve the physiological state of the animal.

Clinical characteristics of milk are generally the first signs for producers to diagnose clinical mastitis, as well as one of the key signs to indicate that a clinical infection has passed. It is also a good method to determine if both antibiotic and supportive treatments are helping to mitigate the signs of mastitis. In the present study, there was no difference between treatments on the clinical scores of milk throughout the study, which is consistent with previous endotoxin-challenge studies (Zimov et al., 2011). This could potentially be due to the short-acting effects of the endotoxin infusion, where the benefits of the NSAID could not be exhibited in this period of time. In addition, the current study only used the LPS endotoxin of *E. coli*, and not the whole cell, so the efficacy of the drug to alleviate clinical signs of the milk could be altered with a naturally-occurring case of clinical mastitis.
Edema scores provide a visual indication of pain due to clinical mastitis. For the current study, at 3, 6 and 24 h after endotoxin-challenge, cows that were administered meloxicam appeared to have a lower, and therefore better edema score than their placebo counterparts. This is similar to another challenge study, which observed the use of meloxicam with acute cases of clinical mastitis (Friton et al., 2002). In their study, the severity of inflammation for affected animals was decreased 2 d following the identification of a case of mastitis with the use of meloxicam. This differs from a previous study, which looked at the effects of meloxicam on naturally-occurring cases of mastitis, where there was no treatment effect on edema score (McDougall et al., 2009). A potential explanation for this discrepancy is due to the differences in study design between the work done by McDougall et al. (2009) and the current study. For the present study, animals experienced signs from severe cases of mastitis, while cows from the previous study experienced signs from mild or moderate cases of mastitis. In addition, the observations of edema scores were taken at identical time points for each cow for the present study, while edema scores in the previous study were taken after the diagnosis of mastitis, not the onset, thus varying between cows. These variations of signs and visual observations in the previous study could be masking the true effect that meloxicam could have on the edema of infected quarters.

It was not hypothesized that temperature by both day, as found with the continual measures of core body temperatures, and by time after endotoxin challenge, as found using rectal temperatures, would be affected by treatment. This is due to the fact that NSAIDs that predominately inhibit COX-1 enzymes are selected more on their anti-
pyretic qualities than COX-2 inhibiting NSAIDs, such as meloxicam (Vangroenweghe et al., 2005). Therefore, it was not surprising that there were no significant differences in daily temperature by treatment. This result was consistent with a previous study that was conducted using meloxicam during an experimentally-induced acute mastitis challenge (Banting et al., 2000). This could potentially be explained by the localized action on areas of inflammation by COX-2 inhibiting NSAIDs, as compared to the more systemic action of NSAIDs that predominately inhibit COX-1 enzymes (Hawkey, 2001). However, there was an unexpected effect of treatment by hour, where at 3 - 5 h after endotoxin challenge, meloxicam cows had significantly lower body temperatures than their placebo counterparts. Banting et al. (2000) did take body temperature measurements at 2-h intervals, but did not report any significant results from these findings. Future research should be conducted to explore the short-acting effects of meloxicam on the body temperature of an animal experiencing experimentally-induced clinical mastitis.

Somatic Cell Counts are the most predictive measures of udder health of a mastitic quarter (Harmon, 2001). Monitoring of this variable can help to identify problem animals in the herd, and help with management decisions for the producer. In previous challenge studies, it was found that both flunixin meglumine (Anderson and Hunt, 1989; Zimov et al., 2011) and carprofen (Vangroenweghe et al., 2005) did not decrease the SCC in treated cows. However, when monitoring SCC during naturally-occurring mastitis, it was found that meloxicam treatment appeared to have a beneficial effect on SCC, as these animals had lower SCC after NSAID administration (McDougall et al., 2009), which at the time, was a novel finding. In the current study, treatment did not have an
effect on SCC. After statistical analysis, it was found that SCC, along with the acute phase proteins monitored in the current study, were not monitored for a long enough duration to reach peak levels, which was not expected when developing the study protocol. Therefore it is difficult to determine whether there would have been a beneficial effect of meloxicam on SCC, as seen in previous research. In future research physiological parameters should be monitored for an increased period of time, to get a better understanding of the full efficacy that the NSAID provides.

