Pain Mitigation in Cattle Following Soft Tissue Surgery

A Thesis Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Amber Diane Futrell

May 2019

Dedication

I dedicate this work to my husband who moved across the state while I worked on this degree, and my family who have supported me through all of my journey.

Acknowledgements

I have been blessed with so many opportunities to further my education and thank God for his guidance and wisdom for keeping me on this path. Although I have no idea what I am called to do or what the future may hold, my trust lies on Christ alone.

This whole journey, from college to vet school to this master's degree and back home, would not have been possible without the support of my family. My husband, bless him, had no ideas what he was getting into when we started dating. I did tell him that school came first, but I'm not sure he realized it meant for vet school and a master's degree. He has been very support on this whole journey, left the country and moved to Knoxville for a year, and constantly reminded me to get my work done. My mom has been a rock in these trying times, encouraging me to stick it out, but also calling attention to my procrastination when I made things harder on myself. My dad was there for moral support through it all and was always good for a joke to break some tension. My brother and sisters were always there to remind me of my goals.

I would like to thank my mentor, Dr. Marc Caldwell, for his wisdom and support. This was not something I ever saw myself doing but he saw my potential and took a chance on me. I'd like to thank my committee members, Drs. Strickland, Anderson, Krawczel. They have been the most understanding from tailoring my degree to my needs to last minute meetings.

I can’t thank Drs. Andi Lear and Meghan Graves enough for letting me crash in their office. I had no office home and they gave me a place to land and kept me motivated and had some great talks.

Thank you to the UTCVM Large Animal Clinical Science Department and the UTAI Animal Science Department for allowing me to try and navigate this new process and blaze a trail for those to follow me.

I’d like to thank all of the wonderful graduate students and veterinary students that help with this project. It was a massive undertaking and those students made it all possible. I have grown close to many of them and hope to continue to do so in the future.

Finally, thank you to Boehringer Ingelheim for your financial support. You have given me an opportunity to explore research in a way that not many veterinarians are able to and have opened many doors through this experience that would not have happened without this project.

Abstract

Pain mitigation for surgical procedures is a topic of concern for the public, producers, and veterinarians. The objective of this study was to determine the efficacy of meloxicam for pain mitigation in adult lactating dairy cattle following a right-side laparotomy with omentopexy. Twenty-four dairy cattle (mean age: 2.51 +/- 0.54 years) were enrolled. Cattle were assigned blocks based on parity, days in milk, milk yield, and pregnancy status, and randomly allocated to groups Meloxicam (MEL) or placebo treated control (CON). The study had two phases; sham (day 0-14) and surgery (day 15-28). On day 0, cattle were prepared for surgery. Injectable meloxicam (MEL) or saline placebo (CON) was administered (dose: 0.5 mg/kg) 5 minutes before simulated surgery (restraint for 30 minutes). On day 15, the surgical procedure was performed. Meloxicam or saline were administered prior to surgery. A right flank laparotomy, brief abdominal exploration, and omentopexy was performed on all animals. Blood was collected via jugular catheter at hours 0, 2, 4, 8, 12, 24, 36, 48, 60, & 72 during both phases for cortisol, and at hours 0, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, & 168 for haptoglobin, PGE2, and fibrinogen. Mechanical nociceptive threshold (MNT) was measured using an algometer and collected at hours 0, 1, 4, & 8 after sham and hours 0, 1, 2, 4, 8, 12, 24, 36, 48, 60, & 72 after surgery. Infrared thermography (IRT) was taken of the incision site at hours 0, 1, 4, & 8 hours after sham and 0, 2, 4, 8, 12, 24, 36, 48, 60, & 72 after surgery. PGE2 concentrations displayed a treatment by time interaction where concentrations were higher in the CON animals (P = 0.003). Total cortisol concentrations were significantly increased in

CON 4 hours post-operatively (P=0.004). Haptoglobin was significantly increased in CON 72 and 96 hours post-operatively (P< 0.001). There was no difference for fibrinogen (P=0.43), MNT (P=0.24) or IRT (P=0.68). This study indicates using meloxicam significantly reduces biomarkers of inflammation and indirect measures of pain and suggests meloxicam is effective in mitigating post-operative pain in adult lactating dairy cattle.

Table of Contents

Introduction 1
Basic Mechanism of Pain 2
What is pain? 2
What is the function of pain? 3
What is the basic pain pathway? 3
Pain management for cattle 5
What is currently available? 5
Assessment of pain in other species 6
Assessment of pain in cattle 8
What are the methods? 8
Physiologic parameters 11
PGE2 11
Cortisol and CBG 13
Haptoglobin and MMP-9 16
Fibrinogen 19
Mean Nociceptive Threshold 20
Thermography 24
Meloxicam 28
Chapter 1 Markers of Pain Mitigation in Cattle Following Soft Tissue Surgery 31
Abstract 32
Introduction 33
Material and methods 37
Animals 37
Phase 1: Sham Procedure 39
Phase 2: Surgical Procedure 40
Prostaglandin E2 (PGE2) 42
Cortisol and Corticosteroid Binding Globulin 42
Fibrinogen 43
Haptoglobin and MMP 9 44
Study conclusion 44
Statistical analysis 44
Results 45
PGE2 45
Cortisol and Cortisol Binding Globulin 46
Fibrinogen 47
Haptoglobin and MMP 9 48
Discussion 49
Surgical Model 49
PGE2 50
Cortisol 52
Conclusion 57

Chapter 2 Non-Invasive Assessment of Pain Mitigation in Cattle Following Soft Tissue Surgery....59
Abstract....60
Introduction....61
Material and methods....65
Animals....65
Phase 1: Sham Procedure....67
Phase 2: Surgical Procedure....68
MNT....70
IRT....71
Statistical analysis....71
Results....72
Mechanical Nociceptive Threshold....73
Infrared Thermography....73
Discussion....74
Conclusions....79
Conclusions....80
Appendix....102
Vita....126

List of Tables

Table 1 - Maximum, Mean and Minimum skin surface temperature within 72 hours of elective right flank laparotomy with prophylactic omentopexy.....125

List of Figures

Figure 1 - – Sham PGE2 concentrations by treatment group 103
Figure 2 - Sham PGE2 concentrations over time in all cattle.....103
Figure 3 - Surgical PGE2 concentrations in meloxicam and placebo-treated cattle.....104
Figure 4 - Sham total cortisol over time for all cattle 105
Figure 5 - Sham CBG concentration over time for all cattle 105
Figure 6 - Surgical cortisol concentration in meloxicam and placebo treated cattle 106
Figure 7 - Surgical Cortisol Binding Globulin concentration in all cattle over time 107
Figure 8 - Surgical Cortisol Binding Globulin (CBG) in meloxicam and placebo treated cattle 108
Figure 9 - Free cortisol index (FCI) for all cattle following elective laparotomy .109
Figure 10 - Free Cortisol Index in meloxicam and placebo-treated cattle over time 110
Figure 11 - Sham Fibrinogen concentration in all cattle over time 111
Figure 12 - Surgical Fibrinogen concentration in all cattle over time.....111
Figure 13 - Surgical Fibrinogen concentrations in meloxicam and placebo-treated cattle.....112
Figure 14 - Sham Haptoglobin concentrations over time for all cattle 113
Figure 15 - Sham Haptoglobin and Matrix Metalloproteinase 9 complex concentration in all cattle over time.....113
Figure 16 - Surgical Haptoglobin concentration in meloxicam and placebo-treated cattle 114
Figure 17 - Surgical Haptoglobin and Matrix Metalloproteinase 9 complex concentration in meloxicam and placebo-treated cattle 115
Figure 18 - Surgical Matrix Metalloproteinase concentration in all cattle over time 116
Figure 19 - FD/S-3 conical steel tip used for MNT 116
Figure 20 - Example of algometry sites around the incision. Sites 1, 2, and 3 are cranial sites and 4, 5, and 6 are caudal sites 117
Figure 21 - Mechanical nociceptive threshold following a right flank laparotomy and prophylactic omentopexy (including times 0 and 1 hour) 118
Figure 22 - Mechanical nociceptive threshold following a right flank laparotomy and prophylactic omentopexy in cattle treated with meloxicam or placebo (excluding times 0 and 1 hour).....119
Figure 23 - Combined mean MNT for all cranial and caudal test sites for all cattle 120
Figure 24 - Maximum, Mean, and Minimum skin surface temperatures collected by infrared thermography on all animals over time 121

Figure 25 - Maximum skin surface temperatures collected by infrared thermography following a right flank laparotomy and prophylactic omentopexy in cattle treated with meloxicam or placebo.....122
Figure 26 - Mean skin surface temperatures collected by infrared thermography following a right flank laparotomy and prophylactic omentopexy in cattle treated with meloxicam or placebo 123
Figure 27 - Minimum skin surface temperatures collected by infrared thermography following a right flank laparotomy and prophylactic omentopexy in cattle treated with meloxicam or placebo.....124

Introduction

Whether for a simple headache or a highly invasive surgery, most humans would need and demand the use of pain medications to alleviate pain and return to a comfortable state. This has also become the expectation for our beloved house pets: dogs, cats, bird, etc. But only recently has the need for approved analgesia for food and fiber animals become a concern, receiving attention from not only veterinarians and producers but by the general public as well. Many surveys have been conducted asking consumers about their perceptions of food and fiber animals' well-being. In a survey to the general public about their perspective on the ideal pig farm, respondents cited humane treatment as an important aspect to include (Sato et al., 2017). In a survey polling Australians, respondents from the general public had a better perception of castrations and disbudding when some form of pain management was given (Phillips et al., 2009). Routine husbandry practices such as castration and dehorning have been a part of normal production for many years, but are now perceived as painful procedures worthy of pain mitigation by the public, producers and veterinarians. An approved, economical, pain management modality is needed now and is both desired and demanded by all aspects of the industry in animal agriculture.

Basic Mechanism of Pain

What is pain?

Pain is be defined as an unpleasant sensory experience, resulting from a noxious stimuli, arising from tissue damage caused by disease, inflammation or acute injury. Specialized sensory neurons within tissues are excited by their respective stimuli, which activate the pain pathway and the ultimate perception of the painful process (Millman, 2013). The International Association for the Study of Pain defines pain as “an aversive sensory experience caused by actual or potential injury that elicits progressive motor and vegetative reactions, results in learned avoidance behaviors, and may modify species specific behavior, including social behavior.”

Pain can further be categorized into distinctive types: physiologic and pathologic. Physiologic pain is characterized by pain caused by a noxious stimuli causing tissue damage. This type of pain serves as a warning signal and is part of the body’s defense mechanism to prevent tissue damage. It is well localized, rapidly transmitted, and only exists for a brief time. The second type of pain is pathologic pain. Pathologic pain is the pain that occurs after the tissue damage has occurred. It is can be experienced in a number of different ways including causalgia (a dull, burning sensation), hyperalgesia (exaggeration of sensation to a noxious stimuli), and allodynia (exaggeration of a sensation that normally does not cause pain). Tissue damage followed by inflammation and nerve damage is accompanied by persistent pain, or pain that exists even after the noxious stimuli

has been removed. Pathologic pain is further divided into acute and chronic pain based on duration of the sensation. Acute pain is associated with withdrawal reflexes and protection of the affected areas. Typically, this type of pain is associated with soft tissue injury and inflammation. An example of acute pain is the pain present after an injury that creates a behavioral modification that prevents overexertion and incidentally, re-injury. Chronic pain is when the expected pain persists for longer than anticipated. Cancer pain, osteoarthritic pain and phantom limb pain are all considered chronic pain events (Lamont et al., 2000).

What is the function of pain?

Millman et al. (2013) states that pain's functions are to “warn the animal of actual damage to its tissues, predict when tissue damage is likely to occur, to warn conspecifics of the presence of danger.” (Millman, 2013). The first function is most closely associated with physiologic pain, while the later describe changes in behavior and are more appropriate descriptions of pathologic pain. Pain leads to physiologic and behavioral responses such as fight, flight or freezing.

What is the basic pain pathway?

Nociception is the term used to refer to the physiologic components of the pain pathway (Anderson & Muir, 2005a). The pain pathway consists of five distinct steps which occur within various areas of the peripheral and central

nervous system: Transduction, Transmission, Modulation, Projection and Perception.

Transduction is the conversion of a noxious stimuli (thermal, mechanical, or chemical) into an action potential by nerve fibers present at the source, called nociceptors. Nociceptors are classified into two groups: A-fibers and C-fibers. When A-fibers are activated, the signal transmitted is associated with a sharp pricking sensation. This is referred to as the “first pain”. When C-fibers are activated, the resulting sensation is a diffuse, dull, burning sensation and is referred to as the “second” or “slow pain” (Lamont et al., 2000; Anderson & Muir, 2005b).

These action potentials originate in the nociceptors and are then *transmitted* to the central nervous system (CNS) by their corresponding afferent nerve fibers. A-delta nerves are myelinated, larger fibers that rapidly conduct action potentials. C nerves are smaller and unmyelinated and conduct signals much slower than A-delta (Lamont et al., 2000).

Action potentials reach the CNS via their respective fibers and are *modulated* at the level of the spinal cord. Modulation of an action potential is facilitated by descending inhibitory neurons, which occurs in the dorsal horn of the spinal cord. These neurons amplify or depress the signal based on several external variables including pharmaceutical effect. Once modulated, action potentials are *projected* to the brain where they are *perceived* as pain (Anderson & Muir, 2005b).

Pain management for cattle

What is currently available?

United States Department of Health and Human Services Guidance for Industry (GFI) #123 states that for label claims of pain alleviation, the FDA "recommends that this indication be based on the control of clinical signs of pain associated with a disease." This GFI further states "We encourage the use of validated methods of pain assessment in the target species." Although guidelines are in place, limited validated methods for pain assessment in cattle exist, making new drug labeling increasingly difficult (FDA, 2006).

One product has been introduced to the market with a label specifically for pain. Transdermal flunixin meglumine (Banamine Transdermal by Merck) is the first drug ever for cattle to have a label indication for pain and was released in 2017. The label specifies that this product is indicated for the treatment of pain associated with foot rot, but is not indicated for use in cattle over 20 months of age. Despite this important advance, there still is no approved products for post-operative pain in adult lactating dairy cattle. Moreover, due to the narrow indications for its approval the use of Banamine Transdermal for other etiologies of pain is considered extra-label use.

Because cattle are food animals, restrictive guidelines exist to prevent violative residues of medications and their metabolites from entering the food supply. The Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994 was created to provide veterinarians the opportunity to determine appropriate extra-

label uses of pharmaceuticals for veterinary species, based on their clinical judgment. One stipulation of extra-label drug use in food and fiber species is the lack of allowable tolerance or zero tolerance of detectable residues in meat or milk. This means when veterinarians use drugs in an extra-label manner, any detection of that drug or its metabolites constitutes a violation (AMDUCA, 1994).

Assessment of pain in other species

Pain assessment indicators should be specific, repeatable and sensitive. This means that an indicator should be measuring pain and no other conditions (specific), repeatable within and between observers, and able to detect pain even when in low amounts (sensitive). Assessing the accuracy and precision of pain indicators, like other diagnostic tests, should be conducted by comparing them to a gold standard. However, there is not a gold standard for comparison when assessing pain in animals. Therefore, many experimental studies derive efficacy of pain management though comparison to animals receiving pain medication to those that not, or who have received varying dosages of pain medication (Ison et al., 2016).

Pain in other species of animals has been validated and study extensively. Human medicine has identified multiple indicators of pain. Human pain researchers have the advantage of patients that self-report pain. For non-verbal patients such as infants, the elderly, or patients with language barriers, other mechanisms including facial grimace scales such as the Wong/Baker faces

rating scale can be used. Other non-verbal cues of pain in humans include changes in vital signs, vocalization, and muscle tension (Fink, 2000).

There is some controversy over whether animals experience pain in the same way as humans. Animals and humans share similar anatomical pathways as well as similar CNS responses evoked by pain. This suggests the basis for comparative pain perception and physiology.

In mice, a validated grimace scale, has been developed that takes into account orbital tightening, nose bulge, cheek bulge, ear position, and whisker changes as collective indicators of pain (Langford et al., 2010). Similar scales have also been developed in rabbits (Keating, Thomas, Flecknell, & Leach, 2012), sheep (Hager et al., 2017) and horses (Dalla Costa et al., 2014).

In pigs, consistent behavioral changes were seen following castration, tail docking, and needle teeth clipping including trembling, tail wagging, and head shaking, respectively. Removal or reduction of these behaviors upon treatment also suggests validity in the assessment of painful conditions, such as the reduction of escape behaviors seen in piglets following administration of a local anesthetic prior to castration. Attempts to quantify the pain threshold in pigs have been developed through the use of nociceptive threshold testing, either by thermal or mechanical means. In this method, a noxious stimulus is applied and the force or length of time applied is measured as an indirect assessment of the animal's tolerance of the stimulus. Physiologic markers such as Fos-positive

neurons, cortisol, substance P, and prostaglandin E2 have also been evaluated as objective measures of pain in multiple species (Ison et al., 2016).

Several issues sited in studies of pain are the unwillingness of the animal to show pain because an observer is present or because of sampling methods. Many of the domesticated food animals are prey species and have the inherent need to hide pain for their survival.

Assessment of pain in cattle

What are the methods?

Ontologically cattle are a prey species that often hide pain in order to avoid predation. An example of this is the feedlot steer with respiratory disease that clearly elicits clinical illness when the observer is out of sight, but may override these signs so successfully as to be unrecognized as ill when the observer is present. Likewise, pain can be masked when fear overrides its physiologic manifestation. Cattle fear responses to humans are also influenced by breed and management conditions the animal has experienced. These responses therefore can be attenuated through positive interactions with human caregivers, typically from a young age, or conditioning through highly repetitive exposures. For example, a hand raised dairy calf is less likely to exhibit a fear response to a novel human than a calf that has never seen a human.

To compound the issue, signs of pain in cattle are subject to interpretation by each observer. Subjective measures of pain and cattle wellness vary by

observer training, experience, age and gender (Coetzee, 2013). When signs of pain are overt and point to a specific limb or body area the assessment becomes more reliable, but assessment of generalized pain can be difficult (Le Bars et al., 2001). The most common types of pain assessment in veterinary medicine are visual assessments where observers attempt to discern behavioral changes in attitude, posture, or disposition. These subjective assessments can be improved with the addition of categorization and appraisal of focal points such orbital tightening, head and neck position, and ear position. Locomotion scoring provides a good example where the scale for severity is divided into 4 - 5 categories and focal points of interest are back arch, stride length, and head position (Grégoire et al., 2013; Groenevelt et al., 2014).

To directly measure pain would be to directly measure the physiologic changes of the pain pathway. For example, measuring the frequency and amplitude of action potentials leaving the area of the noxious stimuli as well as its influence on structures within the central nervous system. Nerve conduction studies and electroencephalograms can provide some approximation of this data, but their impracticality limit implementation on a wider scale. Moreover, these methods do not capture the impact of pain on the affective state and therefore assessment of pain in cattle can truly only be measured indirectly.

Objective measures of pain are quantifiable and are not biased by the observer. These methodologies produce discrete observations within animals overtime with response variables that can be compared across treatment groups.

Objective measures of pain include frequency monitoring of specific behaviors, blood biomarkers, pressure algometry, infrared thermography, heart rate monitors and remote activity monitors such as accelerometers or real-time location systems. Each of these methods have been used extensively in monitoring cattle wellness and stress.

Subjective measures are those assessed by proxy and vary based on observer experience and knowledge. These are often in the form of scales, such as the locomotion scoring system or grimace scales. Objective measures are those that are not biased by the observer. These include measures such as blood metabolites and heart rate. Some indicators are somewhere in the middle. An example would be in the case of mean nociceptive threshold (MNT). This measures the force excreted to cause a pain reaction. Although the output is an objective measure, the ability of the observer to recognize the painful reaction would be subjective, making the overall observation open to possible bias due to knowledge and experience.

Numerous indirect methods have been used to measure pain in cattle associated with lameness, metritis, castration, and dehorning. Methods have been both subjective and objective measures of inflammatory mediators and stress hormones, physiologic parameters, production data, and behavioral data. Inflammatory mediators include cortisol, haptoglobin, fibrinogen, substance P, and serum amyloid A. Physiologic parameters include rectal temperature and heart rate variation. Production data includes feed intake, average daily gain,

morbidity and mortality, dry matter intake and milk yield. Finally behavioral data includes lying time, step lengths, feed bunk aggression/displacement, and chute exit velocity (J. F. Coetzee, 2013a).

Physiologic parameters

PGE2

Prostaglandin E2 is a positive acute phase protein and is one of the most important mediator for inflammatory pain (Kawabata, 2011). Prostaglandins are produced through the arachidonic acid pathway through cyclooxygenase (COX). Cyclooxygenase 1 is found across the mammalian body in peripheral tissues and the central nervous system. COX-1 is important for renal and gastrointestinal homeostasis, and its expression is increased by inflammation and pain. Cyclooxygenase 2 is also found in the central nervous system and is present in the cell in low numbers until the proper stimulus is provided (factors released by dying or damaged cells) (Coetzee, 2011). Cyclooxygenase 1 and 2 convert arachidonic acid to prostaglandin G2 which is converted further to prostaglandin H2 by a peroxidase. This prostaglandin H2 is converted to a number of different prostaglandins including PGE2, PGI2, TXA2, PGD2, and PGF2a (Chandrasekharan & Simmons, 2004).

Prostaglandin E2 has an effect on the central nervous system, vascular smooth muscle, platelets and kidneys, and is generated from PGH2 by 3 separate isomerases: cytosolic PGE synthase, microsomal PGE synthase-2, and

microsomal PGE synthase-1. Both cPGES and mPGES-2 are widely expressed and are important for renal homeostasis and gastrointestinal protection. The mPGES-1 is upregulated with COX-2 in response to an inflammatory mediators leading to the production of PGE2 that creates the inflammation (Kawabata, 2011). COX-1 is responsible for the initial release of prostaglandins and is followed in 2-8 hours by the COX-2 mediated release. Prostaglandins cause the neurons' threshold to lower, allowing for an increase in nociceptive activation. PGE2 is responsible in part for the central hyperalgesia that is seen with increased dorsal root excitability (Coetzee, 2011).

Most research on PGE2 is focused on the reduction of this molecule in tissue through the inhibition of the cyclooxygenase pathway. Non-steroidal anti-inflammatory drugs are used to decrease the inflammatory process by stopping the production of prostaglandins and other pro-inflammatory molecules by inhibiting the cyclooxygenases. NSAIDS will be discussed in further detail at the end of this review.

Novel ways of inhibiting the production of PGE2 without the side effects of NSAIDS are being explored. These possible avenues involve blocking other areas in the cyclooxygenase pathway such as inhibiting mPGES-2 or selectively binding PGE2 receptors antagonist. A study in guinea pigs showed that by selectively inhibiting mPGES-1, PGE2 production is decreased apart from the other prostaglandins involved in this pathway (Xu et al., 2008). More specifically, a human study concluded that PGE2 is involved in the mediation of visceral pain

by inhibiting the receptors specific to PGE2 and mitigating the pain felt by subjects (Sarkar et al., 2003).

Prostaglandin E2 can be measured from serum or urine. Because the PGE2 molecule is not stable, commercial assays are available to measure the metabolites and form an estimation of the PGE2 level.

Cortisol and CBG

Cortisol is used as a marker of stress in both humans and animals and is produced via activation of the hypothalamic-pituitary- axis (HPA). This axis is regulated by the hypothalamus releasing corticotrophin-releasing factor (CRF) and vasopressin (AVP). This activates the release of adrenocorticotrophic hormone (ACTH) from the pituitary. ACTH acts on the adrenal glands, specifically the zona fasciculata of the adrenal cortex, to release glucocorticoids such as cortisol. Glucocorticoids then act as negative feedback on the secretion of CRF and AVP from the hypothalamus as well as directly on the pituitary corticotropes to inhibit the secretion of ACTH (Pariante & Lightman, 2008).

