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**An investigation of pressure algometry and thermal sensitivity tests for assessing pain associated with a sow lameness model and calf disbudding**

by

**Kathleen Renae Tapper**

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Biomedical Sciences (Physiology)

Program of Study Committee:
Suzanne T. Millman, Major Professor
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Ames, Iowa

2011

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**TABLE OF CONTENTS**

## LIST OF TABLES

## LIST OF FIGURES

## THESIS ABSTRACT

The first objective for this thesis was to examine mechanical, including pressure algometry (PA) and von Frey filaments (VF), and thermal sensitivity (TS) nociceptive tests as objective non-invasive measures of pain in swine and cattle. The second objective of this thesis was to examine novel pain mitigation agents to alleviate pain in swine and cattle.

The first research study assessed the validity of the PA and TS pain tests by comparing differences in nociceptive threshold in cull sows when sound and when induced-lame in one hind leg. This lameness study also assessed sodium salicylate and flunixin meglumine (Banamine®) as treatments for pain associated with lameness. Results from this study indicate that PA is a valid non-invasive method to objectively quantify the mechanical nociceptive threshold (MNT) when sound as well as when sows were induced-lame in one hind leg. This test was valid because no differences were detected between the sound and designated lame hind leg prior to induction, and reduced MNTs were observed on the induced lame limb post induction. No improvement was detected with either analgesic treatment as assessed with PA. Due to the high variability in TS latencies from sound to lame leg at all trial days, TS was not a valid pain assessment tool in this sow lameness pain model.

Objectives for the calf cautery disbudding trial were to evaluate VF and TS for assessing disbudding pain in calves relative to PA, which has been previously validated. The effectiveness of ethanol or a depot formation of lidocaine for extended analgesia during disbudding was measured relative to a control lidocaine cornual nerve block. In this experimental design, neither VF nor TS pain tests were practical pain assessment tools. Results from PA indicated that the ethanol anesthetized calves displayed elevated MNTs relative to the control calves, and depot-treated calves tolerated reduced MNTs relative to the control from +1 hour post-disbudding through +83 hours post-disbudding, which indicates that ethanol provided extended anesthetic relief, and that this depot formation of lidocaine is not a suitable anesthetic treatment for cautery disbudding calves.

In conclusion, this research has validated the use of PA as an objective pain assessment tool for both a transient-induced sow lameness and a cautery disbudding pain model. Results from these studies provide promising evidence as to the range of research capabilities offered by PA. In both experimental models, TS was not an appropriate measure of nociception. Further research and refinement is required for this TS test to be applicable in a transient-induced sow lameness and calf cautery disbudding pain model.

# CHAPTER 1: GENERAL INTRODUCTION

## 1.1 Thesis Organization

This thesis is organized with each research project as a separate but cohesive chapter. Information in Chapter 1 introduces the study objectives and expected outcomes. A literature review focusing on animal pain and objective pain assessment is found in Chapter 2. Chapters' 3 and 4 detail each research project and animal pain model, with more specific introductory and background information included within the research chapters. Chapter 3 focuses on the sow lameness research trial, and is formatted following the Journal of Animal Science guidelines. Chapter 4 focuses on the calf disbudding research, and is also formatted following the Journal of Animal Science guidelines. Chapter 5 is a summary of the research results and a general discussion of how the results apply to the study of animal pain and pain assessment.

## 1.2 Study Objectives and Expected Outcomes

The first objective for this thesis was to examine mechanical nociceptive threshold pain tests, including pressure algometry (PA) and von Frey filaments (VF), and also a thermal sensitivity (TS) nociceptive threshold pain test as objective non-invasive measures of pain in swine and cattle. The expected outcome of this thesis objective was to validate objective pain assessment tests in controlled animal pain models. The second objective of this thesis was to examine novel pain mitigation agents, either non-steroidal anti-inflammatory drugs (NSAIDs) or local anesthetics, for alleviating pain in swine and cattle. The expected outcome of this objective is that in each animal pain model, the pain mitigation agents will alleviate pain as assessed by nociceptive thresholds tests.

Objectives of the first research trial, the sow lameness study, were to evaluate PA and TS as objective pain assessment tools for identifying a transient-induced lameness. Both PA and TS pain assessment tests have been validated for quantifying pain in other species and other pain models, and the expected outcome of this study was that each pain assessment test would objectively differentiate sows when sound and lame. This study also compared the analgesic effect of sodium salicylate and flunixin meglumine (Banamine®) on pain associated with sow lameness as assessed by the pain assessment tests. The expected outcome of this objective was that each analgesic treatment would mitigate pain associated with lameness.