Acute phase proteins are effective physiologic indicators of disease in dairy cattle, especially with acute disorders, such as with cases of coliform mastitis (Erskine et al., 2003). When a case of acute coliform mastitis occurs, one of the most common practices is to administer NSAIDs as a supportive therapy, which will help mitigate the physiological effects of this systemic shock (Erskine et al., 2003). However, results from the present study indicate that the use of meloxicam did not have a beneficial effect on the acute phase proteins measured, haptoglobin and serum amyloid A. This is consistent with a study looking at the use of meloxicam in pigs after an endotoxin challenge, where levels of acute phase proteins, including haptoglobin, were not improved in animals that received meloxicam, as compared to the animals that received placebo (Friton et al., 2006). This result is similar to studies which observed the effects of flunixin meglumine on endotoxin challenge, where milk levels of serum amyloid A did not differ between treatments (Zimov et al., 2011). However, this outcome differs in other research, which used carprofen as an NSAID during cases of experimentally-induced mastitis (Vangroenweghe et al., 2005). In this study, levels of serum albumin were decreased with
the use of carprofen treatment. This alternate outcome could be explained due to the type of acute phase protein that was tested, as serum albumin is a negative phase protein, and haptoglobin and serum amyloid A are positive phase proteins, which have different responses to inflammation (Murata et al., 2004).

To the knowledge of the authors, there has been no previous work conducted using the pressure algometer to quantify the efficacy of NSAIDs on pain during cases of clinical mastitis. However, this tool has been used in previous work, where the algometer was used to determine the efficacy of meloxicam as an NSAID at the time of dehorning of calves (Heinrich et al., 2010). In their study, it was found that following dehorning, more pressure could be applied to the area of dehorning for the calves that received meloxicam, as compared to the calves that received a placebo treatment. It was also found that the control calves had approximately twice the increase in sensitivity after dehorning, as compared to the meloxicam calves. These results are quite similar to our mastitis model, as there were significant differences between treatments after the endotoxin-challenge. Specifically, control cows had an increased pain sensitivity after the inoculation, while the cows that received meloxicam appeared to have a decreased pain sensitivity after inoculation.

The algometer readings were always conducted first on the control quarter, followed directly by the challenged quarter. This was done to try and minimize the chance of increased sensitivity readings in the control quarter, if the animal was experiencing pain in her challenged quarter. Although this method decreased the
probability of pain sensitivity in the control quarter being inaccurately high, there was also a risk of the animal being desensitized to the pressure placed on her challenged quarter, because she was already exposed to the algometer on her control quarter. However, since this was such an acute inflammation, it was hypothesized that the animals would not be desensitized in the challenge quarter, and the pain sensitivity readings would be accurate.

In previous endotoxin challenge studies examining pain management strategies, the pain that the cow experiences due to a case of mastitis has been studied in various ways. In a study that observed the efficacy of ketoprofen as an NSAID therapy, pain was monitored through palpation of the udder, and pain was scored based on a visual analog scale (Banting et al., 2008). It was found that animals that received ketoprofen, which was administered 2 h after endotoxin challenge, had a peak pain score at 4 h after challenge, which rapidly declined thereafter. In contrast, the control animals had a peak pain score at 6 h after endotoxin challenge, and pain score decreased more gradually, much like what occurred with our control animals. Other researchers, who observed the effects of carprofen on experimentally-induced mastitis, identified pain by calculating the inflammatory response in the quarter, which was done by palpation of the udder (Lohuis et al., 1991). It was found that animals that received carprofen had decreased swelling, and indirectly less pain, by approximately 5-13 h after NSAID injection, as compared to their control counterparts. A comparable study also looked at the use of meloxicam as a pain management therapy for experimentally-induced mastitis (Banting et al., 2000). After taking pain scores of the affected quarter, it was found that there was more of a
rapid decrease of the animal’s pain score after endotoxin challenge in the animals that received meloxicam, as compared to the control animals, which generally coincides with our findings. Finally, a previous study observed the use of meloxicam as an NSAID for clinical cases of mastitis, where mechanical stimulus was applied on the hind legs of the affected animals to determine their pain threshold responses (Milne et al., 2004). It was found that animals treated with meloxicam returned to their normal pain thresholds at a much quicker rate than their untreated counterparts, which suggests that the pain that they are experiencing due to mastitis is being alleviated. For all of these studies, the subjective procedures to determine pain may be impeding on what the cow is actually experiencing, but they all generally follow the same trend as with the present research, that the use of NSAIDs does help to mitigate the pain that a cow experiences during a case of clinical mastitis.