The HPA axis is activated in response to physical and psychological stressors. Cortisol's role in the body focuses primarily on regulating metabolism of the cells and reducing inflammation (Ison et al., 2016). Excessive, sustained amounts of cortisol in the blood is referred to as hypercortisolism, and is commonly known as Cushing's disease. An insufficient level of cortisol in the body is known as hypocortisolism, and is commonly known as Addison's or Nelson's disease.

Cortisol levels are affected by the intensity, duration and site of the noxious stimuli, and are not a measure of pain directly, but is an evaluation of the body's response to distress. Activation of the HPA is prompted by a variety of physical, emotional and physiological challenges including surgical procedures, anxiety, unusual handling, extreme temperature changes, vigorous exercise and many other stimuli (Mellor et al., 2000). Other stressors affecting the plasma cortisol levels in dairy cattle include age, diet, milk yield, and environmental factors (Dunlap et al., 1981). Cortisol may have a maximum level it can reach in the body. In a study by Coetzee et al. 2008, simulated castration and surgical castration had similar plasma cortisol levels, which suggest that handling alone can reach the maximum threshold for cortisol levels. Since cortisol can rise from the handling alone, sample collection could cause an increase and lead to confounding results. To further complicate cortisol evaluation, endogenous cortisol secretion has a diurnal rhythm of secretion and is variable between individuals (Coetzee et al., 2008). After administration of exogenous cortisol, beef cattle's clearance rate of the cortisol was approximately 30 minutes (Dunlap et al., 1981).

Cortisol levels are also affected by the use of local anesthetics. In a study evaluating cortisol and dehorning, calves dehorned with the use of a local anesthetic had a slight rise in cortisol as a result of handling and had a second, large rise in cortisol after the local anesthetic wore off (Mellor et al., 2000).

Corticosteroid-binding globulin (CBG) is the major binding protein in the blood for cortisol and is produced by the liver. CBG has a high affinity for cortisol and binds 75% of total cortisol. Free cortisol makes up 10% of total cortisol levels, with another 15% bound to albumin. CBG can bind up to approximately 25 µg/dL of cortisol in the plasma. Once this level of binding is reached, the free cortisol level increases rapidly to exceed the usual 10%. This unbound portion of cortisol is the active cortisol that is regulated through the HPA axis. In humans, CBG is increased during pregnancy, hyperthyroidism, diabetes and some genetic disease and is decreased in hypothyroidism and protein deficiencies seen with severe liver disease (Carroll et al., 2011).

In the face of inflammation, CBG has been defined as a negative APP, meaning it decreases in response to inflammation. This decreases the bound cortisol carrying capacity in the blood and would increase the amount of free cortisol. Because free cortisol has a short half-life, the total cortisol level may not change or are underestimated if CBG is decreased (Trevisi et al., 2013).

Free Cortisol Index (FCI) is the total cortisol to CBG ratio. This has been used as a correlate of serum free cortisol. In a human study following healthy adults through major elective surgeries, FCI increased by 130% while total cortisol rose 55% and CBG fell 30%. This measure takes into account CBG's role in cortisol levels and its effect on cortisol measurement and interpretation (le Roux et al., 2003).

Haptoglobin and MMP-9

Haptoglobin (Hp) is a major positive acute phase protein (APP) in cattle produced by the liver and is one of the most specific APPs for inflammation and infection. In healthy adults, Hp is present at less than 0.1 g/L. In acutely ill adults, Hp levels increase over 100-fold and reach maximum levels between 48 and 96 hours (Bannikov et al., 2011). Bovine Hp is made of an alpha and beta chain linked by a disulfide bond (Ceciliani et al., 2012) Hp increases during the acute phase of both infectious and inflammatory conditions such as those following surgical trauma (Chan et al., 2004). Hp also increased in times of stress due to increased levels of circulating cortisol (Guzelbektes et al., 2010). It has also been used to distinguish between chronic and acute inflammation due to the significantly higher levels in the acute phase of inflammation (Paulina & Tadeusz, 2011).

A major function of Hp is to scavenge hemoglobin released by damaged red blood cells. Once bound, the two proteins form a stable complex that is taken up by macrophages for breakdown (Alayash, 2011). This allows haptoglobin to act as an anti-oxidant against the oxidative damage that hemoglobin can cause. Hp is also responsible for some regulation of the innate immune response of white blood cells and has bacteriostatic effects (Ceciliani et al., 2012). It is essentially an anti-inflammatory during this process. Macrophages upregulated anti-inflammatory mediators in response to hemoglobin-haptoglobin complexes binding to CD163 receptors. The anti-inflammatory mediators released include

IL10 and heme oxygenase-1 which activate the anti-inflammatory response further. Haptoglobin also downregulate neutrophil activity directly by inhibiting lipoxygenase and cyclooxygenase or by inhibiting the respiratory burst. Hp further inhibits the Th2 cytokine release to suppress T cell proliferation. Finally, bacteriostatic activity of Hp is achieved when bacteria that need iron to grow are inhibited by Hp due to its scavenging of free hemoglobin. Bacteria can overcome the binding to utilize the heme if they are have an iron acquisition system (Ceciliani et al., 2012).

In cattle, haptoglobin is used for the diagnosis and prognosis of several diseases including mastitis, enteritis, peritonitis, pneumonia, and metritis. The reproductive tracts of cattle also have Hp expression that some have hypothesized are part of the normal physiology of the tract. Hp also increases in response to disease of the reproductive tract. Dairy cattle with increased metabolic stress (measured through beta-hydroxybutyric acid) at the time of calving were found to have increase Hp. Cows with retained fetal membranes and those multiparous cows with assisted calving also had increases in haptoglobin levels (Pohl et al., 2015).

Hp is expressed in high rates in the liver but is also seen in the abomasum and forestomaches. Because the forestomach is continuously exposed to microbes, this explains the need for Hp as a part of the immune system dealing with possible breaches in the epithelium (Dilda et al., 2012).

In cattle, increased expression of Hp is seen with LPS infusion into the mammary gland as well as *E. coli* infections. Haptoglobin has been used as an indicator of disease in cattle for many years. A study measuring haptoglobin in milk verses serum found that animals administered LPS directly into the mammary gland showed an increase in Hp both in the blood and in the milk (Hiss et al., 2004).

In the future, Hp may be a useful tool in slaughterhouses to assist with the meat inspection process. In a study comparing Hp levels of dairy cows with infection and metabolic disease, Hp levels were 6-fold higher than in animals with minor lesions. In a separate study, Hp was 40-fold higher dairy cows with acute lesions than healthy animals (Eckersall & Bell, 2010).

Matrix Metalloproteinase 9 (MMP 9) is a zinc dependent proteinase in the gelatinase B group. They are stored in neutrophils and released during degranulation. They are able to breakdown the extracellular matrix and components of the basement membrane which increases white blood cell migration during inflammation. MMP 9 is also capable of activating IL-8 creating a positive feedback loop for more neutrophil recruitment. The Hp-MMP 9 complex forms in the neutrophil and are stored here until degranulation. The release of this complex represents neutrophil activation. Measuring the Hp-MMP 9 complex allows to differentiation of acute and chronic inflammation since it is only released by activated neutrophils in response to acute inflammation (Hanthorn et al., 2010). A study compared ELISAs for free Hp, free MMP 9 and

Hp-MMP 9 complexes in acute infection, chronic infection and healthy cattle. The results showed Hp-MMP 9 was not detectable in healthy animals and was the highest in animals with acute inflammation (Bannikov et al., 2011).

Fibrinogen

Fibrinogen is a moderate positive APP in cattle and found in healthy animals between 1.58-2.94 g/L (Ceciliani et al., 2012). The liver is the major organ for Fb production, where liver parenchymal cells produce and store Fb until it is needed. The major role of Fb in the body is as a precursor for fibrin formation and a molecule in the coagulation cascade. Fb levels following tissue damage increase within 24 hours and decreases once its maximum concentration is met. In cases of disease causing Fb to be increased, levels remain high until the disease has subsided (McSherry et al., 1970).

In a study by McSherry 1970, 9 cows presented with displaced abomasa (DA). Three of these had fibrinogen levels greater than the reference range (range: 9.0-10.25 g/L). The six remaining cows were within the reference range (3.1-8.0 g/L) (McSherry et al., 1970). This was also seen in several other studies looking at fibrinogen in response to naturally occurring DAs. In a study by Jawor, seven cows that presented with a DA had fibrinogen levels within the physiologically normal range with only one elevated. This animal also had bronchitis as a concurrent disease (Jawor et al., 2009).

In a study of naturally occurring abdominal disorders (LDA, RDA and dystocia), Fb levels were within the reference range (3.0-7.0 g/L) prior to surgery.

Furthermore, surgical intervention did not elevate the levels of Fb on a systemic level. This study also evaluated naturally occurring traumatic reticulo-peritonitis. These animals had a significantly higher concentration of Fb than all other groups (mean: 11.6 g/L) which increase slightly following surgery and then lowered over time. These authors further suggest that fibrinogen is more specific to infectious causes of disease and that an increase after surgery would be more indicative for peritonitis associated with surgery (Hirvonen & Pyorala, 1998).

Mean Nociceptive Threshold

Mean Nociceptive Threshold (MNT) is measured by the application of a continuously increasing stimuli applied to tissues that reaches a pain tolerance threshold and causes a withdrawal or avoidance response. This threshold represents the maximum pressure (or pain) the subject is willing to suffer before a response is occurs. Several stimuli have been used including electrical, thermal, chemical and mechanical. These stimuli are applied to the tissues and produces a quantifiable outcome that is repeatable and is non-invasive.

Electrical stimulation meets the criteria for repeatability, quantification and non-invasiveness. However, this type of stimulus stimulates not only nociceptive fibers but also large diameter fibers used for hot and cold sensation. This stimulus is not typically found in the animal's natural environment and effectiveness of the test may vary due to difference in impedance of different tissues. Thermal stimulation also meets the criteria needed for an MNT method. This type of stimulation does cause the activation of thermoreceptors as well as

nociceptors. One of the disadvantages see with this type of stimulation is the difference seen over black or darker areas of skin. This was demonstrated in a study of guinea pigs where black animals had a nociceptive threshold 8% lower than white animals when radiant heat was used. For chemical stimulation, an algogenic agent is administered and causes a slow stimulus over a longer period of time than the other stimuli listed. This type of stimulus is unique from others because the measured outcome is a measure of behavioral changes in response to an inescapable stimulus (Pongratz & Licka, 2017).

The final type of nociception stimulus, and the focus of this section, is mechanical stimulation. An algometer is used to quantify the threshold based on a force exerted on the tissue. This method's use in damaged tissues is based on the association of inflammation from tissue damage causing hyperalgesia or allodynia. (Di Giminiani et al., 2016). This type of stimulus will also activate mechanoreceptors in the tissues as well as nociceptors (Pongratz & Licka, 2017).

The mechanical stimuli are applied until a response is obtained. Examples of responses to stimuli in mice and rates include tail flicking or paw withdrawals. In cattle, the responses measured include withdrawing the head after dehorning (Heinrich et al., 2010), moving away from the device, looking back at the device, tail flicks, and kicking.

Several principles have been demonstrated when using an algometer as a measure of pain. Tissues that are damaged whether from disease or surgical

trauma will have a lower threshold than those tissues that are healthy. Smaller algometry tips result in a lower MNT while larger tips result in a higher MNT (Pongratz & Licka, 2017). A certain level of variability is always present and is multifactorial.

In a study of pain in dogs undergoing ovariohysterectomy and ovariectomy, algometry was used to measure cutaneous pain of the incision site. During this study a response was classified as a sudden movement away from the algometry unit, attempting to stand, looking at the algometry unit, vocalization, and attempting to bite. This study showed no difference in the two surgeries when comparing algometry reading, but the authors do state there is not an effective way of testing pain from within the peritoneal cavity at this time (Tallant et al., 2016).

In a study of horse back pain, algometry tips were compared based on the diameter and how they elicited a response. This study found that tips with a contact area of 1 cm^2 produced more similar results than larger or smaller tips. Furthermore, the shape of the tip was examined. Rounded (hemispheric shape) rips resulted in a higher pain threshold than cylindrical, flat tips. This study also compared measurements in the thoracic region verses the lumbar region of live horses. Because there is increased tissue thickness in the lumbar region, this area had a higher threshold than the thoracic region (Pongratz & Licka, 2017).

In a study assessing the effects of meloxicam on dairy calves after cautery dehorning, calves were tested with a pressure algometer. Calves treated with

meloxicam were less sensitive to algometry 4 hours after dehorning when compared to calves without meloxicam (Control MNT = 1.62 ± 0.13 kg of force; Meloxicam MNT = 2.13 ± 0.15 kg of force) (Heinrich et al., 2010).

This method has further been used as a way of objectively determining claw pain relation to locomotion scoring in dairy cattle. In this study, algometry was used to measure the pain in claws. The response to a stimulus in this study was withdrawal of the foot (Dyer et al., 2007).

Algometry has several factors that make the results less reliable. Individual variation is one of the biggest factors affecting results for algometry. An individual's sex, breed, age, body condition, and overall pain tolerance effect its MNT. The stage of disease or stage in the healing process affect the response to stimuli such as pressure readings. Algometry is also affected by the operator. In most studies, algometry is carried out by one operator who has been trained on the possible responses the subject by exhibit that require removal of the pressure. Algometry is limited by the need for repeated measurements. While triplicates are required for many statistical readings, subjects may become habituated to these readings over time. One of two scenarios could occur. One, the animal becomes habituated to the noxious stimuli, and its threshold will be falsely increased. Two, the animal begins giving the desired avoidance response earlier than originally intended in order to avoid the experience all together causing a false decrease in the threshold.

Thermography

"Running a fever" has long since been a telltale sign of sickness. Hippocrates speculated that when a patient was covered in mud, areas that dried faster had greater heat and were therefore diseased. Since the invention of the thermometer in the 17th century, body temperature has been used as an objective and quantitative means to measure sickness and its severity. An increased body temperature can tell physicians that body is fighting off infections, has an increase in inflammation, or is overheating due to the environment.

Infrared thermography is being used in many fields to distinguish temperature differences, from law enforcement using this technology to rescue people to building engineers using this to detect heating leaks from a building. Now medical personnel are exploring the possibilities of how infrared thermography can help diagnose and monitor medical conditions.

This modality is helpful in measuring the sympathetic adrenomedullary system. In a fight or flight response, skin temperature decreases as blood vessels constrict in the periphery to move more blood to the muscle and internal organs. IRT is also helpful in measuring inflammation and pyrexia. The five cardinal signs of inflammation are heat, redness, swelling, pain, and loss of function. Temperature in inflammation increases due to vasodilation of the vessels at the area to increase blood flow which is also accompanied by inflammatory mediators such as cytokines, eicosanoids, and complement

proteins. These mediators work to activate PGE2 that then acts on the preoptic area in the brain to increase overall body temperature (Bradford et al., 2015).

Thermal imaging is being used as a screening tool in animals and humans for a change in overall body temperature and the assessment of local inflammation. This method allows for assessing temperatures noninvasively and requires no physical contact between subjects and screeners. Thermal imaging was used to screen people at airports for increases in body temperature during the avian influenza outbreaks. It has been used experimentally for the detection and monitoring for foreign animal disease such as foot-and-mouth disease virus and bluetongue virus, monitoring wildlife for infectious diseases such as rabies, and decreasing the time needed for testing for tuberculosis in cattle (Rekant et al., 2016).

Many studies have demonstrated the use of IRT for screening patients for increased body temperature [due to a febrile state]. In a study in calves inoculated with a high virulent type 2 Bovine Viral Diarrhea Virus (BVDV), infrared thermography of the eye was used to detect disease as early as 1 day post experimental inoculation, which was significantly different from pre-inoculation temperatures, and coincided with the expected course of disease. This correlated with changes in rectal temperature. In this study, thermal images were taken of the side, back, hooves, ears, nose and eyes as well as samples of serum cortisol, haptoglobin and salivary IgA levels. Ocular infrared temperatures were the most reliable and consistent for detection of disease (A. L. Schaefer et

al., 2004). Because of this work, any disease that causes an animal to become febrile (bovine respiratory disease complex, metritis, peritonitis, etc.), can be detected and monitored earlier than by other traditional method without having a direct interaction with the patient.

Thermal imaging has also been used for the detection and monitoring of localized inflammation in cattle such as hoof lesions or mastitis. These localized inflammatory processes are characterized by the dilation of blood vessels, hyperemia, swelling, and hyperthermia. (Rekant et al., 2016). In a study of dairy cattle, IRT was compared to the California Mastitis Test (CMT) on its ability to detect subclinical mastitis. The udder skin surface temperature (SST) and CMT were positively correlated, while rectal temperature had a weak correlation to both (Colak et al., 2008). In a study of lameness in dairy cattle, temperature increases in the hoof with a lesion were observed before behavioral signs became evident and decreased once corrective trimming was performed to alleviate the lesion (Wood et al., 2015).

Thermography's use in detecting inflammation has become even more important when assessing the welfare of animals. Inflammation in the limbs of gaited horses caused by soring is being detected by governing official using IRT as an objective adjunct tool.

One of the greatest advantages of IRT is the ability to collect individual data by a remote, noninvasive means. Observers are able to essentially snap a

picture of a location of interest and have a diagnostic picture of the animal's health or a screening tool for monitoring herd health.

Cow factors that would affect the quality of the thermal image include hair vs non-haired skin, color of skin, and age. Hair is an insulator, which holds heat to the body and out of sight from the IRT camera. For this reason, haired skin tends to appear cooler than non-haired skin. This remains true in the case of clipping for a surgical procedure. Black also absorbs heat more than lighter colors. In black and white colored cows, the black areas will be warmer than white areas. Finally, age has been shown to have a possible effect on body temperature of the eye in human subjects. Other areas where not studies but can be inferred to also affect theses as well.

Environmental factors that can affect the quality of the thermal image include ambient temperature, air movement, sunlight, rain, and various other weather conditions. With the great variety of climate changes throughout a day and the small window of precision that the IRT camera has, the likelihood that the environment may alter outcomes is relatively high. For this reason, evaluating animals in controlled environmental conditions is imperative for the evaluation of thermal images. Further factors in the environment are the presence of moister and debris on the subject. These can limit the use of IRT in veterinary medicine and in practical implication in the field (Rekant et al., 2016).

Meloxicam

Non-steroidal anti-inflammatory drugs (NSAID) are one of the options veterinarians have for treating pain and inflammation in cattle. NSAIDs inhibit cyclo-oxygenase enzymes (COX) from acting on the arachidonic acid pathway and producing prostaglandins as well as other inflammatory mediators, thereby decreasing pain and inflammation. COX is found in two isoforms: COX-1 and COX-2. COX 1 is primarily related to homeostats of the abomasal mucosa and renal perfusion and is constantly expressed in the CNS and PNS. Long term alteration in COX-1 expression can lead to effects of the medications such as ulceration of the abomasum. COX-2 is found in the CNS constantly, but is induced by release of factors from injured tissue. This leads to its role as a major enzyme in prostaglandin production. After a tissue insult, COX-2 mRNA expression takes 2-8 hours to reach maximum levels. For this reason, COX-1 is predominantly responsible for the initial release of prostaglandins (Coetzee, 2013b). Medications that are more selective for COX-2 are thought to be less potent (Anderson & Edmondson, 2013)

Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) in the oxicam class. Meloxicam is more selective for the COX-2 isoform. The dose approved by the European Union is currently 0.5 mg/kg IM or SC and has a 15-day meat withdrawal and a 5-day milk withdrawal time.

Meloxicam is currently approved in the USA for use in dogs and cats. In dogs, meloxicam is approved for use to control pain and inflammation associated

with osteoarthritis ("Metacam Injectable for Dogs"). In cats, meloxicam is labeled for control of post-operative pain and inflammation from spays, neuters, and orthopedic surgery. This label does have a box warning stating that repeated doses in cats is associated with acute renal failure and death ("Metacam Injectable for Cats").

There are a number of studies evaluating meloxicam's efficacy in cattle for several different indications. The American Association of Bovine Practitioners guidelines for castration and dehorning sites meloxicam as a possible long-acting pain medication to mitigate pain associated with these procedures (Practitioners, 2014). Allen and colleagues (Allen et al., 2013) found that calves treated with meloxicam had significantly lower cortisol levels at 4 hours after dehorning and substance P levels at 120 hours than control calves regardless of time of meloxicam administration (either 12 hours pre-procedure or at time of procedure). Heinrich and colleagues (Heinrich et al., 2010) found that calves treated with meloxicam 10 minutes prior to dehorning flicked their ears less during the first 44 hours, had less head shaking during the first 9 hours, and were less sensitive to pressure algometry at 4 hours after the procedure than the control calves. Barrier and colleagues (Barrier et al., 2014) found that beef cows given meloxicam following caesarean section spent more time lying and had more lying bouts than control animals, suggesting that increased lying times are representative of increased comfort.

Coetzee and colleagues (Coetzee et al., 2009) evaluated the pharmacokinetics of meloxicam in ruminant calves receiving the medication either orally (1 mg/kg) or intravenously (0.5 mg/kg). Oral meloxicam had a mean peak plasma concentration of 3.10 µg/mL at 11.64 hours with a half-life of 27.54 hours. Intravenous administration of meloxicam had a half-life of 20.35 hours. Malreddy and colleagues evaluated the pharmacokinetics of meloxicam in lactating dairy cattle when given orally with two different dosing levels of gabapentin. Oral meloxicam had mean peak plasma concentration of 2.89 µg/mL at 11.33 hours. The mean peak milk concentration was 0.41 µg/mL at 9.33 hours (Malreddy et al., 2013).

Chapter 1

Markers of Pain Mitigation in Cattle Following Soft Tissue Surgery

Amber F. Moore, DVM[1]; Marc Caldwell, DVM, PhD[1,*]; Brian K. Whitlock, DVM, PhD[1]; Lew Strickland, DVM, MS[2]; Peter Krawczel, MS, PhD[2]; Liesel Schneider, PhD[2]; David E. Anderson, DVM, MS[1]

[1] Department of Large Animal Clinical Sciences, University of Tennessee, Knoxville, TN 37996

[2] Department of Animal Science, University of Tennessee, Knoxville, TN 37996

* Corresponding author: mcaldwell@utk.edu

Abstract

Mitigation of pain for surgical procedures has become a topic of concern for the public, producers, and veterinarians. The objective of this study was to determine the efficacy of meloxicam for pain mitigation in adult lactating dairy cattle following a right-side laparotomy with omentopexy. Twenty-four dairy cattle (mean age: 2.51 +/- 0.54 years), between 50 and 188 days in milk (median: 117 days +/- 43.15 days) were enrolled. Cattle were administered a 7-day acclimation period to the new environment and social hierarchy and assigned blocks based on parity, days in milk, milk yield, and pregnancy status, and randomly allocated to groups Meloxicam (MEL) or placebo treated control (CON). The study had two phases; sham (day 0-14) and surgery (day 15-28). The objective of the sham phase was to collect baseline behavioral and physiologic data and permit cows to become acclimated to human intervention during the intensive sampling periods. On day 0, cattle were prepared for surgery including local blocks with lidocaine.

Injectable meloxicam (MEL) or saline placebo (CON) was administered (dose: 0.5 mg/kg) 5 minutes before simulated surgery (restraint for 30 minutes) and then returned to their home pen for data collection. On day 15, after a 14-day washout period, the surgical procedure was performed. Meloxicam and saline were administered prior to surgery to each respective group. A right flank laparotomy, brief abdominal exploration, and omentopexy was performed on all animals. Blood was collected via jugular catheter at hours 0, 2, 4, 8, 12, 24, 36, 48, 60, & 72 during both phases for cortisol, and at hours 0, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, & 168 for haptoglobin, PGE2, and fibrinogen. PGE2 concentrations displayed a treatment by time interaction where concentrations were higher in the CON animals ($P = 0.003$). Total cortisol concentrations were significantly increased in CON 4 hours post-operatively ($P=0.004$). Haptoglobin was significantly increased in CON 72 and 96 hours post-operatively ($P< 0.001$). There was no difference for fibrinogen ($P=0.43$). This study indicates the use of meloxicam significantly reduces biomarkers of inflammation and indirect measures of pain and suggests meloxicam is effective in mitigating post-operative pain in adult lactating dairy cattle.