Objectives for the calf disbudding chapter were to determine the effectiveness of ethanol or a depot formation of lidocaine for extended analgesia during disbudding relative to a control lidocaine cornual nerve block. The second objective of the disbudding chapter was to evaluate nociceptive threshold tests, VF and TS, for assessing disbudding pain in calves relative to PA which has previously been validated for detecting pain in a cautery disbudding model. The expected outcome of the first objective of this disbudding research trial was that both ethanol and a depot formation of lidocaine, administered as a cornual nerve block, would decrease the pain sensitivity as assessed by PA, VF, and TS, compared to the control cornual nerve block of lidocaine 2% hydrochloride. The second expected outcome of this research study was that both TS and VF would accurately assess differences in pain sensitivity for disbudded calves compared to each calves' baseline nociceptive thresholds.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Introduction

In production animal agriculture, there are routine management procedures such as castration, tail docking, branding, dehorning, and disbudding that cause short-term pain and can affect animal welfare. Pain associated with injuries such as hoof lesions or inflammation may cause distress and may decrease the welfare of animals. The assessment of animal pain is a crucial aspect of veterinary medicine and animal welfare research. However, pain is an individualistic experience and its measurement is extremely difficult (O'Callaghan et al., 2003).

The objectives of this literature review are to provide an overview of the pertinent published research literature regarding pain, pain assessment, and pain mitigation, as well as to provide an in-depth background for the thesis projects analyzing the validity of objective pain assessment tools in two livestock pain models, a chronic sow lameness model and an acute cautery disbudding model.

## 2.2 Pain and Nociception

Pain has been defined by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP, 1994). Animal pain is an aversive sensory and emotional experience representing awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal's physiology and behavior to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery (Molony and Kent, 1997). Pain is essentially a perceptual process that arises in response to nociception, which is defined as the detection of noxious stimuli and the subsequent transmission of encoded information to the brain (Kidd and Urban, 2001).

Pain can be categorically classified based on the cause, location, duration, or intensity of pain. Visceral pain refers to pain that arises from the viscera in the abdominal or thoracic cavity. Somatic pain refers to pain arising from the periphery, such as muscle or skin (Stasiak et al., 2003). Neuropathic pain results from an injury to the nervous system and can be central or peripheral. Adaptive pain protects the animal from injury and promoting healing. Maladaptive pain, by contrast, is pathological pain that persists long after the initiating

causes have resolved (Anderson and Muir, 2005a). Depending on duration, pain can be either acute or chronic. Acute pain is from a known cause, such as injury or surgery, has a predictable course and duration, and is usually alleviated by analgesic measures (Robertson, 2002). Acute pain serves a protective function, causing the animal to withdraw from the stimulus, and also in reducing activity, increasing sleep and promoting healing that may last minutes to hours (Chapman and Gavrin, 1999). Chronic pain can result from inflammation and nerve damage and is mediated by structural, physiological and functional changes in the central nervous system as result of damage (Garry et al., 2004).

Physiologic pain is initiated by specialized sensory nociceptors that detect noxious thermal, mechanical or chemical stimuli. Physiologic pain in essence is the alarm system guarding from chronic pain (Besson and Chaouch, 1987). Pain becomes pathologic when it is associated with tissue injury and is usually the result of inflammation (Woolf and Salter, 2000; Stasiak et al., 2003). Pathologic pain may involve the development of peripheral sensitization, central sensitization, and neural plasticity (Anderson and Muir, 2005a; Woolf and Salter, 2000).

Nociception is the physiologic component of pain processing involving the transduction, transmission, and modulation of signals generated by stimulation of specialized cutaneous peripheral nociceptors (Lamont, 2008; Garry et al., 2004). Nociception is the sensation of stimuli and the process of generating the sensory signal to the brain. Noxious thermal, chemical or mechanical stimulation activate nociceptors in the skin which then convey this information to the first synaptic relays in the dorsal horn of the spinal cord (Kidd and Urban, 2001). Within the peripheral terminals of nociceptors, specialized receptors are activated and generate depolarizing currents in response to noxious stimuli (Woolf and Salter, 2000). Once generated, impulses propagate along small-diameter (C and A-delta) ascending afferent nerve fibers to the dorsal root ganglian (DRG) of the spinal cord (Raja et al., 1988; Chen et al. 2006). Nociceptors can be classified by their response to noxious and conduction velocities. Cutaneous nociceptors may be rapidly conducting myelinated A-fibers or slower conducting unmyelinated C-fibers (Besson and Chaouch, 1987; Raja et al., 1988; Stasiak et al., 2003). Once the signal is conducted to the DRG, neurotransmitters, primarily glutamate, are released from the afferent nerve, which chemically convey the nociceptive input signal through the spinal ascending tract to the brain cortex. The processing of nociceptive information by the brain allows for the perception of pain, emotional