Rumination has been shown to be a good behavioural monitoring tool in both research and in industry, as decreases in daily rumination time can be indicative of disease, particularly with cases of mastitis (Siivonen et al., 2011). Specifically, with experimentally-induced cases of mastitis, cows appear to exhibit lower rumination on the day of endotoxin challenge, as compared to the day before and after endotoxin challenge (Fogsgaard et al., In Press). In previous studies that have monitored the use of NSAIDs during endotoxin induced mastitis, the primary methods of monitoring rumination are subjective, observational methods, in the form of rumination sounds and cud chewing. The automated rumination monitoring device used in the current study is an objective
method of determining changes in rumination patterns, and can accurately detect the
decreases in rumination directly after endotoxin challenge.

In studies that observed the effects of flunixin meglumine (Lohuis, Van Leeuwen,
Verheijden, Brand and Van Miert, 1989b; Wagner and Apley, 2004; Zimov et al., 2011),
flurbiprofen (Lohuis, Van Leeuwen, Verheijden, Brand and Van Miert, 1989b), carprofen
(Vangroenweghe et al., 2005) and ketoprofen (Banting et al., 2008) on rumen behaviour
after a mastitis challenge, all treatments had beneficial impacts on rumen sounds, cud
chewing, and rumen motility. With animals that were treated with meloxicam (Banting et
al., 2000), it was also found that there was a much quicker recovery of rumination
activity in the treated animals as compared with the control animals, which contradicts
results in the present study. This discrepancy between the present study and previous
work could be attributed to the method of monitoring rumen activity. For the present
research, rumination was calculated with rumination HR-Tags, which give an objective
and continual reading of rumination throughout the entire trial, while visual observations
only monitor a short period of a cow’s daily rumination activity, and do not entirely
represent what the cow is experiencing during an experimental trial.

Recently, lying and standing behaviour have been investigated in dairy cattle
research as it has the ability to detect discomfort in the animal, particularly from disease,
through deviations from their normal behaviour (Robert et al., 2009). For behaviour
during clinical mastitis, specifically with endotoxin induced mastitis, cows tend to
experience changes in lying behaviour after the time of endotoxin challenge (Siivonen et
al., 2011). There are many commercial technologies that are used as herd management tools, and these devices monitor the cow’s behaviour to detect both estrus behaviour, and the incidence of disease. It has been found in previous research that after cases of naturally-occurring mastitis, cows tend to express laterality of their left side, while there was an increased probability of infection on their right side (Kikkers et al., 2006). This result indicates that cows tend to avoid putting weight on their infected quarters by lying on the opposite side, which is an obvious sign of discomfort.

In previous work with endotoxin challenges, it was found that lying behaviour in cows was not affected by the NSAID, flunixin meglumine (Zimov et al., 2011), which agrees with our data, both on a daily and hourly basis. This could be a result of the short-term activity of the endotoxin, as compared with a long-term infection from a naturally-occurring case of mastitis. As with the lying time, and the time spent on the inoculated quarter, there is a chance that the cows develop this adverse behaviour to lying over the progression of this infection, which would not have been exhibited with an endotoxin challenge. It would be beneficial to observe lying and standing behaviour before and after naturally-occurring cases of mastitis, to see if the use of NSAIDs could help mitigate the adverse behaviour that the animals are experiencing.

Productivity measures are indicative of how a cow reacts during a time of distress, in both research and commercial settings. Specifically, a decrease in DMI is an indicator of poor health, welfare and general well-being of a cow (Weary et al., 2006). With systemic mastitis, which the animals experienced in the present study, it has been shown
that cows can experience a decrease in feed intake of approximately 6.7 kg from the onset of the disease to the time of diagnosis (Bareille et al., 2003). In previous endotoxin challenges, it was found that the use of flunixin meglumine as an NSAID did not have a significant effect on improving DMI (Zimov et al., 2011). However, in previous studies that observed the use of meloxicam in calves both with dehorning (Heinrich et al., 2010) and after the onset of diarrhea (Todd et al., 2010), animals that received meloxicam had an increased feed intake as compared to their control counterparts. This beneficial impact on DMI with meloxicam treatment, as shown in the current study, could be due to the long half-life of the drug. Meloxicam has an active half-life of 17.5 h which is much longer than the half-life of other NSAIDs approved for use in lactating cattle, such as ketoprofen and flunixin meglumine. Because of the longer lasting effects of meloxicam, these cows would be able to adapt much better to an acute case of mastitis, and maintain a more consistent DMI.