Introduction

Cattle undergo painful livestock management procedures every day such as dehorning and castration as well as surgical repairs of displaced abomasum (DA), rumenotomies, and caesarian section (C-section). According to the United

States Department of Agriculture (USDA), there are an estimated 9.4 million dairy cows in the US in 2018 (Cessna, 2018). The incidence of left displaced abomasum (LDA) in lactating dairy cattle is approximately 5% with a projected cost of $250 to $450 per case (van Winden, 2002). Therefore, approximately 470,000 cows undergo surgical correction for LDAs each year. Veterinarians scored abdominal surgeries, such as DA corrections and caesarian sections, as the most painful procedures cattle undergo with an average pain score of 7.3 and 8.0, respectively (Fajt et al., 2011). To address pain mitigation, practitioners used a variety of medications including nonsteroidal anti-inflammatory drugs (NSAIDS), opioids, α2 adrenergic receptor agonist, local anesthetics, or a combination of these for pain in response to surgery.

Although practitioners are aware of the pain induced with abdominal surgery and use a wide variety of medications to alleviate pain, only one medication is currently labeled for the mitigation of pain in cattle. The recently approved transdermal flunixin meglumine (Banamine® Transdermal, Merck Animal Health, 2017) has a label indication for pyrexia associated with bovine respiratory disease and control of pain associated with foot rot. However, this product is not currently labeled for use in dairy cattle over 20 months of age. This obligates practitioners to continue to use medications in an Extra Label Drug Use (ELDU) manner under the guidance of the Animal Medicinal Drug Use Clarification Act (AMDUCA). The cattle industry needs new products labelled specifically for post-operative pain that are safe, effective and cost efficient.

Meloxicam, although not approved for use in cattle in the United States, is widely used for pain and other indications in cattle of many ages and production systems. In Canada, meloxicam (Metacam®20, Boehringer Ingelheim (Canada) Ltd., Burlington ON) is indicated for calf diarrhea, mastitis, and the relief of pain associated with dehorning and abdominal surgery. Meloxicam acts by inhibiting cyclooxygenase enzymes from converting arachidonic acid to prostaglandins and prostacyclins. There are two isoforms of the COX molecule: COX-1 and COX-2. COX-2 is present in low levels within the cell and is upregulated in response to inflammation. Meloxicam preferentially inhibits COX-2 thereby decreasing the inflammatory mediators release and depressing the inflammatory response. Following a single subcutaneous dose, meloxicam displays peak plasma concentrations in 7.7 hours (C_{max} = 2.1µg/ml) and an elimination half-life of approximately 22 hours in young cattle (Stock & Coetzee, 2015). Meloxicam's efficacy in alleviating pain associated with castration and dehorning has been well documented (Allen et al., 2013; Brown et al., 2015; Heinrich, Duffield, Lissemore, & Millman, 2010; Melendez et al., 2017). Barrier et al. recently demonstrated changes in lying time and lying bouts in beef cows undergoing emergency caesarian section following a single subcutaneous dose of meloxicam. Likewise, meloxicam treated cattle displayed a higher dry matter intake and altered lying time after implantation of a rumen fistula compared to ketoprofen treated cattle (Barrier et al. (2014).

A major hurtle in the approval of new pain medications is the ability to objectively measure and quantify pain in cattle to meet FDA specifications ("GFI #123-Target Animal Safety-Approval of NSAIDS,"). Pain is an inherently variable and individual response, influenced by temperament, breed, and each animal's physiologic and/or affective state. Current research methodology attempts to quantify pain indirectly through inflammatory or neuropeptide biomarkers, changes in behavior, or changes in production parameters. Cattle are ontologically prey species, and are behaviorally conditioned to mask pain or disease in order to avert predation. Therefore, the pursuit of systemic biomarkers that are byproducts of the pain or inflammatory response are attractive for evaluating the efficacy of pain therapies in clinical studies. To the authors' knowledge, no previous studies have evaluated the efficacy of meloxicam in alleviating post-operative pain in lactating dairy cattle.

Thus, the objective of this study was to evaluate the efficacy of subcutaneously administered meloxicam in alleviating post-operative pain in lactating dairy cattle following elective laparotomy with prophylactic omentopexy. Our hypothesis was that cattle administered meloxicam would demonstrate lower concentrations of relevant biomarkers associated with pain and inflammation (cortisol, CBG, PGE2, Hp, Hp – MMP9 complex, and fibrinogen) compared to placebo administered cattle.

Material and methods

The University of Tennessee Institutional Animal Care and Use Committee approved all experimental procedures under the supervision of the university veterinarian (Protocol # 2246-0314).

The study was conducted in two phases. Animals were first subjected to a sham surgical procedure to ensure habituation to the intensive sampling procedures followed by application of the surgical procedure after a 14-day wash out period. Data are presented in relationship to each phase.

Animals

Twenty-four adult dairy cattle, greater than 20 months of age (mean age: 2.51 +/- 0.54 years), were housed at the East Tennessee Research and Education Center - Little River Dairy Animal and Environmental Unit (Latitude: 35.772115; Longitude: -83.850182) during the months of June, July and August and were maintained in an ambient temperature. Animals were between 50 and 188 days in milk (median: 117 days +/- 43.15 days) and weighed between 512 kg and 705 kg (mean: 596.4 kg +/- 41.8 kg). Cows were allocated into two equal groups: Meloxicam (n = 12; MEL) and Control (n = 12; CON). Groups were balanced based on days in milk (Median: 111 days MEL; 127 days CON; Range: 50 – 188 days), milk yield (Mean: 74.0 lbs. MEL; 77.5 lbs. CON; Range: 57.6 – 93.1 lbs.), and pregnancy status (n = 2 MEL; n = 2 CON). Exclusion criteria for enrollment in the study included clinical signs associated with potentially systemic inflammation (i.e. mastitis, metritis, ketosis, lameness, etc.) or history or

evidence of previous abdominal surgery. All animals were considered healthy based on a physical exam performed by a veterinarian 1 day prior to the start of the study.

Cattle were housed in a free stall barn approximately 20 m x 12.5 m (800 sq. m) with 24 sand-bedded stalls and 32 headlock stanchions. Stocking density was maintained at 75% and did not exceed 100% during the study (lowest was 22 cows per 24 stalls = 91%). Cattle were milked twice daily (at approximately 7:30 AM and 4:30 PM). Milk was discarded throughout the entire of the experiment. A total mixed ration (TMR) was fed ad libitum during the study and was formulated according to Nutrient Research Council requirements to meet or exceed nutrient requirements of lactating dairy cattle. Nutrient analysis of TMR was conducted prior to and upon completion of the study. Fresh TMR was prepared and dispensed twice daily in parallel with milking. Twice daily at approximately 12:00 PM and 6:00 PM residual TMR was pushed up the headlocks. Waste TMR was collected twice daily immediately prior to the dispensing of each new feeding and weighed to determine pen level feed intake. Throughout the study animals were also provided ad libitum access to water. The cattle were permitted 7 days prior to the initiation of the study to acclimate to the new environment and social hierarchy. During this time, the cattle were also acclimated to handling via halters and grooming to simulate the contact associated with the intense sampling periods.

Phase 1: Sham Procedure

In order to determine the effect of the intense sampling scheme and experimental assessments alone without the influence of the surgical procedure, a sham experimental phase was imposed on the cattle. On day -1, a 14-gauge X 13 cm polyurethane IV jugular catheter (MILACATH-Extended Use, Mila International, Inc. Florence, KY) was placed and were maintained with heparinized saline until 4 days after the procedure, at which point they were removed and subsequent samples taken via direct venipuncture.

On day 0, a sham procedure was performed. Cows were prepared for sham surgery by clipping and sterilely preparing. Cows were blocked with 2% lidocaine (90 ml or 1800 mg, local tissue infusion, VetOne, MWI Animal Health, Boise, ID) in a line block pattern on their right side approximately 15 cm below the transverse process of the 3rd lumbar vertebrae and 10 cm caudal to the costal arch. Meloxicam (0.5 mg/kg, Metacam®20, Boehringer Ingelheim Ltd., Burlington, Ontario, Canada) was administered SQ in the neck to the meloxicam treatment group (MEL) and saline (0.025 mL/kg, 0.9% Sodium Chloride Injection USP, Hospira, Inc. Lake Forest, IL) was administered SQ in the neck to the control group (CON). Ampicillin trihydrate (10 mg/kg, Polyflex®, Boehringer Ingelheim Ltd., St. Joseph, MO) was administered intramuscularly to all cows on the contralateral side of the neck from the treatment.

Four veterinarians were designated as surgeons during the study (MC, DA, BW, and LS). Six replicates of sham procedures occurred with 4 animals per

cohort. Each cohort was equally balanced for MEL and CON cattle (2 MEL and 2 CON). Each surgeon performed six procedures with equal numbers of MEL and CON cattle (3 MEL and 3 CON). A sham surgical simulation was performed with the surgeon standing adjacent to the animal while it was restrained for 30 minutes. Procedure initiation for each cohort was staggered and administration of treatment and sampling time points were relative to each animal's sham procedure start time.

Following the sham procedure, cattle were returned to their home pen for sample collection lasting up to 7 days. Following completion of phase 1, the cattle were provided a 7-day washout period. Therefore, a total of 14 days following administration of the meloxicam or saline placebo elapsed prior to initiation of phase 2.

Phase 2: Surgical Procedure

The methods used for the phase 2 were similar to those executed during phase 1 with the exception of preforming right flank laparotomies with prophylactic omentopexy for all cattle. Briefly, on day -1 before the surgical procedure, cattle were refitted with heart rate monitors and jugular catheter was placed. As previously, jugular catheters were maintained with heparinized saline through the first 96 hours of the sampling period then removed and subsequent blood samples collected via direct venipuncture. On day 0, approximately 5 minutes prior to the initiation of each animals' surgical procedure, meloxicam (0.5 mg/kg) was administered SQ in the neck to the meloxicam treatment group

(MEL) while a saline placebo (0.025 mL/kg) was administered SQ in the neck to the control group (CON). Ampicillin trihydrate (10 mg/kg) was administered intramuscularly to all cows on the contralateral side of the neck from the meloxicam or saline treatment.

The same four veterinarians carried out the surgical procedures in 6 replicates of 4 cows. All treatments were balanced within each cohort and balanced across each surgeon. Each laparotomy, abdominal exploratory and prophylactic omentopexy were performed according to a standardized protocol. The right paralumbar fossa was clipped, the skin was aseptically prepared, and a line block with lidocaine (120mL of lidocaine) was performed as previously described. After aseptic preparation, a 15 cm vertical incision was made starting 10-cm caudal to the caudal curvature of the last rib and 15 cm ventral to the transverse process of the 3rd lumbar vertebrae. The incision progressed through the skin, external abdominal oblique muscle, internal abdominal oblique muscle, transversus abdominus muscle, and peritoneum. The surgeon then placed the left arm into the abdomen and briefly explored the abdomen to identify and palpate the rumen, omentum and abomasum. Similar to the techniques described in Turner and McIlwraith's Techniques in Large Animal Surgery (2013), a standard omentopexy was performed by suturing the omentum, peritoneum and transversus closed using #2 polyglactin 910 (Vicryl, Ethicon, Inc. Somerville, NJ) in a simple continuous pattern. The external abdominal oblique muscle and internal abdominal oblique muscle were also closed using #2 polyglactin 910 in a

simple continuous pattern. Finally, the skin was closed using #4 nylon (Supramid Extra II. S. Jackson, Inc. Alexandra, VA) in a continuous interlocking pattern.

Prostaglandin E2 (PGE2)

Blood samples (5 mL) were collected into a lithium heparin tube at 0 (baseline), 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 after sham/surgery. Plasma was harvested after centrifugation and frozen until analyzed. A commercially available kit was used to measure the PGE2 metabolites (Prostaglandin E Metabolite (Kit # 514531), Cayman Chemical Company, Ann Arbor, MI) following previously described methodology (Fraccaro et al., 2013). Briefly, the stable metabolite of PGE_2 was measured in a competitive assay with a PGE metabolite conjugated with acetylcholinesterase. The concentration of PGE metabolite tracer was determined spectrophotometerically and used to calculate the concentration of free PGE metabolite. Absorbances were read by an ELX808 (BioTek Instruments, Inc., Winooski, VT). Intra- and inter-assay CV (n = 570) of pooled bovine plasma was 10.7% and 4.0%, respectively.

Cortisol and Corticosteroid Binding Globulin

Blood samples (6 mL) were collected into a lithium heparin tube at 0 (baseline), 2, 4, 8, 12, 24, 36, 48, 60, and 72 after sham or surgery. Plasma was harvested by centrifugation and frozen until analyzed. Isolation and purification of corticosteroid-binding globulin from bovine plasma (CBG) and development and validation of an ELISA for its quantification followed the procedures outlined by

Roberts et al. (2003) for porcine CBG. For the CBG assay the absorbances were read by an ELX808 (BioTek Instruments, Inc., Winooski, VT) and data were collected using Gen5 software version 2.03.1 (BioTek Instruments, Inc., Winooski, VT). Intra- and inter-assay CV of pooled bovine plasma was 5.6% and 9.7%, respectively. Total serum cortisol concentration (ng/mL) was determined using the RIA procedure of Coat-A-Count Cortisol (Siemens Medical Solutions Diagnostics, Los Angeles, CA) as performed previously in our lab (Doherty et al., 2007). The free cortisol index (FCI; nmol/mg) was calculated using the ratio of plasma total cortisol (nmol/L) to CBG (mg/L; Le Roux et al., 2003).

Fibrinogen

Blood samples (6 mL) were collected into a lithium heparin tube at 0 (baseline), 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours after the sham and surgery procedures. Serum was harvested by centrifugation and frozen until analyzed. Fibrinogen was measured using the heat precipitation method. Two microhematocrit tubes were filled with plasma. The first tube was centrifuged and the total protein was measured using a refractometer. The second tube was heated to 56 C° for 3 minutes, centrifuged, and the total protein was measured. This precipitates the fibrinogen from the plasma. The second protein measurement from the heated tube was subtracted from the first protein measurement of the unheated tube with the difference being the fibrinogen level. The % CV of all samples (n = 576) was 8.1%.

Haptoglobin and MMP 9

Blood samples (5 mL) were collected into a serum clot activator tube at baseline prior to sham/surgery and hours 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 after sham/surgery. Blood were collected into a serum clot activator tube. Serum was harvested by centrifugation after clotting and frozen until analysis. Hp was analyzed as previously described using a commercially available bovine haptoglobin ELISA test kit following the manufacturer's instructions (Hanthorn et al., 2014).

Study conclusion

A 30-day investigational withdrawal period, mandated by the food-use authorization, was used for edible tissues following the last treatment. In addition, a 10-day investigational milk discard time was used following the last treatment of meloxicam.

Statistical analysis

All statistical analyses were performed in Statistical Analyses System (SAS Version 9.1: SAS Institute Inc., Cary NC; 1991-2001). The level of significance was established to be $P< 0.05$.

Normality test of the data and residuals was performed for each indicator. Surgery total cortisol, fibrinogen, and PGE2 concentrations were normally distributed. Surgery CBC, free cortisol index, Hp-MMP 9 and Haptoglobin concentrations were normally distributed following log transformation. Non-

normally distributed data was log transformed to achieve normal distribution. Data was analyzed using the GLIMMIX procedure of SAS with Tukey adjustment. Fixed effects included treatment group. Time was used as a repeated measure. Animal ID was used as a random effect for all blood markers. Surgeon and time of surgery (surgery block) was analyzed as a random effect, but did not have a significant effect on any blood markers and was therefore removed from the analysis.

Results

Two animals were removed during the course of the study; one due to developing mastitis (CON) and one due to surgical complications (MEL), and have not been included in the statistical analyses. Cows included in the results is n = 22 (n = 11 MEL; n = 11 CON).

Surgeries were performed in the unused free stall pen adjacent to the home pen. The surgery time began when the incision was started and ended when the last suture was tied. Surgeries ranged from 12 to 36 minutes (mean time was 24.41 minutes). If surgeons finished before 30 minutes, cattle were maintained in their surgical position until 30 minutes was completed. Time point 0 hours represents the start of the incision for each animal.

PGE2

During the sham procedure, there was not a treatment by time interaction (P = 0.31). There was an effect of treatment (P = 0.02; Figure 1) and time (P <

0.001; Figure 2) on plasma concentrations of PGE2 such that CON PGE concentrations were greater than MEL.

During the surgery period, there was not an effect of treatment (P = 0.11). However, there was an effect on time (P < 0.001) and a treatment by time interaction (P = 0.003; Figure 3) on plasma PGE2 concentrations, such that the CON group had greater concentrations at 2 and 8 hours after surgery (P= 0.004 and P<0.001, respectively).

Cortisol and Cortisol Binding Globulin

During the sham phase, total cortisol concentration was affected by time (P = 0.016), but not significantly affected by treatment (P = 0.14), nor was there a time by treatment interaction (P = 0.27). However, all cows displayed a decline in total cortisol concentration over the observation period (P = 0.015, Figure 4). CBG displayed a similar pattern were there was a time effect (P < 0.001), but was not affected by treatment (P = 0.08) or a treatment by time interaction (P = 0.58). However, a decline in CBG over time was observed for all cows (P > 0.001, Figure 5). Finally, due to the comparative declines in both total cortisol and CBG, the FCI had no significant time (P = 0.10), treatment (P = 0.25), or time by treatment interaction (P = 0.17, data not shown).

Total cortisol concentration was affected by both a time across treatment groups (P < 0.001) and a time by treatment interaction (P = 0.004) following the surgical procedure. Figure 6 displays the least squares mean concentrations of cortisol by treatment group over time after surgery. Cows that received

meloxicam had significantly lower cortisol concentrations at 4 hours after surgery ($P < 0.001$) than placebo-treated controls.

Unlike the observations made in the sham phase, CBG concentrations demonstrated a significant rise over time in both treatment groups ($P < 0.001$, Figure 7), but these differences were not significant by treatment ($P= 0.14$) or time by treatment interaction ($P = 0.52$, Figure 8). Due to the overall rise in total cortisol in the early post-operative period and the slowly rising CBG concentration in both treatments, FCI demonstrated a significant elevation post-operatively that declined as cortisol normalized, and CBG continued a parallel rise for both treatment groups ($P < 0.001$, Figure 9). However, despite the differences in early total cortisol concentrations, there was no significant treatment effect ($P = 0.34$) or time by treatment interaction ($P = 0.29$, Figure 10).

Fibrinogen

Interestingly, fibrinogen concentrations slowly declined over the observation period during the sham phase ($P < 0.001$), despite the lack of induced inflammation in either group, and as expected there were no treatment ($P = 0.52$) or time by treatment interactions ($P = 0.96$, Figure 11).

During the surgery phase, fibrinogen concentration displayed a rise and plateau between 4-hr and 144-hr post-operatively with a significant time effect ($P < 0.001$, Figure 12), however there was no treatment effect ($P = 0.56$, Figure 13) or time by treatment interaction ($P = 0.43$).

Haptoglobin and MMP 9

During the sham procedure, Hp concentrations exhibited a peak at 72-hr with an overall time effect for all cattle (P = 0.002), but no treatment (P = 0.42) or time by treatment interaction (P = 0.39, Figure 14). Hp-MMP 9 complex concentration likewise, exhibited brief spikes in concentration at 24-hr and 144 – 168-hr during the sham procedure with an overall time effect (P < 0.001), but no treatment (P = 0.15) or treatment by time interaction (P = 0.36, Figure 15).

During the surgery procedure, Hp concentration were observed to have both time effects (P < 0.001) and time by treatment interaction (P < 0.001), but not a treatment effect (P = 0.66, Figure 16). Placebo-treated control animals had significantly higher Hp concentrations are 72 and 96 hours after surgery (P = 0.015 and <0.001, respectively).

Hp-MMP 9 complex concentration, displayed a similar pattern with a peak in concentration around 48-hr for both treatment groups (Figure 17) with a significant time effect and time by treatment interaction (P < 0.001 and P = 0.012, respectively), but no difference between treatments (P = 0.5317). Control cows had a significantly lower Hp-MMP 9 complex concentration at 72 hours post-surgery. Hp-MMP 9 complex concentrations in all cows over time displayed a significant time effect (P < 0.001, Figure 18). Complex concentrations at hours 8, 12, 24, and 48 were significantly higher in all cows following surgery.

Discussion

This study supports the hypothesis that meloxicam is effective in reducing pain and inflammation following soft tissue surgery. The increase in PGE2, cortisol and haptoglobin in the control animals indicate a response to the acute inflammation and pain following an elective laparotomy with prophylactic omentopexy. Meloxicam reduced these markers at varying time points after surgery in treated animals.

Surgical Model

The surgical model chosen for this study was a standing right flank laparotomy with prophylactic omentopexy (simulated correction of an LDA). This model accurately reproduces the sharp, acute pain associated with a surgical procedure as well as the inflammatory pain experienced during the healing process. All the animals enrolled in the present study recovered without complication with the exception of one. This animal developed an intestinal entrapment associated with the omentopexy and was subsequently exclude from the statistical analysis on the basis of the exaggerated inflammatory response she was perceived to be experiencing. An additional animal developed a new intramammary infection during the study and was excluded as well on the basis of the confounding inflammatory process. Cattle enrolled in the present study can be characterized as early to mid-lactation (DIM: 50 – 188 days, median: 121 days). Naturally occurring displaced abomasa often occur at higher incidence earlier in lactation, classically within the first 14 DIM and frequently occur as the

result of or along with concurrent co-morbidities such as ketosis, hypocalcemia, retained fetal membranes, metritis or mastitis. Likewise, cattle with disease processes indicating surgical intervention, such as dystocia requiring Cesarean delivery, may be in an altered physiologic state compared to the cattle represented in the present study. Whether these co-morbidities would have confounded the results demonstrated here is not known, but it probable that NSAID therapy, such as meloxicam, would provide therapeutic benefit for cattle experiencing these types of systemic inflammatory responses. Regardless, these factors should be considered when comparing the results observed here to clinical applications.

PGE2

The reduction of prostaglandins by NSAIDs is the main method of analgesia and anti-inflammatory effects produced by these drugs. PGE2 is one product of the Arachidonic Acid pathway and is upregulated by the COX-2 enzyme increases during inflammation. For this reason, PGE2 is expected to decrease when the patient is given a COX-2 inhibitor such as meloxicam. This has been demonstrated in several studies prior to the current one. Meloxicam significantly reduced PGE2 concentrations in blood and synovial fluid of dogs treated with meloxicam for 21 days. This is consistent with the suppression of the COX-2 enzyme (Jones, Streppa, Harmon, & Budsberg, 2002).. In a human study using whole blood and microsomal assays, meloxicam preferentially inhibited human COX-2 at 0.01 to 1 µmol/L but was as potent of an inhibitor of both COX-

1 and COX-2 at higher concentrations. In the microsomal assay, meloxicam was again highly selective for COX-2 (Churchill et al., 1996). Finally, in a study of cautery dehorning in calves, meloxicam treated calves had a significantly lower PGE2 level than control calves (Allen et al., 2013). The present study follows these same principles of PGE2 reduction in response to meloxicam. The reduction of PGE2 was significant at 2- and 8-hours following surgery. Some studies even suggest that spinal PGE2 may be responsible for the increased excitability in the dorsal root leading to hyperalgesia (J.F. Coetzee, 2011). Because PGE2 is a known inflammatory mediator and cause pain, regulating the magnitude of this response mitigates pain.