experience of pain, memory development of pain, and the autonomic changes associated with pain (Stasiak et al.,2003). The perception of pain involves interpreting the sensory information and including emotions and pain memories. The sensory signal travels to the brain primarily via the spinothalamic tract, through the medulla, with synapses in the reticular formation which controls physical behaviors, and synapses in the thalamus which is the brain's relay center. Nerves from the thalamus relay the signal to various areas of the brain's somatosensory cortex, which encode the emotional experience and perception of pain, the limbic system for memory formation, and the autonomic nervous system which aids in the fight or flight response and homeostasis. Within the spinal cord, a second physiologic route for the electrical signal is the transmission of information through the descending efferent motor neurons to elicit a spinally-mediated withdrawal reflex which allows the body to rapidly withdraw from the noxious stimuli (Stasiak et al., 2003). This spinally mediated quantifiable reflex withdrawal response is the basis for nociceptive threshold assessments. The anatomical basis for the protective nociceptive withdrawal reflex is the reflex arc consisting of the nociceptor, a primary afferent nerve fiber, spinal cord synapse, and efferent motor neuron leading to effector organs such as skeletal muscles (Kitchell and Guinan, 1990).

Using electrophysiological techniques, nociceptors have been identified in chickens (Gentle, 1992, 1997) and mammals (Cottrell and Molony, 1995; Yeoman and Proudfit, 1996). Electrophysiological research conducted by Gentle (1997) studied the effects of a chemical-induced arthritis in chickens by injecting sodium urate into the ankle joint. Mechanoreceptors were recorded through electrophysiological recordings immediately following intra-articular injection of sodium urate, which caused nociceptive sensitization through an increased nociceptive receptive field size, decreased threshold, and increased spontaneous activity (Gentle, 1997). Through electrophysiological recording, it has been shown that castration of lambs with rubber rings produces significant increases in the afferent activity from nociceptors in the testes, which was rapidly blocked with an intra-testicular injection of local anesthetic (Cottrell and Molony, 1995). Electrophysiological recordings of a rat foot withdrawal response through thermal stimulation have provided evidence that different nociceptive afferents are activated at different heating temperatures (Yeomans and Proudfit, 1996).

According to Bussieres and colleagues (2008), animals experiencing pain have increased sensitivity to aversive stimuli and, consequently, a lowered threshold to subsequent stimulation. Nociceptors produce

responses that warn and protect the host from impending tissue damage, thereby helping to maintain bodily integrity and survival (Craig, 2003). Inflammation and repetitive stimulation sensitize both the activation threshold of the peripheral nerves and the conduction of the cells for spinal transmission, thus lower firing thresholds (Chapman and Gavrin, 1999). The sensitization of nociceptors under pathological conditions contributes to chronic pain. Following tissue injury, changes in tissue pH and electrolyte composition as well as production of inflammatory mediators and up-regulation of pro-inflammatory enzymes sensitize peripheral nociceptors toward noxious and non-noxious stimuli (Driessen and Zarucco, 2007). Some prostaglandins (PGs) and leukotrienes are produced during inflammation and sensitize the nociceptors leading to lower threshold for their activation, and hyperalgesia (FELASA, 1994; Anderson and Muir, 2005b).

Tissue injury results in the release of inflammatory mediators from damaged cells including ions (K+, and H+), bradykinin, histamine, 5-hydroxytryptamine (5-HT), ATP and nitric oxide (Kidd and Urban, 2001), leading to inflammation, pain, and peripheral sensitization of nociceptors. Inflammatory cytokines induce the synthesis of cyclooxygenase (COX-2) enzyme resulting in the production and release of prostaglandins (Maier et al., 1990). The release of PGs in conjunction with other inflammatory mediators and proteases result in inflammation (Mitchell et al., 1994) and an inflammatory cascade. Peripheral sensitization is caused by the release and accumulation of inflammatory mediators into the extracellular environment which may activate sensory nerve endings or sensitize high-threshold nociceptors to mechanical, thermal, or chemical stimuli (Muir and Woolf, 2001).