3.5 CONCLUSIONS

In conclusion, the use of meloxicam as a NSAID therapy for cases of endotoxin-induced mastitis had beneficial effects on pain sensitivity, edema score of the udder and DMI following the endotoxin challenge. It did not have an effect on physiological indicators, such as SCC and acute phase proteins; however, increased monitoring of these parameters on the days following the endotoxin challenge may have demonstrated an efficacious effect of this drug, as shown in previous work. Behavioural monitoring should be conducted with naturally-occurring cases of mastitis to determine if meloxicam can mitigate the adverse behaviours caused by the progression of an intramammary infection.
In summary, with this work, and previous research focusing on meloxicam use with cases of clinical mastitis, it can be concluded that meloxicam can help to mitigate the signs of pain, discomfort and stress that are associated with mastitis.
**Table 3.1.** Five-point clinical mastitis classification scale to determine the clinical score of milk in quarters inoculated with *E. coli* lipopolysaccharide. Chart is adapted by Wenz et al. (2006) and Zimov et al. (2011).

<table>
<thead>
<tr>
<th>Clinical Mastitis Score</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal mammary gland and normal milk</td>
</tr>
<tr>
<td>1 CMT Positive</td>
<td>Normal mammary gland and normal milk, but positive for California Mastitis Test</td>
</tr>
<tr>
<td>2 Mild</td>
<td>Normal mammary gland and abnormal milk (clots, clumps, changes in milk color)</td>
</tr>
<tr>
<td>3 Moderate</td>
<td>Abnormal mammary gland and abnormal milk (clots, clumps, changes in milk color)</td>
</tr>
<tr>
<td>4 Severe</td>
<td>Swollen mammary gland, abnormal milk and systemic signs (such as elevated rectal temperature, depression, dullness, etc.)</td>
</tr>
</tbody>
</table>
Table 3.2. Five-point edema classification scale to determine the edema score of both control quarters and quarters inoculated with *E. coli* lipopolysaccharide. Chart is adapted by Nestor, Jr. et al. (1988).

<table>
<thead>
<tr>
<th>Edema Score</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No edema</td>
</tr>
<tr>
<td>2</td>
<td>Slight edema, edema in the base of the udder and around the teats</td>
</tr>
<tr>
<td>3</td>
<td>Moderate edema, swelling covering the lower half of the udder</td>
</tr>
<tr>
<td>4</td>
<td>Severe edema, almost the entire udder edematous</td>
</tr>
<tr>
<td>5</td>
<td>Very severe edema, the entire udder edematous and some edema on the brisket, thighs or both</td>
</tr>
</tbody>
</table>
**Table 3.3.** Least square mean (±SE) edema scores in challenged quarters of 24 Holstein dairy cows treated with either meloxicam (n=12) or placebo (n=12) before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.

<table>
<thead>
<tr>
<th>Time Interval Relative to Endotoxin Challenge (h)</th>
<th>Meloxicam Edema Score</th>
<th>Placebo Edema Score</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.0 ± 0.03</td>
<td>1.0 ± 0.03</td>
<td>0.32</td>
</tr>
<tr>
<td>3</td>
<td>2.2 ± 0.2***</td>
<td>2.8 ± 0.2***</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>2.8 ± 0.2***</td>
<td>3.5 ± 0.2***</td>
<td>0.05</td>
</tr>
<tr>
<td>12</td>
<td>2.6 ± 0.2***</td>
<td>2.9 ± 0.2***</td>
<td>0.30</td>
</tr>
<tr>
<td>24</td>
<td>1.4 ± 0.2</td>
<td>2.0 ± 0.2***</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Significant differences within treatment groups as compared with baseline values are denoted by symbols (*** = $P \leq 0.001$).
Table 3.4. Least square mean (±SE) pressure (lbf) in challenged quarters of 24 Holstein dairy cows treated with either meloxicam (n=12) or placebo (n=12) before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.