Inhibition of prostaglandin and prostacylin production is the primary target of NSAID therapy. Reduction in these products, reduces the downstream mediation of inflammation and pain. PGE2 is one product of the arachidonic acid pathway and is increased upon induction of the cyclooxygenase-2 (COX-2) enzyme during inflammation. For this reason, PGE2 is expected to decrease when the patient is given a COX-2 inhibitor such as meloxicam. In fact, multiple COX-1 and COX-2 inhibitors such as, acetylsalicylic acid, flunixin meglumine and a celecoxib have been shown to reduce PGE2 production in *in vitro* isolated bovine peripheral mononuclear cells (Myers et al., 2010). Because PGE2 is a well described marker of inflammatory pain, regulating the magnitude of this response mitigates pain. Moreover, it has been suggested in cattle that spinal PGE2 may play a role in increased excitability in the dorsal root of the spinal

cord, leading to hyperalgesia, therefore systemic administration of meloxicam and reduction of incipient PGE2 production may decrease the overall transduction and perception of pain (Coetzee, 2011).

In the present study, meloxicam suppressed the synthesis of PGE2 at 2- and 8-hours following surgery. Although the plasma kinetics of meloxicam were not directly measured in these animals, the reduction in PGE2 concentrations parallel previously reported pharmacokinetics data following subcutaneous administration of meloxicam. Other studies have demonstrated meloxicam C_{max} was reached between 6 – 8 hours following administration. Allen et al (2013) observed a decline in PGE2 concentrations in dairy calves treated with oral meloxicam at the time of cautery dehorning that extended out 48 hr. after the procedure. Likewise, cattle subjected to a tissue cage implantation model, demonstrated a 48 hr. reduction in PGE2 in the cage exudate when treated with meloxicam following a sterile inflammatory stimulus. Other studies evaluating meloxicam however, have not observed a concomitant decrease in PGE2 following the induction of similar painful or inflammatory conditions, perhaps indicating the timing of meloxicam administration or the severity of the stimulus is relevant to induction of COX-2 enzyme (Fraccaro, 2013).

Cortisol

Cortisol has been studied as a marker of the stress response associated with pain with several models including dehorning and castration. It is an indirect measure of the HPA axis and autonomic nervous system activation. Two studies

evaluating the impact of meloxicam on cortisol concentration following cautery dehorning demonstrated a peak in cortisol within 4 – 6 hours after the procedure. This time frame suggests the pain response and activation of the HPA axis is delayed until the effect of local analgesics had dissipated. Both groups in each study had the benefit of local anesthesia yet, in spite of the immediate effect on cortisol concentration in both groups, calves treated with meloxicam continued to demonstrate significantly lower cortisol responses compared to non-treated calves. (Allen et al., 2013; Heinrich et al., 2009). In the present study, peak cortisol concentration for placebo-treated control cows at 4 hours post-operatively displayed a similar pattern, indicating a similar delay in induction of the stress response after return of sensation to the previously anesthetized area. Meloxicam treatment, however, abrogated the rise in cortisol during that time frame and treated cattle maintained significantly lower cortisol concentration. This suggests that, in addition to the pain mitigation of local anesthesia, meloxicam alleviated pain sufficiently to blunt the stress response in treated cattle.

CBG is the major binding protein of cortisol in the blood. In the face of inflammation, CBG concentrations decrease in response to an increase of elastase from activated neutrophils (Lewis & Elder, 2014). The degradation of CBG releases bound cortisol, making it available at the site of inflammation, and increasing free cortisol concentrations (Bladon et al., 1996). However, because free cortisol has a short half-life of approximately 2 minutes and is rapidly

metabolized, total cortisol concentrations may be unchanged or underestimated when CBG is low (Trevisi et al., 2013). For this reason, a higher than normal total cortisol can be assumed to be correct, whereas a lower cortisol may not be an accurate assessment of inflammation and ongoing stress.

In this study, CBG was not significantly different between groups but did increase over time after surgery. As a negative acute phase protein, CBG would have been expected to decrease throughout the observation period. A rising CBG concentration is contrary to the currently established interactions of CBG and the inflammatory process. In humans evaluated post-operatively or during sepsis, CBG characteristically decreases in response to inflammation (le Roux et al., 2003: Bladon et al., 1996; Ingenbleek & Young, 1994). In these reports, CBG measurement was made immediately post-operatively and may not accurately reflect ongoing changes in those patients. Relevant examples of the change in CBG in response to inflammation over time in cattle are limited, but Sharma et al. observed a static to slightly increasing CBG concentration during the clinical phase of anaplasmosis in cattle. (Sharma, 1986) When considered with results from the present study, it suggests that CBG may have different induction stimuli and more research into CBG alternations during inflammation and surgery are warranted.

The free cortisol index is the ratio of total cortisol to CBG. This ratio permits the assessment of cortisol that is biologically active in the context of variations in CBG and reflects free cortisol more accurately than total cortisol

concentrations (Dhillo et al., 2002). In the present study, FCI was did not significantly differ between groups. However, there was a trend toward increased FCI in non-treated cattle at 4 hours post-operative, paralleling a rise in total cortisol at the same time point. In the face of concurrent static CBG concentrations, this suggests a rise in free cortisol and stronger induction of the HPA axis in non-treated cattle compared to meloxicam treated cattle.

Fibrinogen was not significantly different between treatment groups, however there was a significant interaction between concentration and time. Despite an elevation for both treatment groups between 4 – 120 hours, all cattle remained within or below established reference ranges (300-800 mg/dL). This finding is consistent with other studies where Fb was not elevated in cattle affected with displaced abomasa and surgical correction. McSherry et al in a review of Fb concentrations in cattle following DA correction, animals that developed post-operative complications such as peritonitis where the only individuals with concentrations above reference ranges, while the majority of cattle remained normal. (McSherry et al., 1970) This suggests that the inflammation associated with laparotomy alone is insufficient to induce a profound Fb response. Additionally, a retrospective analysis of cattle presenting with naturally occurring dystocia or right- or left- sided DA, surgical intervention did not influence Fb concentration. (Hirvonen & Pyorala, 1998). In the present study, with the exception of one animal whose data were discarded for analysis,

there was minimal sequalae associated with surgery and perhaps limited induction of Fb.

Haptoglobin is a positive APP produced in the liver. In healthy adults, Hp is present at less than 100 µg/mL. However, in acute inflammation, Hp concentration can greater than 100-fold within 48 to 96 hours (Bannikov et al., 2011). The limitations of Hp as a diagnostic indicator include a lack of specificity as to the source of inflammation and extended half-life with limited discrimination between acute and chronic inflammation (Bannikov et al., 2011). A study by Mainau et al. (2014) found that the administration of meloxicam to naturally calving dairy cattle, not experiencing dystocia, had no effect on Hp concentration (Mainau et al., 2014). The lack of effect was, in their estimation, due to an imprecise timing of administration in relation to the delivery process and an overall lack of inflammatory response in normal births. In addition, they concluded that cattle experiencing dystocia may still benefit from meloxicam treatment, despite a lack of response in normal births. In the present study, Hp concentrations were significantly increased at 72 and 96 hours after surgery in placebo-treated control cows compared to meloxicam treated cows. All animals were evaluated daily by blinded, experienced personnel and consistently displayed low clinical illness scores, suggesting elevations in Hp were solely induced by the experimental laparotomy and was significantly reduced by the administration of meloxicam.

The Hp-MMP 9 complex is found specifically in activated neutrophils and is release during acute inflammation. Bannikov et al. (2011), evaluated the diagnostic specificity of Hp, MMP-9 and the Hp-MMP 9 complex in correlation with the severity of naturally occurring diseases. Hp concentration was increased in cows with both acute and chronic inflammatory conditions, while un-complexed MMP-9 concentration varied greatly and was not strongly correlated severity of inflammation or Hp concentration. The combined Hp-MMP 9 complex however, was elevated in acute inflammatory conditions but not in more chronic cases and therefore, was a more accurate predictor of timing of the response than each analyte independently. In the current study, the Hp-MMP 9 complex concentration was significantly lower at 72 hours for control cows, in contrast to the predicted outcome, although all cattle demonstrated a significant time effect at 8, 12, 24 and 48 hours. These observations indicate that the experimental surgical procedure did induce complex formation, but was not affected by meloxicam administration.

Conclusion

Although NSAIDs, specifically meloxicam, are known to have analgesic effects in dairy cattle, there are limited options for alleviating pain in livestock specifically, for post-operative pain or in lactating dairy cattle. Our results demonstrate that administration of meloxicam at a dose of 0.5 mg/kg SQ abrogated relevant markers of pain and inflammation, namely cortisol,

haptoglobin, and PGE2, in lactating dairy cattle following soft tissue surgery compared to placebo-treated control animals.

The attributes of the elective surgical model described here include uniformity in the induction of the pain and inflammatory stimulus and a more controlled temporal comparison of treatments. Collectively, these suggest that laparotomy with prophylactic omentopexy a promising model for the study of post-operative pain in cattle. Pain and inflammation are common responses to surgical procedures in cattle, and this model provides an avenue to investigate other therapeutic techniques in the future.

Chapter 2
Non-Invasive Assessment of Pain Mitigation in Cattle Following Soft Tissue Surgery

Amber F. Moore, DVM[1]; Marc Caldwell, DVM, PhD[1,*]; Brian K. Whitlock, DVM, PhD[1]; Lew Strickland, DVM, MS[2]; Peter Krawczel, MS, PhD[2]; Liesel Schneider, PhD[2]; David E. Anderson, DVM, MS[1]

[1] Department of Large Animal Clinical Sciences, University of Tennessee, Knoxville, TN 37996

[2] Department of Animal Science, University of Tennessee, Knoxville, TN 37996

* Corresponding author: mcaldwell@utk.edu

Abstract

Mitigation of pain for surgical procedures has become a topic of concern for the public, producers, and veterinarians. The objective of this study was to determine the efficacy of meloxicam for pain mitigation in adult lactating dairy cattle following a right-side laparotomy with omentopexy. Twenty-four dairy cattle (mean age: 2.51 +/- 0.54 years), between 50 and 188 days in milk (median: 117 days +/- 43.15 days) were enrolled. Cattle were administered a 7-day acclimation period to the new environment and social hierarchy and assigned blocks based on parity, days in milk, milk yield, and pregnancy status, and randomly allocated to groups Meloxicam (MEL) or placebo treated control (CON). The study had two phases; sham (day 0-14) and surgery (day 15-28). The objective of the sham phase was to collect baseline behavioral and physiologic data and permit cows to become acclimated to human intervention during the intensive sampling periods. On day 0, cattle were prepared for surgery including local blocks with lidocaine. Injectable meloxicam (MEL) or saline placebo (CON) was administered (dose:

0.5 mg/kg) 5 minutes before simulated surgery (restraint for 30 minutes) and then returned to their home pen for data collection. On day 15, after a 14-day washout period, the surgical procedure was performed. Meloxicam and saline were administered prior to surgery to each respective group. A right flank laparotomy, brief abdominal exploration, and omentopexy was performed on all animals. Mean nociceptive threshold (MNT) was measured using an algometer and collected at hours 0, 1, 4, & 8 after sham and hours 0, 1, 2, 4, 8, 12, 24, 36, 48, 60, & 72 after surgery. Infrared thermography (IRT) was taken of the incision site at hours 0, 1, 4, & 8 hours after sham and 0, 2, 4, 8, 12, 24, 36, 48, 60, & 72 after surgery. There was no difference for MNT (P=0.24) or IRT (P=0.68). This study indicates that meloxicam does not significantly affect these measures and that the use of these technologies need to be studied further for its usefulness in accessing pain in cattle.

Introduction

Cattle are frequently subjected to painful procedures related to health interventions or production management. These may include dehorning, castration, or surgical correction of displaced abomasum and caesarean sections. Current practitioner and producer surveys indicate changing perspectives on the inherent benefit of pain mitigation for livestock species. This underscores the need for evidenced based recommendations and FDA approved medications. At present, there are no treatments approved for the alleviation of

pain due to soft tissue surgery in the US. Recently a transdermal flunixin meglumine product was approved for use in treating pain associated with foot rot in beef cattle and dairy calves, but is not approved for adult dairy cattle greater than 20 months of age.

A major roadblock in evaluating the efficacy of pain management under experimental conditions is the prey nature of cattle. Cattle demonstrate a profound behavioral modification involving the masking signs of pain and distress in order to appear fit in the presence of danger. Therefore, accurate assessment of pain can be difficult and requires specialized equipment and training to detect subtle behavioral cues. The cattle industry needs innovative, noninvasive methods of detecting pain and distress in cattle. Two possible methods are mean nociceptive thresholds and infrared thermography.

Mechanical Nociceptive Threshold (MNT) is the mean threshold at which an animal will respond to a painful stimulus. Another interpretation is the maximum pain stimulus an animal will endure before altering its behavior. MNT is measured by pressure algometry were and observer consistently applies increasing force applied to specific tissues. When a pain tolerance threshold is breached, the stimulus induces a withdrawal or avoidance response. This threshold can be highly modulated based on factors within the tissue such as trauma as well as external factors such as physiologic and psychologic states as well as the use of desensitizing medications. Following a surgical disruption of tissue, inflammation is induced at the surgical site and is a necessary precursor

to healing. An exaggerated or uncontrolled inflammatory process can potentially create more sensitivity and pain. Therefore, MNT can provide an objective assessment of the nociceptive threshold and provide a useful means of determining the efficacy of pain therapy.

MNT has been used primarily in the detection of lameness and monitoring sensitivity of hoof lesions in cattle. Laven et al described differences in MNT comparing corrective trimming and/or NSIAD therapy on allodynia inducing hoof lesions in dairy cattle (Laven et al., 2008). Raundal et al (2014) later compared the accuracy and precision of hand held algometers in loose-housed dairy cows and concluded that these devices poor reproducibility between observers, suggesting that future studies habituate cows prior to application (Raundal et al., 2014) . In a follow up study, pre-habituation prior to MNT testing, such stroking in an attempt to remove fear of the applicator and anticipation, increased the reliability of the test in dairy cattle (Raundal et al., 2015). These studies indicate that while MNT can be useful to assess the pain threshold in cattle, attention to the experimental procedure and technique is necessary to produce valid measurements.

Infrared thermography is an imaging technique that detects radiation in the long-infrared spectrum and translates that data into a color map or thermogram. The amount of radiation emitted by a surface is affected by its intrinsic temperature. In warm-blooded animals, physiologic changes, such as inflammation or infection, that alter vascular resistance and increase blood flow

to a tissue, subsequently increase the surface temperature of the overlying cutaneous tissues. These changes can be monitored and provide a non-invasive tool for clinical assessment. In cattle, thermography has been used in the detection of hoof lesions (Stokes et al., 2012), to estimate tick infestation on cattle (Barbedo et al., 2017), in the detection of diseases(Schaefer et al., 2012) (Polat et al., 2010), to estimate live bull weight (Stajnko, Brus, & Hočevar, 2008), to determine body condition score in dairy cattle (Halachmi, Klopčič, Polak, Roberts, & Bewley, 2013), to measure stress in dairy cattle (Stewart et al., 2007), and in monitoring health and welfare of dairy cattle (Stewart, Wilson, Schaefer, Huddart, & Sutherland, 2017).

The objectives of the present study were to: 1) evaluate the efficacy of MNT and IRT in assessing pain and inflammation following elective right flank laparotomy with prophylactic omentopexy in adult lactating dairy cattle and; 2) use MNT and IRT to determine the efficacy of meloxicam in reducing pain and inflammation following soft tissue surgery in adult lactating dairy cattle. Our hypothesis was that an experimental laparotomy will induce sufficient inflammation to reduce the MNT and increase the skin surface temperature adjacent to the laparotomy incision. A secondary hypothesis was that the administration of meloxicam will significantly reduce the pain and inflammation induced by the procedure such that MNT and IRT will discriminate between treated and non-treated control animals.

Material and methods

The University of Tennessee Institutional Animal Care and Use Committee approved all experimental procedures under the supervision of the university veterinarian (Protocol # 2246-0314).

The study was conducted in two phases. Animals were first subjected to a sham surgical procedure to ensure habituation to the intensive sampling procedures followed by application of the surgical procedure after a 14-day wash out period. Data are presented in relationship to each phase.

Animals

Twenty-four adult dairy cattle, greater than 20 months of age (mean age: 2.51 +/- 0.54 years), were housed at the East Tennessee Research and Education Center - Little River Dairy Animal and Environmental Unit (Latitude: 35.772115; Longitude: -83.850182) during the months of June, July and August and were maintained in an ambient temperature. Animals were between 50 and 188 days in milk (median: 117 days +/- 43.15 days) and weighed between 512 kg and 705 kg (mean: 596.4 kg +/- 41.8 kg). Cows were allocated into two equal groups: Meloxicam (n = 12; MEL) and Control (n = 12; CON). Groups were balanced based on days in milk (Median: 111 days MEL; 127 days CON; Range: 50 – 188 days), milk yield (Mean: 74.0 lbs. MEL; 77.5 lbs. CON; Range: 57.6 – 93.1 lbs.), and pregnancy status (n = 2 MEL; n = 2 CON). Exclusion criteria for

enrollment in the study included clinical signs associated with potentially systemic inflammation (i.e. mastitis, metritis, ketosis, lameness, etc.) or history or evidence of previous abdominal surgery. All animals were considered healthy based on a physical exam performed by a veterinarian 1 day prior to the start of the study.

Cattle were housed in a free stall barn approximately 20 m x 12.5 m (800 sq. m) with 24 sand-bedded stalls and 32 headlock stanchions. Stocking density was maintained at 75% and did not exceed 100% during the study (lowest was 22 cows per 24 stalls = 91%). Cattle were milked twice daily (at approximately 7:30 AM and 4:30 PM). Milk was discarded throughout the entire of the experiment. A total mixed ration (TMR) was fed ad libitum during the study and was formulated according to Nutrient Research Council requirements to meet or exceed nutrient requirements of lactating dairy cattle. Nutrient analysis of TMR was conducted prior to and upon completion of the study. Fresh TMR was prepared and dispensed twice daily in parallel with milking. Twice daily at approximately 12:00 PM and 6:00 PM residual TMR was pushed up the headlocks. Waste TMR was collected twice daily immediately prior to the dispensing of each new feeding and weighed to determine pen level feed intake. Throughout the study animals were also provided ad libitum access to water. The cattle were permitted 7 days prior to the initiation of the study to acclimate to the new environment and social hierarchy. During this time, the cattle were also

acclimated to handling via halters and grooming to simulate the contact associated with the intense sampling periods.

Phase 1: Sham Procedure

In order to determine the effect of the intense sampling scheme and experimental assessments alone without the influence of the surgical procedure, a sham experimental phase was imposed on the cattle. On day -1, a 14-gauge X 13 cm polyurethane IV jugular catheter (MILACATH-Extended Use, Mila International, Inc. Florence, KY) was placed and were maintained with heparinized saline until 4 days after the procedure, at which point they were removed and subsequent samples taken via direct venipuncture.

On day 0, a sham procedure was performed. Cows were prepared for sham surgery by clipping and sterilely preparing. Cows were blocked with 2% lidocaine (90 ml or 1800 mg, local tissue infusion, VetOne, MWI Animal Health, Boise, ID) in a line block pattern on their right side approximately 15 cm below the transverse process of the 3rd lumbar vertebrae and 10 cm caudal to the costal arch. Meloxicam (0.5 mg/kg, Metacam®20, Boehringer Ingelheim Ltd., Burlington, Ontario, Canada) was administered SQ in the neck to the meloxicam treatment group (MEL) and saline (0.025 mL/kg, 0.9% Sodium Chloride Injection USP, Hospira, Inc. Lake Forest, IL) was administered SQ in the neck to the control group (CON). Ampicillin trihydrate (10 mg/kg, Polyflex®, Boehringer Ingelheim Ltd., St. Joseph, MO) was administered intramuscularly to all cows on the contralateral side of the neck from the treatment.

Four veterinarians were designated as surgeons during the study (MC, DA, BW, and LS). Six replicates of sham procedures occurred with 4 animals per cohort. Each cohort was equally balanced for MEL and CON cattle (2 MEL and 2 CON). Each surgeon performed six procedures with equal numbers of MEL and CON cattle (3 MEL and 3 CON). A sham surgical simulation was performed with the surgeon standing adjacent to the animal while it was restrained for 30 minutes. Procedure initiation for each cohort was staggered and administration of treatment and sampling time points were relative to each animal's sham procedure start time.

Following the sham procedure, cattle were returned to their home pen for sample collection lasting up to 7 days. Following completion of phase 1, the cattle were provided a 7-day washout period. Therefore, a total of 14 days following administration of the meloxicam or saline placebo elapsed prior to initiation of phase 2.

Phase 2: Surgical Procedure

The methods used for the phase 2 were similar to those executed during phase 1 with the exception of preforming right flank laparotomies with prophylactic omentopexy for all cattle. Briefly, on day -1 before the surgical procedure, cattle were refitted with heart rate monitors and jugular catheter was placed. As previously, jugular catheters were maintained with heparinized saline through the first 96 hours of the sampling period then removed and subsequent blood samples collected via direct venipuncture. On day 0, approximately 5

minutes prior to the initiation of each animals' surgical procedure, meloxicam (0.5 mg/kg) was administered SQ in the neck to the meloxicam treatment group (MEL) while a saline placebo (0.025 mL/kg) was administered SQ in the neck to the control group (CON). Ampicillin trihydrate (10 mg/kg) was administered intramuscularly to all cows on the contralateral side of the neck from the meloxicam or saline treatment.

The same four veterinarians carried out the surgical procedures in 6 replicates of 4 cows. All treatments were balanced within each cohort and balanced across each surgeon. Each laparotomy, abdominal exploratory and prophylactic omentopexy were performed according to a standardized protocol. The right paralumbar fossa was clipped, the skin was aseptically prepared, and a line block with lidocaine (120mL of lidocaine) was performed as previously described. After aseptic preparation, a 15 cm vertical incision was made starting 10-cm caudal to the caudal curvature of the last rib and 15 cm ventral to the transverse process of the 3rd lumbar vertebrae. The incision progressed through the skin, external abdominal oblique muscle, internal abdominal oblique muscle, transversus abdominus muscle, and peritoneum. The surgeon then placed the left arm into the abdomen and briefly explored the abdomen to identify and palpate the rumen, omentum and abomasum. Similar to the techniques described in Turner and McIlwraith's Techniques in Large Animal Surgery (2013), a standard omentopexy was performed by suturing the omentum, peritoneum and transversus closed using #2 polyglactin 910 (Vicryl, Ethicon, Inc. Somerville,

NJ) in a simple continuous pattern. The external abdominal oblique muscle and internal abdominal oblique muscle were also closed using #2 polyglactin 910 in a simple continuous pattern. Finally, the skin was closed using #4 nylon (Supramid Extra II. S. Jackson, Inc. Alexandra, VA) in a continuous interlocking pattern.

MNT

Mechanical nociceptive threshold was measured using a pressure algometer (Wagner Mark-10 M3, Wagner Instruments, Greenwich, CT) using a conical steel tip (FD/S-3, Wagner Instruments, Greenwich, CT, Figure 19). During the sham phase, MNT was measured at hours 0, 1, 4, and 8 respective to time 0 hours beginning immediately prior to the sham procedure and during the surgical phase at hours 0, 1, 2, 4, 8, 12, 24, 36, 48, 60, and 72 hours respective to time 0 hr. prior to the surgical procedure. For each time point and for both phases, the right flank adjacent to the mock or actual incision was divided into 6 test locations (Figure 20). Cows were randomly assigned to one of two starting positions where measurements were initiated. These were either the cranial group, where the sequence of measurements proceeded as 1, 2, 3, 4, 5, 6 or the caudal group where the sequence proceeded 4, 5, 6, 1, 2, 3. The assignment and sequence remained constant for each cow and all MNT measurements and were balanced across treatment groups. Immediately prior to measurement, the cattle were restrained using a halter or headlocks within the pen. The observer placed a hand gently on the incision to habituate the cow to their presence before the measurement was taken. The algometer was applied at a steady force of

approximately 1 kgf/s to each of location until the cow presented an avoidance response. These responses included movement away from the pressure, tail flicking, ear twitching, looking back and kicking. All MNT measurements were carried out by the same individual who was blinded to treatment.