**2.3 Pain Mitigation**

Analgesia is defined by the International Association for the Study of Pain as the absence of pain in response to stimulation which would normally be painful (IASP, 1994). Analgesia can be achieved pharmacologically through the use of opioids, alpha-2 agonists, local anesthetics, or nonsteroidal anti-inflammatory drugs (NSAIDs). Opioids, alpha-2 agonists and local anesthesics act to induce analgesia by inhibiting the transmission of nociceptive signals throughout the nociceptive pathway. The modes of action for NSAIDs primarily reduce inflammation which reduces pain. Research from this thesis measured the analgesic effect of NSAIDs to mitigate pain associated with sow lameness, as well as extended anesthetic efficacy of

novel local anesthetic treatments for use with bovine disbudding. Specific research pertaining to NSAIDs' effect on lameness and disbudding pain alleviation with use of local anesthetics is detailed later in the literature review. This section will detail the modes of action for NSAIDs and local anesthetics.

### 2.3.1 Local or Regional Anesthetics

Local or regional anesthetics reversibly block the nociceptive signal generation in primary afferent terminals. Graf and Senn (1999) found the effect of the a 2% lidocaine anesthetic to significantly decrease the frequencies of pain related behaviors in placebo-treated calves and an absence of pain related behaviors in calves that received anesthesia when sham or cautery disbudding. Plasma ACTH and cortisol concentrations immediately and rapidly spiked in placebo-treated calves that were cautery disbudded as opposed to calves locally anesthetized with 2% lidocaine in the Graf and Senn study. Local anesthetics interfere with conduction of impulses by preventing generation and conduction of the nerve impulse (Riebold, 1995). Results of a study conducted by Hollmann and colleagues (2001) suggest the G-protein-coupled receptors may be the common targets for local anesthetics as tested with lidocaine.

During dehorning or disbudding of cattle, local anesthetic agents such as lidocaine or bupivacaine are injected into the cornual nerve of the calf to block the sensory perception from the horn and surrounding tissue. The concern with local anesthetics as a sole pain mitigation treatment is that it delays pain by alleviating pain in the short term which decreases the total pain rather than eliminating all pain associated with the procedure. In disbudding for example, the acute pain response to the disbudding event is abolished by local anesthetics, but local anesthetics do not have the pharmacodynamic capabilities to relieve the inflammatory pain that develops several hours after a tissue injury.

Ethanol, in animals, can cause immobility in response to a noxious stimulus (Wong et al., 1997). Ethanol effects in concentrations relevant to general anesthesia were studied within the spinal cord in rats in which action potentials were evoked through electrodes that stimulated the dorsal root, research of which was conducted by Wong and colleagues (1997). Results from Wong and colleagues (1997) provide evidence that ethanol depresses the response of excitatory postsynaptic potential (EPSPs) which suggests that ethanol depresses synaptic transmission and the amplitude of the monosynaptic reflex which may be related to ethanol's

anesthetic and analgesic properties. Limited research has been conducted on ethanol as an anesthetic for use in animal pain models.

### 2.3.2 Non-steroidal Anti-Inflammatory Drugs (NSAIDs)

The most established mechanism of action of NSAIDs is the inhibition of cyclooxygenase (COX) enzymes and the subsequent reduction in the generation of pro-inflammatory PGs and other inflammatory mediators (Mitchell et al., 1997; Curry et al., 2005). Results from Mitchell and colleagues (1994) provide evidence that the anti-inflammatory and therapeutic effect of NSAIDs are due to their ability of inhibit the isoform COX-2, while some of the side effects correlated to NSAID therapies are due to the inhibition of the COX-1 enzyme isoform including gastrointestinal irritation caused by inhibiting PG synthesis in the gut which alters secretion of mucus and decreased gastric cell protection, or renal damage by inhibiting PG synthesis in the kidney. Each NSAID dosage is species-dependent as well as dependent on the therapeutic objective to be attained.

Salicylic acid derivatives, which include sodium salicylate and acetylsalicylic acid (aspirin), are widely used as analgesic, antipyretic and anti-inflammatory agents (USP, 2004). However, the U.S. Food and Drug Administration (FDA) have not specifically approved salicylates for these purposes for use in animals. Salicylates inhibit prostaglandin synthesis by non-selectivey binding COX-1 and COX-2.