<table>
<thead>
<tr>
<th>Time Interval Relative to Endotoxin Challenge (h)</th>
<th>Meloxicam Pressure (lbf)</th>
<th>Placebo Pressure (lbf)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>13.87 ± 0.83</td>
<td>14.17 ± 0.83</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>14.83 ± 1.32</td>
<td>11.22 ± 1.28**</td>
<td>0.04</td>
</tr>
<tr>
<td>6</td>
<td>15.88 ± 1.21</td>
<td>11.50 ± 1.21**</td>
<td>0.008</td>
</tr>
<tr>
<td>12</td>
<td>13.07 ± 1.35</td>
<td>12.73 ± 1.35</td>
<td>0.85</td>
</tr>
<tr>
<td>24</td>
<td>13.13 ± 1.19</td>
<td>11.20 ± 1.19**</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Significant differences within treatment groups as compared with baseline values are denoted by symbols (** = *P* ≤ 0.01).
Table 3.5. Least square mean (±SE) dry matter intake (kg) of 24 Holstein dairy cows treated with either meloxicam (n=12) or placebo (n=12) before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.

<table>
<thead>
<tr>
<th>Time Interval Relative to Endotoxin Challenge (h)</th>
<th>Meloxicam DMI (kg)</th>
<th>Placebo DMI (kg)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>20.5 ± 0.7</td>
<td>20.8 ± 0.7</td>
<td>0.75</td>
</tr>
<tr>
<td>0</td>
<td>20.0 ± 0.7</td>
<td>19.2 ± 0.7**</td>
<td>0.41</td>
</tr>
<tr>
<td>1</td>
<td>20.6 ± 0.7</td>
<td>20.3 ± 0.7</td>
<td>0.77</td>
</tr>
<tr>
<td>2</td>
<td>20.7 ± 0.7</td>
<td>19.8 ± 0.7</td>
<td>0.35</td>
</tr>
<tr>
<td>3</td>
<td>20.0 ± 0.7</td>
<td>20.0 ± 0.7</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Significant differences within treatment groups as compared with baseline values are denoted by symbols (** = P ≤ 0.01).
Figure 3.1. Least square mean (±SE) clinical scores in challenged quarters of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.
Figure 3.2. Least square mean (±SE) edema scores in challenged quarters of 24 Holstein dairy cows treated with either meloxicam (n=12; ▐) or placebo (n=12; □) before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.

Significant differences between treatment groups are denoted by symbols (* = P ≤ 0.05; ** = P ≤ 0.01).
Figure 3.3. Least square mean (±SE) rectal temperature (°C) of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.
Figure 3.4. Least square mean (±SE) core body temperature (°C) of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) before (baseline, average of 2 d prior to inoculation), on the day of (d 0) intramammary inoculation of *E. coli* lipopolysaccharide, and the days following intramammary inoculation.
Figure 3.5. Least square means (±SE) core body temperature (°C) of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) on the day of intramammary inoculation of *E. coli* lipopolysaccharide. Experimental days were adjusted to start at 0800 h, time when all cows were inoculated.

Standard errors are too small to be visible.

Significant differences between treatment groups are denoted by symbols (** = *P* ≤ 0.01).
Figure 3.6. Least square mean (±SE) somatic cell scores in challenged quarters of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.
Figure 3.7. Least square mean (±SE) haptoglobin levels (g/L) of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.
Figure 3.8. Least square mean (±SE) serum amyloid A levels (mg/L) of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.
Figure 3.9. Least square mean (±SE) pressure (lbf) in challenged quarters of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.

Significant differences between treatment groups are denoted by symbols (* = P ≤ 0.05; ** = P ≤ 0.01).
Figure 3.10. Least square mean (±SE) pressure (lbf) in control quarters of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) before (baseline, average of 3 d and directly before inoculation) and 3 to 24 h after the intramammary inoculation of *E. coli* lipopolysaccharide.
Figure 3.11. Least square mean (±SE) rumination time (min) of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) before (baseline, average of 2 d prior to inoculation), on the day of (d 0) intramammary inoculation of *E. coli* lipopolysaccharide, and the days following intramammary inoculation.
**Figure 3.12.** Least square means (±SE) rumination time (min/2 h) of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) on the day of intramammary inoculation of *E. coli* lipopolysaccharide. Experimental days were adjusted to start at 0800 h, time when all cows were inoculated.