IRT

Thermograms were obtained of each cow at hours 0, 1, 4, and 8 hours beginning immediately prior to the sham procedure and at hours 0, 1, 2, 4, 8, 12, 24, 36, 48, 60, and 72 hours beginning immediately prior to the surgical procedure. Cows were restrained using a halter or headlocks located within the pen. The camera (Med2000^{TM}, Meditherm, Inc. Fort Myers, FL) was positioned approximately one-meter distance from the incision so that the entire incision and paralumbar fossa was included in the image. Triplicate images were taken for each time point and saved for future analysis. Using the accompanying software (IRIS 7.5, WinTes II, Meditherm, Inc. Fort Myers, FL), the incision was isolated from the image using the programs cropping function, and the maximum, average, and minimum temperatures were recorded for each thermogram.

Statistical analysis

All statistical analyses were performed in Statistical Analyses System (SAS Version 9.1: SAS Institute Inc., Cary NC; 1991-2001). The level of significance was established to be $P< 0.05$.

Normality test of the data and residuals was performed for each indicator. MNT was normally distributed following log transformation then was analyzed using the GLIMMIX procedure. All sites were averaged to give one reading per time period to get the average reading. During the analysis, hours 0 and 1 were removed from the time by treatment interaction. Animal ID and cranial site vs caudal sites were used as a random effect. Surgeon and surgery block were analyzed as random effect but had no significant effect and were removed from the model. Fixed effects included treatment group. Time was used as a repeated measure. Next, the three cranial sites and three caudal sites were divided, averaged and analyzed. Tukey adjustment was used for MNT.

Maximum, mean and minimum temperatures for IRT were normally distributed. Fixed effects included treatment group. Time was the repeated measure. Animal ID was used as a random effect. Protected LSD was used for adjustment. Surgeon and time of surgery (surgery block) was analyzed as a random effect, but did not have a significant effect on any blood markers and was therefore removed from the analysis.

Results

Two cows were removed from the study and not included in the results. Cows included in the results is n = 22.

Mechanical Nociceptive Threshold

All cattle demonstrated a time effect with a significantly higher tolerance of pressure observed at 0 and 1 hr. following the surgical procedure ($P < 0.001$, Figure 21). When the 0 and 1 hr. time points were excluded from the analysis in an attempt to isolate responses that occurred without the influence of lidocaine, the significance observed over time was removed ($P = 0.24$, Figure 22). Numerically, cattle treated with meloxicam to demonstrate a higher tolerance of pressure at 24- and 36-hours following surgery, but this effect was not significant ($P = 0.37$). Location with respect to the incision on the MNT demonstrated a significant difference, with the cranial sites requiring less force to elicit a response (lower MNT) than the caudal sites ($P = 0.03$, Figure 23Figure). However, randomization of the start site had no effect on the overall MNT and therefore was not included as a variable in the model.

Infrared Thermography

A significant time effect was observed for the maximum, average, and minimum IRT observations for all animals ($P< 0.001$ for each corresponding variable, Figure 24). There were no significant differences observed between treatments or time by treatment interaction of the maximum skin surface temperatures ($P = 0.46$ and 0.71 respectively, Figure 25). Cattle administered meloxicam had slightly lower average and minimum surface temperatures at 2 hours following the surgery, but an overall treatment and time by treatment effect was not observed ($P = 0.91$ and 0.67; $P = 0.42$ and 0.63, respectively, Figure 26

and Figure 27). Table 1 displays the values for maximum, mean, and minimum temperatures.

Discussion

In the present study, pressure algometry and IRT were used to evaluate pain sensitivity and inflammation following an elective right flank laparotomy with prophylactic omentopexy. Each technology successfully detected changes over time compared to baseline observations. The administration of meloxicam however, did not alter pain sensitivity or skin surface temperature sufficiently to discriminate between treated and non-treated cattle.

In our study, the meloxicam treated cattle had significantly higher MNT recordings than control cattle at baseline and 1-hour post-operative. This could be the result of type 1 error, despite efforts to randomize cattle to groups. The baseline measurements were taken prior to the administration meloxicam and lidocaine and based on the reported pharmacokinetics of meloxicam, it's doubtful therapeutics concentrations were present at 1-hour post-administration. When these time points were removed from the analysis, no significance was found at all other clinically relevant time points. However, the time effect for all animals does demonstrate that the surgical model created surgical and inflammatory pain that was quantifiable using algometry.

Another finding in the present study was the differences observed between sites cranial and caudal to the incision. We believe this is due to the severing of the superficial innervation and loss of sensory input in the caudal

area as the incision was made. Although innervation was not completely removed as evidence by reduced MNT for all caudal measurements compared to baseline, the fact that some sensation was removed is interesting and selection of response sites should be considered carefully for future studies.

Several studies have evaluated MNT changes in cattle following dehorning.(Allen et al., 2013; Heinrich et al., 2010; Stock et al., 2016; Tapper et al., 2011) A common experimental design of these studies has been to collect baseline observations prior to induction of the painful procedure, as has been done here. In each of these studies, a significant reduction in MNT was observed from baseline samples prior to dehorning compared to post-dehorning observations. Another frequent design aspect is to maintain an independent cohort that receives a sham procedure concurrently with animals receiving the actual procedure. In the study presented here, the sham procedure was conducted on the principal animals 2 weeks prior to initiation of the surgical procedure. The advantages of this approach are the habituation of the animal to sample collection and the direct comparison of results within the same animal. Both approaches provide consistent application of the methodology and comparison between affected and non-affected groups.

It appears that pressure algometry and MNT are most useful in the presence of inflammation and active lesions. This bears true when the technique has been applied to naturally occurring and experimentally induced lameness. Whay et al. (1997) found a significant correlation between the severity of visually

assessed lameness scores and pressure sensitivity in primiparous Holstein heifers (Whay et al., 1997). Cutler et al (2013) demonstrated that digital dermatitis lesions could be accurately identified by MNT as active, healing, or healed lesions (Cutler et al., 2013). Likewise, Mohling et al. (2014) observed a reduction of as much as 50% MNT in sows being subjected to chemically induced lameness (Mohling et al., 2014). On the other hand, Wheeler et al (2013) found that the probability of healthy calves to respond to pressure externally applied to joints did not correlate with other indicators of inflammation and therefore pressure algometry was not recommended for the assessment of lameness in young calves (Wheeler et al., 2013).

Millman (2013) discusses the need to refine this technique by providing proper restraint and blind folding calves to avoid anticipation and fear-based responses. This would lead to more reliable testing for both intraobserver and interobserver measurements. Raundal et al. found a high level of individual animal variation and low agreement of MNT readings between observers (Raundal et al., 2014). Tallant et al., found dogs became accustomed to algometry and tolerance varied by position and observer (Tallant et al., 2016). Blindfolding was not performed in the current study and the only attempt to acclimate and normalize responses was the placement of the observer's hand near the incision. Perhaps had additional efforts been used to remove the anticipatory effects of the approaching observer, greater differences among the treatment groups would have been observed.

Changes in the maximum, mean and minimum skin surface temperatures at the surgical site were observed over time for both treatments, suggesting the successful induction of physiologic mechanisms that influence these temperatures. Maximum and mean temperatures increased from baseline and remained elevated throughout, while the minimum temperatures decreased from the time of surgery. This was likely the result of post-operative inflammation and was an expected response as has been described with IRT mapping of surgical wounds (Celeste et al., 2013).

The administration of meloxicam did not appear to alter the inflammatory process at the level of the incision sufficiently to induce detectable differences in skin temperature. However, there was a numerical trend for treated cows to have lower mean and minimum skin temperatures at 2 hours. In most recent studies, utilizing IRT in cattle, the technique is more often used to monitor periocular temperature and systemic autonomic responses to painful conditions (Coetzee et al., 2012; Schaefer et al., 2012; Stewart et al., 2009; Stewart et al., 2010). In many of these studies, NSAID therapy has reduced periocular temperature and stabilized the autonomic or stress induced response. In the current study, periorbital temperature was not recorded and the impact of the changes in the autonomic nervous system on skin temperatures at the surgical site is unknown, but presumably has limited effect.

An unexpected observation made during the analysis of the thermograms revealed a spurious assignment of low temperatures associated with the suture

material in each incision. Each surgeon left an irregular length of suture tag, where longer tags had an influence on the range and minimum surface temperatures assigned in the region of interest, which could not be excluded. However, these variations as well as other animal independent differences were accounted for in the statistical model during final analysis.

To our knowledge, this is the first study to evaluate the efficacy of meloxicam as post-operative pain management for cattle following a simulated surgical DA repair. Barrier et al. (2014) evaluated beef cattle undergoing cesarean section and found that meloxicam treated cattle had greater lying times in the first 16 hours after delivery. An additional head to head to clinical trial comparing meloxicam and ketoprofen in cattle after rumen fistulation surgery, found evidence of pain after surgery, but no differences between treatments. These studies suggest a limited efficacy of meloxicam in soft tissue surgery, but this appears to be in stark contrast to studies evaluating its efficacy in other painful conditions. For example, multiple studies have revealed a benefit from meloxicam treatment in reducing stress and inflammatory biomarkers, improving pressure tolerance and MNT, and improving behavioral responses of cattle undergoing cautery dehorning, castration, mastitis and transportation. Therefore, care should be used when drawing the conclusion that meloxicam therapy is ineffective for pain mitigation associated soft tissue surgery.

Conclusions

Although a difference between treatment groups was not detected, MNT and IRT are promising noninvasive technologies for inflammatory pain detection. These technologies would be ideal for monitoring pain sensitivity and inflammation following surgical interventions and would be useful in assisting veterinarians and producers in making decision about pain management.

Conclusions

Approximately 470,000 cows undergoing painful surgical procedures every year, producers and veterinarians are seeing the effects of pain first hand. Objective measurements are need to quantify this pain as well as a model to research this pain and analgesic methods.

This study presents several possible methods of measuring pain including blood parameters and non-invasive objective technology. The blood biomarkers that demonstrated the highest degree of difference in meloxicam treated cattle compared to placebo-treated control cattle were PGE2, haptoglobin, and cortisol. Cows that received meloxicam had significantly lower PGE2 levels at 2 and 8 hours after surgery ($P= 0.0044$ and $P=0.0001$, respectively), significantly lower haptoglobin levels are 72 and 96 hours after surgery ($P = 0.0153$ and 0.0002, respectively), and significantly lower cortisol levels at 4 hours after surgery ($P < 0.0001$) than placebo-treated controls. Although fibrinogen showed no significant difference between the groups, fibrinogen concentration did show a time effect ($P < 0.0001$), though these changes never increased over a clinically relevant threshold.

This study also evaluated mechanical nociceptive threshold and infrared thermography. MNT measurements in cattle treated with meloxicam trended to demonstrate a higher tolerance of pressure at 24- and 36-hours following surgery, but this effect was not significant ($P = 0.3710$). Cattle administered meloxicam had slightly lower mean and minimum skin surface temperatures at 2

hours following the surgery, but an overall treatment and time by treatment effect was not observed (P = 0.9144 and 0.6736; P = 0.4186 and 0.6277, respectively). While these non-invasive methods did not detect a difference between the treatment groups, it did detect a change in the post-operative period, confirming that this surgical model does in fact create inflammation and pain that is quantifiable. These observations are consistent with the routine inclusion of these technologies in other pain studies and underscores the relevance of their use in pain studies in cattle.

This study demonstrates meloxicam provides analgesia for dairy cattle undergoing soft tissue surgery. Moreover, this study demonstrates that cattle experience pain and should be provided multi-modal analgesia for painful procedures. As responsible advocates of agriculture and animal welfare, it is the animal agriculture community's duty to see that these animals are treated appropriately. We should be at the forefront of these issue and determining the best way to provide the best well-being to these animals based on sound scientific research.

More research is needed to advance pain management for cattle. Because these animals are stoic and difficult to assess pain in, new and innovative methods of detecting pain are needed. This study has found methods, both blood parameters and technologies, that address this issue. More of these techniques are needed to further improve our ability to assess pain. It also demonstrates using a unique surgical model to create and then assess pain in

dairy cattle. Finally, from this study, not only have we provided a number of assessment tools of pain, but have also confirmed the use of meloxicam as an analgesia. There is a major need for research to prove that not only meloxicam, but other analgesic methods are not only useful tools but warranted measures both as a single modality or in a multi-modal approach.

References

Alayash, A. I. (2011). Haptoglobin: old protein with new functions. *Clin Chim Acta, 412*(7-8), 493-498. doi:10.1016/j.cca.2010.12.011

Allen, K. A., Coetzee, J. F., Edwards-Callaway, L. N., Glynn, H., Dockweiler, J., KuKanich, B., . . . Bergamasco, L. (2013). The effect of timing of oral meloxicam administration on physiological responses in calves after cautery dehorning with local anesthesia. *J Dairy Sci, 96*(8), 5194-5205. doi:10.3168/jds.2012-6251

Animal Medicinal Drug Use Clarification Act of 1994, Http://uscode.house.gov/statutes/pl/103/396.pdf (1994).

Anderson, D. E., & Edmondson, M. A. (2013). Prevention and management of surgical pain in cattle. *Vet Clin North Am Food Anim Pract, 29*(1), 157-184. doi:10.1016/j.cvfa.2012.11.006

Anderson, D. E., & Muir, W. W. (2005a). Pain management in cattle. *Vet Clin North Am Food Anim Pract, 21*(3), 623-635, v-vi. doi:10.1016/j.cvfa.2005.07.002

Anderson, D. E., & Muir, W. W. (2005b). Pain management in ruminants. *Vet Clin North Am Food Anim Pract, 21*(1), 19-31. doi:10.1016/j.cvfa.2004.12.008

Astasio-Picado, A., Escamilla Martínez, E., Martínez Nova, A., Sánchez Rodríguez, R., & Gómez–Martín, B. (2018). Thermal map of the diabetic foot using infrared thermography. *Infrared Physics & Technology, 93*, 59-62. doi:10.1016/j.infrared.2018.07.008

Bannikov, G. A., Hinds, C. A., Rajala-Schultz, P. J., Premanandan, C., Rings, D. M., & Lakritz, J. (2011). Serum haptoglobin-matrix metalloproteinase 9 (Hp-MMP 9) complex as a biomarker of systemic inflammation in cattle. *Vet Immunol Immunopathol, 139*(1), 41-49. doi:10.1016/j.vetimm.2010.08.004

Barbedo, J. G., Gomes, C. C., Cardoso, F. F., Domingues, R., Ramos, J. V., & McManus, C. M. (2017). The use of infrared images to detect ticks in cattle and proposal of an algorithm for quantifying the infestation. *Vet Parasitol, 235*, 106-112. doi:10.1016/j.vetpar.2017.01.020

Barrier, A. C., Coombs, T. M., Dwyer, C. M., Haskell, M. J., & Goby, L. (2014). Administration of a NSAID (meloxicam) affects lying behaviour after caesarean section in beef cows. *Applied Animal Behaviour Science, 155*, 28-33. doi:10.1016/j.applanim.2014.02.015

Bladon, P. T., Rowlands, T. E., Whittaker, J. A., & Oakey, R. E. (1996). Serum cortisol binding capacity measured with Concanavalin A-Sepharose in patients with a recent inflammatory response. *Clinica Chimica Acta, 253*(1-2), 9-20. doi:https://doi.org/10.1016/0009-8981(96)06347-4

Bradford, B. J., Yuan, K., Farney, J. K., Mamedova, L. K., & Carpenter, A. J. (2015). Invited review: Inflammation during the transition to lactation: New adventures with an old flame. *J Dairy Sci, 98*(10), 6631-6650. doi:10.3168/jds.2015-9683

Brown, A. C., Powell, J. G., Kegley, E. B., Gadberry, M. S., Reynolds, J. L., Hughes, H. D., . . . Richeson, J. T. (2015). Effect of castration timing and oral meloxicam administration on growth performance, inflammation, behavior, and carcass quality of beef calves. *J Anim Sci, 93*(5), 2460-2470. doi:10.2527/jas.2014-8695

Carroll, T. B., Aron, D. C., Findling, J. W., & Tyrrell, B. (2011). Glucocorticoids and Adrenal Androgens. In D. G. Gardner & D. Shoback (Eds.), *Greenspan's Basic & Clinical Endocrinology* (Vol. 9e): McGraw-Hill.

Ceciliani, F., Ceron, J. J., Eckersall, P. D., & Sauerwein, H. (2012). Acute phase proteins in ruminants. *J Proteomics, 75*(14), 4207-4231. doi:10.1016/j.jprot.2012.04.004

Celeste, C. J., Deschesne, K., Riley, C. B., & Theoret, C. L. (2013). Skin temperature during cutaneous wound healing in an equine model of cutaneous fibroproliferative disorder: kinetics and anatomic-site differences. *Vet Surg, 42*(2), 147-153. doi:10.1111/j.1532-950X.2012.00966.x

Cessna, J. (2018). U.S. quarterly milk production and related data.

Chan, J. P., Chu, C. C., Fung, H. P., Chuang, S. T., Lin, Y. C., Chu, R. M., & Lee, S. L. (2004). Serum Haptoglobin Concentration in Cattle. *Journal of Veterinary Medical Science, 66*(1), 43-46.

Chandrasekharan, N. V., & Simmons, D. L. (2004). Protein family review: The cyclooxygenases. *Genome Biology, 5:241*.

Churchill, L., Graham, A. G., Shih, C.-K., Pauletti, D., Farina, P. R., & Grob, P. M. (1996). Selective Inhibition of Human Cyclo-Oxygenase-2 by Meloxicam. *InflammoPharmacology, 4*(2), 125-135.

Coetzee, J. F. (2011). A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: Practical implications for cattle production within the United States. *Applied Animal Behaviour Science, 135*(3), 192-213. doi:10.1016/j.applanim.2011.10.016

Coetzee, J. F. (2013). A review of analgesic compounds used in food animals in the United States. *Vet Clin North Am Food Anim Pract, 29*(1), 11-28. doi:10.1016/j.cvfa.2012.11.008

Coetzee, J. F., Mosher, R. A., KuKanich, B., Gehring, R., Robert, B., Reinbold, J. B., & White, B. J. (2012). Pharmacokinetics and effect of intravenous meloxicam in weaned Holstein calves following scoop dehorning without local anesthesia. *BMC Vet Res, 8*, 153. doi:10.1186/1746-6148-8-153

Coetzee, J. F. (2013a). Assessment and management of pain associated with castration in cattle. *Vet Clin North Am Food Anim Pract, 29*(1), 75-101. doi:10.1016/j.cvfa.2012.11.002

Coetzee, J. F., KuKanich, B., Mosher, R., & Allen, P. S. (2009). Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. *Veterinary Therapeutics, 10*(4).

Coetzee, J. F., Lubbers, B. V., Toerber, S. E., Gehring, R., Thomson, D. U., White, B. J., & Apley, M. D. (2008). Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. *American Journal of Veterinary Research, 69*(6), 751-762.

Colak, A., Polat, B., Okumus, Z., Kaya, M., Yanmaz, L. E., & Hayirli, A. (2008). Short communication: early detection of mastitis using infrared thermography in dairy cows. *J Dairy Sci, 91*(11), 4244-4248. doi:10.3168/jds.2008-1258

Cutler, J. H., Cramer, G., Walter, J. J., Millman, S. T., & Kelton, D. F. (2013). Randomized clinical trial of tetracycline hydrochloride bandage and paste treatments for resolution of lesions and pain associated with digital dermatitis in dairy cattle. *J Dairy Sci, 96*(12), 7550-7557. doi:10.3168/jds.2012-6384

Dalla Costa, E., Minero, M., Lebelt, D., Stucke, D., Canali, E., & Leach, M. C. (2014). Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine castration. *Plos One, 9*(3), e92281. doi:10.1371/journal.pone.0092281

Dhillo, W. S., Kong, W. M., Le Roux, C. W., Alaghband-Zadeh, J., Jones, J., Carter, G., . . . O'Shea, D. (2002). Cortisol-binding globulin is important in the interpretation of dynamic tests of the hypothalamic–pituitary–adrenal axis. *European Journal of Endocrinology, 146*, 231-235.

Di Giminiani, P., Sandercock, D. A., Malcolm, E. M., Leach, M. C., Herskin, M. S., & Edwards, S. A. (2016). Application of a handheld Pressure Application Measurement device for the characterisation of mechanical nociceptive thresholds in intact pig tails. *Physiology & Behavior, 165*, 119-126. doi:10.1016/j.physbeh.2016.07.006

Dilda, F., Pisani, L. F., Rahman, M. M., Modina, S., Tessaro, I., Sartorelli, P., . . . Lecchi, C. (2012). Distribution of acute phase proteins in the bovine forestomachs and abomasum. *Vet J, 192*(1), 101-105. doi:10.1016/j.tvjl.2011.05.005

Dunlap, S. E., Kiser, T. E., J.J., V., Rampacek, G. B., R.R., K., & Benyshek, L. L. (1981). Clearance of Serum Cortisol in Suckled and Snonsuckled Postpartum Beef Cows. *Journal of Animal Science, 53*(4).

Dyer, R. M., Neerchal, N. K., Tasch, U., Wu, Y., Dyer, P., & Rajkondawar, P. G. (2007). Objective determination of claw pain and its relationship to limb locomotion score in dairy cattle. *J Dairy Sci, 90*(10), 4592-4602. doi:10.3168/jds.2007-0006

Eckersall, P. D., & Bell, R. (2010). Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *Vet J, 185*(1), 23-27. doi:10.1016/j.tvjl.2010.04.009

Fajt, V. R., Wagner, S. A., & Norby, B. (2011). Analgesic drug administration and attitudes about analgesia in cattle among bovine practitioners in the

United States. *Journal of the American Veterinary Medical Association, 238*(6), 755-767.

FDA. U.S. Department of Health and Human Services Food and Drug Administration Center for Veterinary Medicine. (2006.) "GFI #123-Target Animal Safety-Approval of NSAIDS". https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm052663.pdf

Fink, R. (2000). Pain assessment: the cornerstone to optimal pain management. *BUMC Proceedings*, 236-239.

Fraccaro, E., Coetzee, J. F., Odore, R., Edwards-Callaway, L. N., Kukanich, B., Badino, P., . . . Bergamasco, L. (2013). A study to compare circulating flunixin, meloxicam and gabapentin concentrations with prostaglandin E(2) levels in calves undergoing dehorning. *Res Vet Sci, 95*(1), 204-211. doi:10.1016/j.rvsc.2013.01.018

Guzelbektes, H., Sen, I., Ok, M., Constable, P. D., Boydak, M., & Coskun, A. (2010). Serum Amyloid A and Haptoglobin Concentrations and Liver Fat Percentage in Lactating Dairy Cows with Abomasal Displacement. *Journal of Veterinary Internal Medicine, 24*, 213-219.