Flunixin meglumine is widely used as an analgesic, anti-inflammatory, and antipyretic. The exact pharmacologic mode of action is unknown, but the analgesic action may involve blocking pain impulses by non-selectively inhibiting COX-1 and COX-2 which blocks the synthesis of PGs and inhibits other local mediators of the inflammatory response. Flunixin meglumine is approved by the FDA for the treatment of fever and inflammation in cattle, and inflammation and pain in horses (USP, 2004). Banamine-S® is the only form of flunixin meglumine that is approved by the FDA for use in swine, but is only approved for reduction in fever, and not for analgesic effects (Schering-Plough Animal Health Corp., Summit, NJ 07901). In a study conducted by Langhoff and colleagues (2009), pain induced by castration of piglets was reduced by the pre-emptive administration of flunixin meglumine as observed with reduced cortisol concentration for four hours after

castration compared to the control group given no analgesics, as well as a reduction in pain behaviors such as tail wagging, drooping the tail, and changing position.

**2.4 Objective Pain Assessment**

The determination of pain thresholds allow for a more precise assessment and a quantification of thermal and mechanical allodynia and hyperalgesia (Hansson et al., 2007). Nociceptive withdrawal responses are assessed by applying a painful stimulus, the intensity of which is controlled, to the animal and measuring the animal's latency to respond and the nature of its reaction (Veissier, 2000; Hansson et al., 2007). Anil and colleagues (2005) state that if a stimulus is capable of creating a painful sensation in humans, it could be assumed to cause the same sensation in animals. According to Bateson (1991), the criteria that lead to the judgment that a human is in pain can be generalized on the basis of uncovering comparable mechanisms and comparable behavior. If mammals possess the functional features and anatomical structures to detect noxious stimuli, then it could be assumed that the animal is capable of nociception. To suggest pain perception, it must be shown that behavioral or physiological alterations are not only reflexive, but that include higher brain functioning. To examine possible pain perception, indirect measurements of behavior and physiological responses to noxious stimuli or a potentially painful event are measured.

Nociceptive withdrawal threshold testing has been and remains a common method of determining changes in the sensitivity of various tissues to noxious and non-noxious stimuli in addition to the evaluation of analgesic drug efficacy (Bussieres et al., 2008). Chen and colleagues (1999) compared the development of primary hyperalgesia to mechanical and thermal stimuli between two animal pain models, the formalin test and the bee venom test, each of which was subcutaneously administered into the plantar surface of the hindpaw of a rat. Both the formalin and the bee venom injection produced inflammation accompanied by pronounced primary hyperalgesia to mechanical and thermal stimuli.

According to Garry and colleagues (2004), neurons in the dorsal spinal cord that are involved in processing mechanical and thermal sensory inputs can become functionally sensitized when subjected to persistent afferent activity following tissue damage which corresponds to a state of hyperalgesia.

It is important in the diagnosis of pain in animals to appreciate the species, breed and individual differences in their response to injury (Short, 1998). Pain in an individualized and subjective experience and an individual's response can vary as a result of age, stage of development, gender, environment, or prior pain experience. Pain assessment can also be subjective and could be based on the assessors' background and experience assessing animal pain. Another challenge in animal pain assessment is the inability of animals to verbally communicate their level of pain. All of these factors limit the usefulness of subjective assessment measures as a tool to identify and assess animal pain. Objective pain assessment tools are needed to identify and quantify pain in animal pain models.

The follow portion of this literature review details research assessing the validity of objective pain assessment tools. Molony and Kent (1997) state that acute pain assessment is improved by using several indices. The two primary pain assessment techniques analyzed for this thesis are mechanical and thermal nociceptive thresholds assessments. Other pain assessment tools and indicators of pain will be introduced within this literature review and specific research data will be explained, but not detailed to the same level as mechanical or thermal nociceptive threshold assessments.

### 2.4.1 Mechanical Nociceptive Threshold

The mechanical nociceptive threshold (MNT) is the minimum pressure that produces a pain response (Fischer, 1987; Haussler and Erb, 2006). Measuring MNTs and sensitivity to pain using pressure algometry (PA) is a relatively novel method to objectively assess pain in livestock. Pressure algometry was first used when quantifying human MNTs, and has been researched extensively in both healthy subjects and subjects with clinical pain. Giesbrecht and Battie (2005) compared pressure pain thresholds using pressure algometry to healthy non-painful subjects and subjects with chronic low back pain. This study found that the mean pressure pain thresholds were lower at every test location for chronic back pain subjects compared to pain-free subjects. Fibromyalgia (FM) research has also been conducted comparing MNTs of subjects with FM to healthy control subjects, with results indicating that subjects with FM reported lower MNTs compared to healthy control subjects (Maquet et al., 2004)