[] denotes feeding time and ↓ denotes milking time.
Figure 3.13. Least square mean (±SE) lying time (min) of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) before (baseline, average of 2 d prior to inoculation), on the day of (d 0) intramammary inoculation of *E. coli* lipopolysaccharide, and the day following intramammary inoculation.
Figure 3.14. Least square mean (±SE) lying time on inoculated quarter (min) of 24 Holstein dairy cows treated with either meloxicam (n=12; □) or placebo (n=12; □) before (baseline, average of 2 d prior to inoculation), on the day of (d 0) intramammary inoculation of *E. coli* lipopolysaccharide, and the days following intramammary inoculation.
Figure 3.15. Least square mean (±SE) lying bouts of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) before (baseline, average of 2 d prior to inoculation), on the day of (d 0) intramammary inoculation of *E. coli* lipopolysaccharide, and the days following intramammary inoculation.
Figure 3.16. Least square mean (±SE) lying bout duration (min) of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) before (baseline, average of 2 d prior to inoculation), on the day of (d 0) intramammary inoculation of E. coli lipopolysaccharide, and the days following intramammary inoculation.
Figure 3.17. Least square means (±SE) lying time (min) of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) on the day of intramammary inoculation of *E. coli* lipopolysaccharide. Experimental days were adjusted to start at 0800 h, time when all cows were inoculated. ☀ denotes feeding time and ⇢ denotes milking time.
Figure 3.18. Least square means (±SE) lying time on inoculated quarter (min) of 24 Holstein dairy cows treated with either meloxicam (n=12; ■) or placebo (n=12; □) on the day of intramammary inoculation of *E. coli* lipopolysaccharide. Experimental days were adjusted to start at 0800 h, time when all cows were inoculated.

❖ denotes feeding time and ▼ denotes milking time.
Figure 3.19. Least square mean (±SE) dry matter intake (kg) of 24 Holstein dairy cows treated with either meloxicam (n=12; □) or placebo (n=12; ○) before (baseline, average of 4 d prior to inoculation), on the day of (d 0) intramammary inoculation of *E. coli* lipopolysaccharide, and the days following intramammary inoculation.

Significant differences between days for the placebo treatment group are denoted by symbols (** = P ≤ 0.01).
CHAPTER 4: GENERAL SUMMARY AND CONCLUSIONS

4.1 CONCLUSIONS

The first objective of this study was to investigate the use of both a pressure algometer and rumination tags as methods to objectively assess changes in response to pain and discomfort. The second objective used these tools and other methods to evaluate the efficacy of pain management therapy on the physiological and behavioural impacts associated with pain in dairy cattle with experimentally-induced endotoxin clinical mastitis.

To the knowledge of the authors, there has been no previous work using the algometers to monitor the effects of clinical mastitis on pain threshold of cows. It was found that the algometer was able to detect changes in pain sensitivity after the endotoxin challenge. Specifically, there was a significant decrease in the amount of pressure placed on the challenged quarter as compared with baseline readings 3 and 6 hours after inoculation. Automated rumination monitoring tags have been evaluated in the literature, but to the knowledge of the authors, have not been used to monitor systemic discomfort with clinical cases of mastitis. It was found that rumination decreased in the first 8 h after endotoxin challenge, as compared with the same time on the day before endotoxin challenge. From these results, it can be concluded that both the algometer, and rumination tags can successfully be used in a research setting to quantify the change in response to pain and systemic discomfort in dairy cattle.
The second objective used 24 animals to determine the efficacy of meloxicam as a pain management treatment for cows with cases of experimentally-induced clinical mastitis. Animals were administered either meloxicam (n=12) or placebo (n=12) treatments, and a number of variables were recorded to measure physiological and behavioural changes after endotoxin challenge. It was found that there was a beneficial effect of meloxicam on edema scores, pain sensitivity, core body temperature and DMI of the day of endotoxin challenge. Clinical score, SCC, rectal temperature, blood parameters, rumination and lying behaviour were not affected by treatment. These results both coincide and contradict other studies which observed the efficacy of meloxicam with clinical cases of mastitis. Improved edema scores and restored pain-threshold responses were found in previous experimentally-induced mastitis studies, which corresponded with the current study (Friton et al., 2002; Milne et al., 2004). In contrast, in a previous naturally-occurring study, it was found that there was a decreased SCC in animals that were treated with meloxicam, as compared to their placebo counterparts, which differs from the current study (McDougall et al., 2009). However, the animals in the previously mentioned study were monitored for an extensive period of time after the onset of clinical infection, while the animals in the current study were only monitored for 24 hours following the endotoxin challenge. Overall, there was a beneficial effect from the administration of meloxicam to cows with cases of experimentally-induced clinical mastitis.
4.2 PROJECT LIMITATIONS

There were various design elements that may have limited some components of the study. As this was an endotoxin-induced experimental trial, the systemic effects of a case of *E. coli* were not fully exhibited. In naturally-occurring cases, or even an experimental trial with a live pathogen, treatment occurs at the onset of clinical signs of infection, while in the current study, pain mediation treatment was performed directly after the endotoxin challenge. In addition, due to the short-term duration of an endotoxin infection, the systemic effects of a true *E. coli* infection are muted. Because of this, the full effects of treating the pain of these animals may be underestimated.