Hager, C., Biernot, S., Buettner, M., Glage, S., Keubler, L. M., Held, N., . . . Bleich, A. (2017). The Sheep Grimace Scale as an indicator of post-operative distress and pain in laboratory sheep. *Plos One, 12*(4), e0175839. doi:10.1371/journal.pone.0175839

Halachmi, I., Klopčič, M., Polak, P., Roberts, D. J., & Bewley, J. M. (2013). Automatic assessment of dairy cattle body condition score using thermal imaging. *Computers and Electronics in Agriculture, 99*, 35-40. doi:10.1016/j.compag.2013.08.012

Hanthorn, C. J., Dewell, G. A., Dewell, R. D., Cooper, V. L., Wang, C., Plummer, P. J., & Lakritz, J. (2014). Serum concentrations of haptoglobin and haptoglobin-matrix metalloproteinase 9 (Hp-MMP 9) complexes of bovine calves in a bacterial respiratory challenge model. *Bmc Veterinary Research, 10*. doi:10.1186/s12917-014-0285-5

Heinrich, A., Duffield, T. F., Lissemore, K. D., & Millman, S. T. (2010). The effect of meloxicam on behavior and pain sensitivity of dairy calves following cautery dehorning with a local anesthetic. *J Dairy Sci, 93*(6), 2450-2457. doi:10.3168/jds.2009-2813

Heinrich, A., Duffield, T. F., Lissemore, K. D., Squires, E. J., & Millman, S. T. (2009). The impact of meloxicam on postsurgical stress associated with cautery dehorning. *J Dairy Sci, 92*(2), 540-547. doi:10.3168/jds.2008-1424

Hendrickson, D. A., Baird, A. N., & Turner, A. S. (2013). *Turner and McIlwraiths techniques in large animal surgery*. Ames (Iowa): John Wiley & Sons.

Hirvonen, J., & Pyorala, S. (1998). Acute-phase Response in Dairy Cows with Surgically-treated Abdominal Disorders. *The Veterinary Journal, 155*.

Hiss, S., Mielenz, M., Bruckmaier, R. M., & Sauerwein, H. (2004). Haptoglobin Concentrations in Blood and Milk After Endotoxin Challenge and

Quantification of Mammary Hp mRNA Expression. *Journal of Dairy Science, 87*(11), 3778–3784.

Ingenbleek, Y., & Young, V. (1994). Transthyretin (Prealbumin) in health and Disease: Nutritional Implications. *Annual Reviews Nutrition, 14*, 495-533.

Ison, S. H., Clutton, R. E., Di Giminiani, P., & Rutherford, K. M. (2016). A Review of Pain Assessment in Pigs. *Front Vet Sci, 3*, 108. doi:10.3389/fvets.2016.00108

Jawor, P., Stefaniak, T., Steiner, S., & Baumgartner, W. (2009) Dynamics of Selected Acute Phase Proteins in Surgical Abomasum Reposition in Cows.

Jones, C. J., Streppa, H. K., Harmon, B. G., & Budsberg, S. C. (2002). In vivo effects of meloxicam and aspirin on blood, gastric mucosal, and synovial fluid prostanoid synthesis in dogs. *American Journal of Veterinary Research, 63*(11), 1527-1531.

Kawabata, A. (2011). Prostaglandin E2 and Pain-An Update. *Biological and Pharmaceutical Bulletin, 34*(8), 1170 - 1173.

Keating, S. C., Thomas, A. A., Flecknell, P. A., & Leach, M. C. (2012). Evaluation of EMLA cream for preventing pain during tattooing of rabbits: changes in physiological, behavioural and facial expression responses. *Plos One, 7*(9), e44437. doi:10.1371/journal.pone.0044437

Lamont, L. A., Tranquilli, W. J., & Grimm, K. A. (2000). Physiology of Pain. *Veterinary Clinics of North America: Small Animal Practice, 30*(4).

Langford, D. J., Bailey, A. L., Chanda, M. L., Clarke, S. E., Drummond, T. E., Echols, S., . . . Mogil, J. S. (2010). Coding of facial expressions of pain in the laboratory mouse. *Nat Methods, 7*(6), 447-449. doi:10.1038/nmeth.1455

Laven, R. A., Lawrence, K. E., Weston, J. F., Dowson, K. R., & Stafford, K. J. (2008). Assessment of the duration of the pain response associated with lameness in dairy cows, and the influence of treatment. *N Z Vet J, 56*(5), 210-217. doi:10.1080/00480169.2008.36835

le Roux, C. W., Chapman, G. A., Kong, W. M., Dhillo, W. S., Jones, J., & Alaghband-Zadeh, J. (2003). Free cortisol index is better than serum total cortisol in determining hypothalamic-pituitary-adrenal status in patients undergoing surgery. *J Clin Endocrinol Metab, 88*(5), 2045-2048. doi:10.1210/jc.2002-021532

Lewis, J. G., & Elder, P. A. (2014). The reactive centre loop of corticosteroid-binding globulin (CBG) is a protease target for cortisol release. *Mol Cell Endocrinol, 384*(1-2), 96-101. doi:10.1016/j.mce.2014.01.005

Lin, P. H., Echeverria, A., & Poi, M. J. (2017). Infrared thermography in the diagnosis and management of vasculitis. *J Vasc Surg Cases Innov Tech, 3*(3), 112-114. doi:10.1016/j.jvscit.2016.12.002

Mainau, E., Cuevas, A., Ruiz-de-la-Torre, J. L., Abbeloos, E., & Manteca, X. (2014). Effect of meloxicam administration after calving on milk production, acute phase proteins, and behavior in dairy cows. *Journal of Veterinary Behavior: Clinical Applications and Research, 9*(6), 357-363. doi:10.1016/j.jveb.2014.07.007

Malreddy, P. R., Coetzee, J. F., Kukanich, B., & Gehring, R. (2013). Pharmacokinetics and milk secretion of gabapentin and meloxicam co-administered orally in Holstein-Friesian cows. *J Vet Pharmacol Ther, 36*(1), 14-20. doi:10.1111/j.1365-2885.2012.01384.x

McSherry, B. J., F.D., H., & deGroot, J. J. (1970). Plasma Fibrinogen Levels in Normal and Sick Cows. *Canadian Journal of Comparative Medicine*, 191-197.

Melendez, D. M., Marti, S., Pajor, E. A., Moya, D., Gellatly, D., Janzen, E. D., & Schwartzkopf-Genswein, K. S. (2017). Effect of timing of subcutaneous meloxicam administration on indicators of pain after knife castration of weaned calves. *J Anim Sci, 95*(12), 5218-5229. doi:10.2527/jas2017.1978

Mellor, D. J., Cook, C. J., & Stafford, K. J. (2000). Quantifying Some Responses to Pain as a Stressor. In G. P. Moberg & J. P. Mernch (Eds.), *The Biology of Animal Stress: Basic Principles and Implications of Animal Welfare* (pp. 171–198). London, UK: CABI.

Metacam Injectable for Cats. In BIVM-15129 (Ed.).

Metacam Injectable for Dogs. In BIVM-15129 (Ed.).

Millman, S. T. (2013). Behavioral responses of cattle to pain and implications for diagnosis, management, and animal welfare. *Vet Clin North Am Food Anim Pract, 29*(1), 47-58. doi:10.1016/j.cvfa.2012.11.007

Mohling, C. M., Johnson, A. K., Coetzee, J. F., Karriker, L. A., Stalder, K. J., Abell, C. E., . . . Millman, S. T. (2014). Evaluation of mechanical and thermal nociception as objective tools to measure painful and nonpainful lameness phases in multiparous sows. *J Anim Sci, 92*(7), 3073-3081. doi:10.2527/jas.2013-7521

Myers, M. J., Scott, M. L., Deaver, C. M., Farrell, D. E., & Yancy, H. F. (2010). Biomarkers of inflammation in cattle determining the effectiveness of anti-inflammatory drugs. *J Vet Pharmacol Ther, 33*(1), 1-8. doi:10.1111/j.1365-2885.2009.01096.x

Omar, N. A., & McKinley, J. C. (2018). Measurement of temperature induced in bone during drilling in minimally invasive foot surgery. *Foot (Edinb), 35*, 63-69. doi:10.1016/j.foot.2018.02.002

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: classical theories and new developments. *Trends Neurosci, 31*(9), 464-468. doi:10.1016/j.tins.2008.06.006

Paulina, J., & Tadeusz, S. (2011). Acute Phase Proteins in Cattle. *Acute Phase Proteins as Early Non-Specific Biomarkers of Human and Veterinary Diseases, Prof. Francisco Veas (Ed.), InTech,*. doi:10.5772/21154

Phillips, C. J., Wojciechowska, J., Meng, J., & Cross, N. (2009). Perceptions of the importance of different welfare issues in livestock production. *Animal, 3*(8), 1152-1166. doi:10.1017/S1751731109004479

Pohl, A., Burfeind, O., & Heuwieser, W. (2015). The associations between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes, and metritis. *J Dairy Sci, 98*(7), 4544-4551. doi:10.3168/jds.2014-9181

Polat, B., Colak, A., Cengiz, M., Yanmaz, L. E., Oral, H., Bastan, A., . . . Hayirli, A. (2010). Sensitivity and specificity of infrared thermography in detection of subclinical mastitis in dairy cows. *J Dairy Sci, 93*(8), 3525-3532. doi:10.3168/jds.2009-2807

Pongratz, U., & Licka, T. (2017). Algometry to measure pain threshold in the horse's back - An in vivo and in vitro study. *Bmc Veterinary Research, 13*, 8. doi:10.1186/s12917-017-1002-y

Practitioners, A. A. o. B. (2014). Castration and Dehorning Guidelines. In.

RA Laven, K. L., JF Weston , KR Dowson & KJ Stafford. (2008). Assessment of the duration of the pain response associated with lameness in dairy cows, and the influence of treatment. *New Zealand Veterinary Journal*.

Raundal, P. M., Andersen, P. H., Toft, N., Forkman, B., Munksgaard, L., & Herskin, M. S. (2014). Handheld mechanical nociceptive threshold testing in dairy cows - intra-individual variation, inter-observer agreement and

variation over time. *Vet Anaesth Analg, 41*(6), 660-669. doi:10.1111/vaa.12159

Raundal, P. M., Andersen, P. H., Toft, N., Herskin, M. S., Forkman, B., Munksgaard, L., . . . Rushen, J. (2015). Pre-test habituation improves the reliability of a handheld test of mechanical nociceptive threshold in dairy cows. *Res Vet Sci, 102*, 189-195. doi:10.1016/j.rvsc.2015.07.020

Rekant, S. I., M.A., L., J.M., P., J., A., & L.L., R. (2016). Veterinary applications of infrared thermography. *AJVR, 77*(1).

Sarkar, S., Hobson, A. R., Hughes, A., Growcott, J., Woolf, C. J., Thompson, D. G., & Aziz, Q. (2003). The prostaglandin E2 receptor-1 (EP-1) mediates acid-induced visceral pain hypersensitivity in humans. *Gastroenterology, 124*(1), 18-25. doi:10.1053/gast.2003.50022

Sato, P., Hotzel, M. J., & von Keyserlingk, M. A. G. (2017). American Citizens' Views of an Ideal Pig Farm. *Animals (Basel), 7*(8). doi:10.3390/ani7080064

Schaefer, A. L., Cook, N. J., Bench, C., Chabot, J. B., Colyn, J., Liu, T., . . . Webster, J. R. (2012). The non-invasive and automated detection of bovine respiratory disease onset in receiver calves using infrared thermography. *Res Vet Sci, 93*(2), 928-935. doi:10.1016/j.rvsc.2011.09.021

Schaefer, A.L., Cook, N., Tessaro, S. V., Deregt, D., Desroches, G., Dubeski, P. L., . . . Godson, D. L. (2004). Early detection and prediction of infection

using infrared thermography. *Canadian Journal of Animal Science*(84), 73-80. doi:https://doi.org/10.4141/A02-104

Stajnko, D., Brus, M., & Hočevar, M. (2008). Estimation of bull live weight through thermographically measured body dimensions. *Computers and Electronics in Agriculture, 61*(2), 233-240. doi:10.1016/j.compag.2007.12.002

Stewart, M., Stookey, J. M., Stafford, K. J., Tucker, C. B., Rogers, A. R., Dowling, S. K., . . . Webster, J. R. (2009). Effects of local anesthetic and a nonsteroidal antiinflammatory drug on pain responses of dairy calves to hot-iron dehorning. *J Dairy Sci, 92*(4), 1512-1519. doi:10.3168/jds.2008-1578

Stewart, M., Verkerk, G. A., Stafford, K. J., Schaefer, A. L., & Webster, J. R. (2010). Noninvasive assessment of autonomic activity for evaluation of pain in calves, using surgical castration as a model. *J Dairy Sci, 93*(8), 3602-3609. doi:10.3168/jds.2010-3114

Stewart, M., Webster, J. R., Verkerk, G. A., Schaefer, A. L., Colyn, J. J., & Stafford, K. J. (2007). Non-invasive measurement of stress in dairy cows using infrared thermography. *Physiol Behav, 92*(3), 520-525. doi:10.1016/j.physbeh.2007.04.034

Stewart, M., Wilson, M. T., Schaefer, A. L., Huddart, F., & Sutherland, M. A. (2017). The use of infrared thermography and accelerometers for remote

monitoring of dairy cow health and welfare. *J Dairy Sci, 100*(5), 3893-3901. doi:10.3168/jds.2016-12055

Stock, M. L., Barth, L. A., Van Engen, N. K., Millman, S. T., Gehring, R., Wang, C., . . . Coetzee, J. F. (2016). Impact of carprofen administration on stress and nociception responses of calves to cautery dehorning. *J Anim Sci, 94*(2), 542-555. doi:10.2527/jas.2015-9510

Stock, M. L., & Coetzee, J. F. (2015). Clinical Pharmacology of Analgesic Drugs in Cattle. *Veterinary Clinics of North America: Food Animal Practice, 31*(1), 113-138. doi:https://doi.org/10.1016/j.cvfa.2014.11.002Trevisi, E., Bertoni, G., Lombardelli, R., & Minuti, A. (2013). Relation of inflammation and liver function with the plasma cortisol response to adrenocorticotropin in early lactating dairy cows. *J Dairy Sci, 96*(9), 5712-5722. doi:10.3168/jds.2012-6375

Stokes, J. E., Leach, K. A., Main, D. C., & Whay, H. R. (2012). An investigation into the use of infrared thermography (IRT) as a rapid diagnostic tool for foot lesions in dairy cattle. *Vet J, 193*(3), 674-678. doi:10.1016/j.tvjl.2012.06.052

Su, T. Y., Ho, W. T., Chiang, S. C., Lu, C. Y., Chiang, H. K., & Chang, S. W. (2017). Infrared thermography in the evaluation of meibomian gland dysfunction. *J Formos Med Assoc, 116*(7), 554-559. doi:10.1016/j.jfma.2016.09.012

Tallant, A., Ambros, B., Freire, C., & Sakals, S. (2016). Comparison of intraoperative and postoperative pain during canine ovariohysterectomy and ovariectomy. *Canadian Veterinary Journal-Revue Veterinaire Canadienne, 57*(7), 741-746.

Tapper, K. R., Goff, J. P., Leuschen, B. L., West, J. K., & Millman, S. (2011). *Novel techniques for anesthesia during disbudding of calves* (Vol. 89).

Trevisi, E., Bertoni, G., Lombardelli, R., & Minuti, A. (2013). Relation of inflammation and liver function with the plasma cortisol response to adrenocorticotropin in early lactating dairy cows. *J Dairy Sci, 96*(9), 5712-5722. doi:10.3168/jds.2012-6375

van Winden, S. (2002). Displacement of the abomasum in dairy cows -risk factors and pre-clinical alterations.

Whay, H. R., Waterman, A. E., & Webster, A. J. (1997). Associations between locomotion, claw lesions and nociceptive threshold in dairy heifers during the peri-partum period. *Vet J, 154*(2), 155-161.

Wheeler, C. A., White, B. J., Anderson, D. E., Amrine, D. E., & Larson, R. L. (2013). Assessment of biometric tools for quantitative gait analysis in Holstein calves. *Am J Vet Res, 74*(11), 1443-1449. doi:10.2460/ajvr.74.11.1443

Wood, S., Lin, Y., Knowles, T. G., & Main, D. C. (2015). Infrared thermometry for lesion monitoring in cattle lameness. *Vet Rec, 176*(12), 308. doi:10.1136/vr.102571

Xu, D., Rowland, S. E., Clark, P., Giroux, A., Cote, B., Guiral, S., . . . Riendeau, D. (2008). MF63 [2-(6-chloro-1H-phenanthro[9,10-d]imidazol-2-yl)-isophthalonitrile], a selective microsomal prostaglandin E synthase-1 inhibitor, relieves pyresis and pain in preclinical models of inflammation. *J Pharmacol Exp Ther, 326*(3), 754-763. doi:10.1124/jpet.108.138776

Appendix

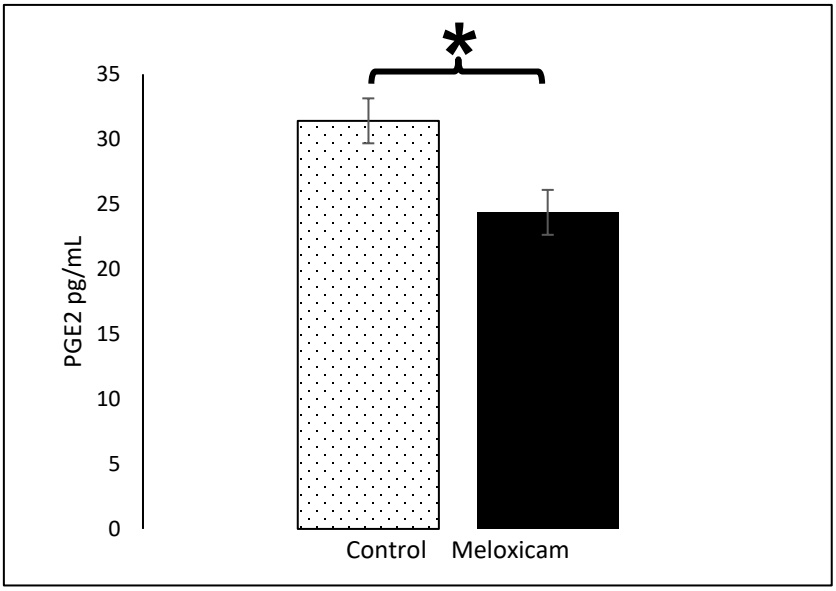

Figure 1 – Sham PGE2 concentrations by treatment group

PGE2 concentration for control cattle (shaded) and meloxicam treated cattle (solid) were significantly different between the treatment groups during the sham prodecure (P = 0.02).

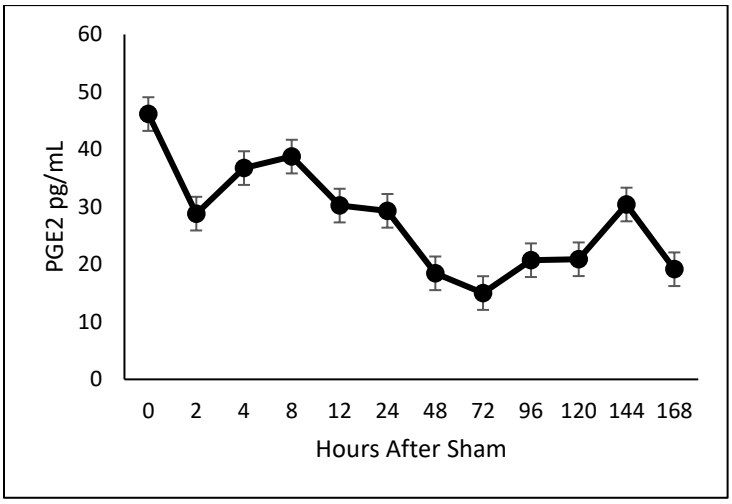

Figure 2 – Sham PGE2 concentrations over time in all cattle

PGE2 concentrations were determined at each time point for all cattle over time during the sham phase. Data represent the mean for all cattle over time. PGE was affected by time (P < 0.001).

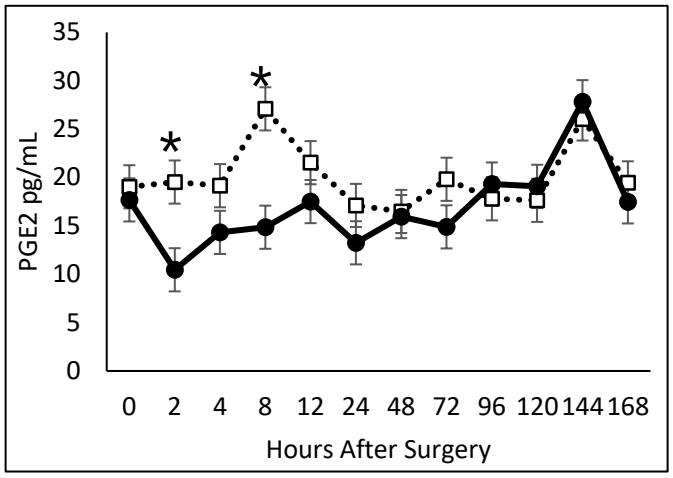

Figure 3 – Surgical PGE2 concentrations in meloxicam and placebo-treated cattle

Least square means PGE2 concentrations in placebo-treated cows (dash line and □) and meloxicam-treated cows (solid line and ●) over time after an elective laparotomy with prophylactic omentopexy. A time by treatment interaction was observed ($P = 0.003$). Control cows had significantly higher PGE2 levels at 2 and 8 hours after surgery than meloxicam cows ($P = 0.004$ and $P < 0.001$, respectively).

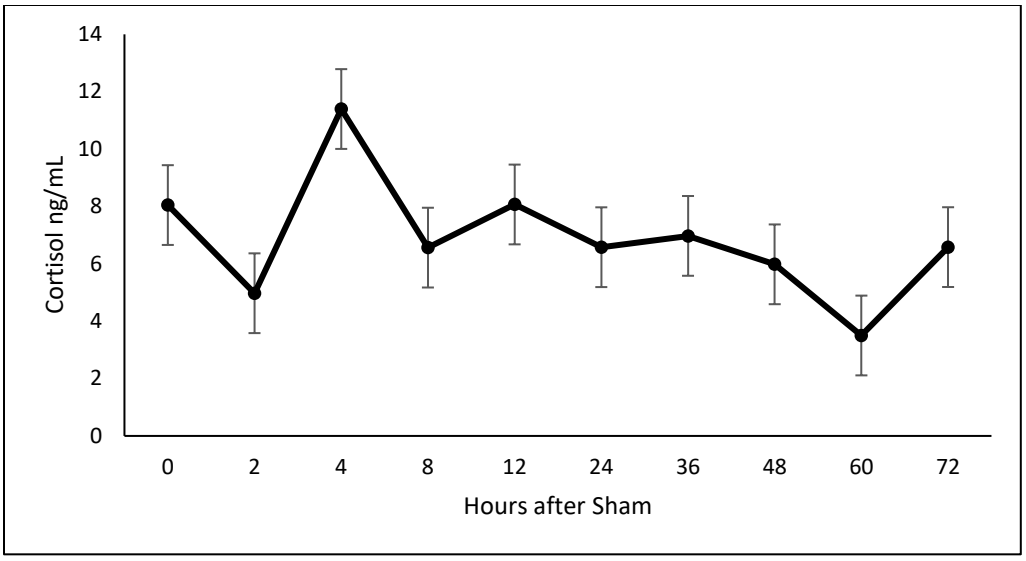

Figure 4 – Sham total cortisol over time for all cattle

Total cortisol concentrations were determined at each time point during the sham procedure. Data represent the LS mean for all cattle over time. There was a significant difference over time (P = 0.015)

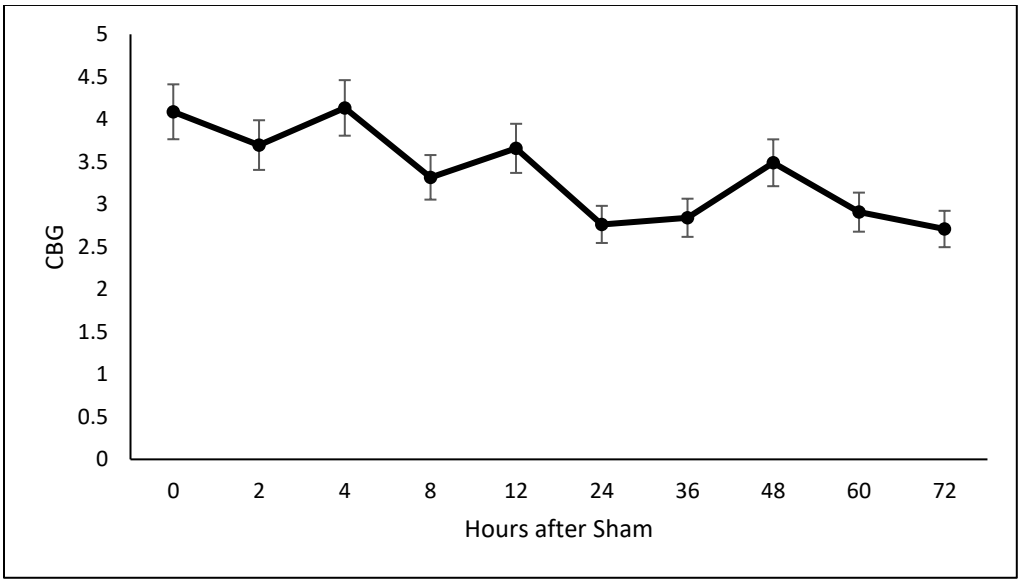

Figure 5 – Sham CBG concentration over time for all cattle

CBG concentration was determined at each time point during the sham procedure. Data represent the LS mean for all cattle over time. There was a significant difference in time points (P > 0.001).

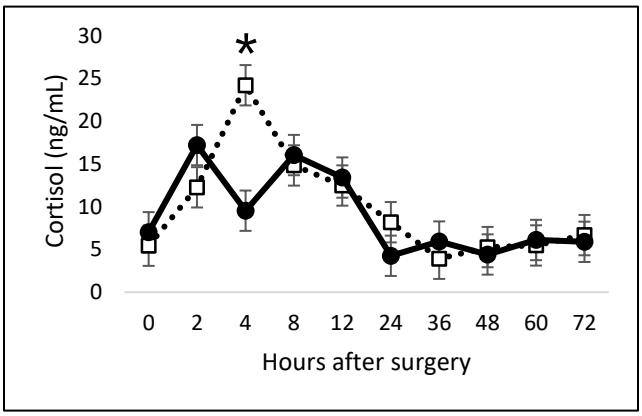

Figure 6 – Surgical cortisol concentration in meloxicam and placebo treated cattle

LS means of cortisol (ng/mL) in placebo-treated cows (dash line and □) and meloxicam-treated cows (solid line and ●) over time after an elective laparotomy with prophylactic omentopexy. Control cows had significantly higher cortisol concentrations than meloxicam cows at 4 hours after surgery ($P < 0.001$).

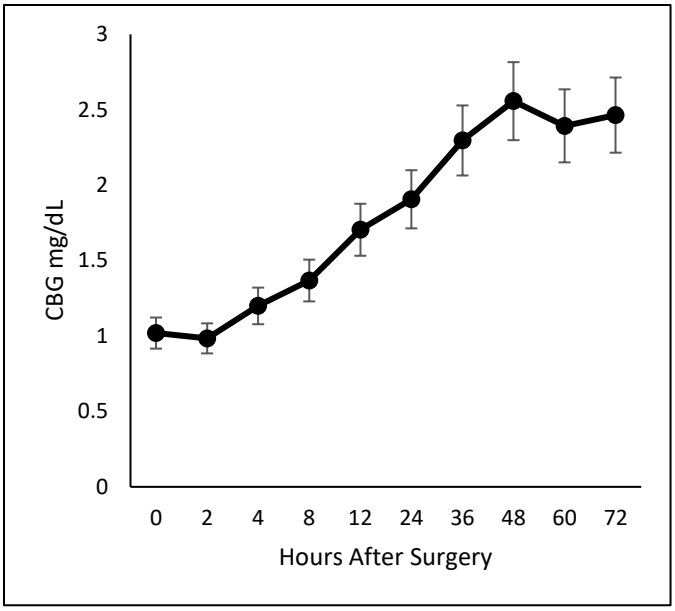

Figure 7 –Surgical Cortisol Binding Globulin concentration in all cattle over time

LS means of CBG for all animals over time following elective laparotomy with prophylactic omentopexy. CBG was affected by time ($P > 0.0001$) but not by treatment group or time by treatment interaction ($P = 0.1396$ and $P = 0.5187$, respectively).

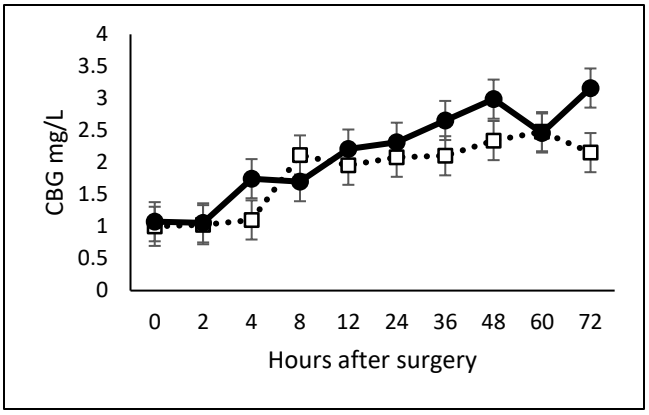

Figure 8 – Surgical Cortisol Binding Globulin (CBG) in meloxicam and placebo treated cattle

LS means of CBG (mg/L) in placebo-treated cows (dash line and □) and meloxicam-treated cows (solid line and ●) over time after an elective laparotomy with prophylactic omentopexy. CBG was affected by time, but there was not difference observed between treatment or time by treatment interaction (P = 0.14 and P = 0.52, respectively)

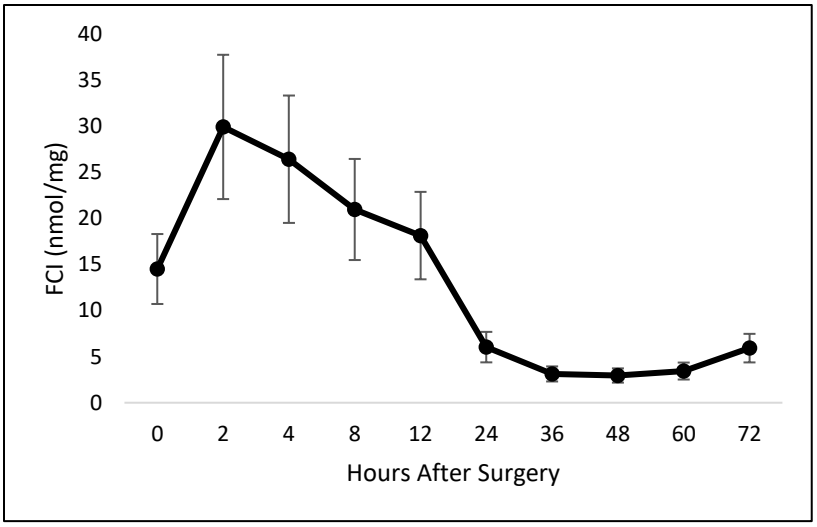

Figure 9 – Free cortisol index (FCI) for all cattle following elective laparotomy

LS means of FCI over time after elective laparotomy with prophylactic omentopexy. FCI was affected by time ($P < 0.001$), but not by treatment or time by treatment interaction ($P = 0.34$ and $P = 0.29$, respectively).

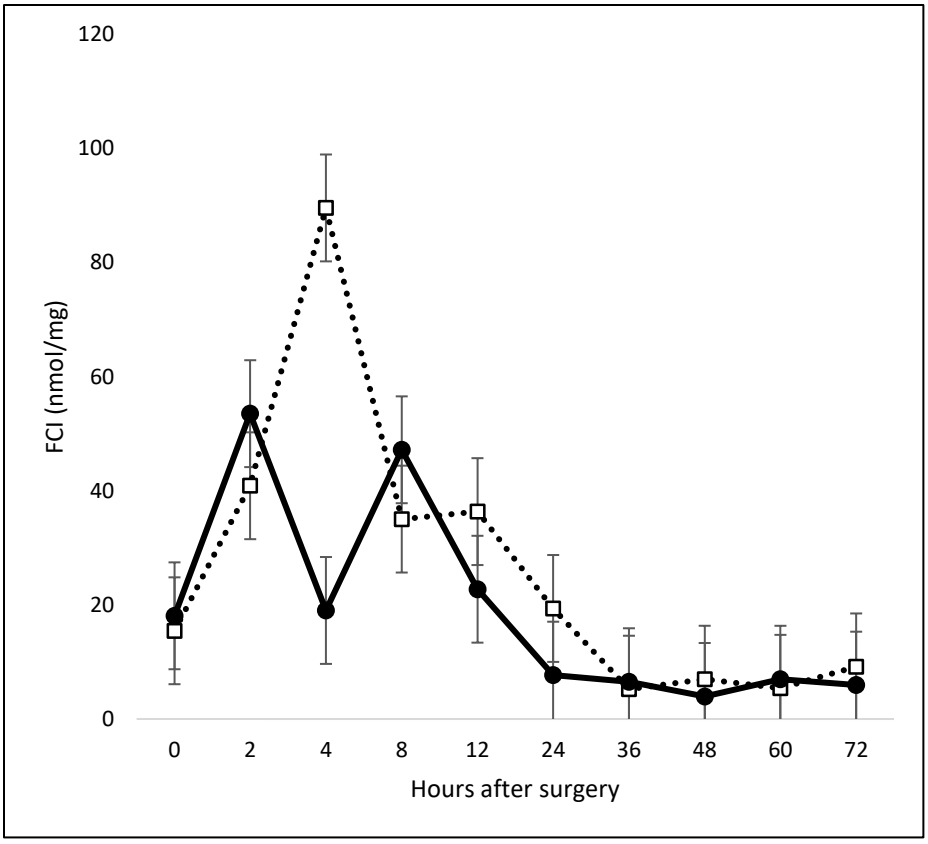

Figure 10 – Free Cortisol Index in meloxicam and placebo-treated cattle over time

LS means of FCI (nmol/mg) in placebo-treated cows (dash line and □) and meloxicam-treated cows (solid line and ●) over time after an elective laparotomy with prophylactic omentopexy. FCI was affected by time, but there was not difference observed between treatment or time by treatment interaction (P = 0.34 and P = 0.29, respectively)

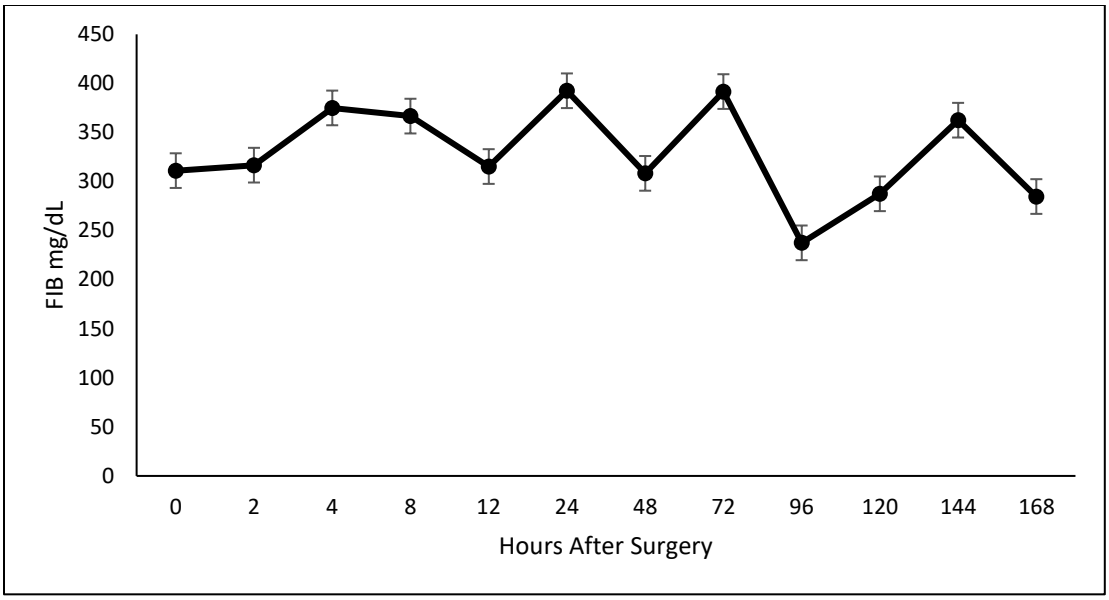

Figure 11 – Sham Fibrinogen concentration in all cattle over time

Fibrinogen concentration (mg/dL) in all cattle over time for all cattle showed a significant time effect (P > 0.001) but did not show a treatment (P = 0.52) or time by treatment interaction (P = 0.96).

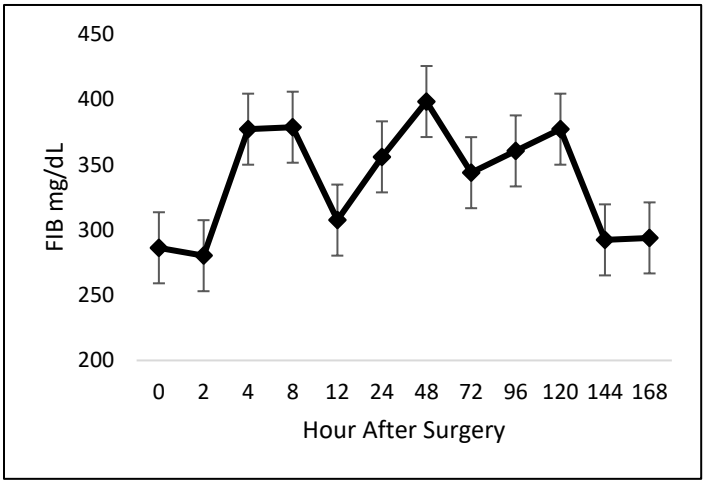

Figure 12 – Surgical Fibrinogen concentration in all cattle over time

LS means of fibrinogen for all animals over time after elective laparotomy with prophylactic omentopexy. A time effect was seen in response to the time of surgery (P < 0.001).

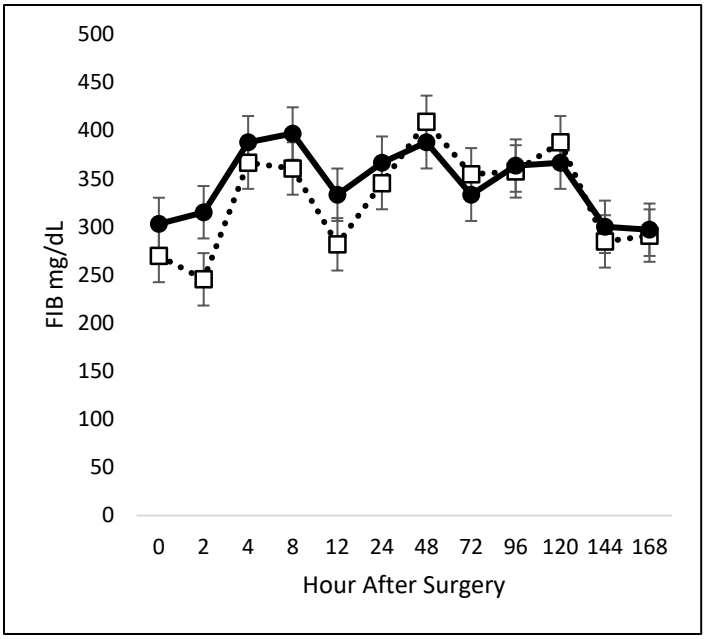

Figure 13 – Surgical Fibrinogen concentrations in meloxicam and placebo-treated cattle

LS means fibrinogen concentration in placebo-treated cows (dash line and □) and meloxicam-treated cows (solid line and ●) over time after an elective laparotomy with prophylactic omentopexy. Treatment groups did not differ (P = 0.56) nor was a time by treatment interaction observed (P = 0.43).

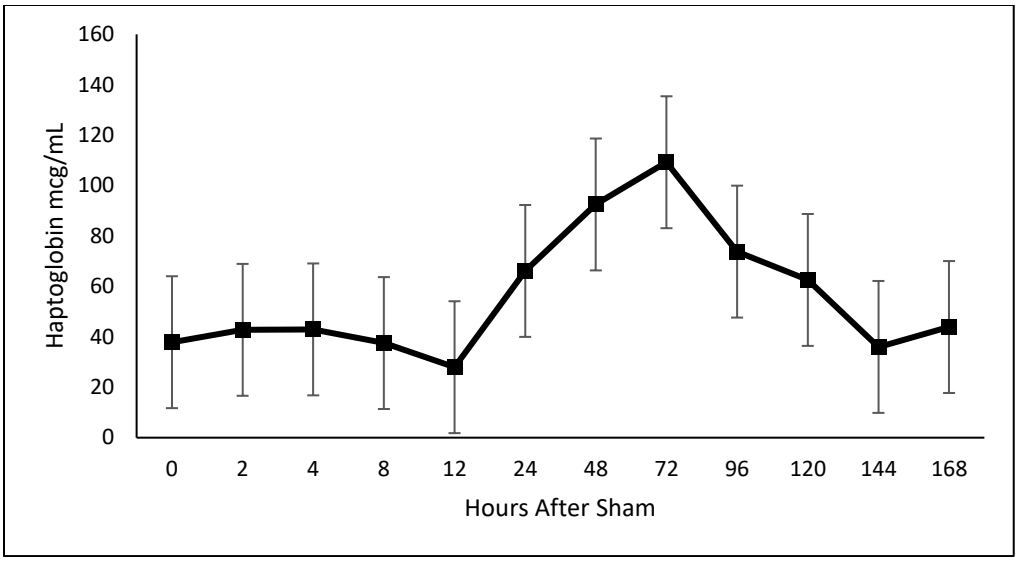

Figure 14 – Sham Haptoglobin concentrations over time for all cattle

Haptoglobin concentration slowly increased after the first 24 hours during the sham procedure over time for all cows (P = 0.0019).

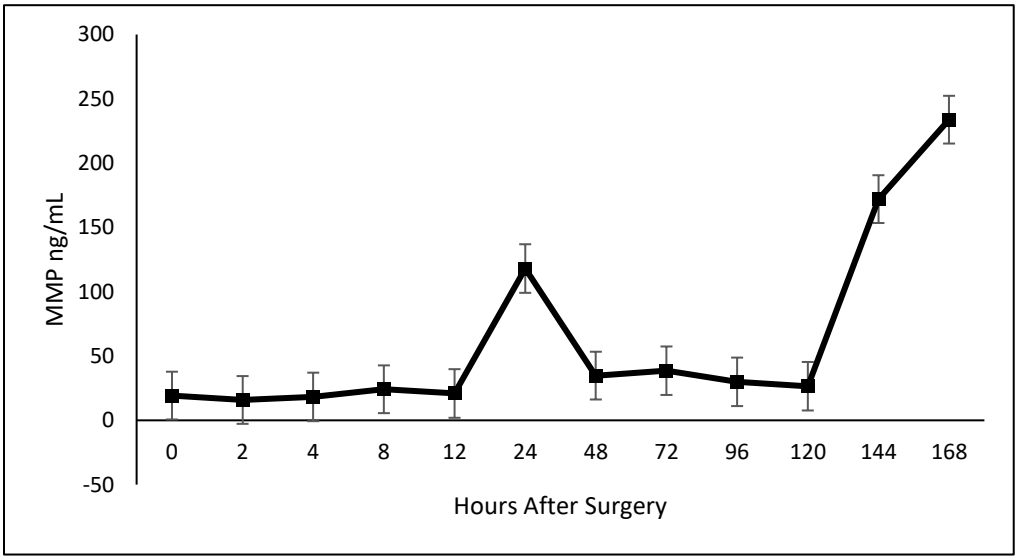

Figure 15 – Sham Haptoglobin and Matrix Metalloproteinase 9 complex concentration in all cattle over time

Hp and MMP 9 complexes displayed spikes at 24 hr. and 120 hr. after sham procedure with an overall a significant time effect (P > 0.0001).

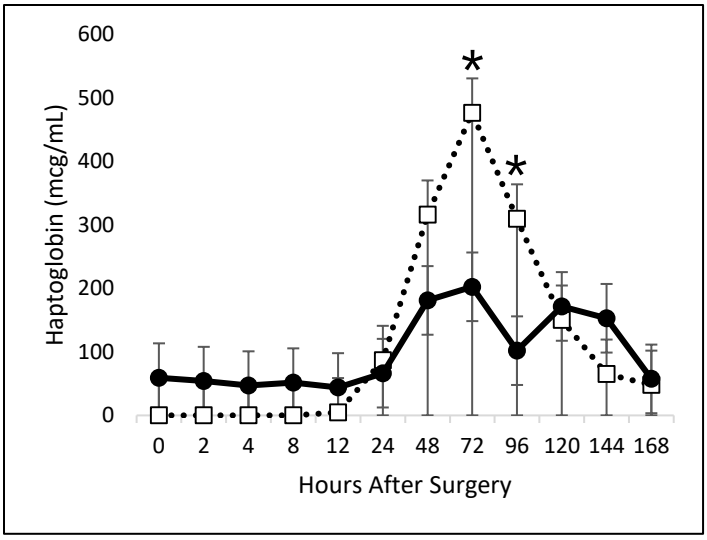

Figure 16 – Surgical Haptoglobin concentration in meloxicam and placebo-treated cattle

LS means haptoglobin concentration in placebo-treated cows (dash line and □) and meloxicam-treated cows (solid line and ●) over time after an elective laparotomy with prophylactic omentopexy. CON had significantly higher Hp concentrations (mcg/mL) at 72 and 96 hours after surgery (P = 0.0153 and 0.0002, respectively).

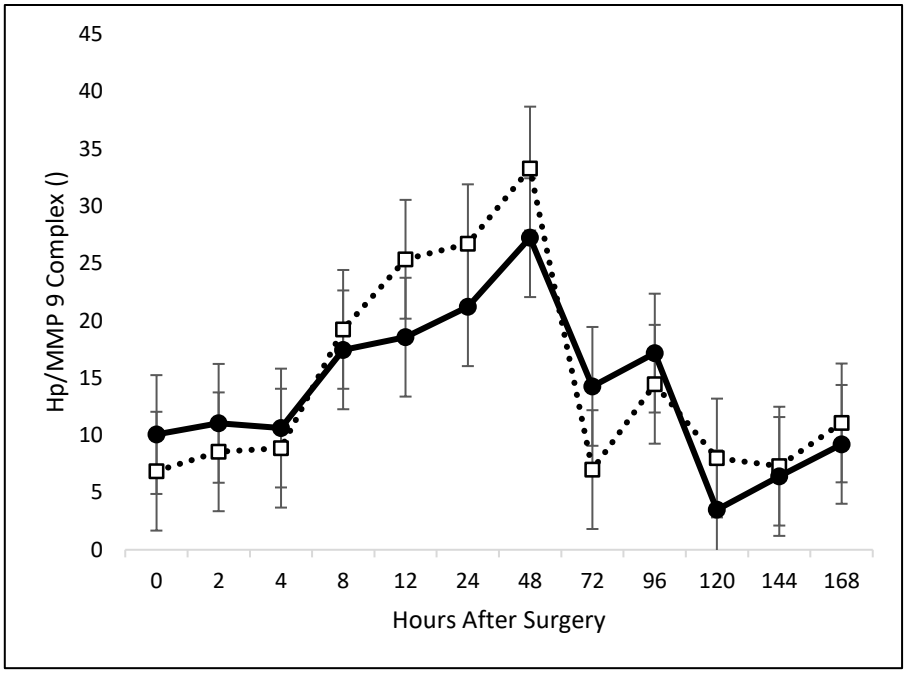

Figure 17– Surgical Haptoglobin and Matrix Metalloproteinase 9 complex concentration in meloxicam and placebo-treated cattle

LS means of Hp-MMP 9 complex concentration in placebo-treated cows (dash line and □) and meloxicam-treated cows (solid line and ●) over time after an elective laparotomy with prophylactic omentopexy. A time effect and a time by treatment interaction was found ($P < 0.0001$ and $P = 0.0107$, respectively) but no treatment effect was observed ($P = 0.5317$). Contol cows had a significantly lower Hp-MMP 9 complex concentration at 72 hours post surgery.