The physiological indicators of inflammation were not monitored for an adequate period of time to determine the full significance of the drug on these parameters. These variables included haptoglobin, SAA and SCC. Unlike the other variables that peaked and declined within the 24 h of observation, these parameters had not yet peaked at the end of the 24-h period. In reality, these parameters most likely peaked in the days following the endotoxin challenge. Because of this, the efficacy of meloxicam on these parameters was misrepresented. For example, SCC was not found to be effected in the current study, however, in a previous study which looked at naturally-occurring cases of mastitis, meloxicam-treated animals had significantly lower levels of SCC than their placebo treated counterparts during the days following the onset of infection (McDougall et al., 2009). If these parameters were monitored for a longer period of time in the current study, perhaps similar results would have been obtained.
There was an error with recording equipment in the study, which was not recognized until after the trial was complete. As a result of this error, daily milk production data were not obtained. Not only is this variable important to monitor in research, but it is one of the main concerns for producers. When conducting research, it is important to include relevant parameters if the research is to be applied on-farm. This would have been an important variable to monitor.

4.3 FUTURE RESEARCH

As the current project not only evaluated novel research tools, but also evaluated a relatively novel product in the Canadian dairy industry, it goes without saying that there are many questions that remain unanswered. For the rumination tags specifically, this equipment is being distributed commercially in the dairy industry, and an on-farm assessment of its use would be beneficial for a better understanding of the tag’s health detection potential. Further research should be conducted on commercial farms to monitor the changes in both rumination and activity in relation to stress or disease.

As previously stated, there have been a variety of studies worldwide that have evaluated the efficacy of meloxicam as a pain management therapy for cases of both mild and moderate naturally-occurring cases of mastitis (McDougall et al., 2009) and severe and systemic experimentally-induced cases of mastitis (Banting et al., 2000; Friton et al., 2002; Milne et al., 2004). These studies have been successful in identifying the beneficial effects of meloxicam, however, further research should be completed looking at severe and systemic cases of naturally-occurring mastitis. Future studies should be conducted on
commercial facilities to monitor the efficacy of this drug with different management practices. Since producers would be more likely to use meloxicam as a pain management therapy for severe cases of mastitis, as opposed to mild or moderate cases, it is important to investigate both the short-term and long-term effects of meloxicam on these severe naturally-occurring cases.

From the success of the use of meloxicam in clinical cases of mastitis, there is a potential to use this product in lactating dairy cows with other diseases, such as transition diseases, lameness or dystocia. Currently in the EU, this drug has been approved for both acute cases of clinical mastitis, and acute respiratory infections in lactating cattle, but there is a potential for its use with other diseases. This research should be conducted in both a controlled setting, such as with research herds, to monitor a variety of physiological parameters, and on-farm, to monitor the efficacy of this drug with different management practices.

4.4 **IMPLICATIONS**

The current study has found that both the algometer and rumination tags were able to quantitatively assess changes in response to pain and systemic discomfort in cows after an experimentally-induced endotoxin challenge. The algometer was able to detect increases in pain sensitivity approximately 3-6 hours after endotoxin challenge, which is when the majority of physiological parameters are most affected by the challenge. This tool has the potential to detect pain in dairy cattle research with a variety of diseases and management procedures. The rumination tags were able to detect significant decreases in
rumination immediately after endotoxin challenge, as compared to the days before challenge. This monitoring device has potential to be used in commercial herds to detect systemic discomfort associated with disease. When considering the use of meloxicam as a pain management therapy, there were many significant benefits with the use of this NSAID after endotoxin challenge. Specifically, there were beneficial effects on pain sensitivity, edema score of the udder and DMI following the endotoxin challenge. From the results from the current study, and from previous studies on the beneficial effects of meloxicam, it is evident that there is a need for this product to be available in Canada for producers, to help mitigate the pain and discomfort experienced during cases of clinical mastitis.
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APPENDICES
APPENDIX 1: SOP FOR FORCE TEN™ FDX 50 ALGOMETER