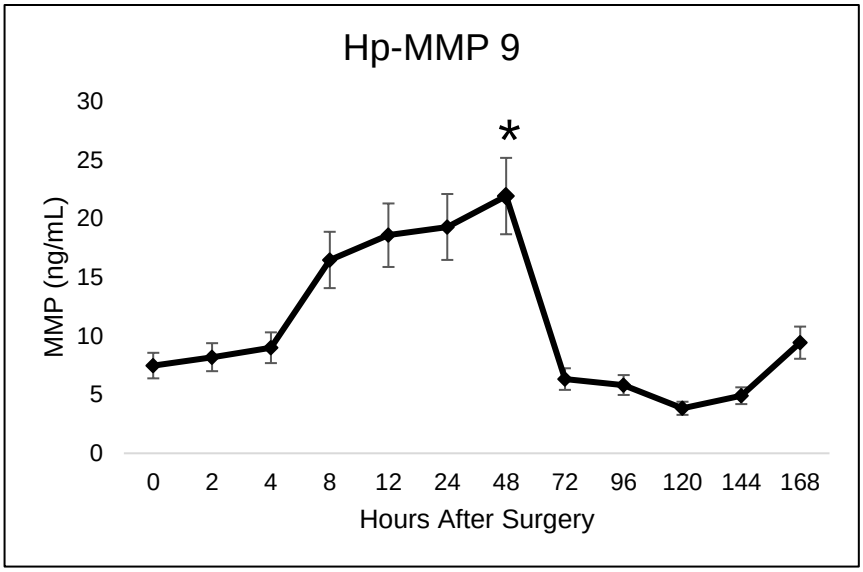

Figure 18 – Surgical Matrix Metalloproteinase concentration in all cattle over time

LS means of Hp-MMP 9 complex concentrations in all cows over time after elective laparotomy with prophylactic omentopexy. A significant time effect was observed ($P < 0.0001$). Hours 8, 12, 24, and 48 were significantly higher in all cows after surgery.

Figure 19 - FD/S-3 conical steel tip used for MNT

Figure 100 - Example of algometry sites around the incision. Sites 1, 2, and 3 are cranial sites and 4, 5, and 6 are caudal sites.

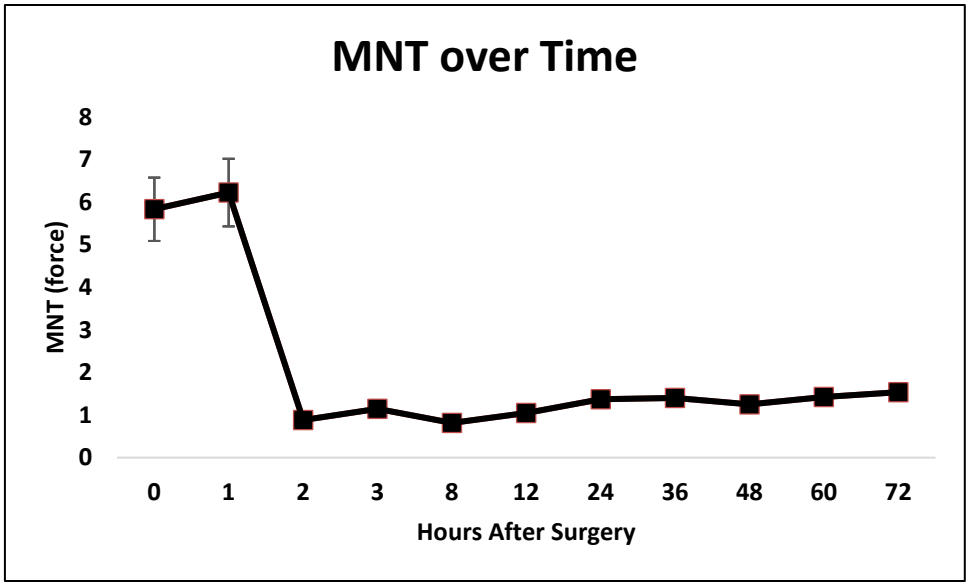

Figure 21 – Mechanical nociceptive threshold following a right flank laparotomy and prophylactic omentopexy (including times 0 and 1 hour)

There was a significant time effect observed with hours 0 and 1 being significantly higher than all other hours ($P < 0.001$). Prior to the surgical procedure, lidocaine was used to induce local anesthesia. The significant decrease in the pain threshold at hour 2 demonstrates the change in sensitivity to pressure due to inflammation and surgical pain without the influence of lidocaine.

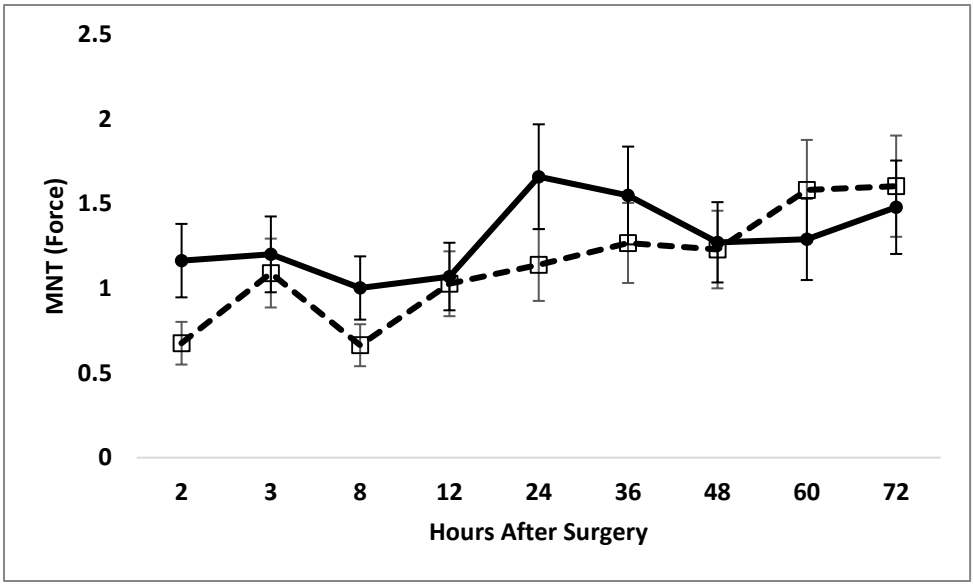

Figure 22 - Mechanical nociceptive threshold following a right flank laparotomy and prophylactic omentopexy in cattle treated with meloxicam or placebo (excluding times 0 and 1 hour)

There was no significant difference between treatment groups (MEL; solid line with circles, CON; dashed line with open squares, P = 0.37). No time by treatment interaction was observed (P = 0.24)

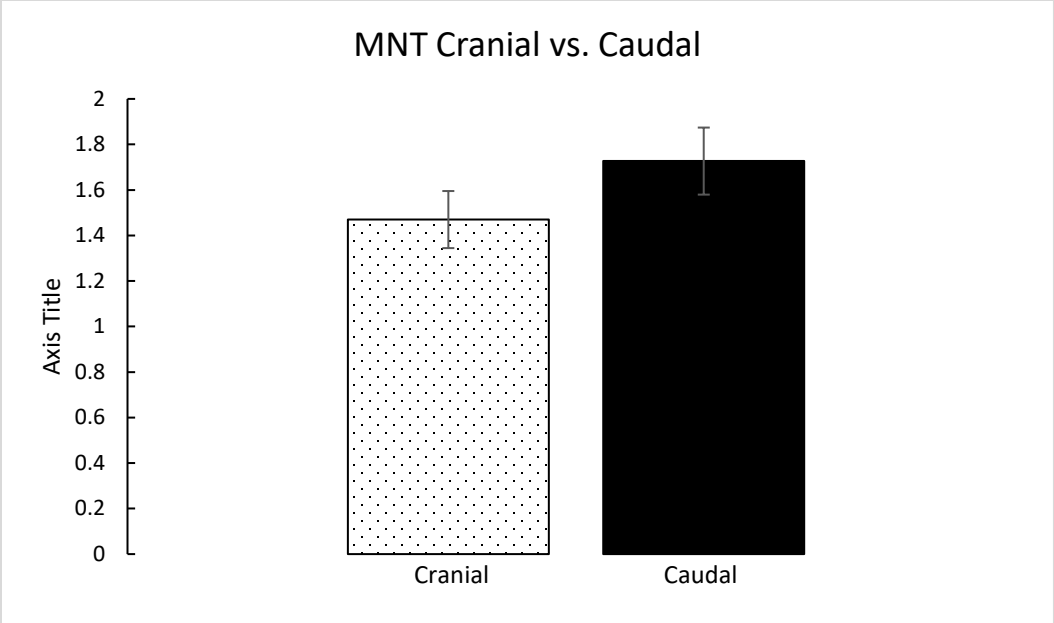

Figure 23 - Combined mean MNT for all cranial and caudal test sites for all cattle

There was a significant difference between the cranial and caudal readings across all cows with the cranial sites requiring less force to elicit a response (lower MNT) than the caudal sites (P = 0.031).

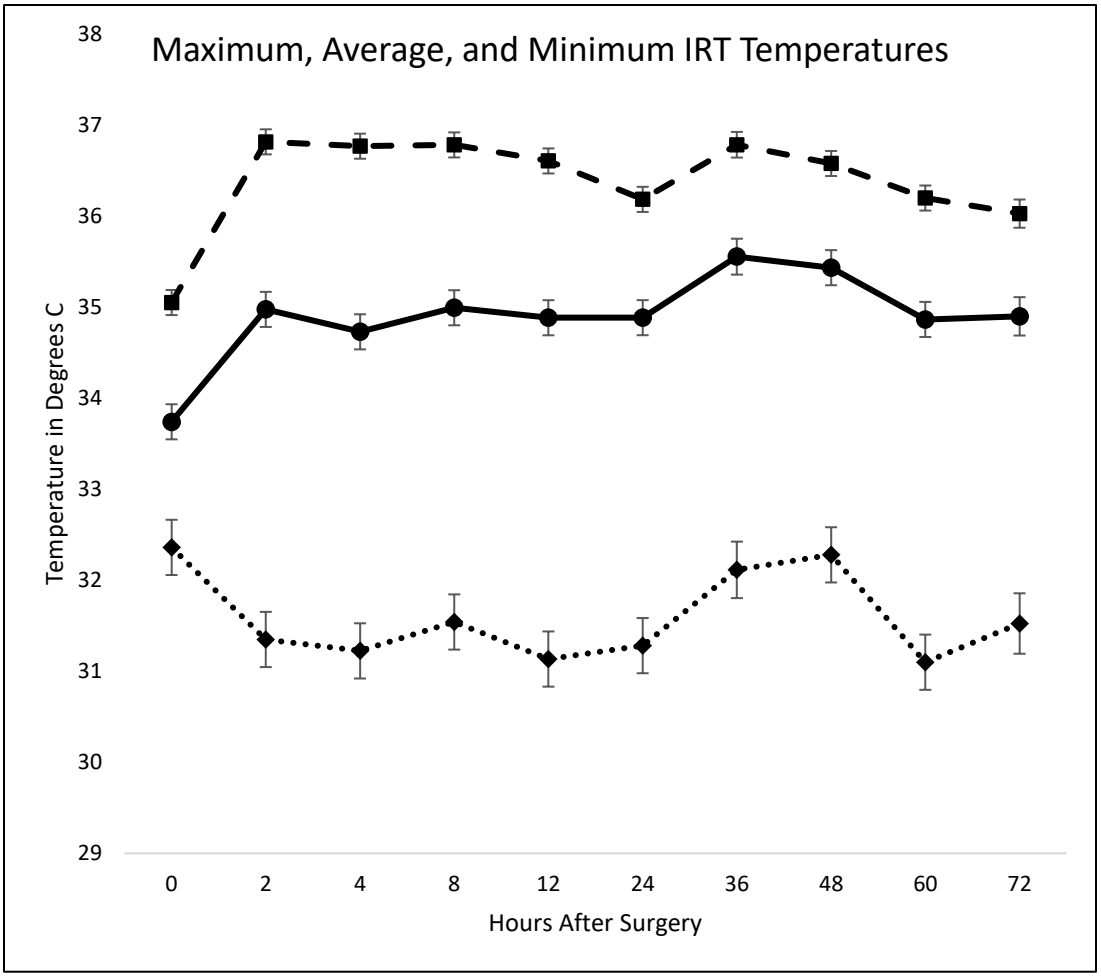

Figure 24 – Maximum, Mean, and Minimum skin surface temperatures collected by infrared thermography on all animals over time

Maximum (dashed with squares), mean (solid with circles), and minimum (dotted with diamonds) temperatures displayed a significant time effect

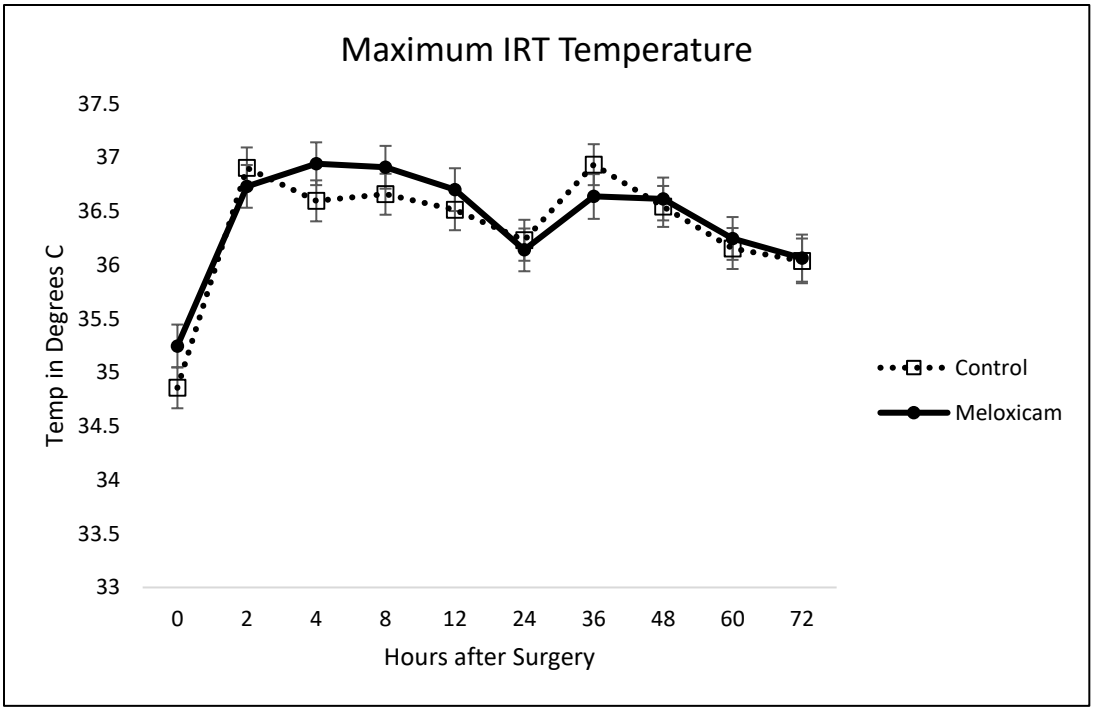

Figure 25 - Maximum skin surface temperatures collected by infrared thermography following a right flank laparotomy and prophylactic omentopexy in cattle treated with meloxicam or placebo.

The least square mean of the maximum IRT temperatures were affected by time (P< 0.0001), but showed no significant difference between treatment and time by treatment interaction (P = 0.46 and 0.71 respectively).

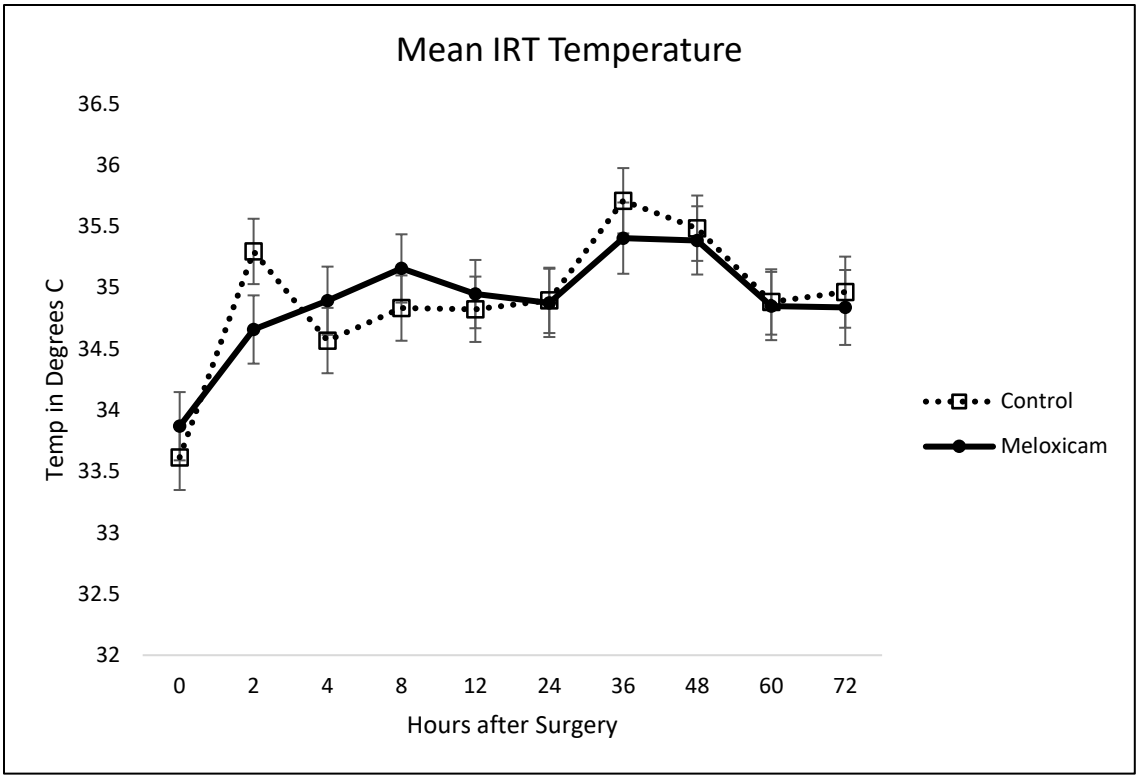

Figure 26 - Mean skin surface temperatures collected by infrared thermography following a right flank laparotomy and prophylactic omentopexy in cattle treated with meloxicam or placebo.

The least square mean of the mean surface temperatures was affected by time ($P < 0.001$), but showed no significant difference between treatment and time by treatment interaction ($P = 0.91$ and 0.67 respectively).

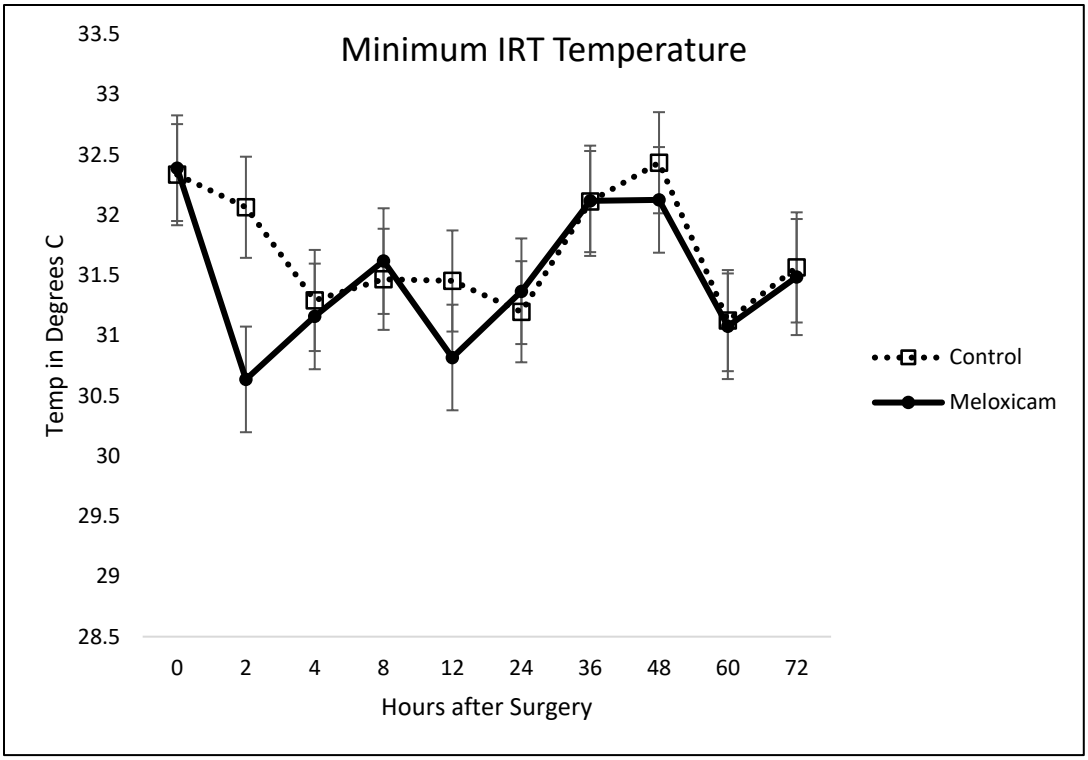

Figure 27 - Minimum skin surface temperatures collected by infrared thermography following a right flank laparotomy and prophylactic omentopexy in cattle treated with meloxicam or placebo.

The least square mean of the minimum IRT temperatures were affected by time ($P < 0.0001$), but showed no significant difference between treatment and time by treatment interaction ($P = 0.42$ and 0.63 respectively).

Table 1 – Maximum, Mean and Minimum skin surface temperature within 72 hours of elective right flank laparotomy with prophylactic omentopexy
a-d represent significant differences between LS means of temperature within columns over time for all cows

	Maximum Temperatures		Average Temperatures		Minimum Temperatures	
Hours After Surgery	LS Mean of Temperature	Standard Error	LS Mean of Temperature	Standard Error	LS Mean of Temperature	Standard Error
0	32.3619 *a*	0.3037	33.7420 *d*	0.1928	32.3619 *a*	0.3037
2	31.3499 *cd*	0.3037	34.9772 *bc*	0.1928	31.3499 *cd*	0.3037
4	31.2245 *d*	0.3037	34.7308 *c*	0.1928	31.2245 *d*	0.3037
8	31.5420 *bcd*	0.3037	34.9952 *bc*	0.1928	31.5420 *bcd*	0.3037
12	31.1350 *d*	0.3037	34.8856 *c*	0.1928	31.1350 *d*	0.3037
24	31.2822 *d*	0.3037	34.8861 *c*	0.1928	31.2822 *d*	0.3037
36	32.1091 *abc*	0.3111	35.5564 *a*	0.1974	32.1091 *abc*	0.3111
48	32.2800 *ab*	0.3037	35.4355 *ab*	0.1928	32.2800 *ab*	0.3037
60	31.1001 *d*	0.3037	34.8668 *c*	0.1928	31.1001 *d*	0.3037
72	31.5238 *abcd*	0.3331	34.9008 *bc*	0.2113	31.5238 *abcd*	0.3331

Vita

Amber Futrell Moore was born in Gadsden, TN. From a very young age, she has wanted to be a veterinarian. She attended Crockett County High School and graduated in 2010. She then attended the University of Tennessee at Martin where she earned her Bachelor of Agriculture in 2013. She then attended the University of Tennessee College of Veterinary Medicine and earned her D.V.M in 2017. She then went on to pursue a Master's of Animal Science. She works at Bells Animal Clinic as a veterinarian and is also teaching introductory animal science classes as adjunct faculty for the University of Tennessee Martin.