• A Force Ten™ FDX 50 Algometer (Wagner Instruments, Greenwich, CT, USA), with a pain threshold capacity of 50 lbs. should be used (see figure below)
• The algometer should be set to record pain threshold in lbs. as the unit of measurement, and should be programmed to record peak readings of each pain tolerance test. More detailed information on how to program the algometer to fit these settings can be found in the Wagner Operations Manual for the Force Ten™ FDX Compact Digital Force Gage
• The quarter detected with clinical mastitis, as well as the ipsilateral quarter not affected with mastitis will be evaluated to determine the level of pain in both the affected and non-affected glad of each cow enrolled in the study
• The Force Ten™ Algometer will apply digital pressure on the point approximately 15 cm ventral to the udder attachment (as shown in the figures below)
• The ipsilateral quarter will be tested first, followed directly by the infected quarter
• The operator of the algometer will apply even pressure on each quarter, and will continue to apply increased pressure to the area until:
  o They can no longer apply pressure OR
  o Have an adverse reaction from the cow (e.g. tail swishing, kicking, shifting weight)
• As the algometer is set to record the peak reading of each test, the value on the algometer once you have removed it from the quarter represents the maximum amount of pressure that you have put on the affected area.
APPENDIX 2: SOP FOR VEMCO MINILOG 8 TEMPERATURE DATA LOGGER

Programming the Temperature Loggers:

- Open Minilog software on computer.
- Put logger onto the read out unit and connect it to PC.
- Choose “Initialize New Study” (green arrow) in menu. This will recognize the logger and open a window which shows you details of the last study. If you did not download the last study, Minilog will warn you at this point.
- Name your study in “Study ID”.
- Choose “Delayed Start” to define the date and time your study starts.
- Choose a 1 minute interval in which the temperatures should be taken.
- Press “OK” after finishing these steps.
- The logger will get initialized. The logger will flash every 5 sec, if it is initialized successfully.

Preparing Logger and CIDR:

- Soak the CIDRs in 70 or 99% isopropyl alcohol to try and extract as much progesterone from the device as possible.
- Cut the end of the CIDR with a saw.
- Move the rubber layer in direction of the end of the T.
- Cut the plastic part with a saw.
- Attach the logger with the probe pointing to the T to the CIDR and move the rubber layer over the logger. This will fix the logger onto the modified CIDR T.
- Take a surgical glove and bring the thumb, ring and little finger into the glove, leaving the index and middle finger out.
- Place the two ends of the T of the CIDR into the index and middle fingers of the glove.
- Wrap the excess material of the glove around the CIDR, and secure at the bottom, and directly under the T with medical tape.
- Tie fishing line to the logger.
- Put the prepared logger into a bucket of warm water with iodine.
- Put the CIDR applicator into the same bucket.
Insert Logger Into a Cow:

- Remove the plastic packaging around a cardboard speculum, and insert the CIDR into the end. Insert the logger end into the speculum first so it is the first part to enter the cow. Bend the T part of the CIDR backwards to it fits.
- Insert the speculum/logger into the vagina
  - Hold the speculum only on the ring.
  - The second person should take the vulva on 1 side for easier insertion.
- The person who is inserting the logger should hold the vulva on the other side with one hand and insert the speculum into the vagina with the other hand. The speculum should not touch any skin. While inserting the speculum first has to point to the cows back (dorsal). After a barrier is reached the direction has to be changed to ventral. A little rotation during the insertion will make it easier.
- After the speculum is completely inserted the logger should be pushed through the speculum with the CIDR applicator.
- Gently remove both instruments from the vagina.
- Tie the fishing line to the ear tag.

Check the Logger 2 to 3 Times a Day While the Study is Running.

To Remove the Logger:

Pull gently on the fishing line. If this rips restrain and clean the cow as described above, wear an insemination glove, disinfect it with diluted iodine and enter the vaginal cavity to remove the logger. Clean the logger with warm water and soap.

Read Out the Logger:

- Follow the steps described above to enter the Minilog software.
- Choose “Read Data From Logger” (Red arrow)
- Choose “View Data”
- Choose “Create ASCII Subject” in Edit
- Save the text file
- You can import these text files into Excel