Several published studies have researched the reliability and repeatability over consecutive days, and found that the pain threshold does not change in healthy human subjects over consecutive testing days (Nussbaum and Downes, 1998; Persson et al., 2004; Ylinen et al., 2007). Jones and colleagues (2007) evaluated the reliability of the pressure algometer over four consecutive days, tested on healthy women, and reduced MNT values were reported on consecutive days compared to the initial baseline values at eight separate testing locations on the test subject's torso and upper arm. However, this study found the pressure algometer test to provide consistent, albeit declining, means across test subjects and testing locations on each trial day. Inter-examiner variability has also been tested using pressure algometry and results found good inter-rater reliability (Antonaci et al., 1998; Chesterton et al., 2007). Chesterton and colleagues (2007) concluded that inter-rater reliability has greater within-observer mean values than the first MNT value, suggesting that MNT means rather than single measurements should be used in multiple-observer studies of MNT.

Heinrich and colleagues (2010) first published research validating pressure algometry (PA) as a pain assessment tool for use in disbudding dairy calves. Dyer and colleagues (2007) quantified claw pain in dairy cattle and its relationship to limb locomotion using PA. The results of Dyer's study support PA as an objective measure of pain for assessing claw pain in lame dairy cows. The data from Dyer's study demonstrated that the magnitude of claw pain correlated to the number and severity of lesions and locomotor disturbances. Stubsjøen and colleagues (2009, 2010) measured mechanical nociceptive thresholds to compare the level of pain induced by inflation of a tourniquet on lamb forelimbs with an electronic PA, although was not able to detect differences due to the short duration of tourniquet administration. Haussler and colleagues (2006, 2007, 2008) have quantified pain-pressure thresholds in equines and found the PA to be a useful measure of pain thresholds in induced back pain and induced osteoarthritis equine models, as well as quantified reference MNT as objective standards for inspection and detection of possible leg irritants in Tennessee Walking Horses. Varco-Cocks and colleagues (2006) quantified the intensity muscle pain in racehorses suspected to have sacroiliac dysfunction (SID) and found a significant correlation between the MNT and suspected SID grade and manual palpation response, confirming that PA is a repeatable test that can objectively measure muscle pain in horses. Pressure nociception has also been researched for evaluation of analgesia in cats (Dixon et al., 2007) and rats (Andrew et al., 1999; Chen et al., 1999).

Lame sheep (Ley et al., 1989) and horses (Chambers et al., 1994) demonstrated an increased sensitivity to a noxious stimulus, indicating that lame animals were in a hyperalgesic state. In the lame sheep study, ewes were suffering from foot rot, and when a local anesthetic block was injected into the affected foot, the MNT values increased similar to those of non-lame ewes. According to a study from Whay and colleagues (1997), as lameness increased, the MNT significantly decreased indicating sensitization in dairy heifers whom had developed sole lesions in the hind claws. No published research has been conducted validating MNT testing for use in swine lameness.

The VF nociceptive test also measures MNTs by gradually increasing the amount of pressure until a withdrawal response is seen. The VF tests detect more sensitive differences in MNT then PA by stimulating fewer nociceptors with a smaller force diameter. Chaplan and colleagues (1994) measured allodynia using VF and lightly touching the rats' paw to study neuropathic pain. KuKanich and colleagues (2005) objectively evaluated the efficacy of morphine using VF in beagles, and found that the VF device was technically simple to use, caused no apparent tissue damage and was able to discriminate the antinociceptive effects of morphine. Reduá and colleagues (2002) provided evidence that a VF device was able to quantify cutaneous sensitivity and detect differences in the nociceptive threshold from mares with an incision on one leg versus the nonincised leg.

### 2.4.2 Thermal Nociceptive Threshold

Rats and mice have been tested extensively with thermal nociceptive threshold tests (Andrew et al, 1999; Chen et al, 1999; Hargreaves et al, 1988). Andrew and colleagues (1999) assessed the mechanical and thermal responses of single fiber cutaneous nociceptors of the rat hindpaw after induction of acute inflammation. Results of the Andrew and colleagues study provide evidence that mechanical hyperalgesia caused by peripheral inflammation could be explained by nociceptor sensitization. Chen and colleagues (1999) quantified hyperalgesia to mechanical and heat stimuli following subcutaneous administration of bee venom or formalin to compare the duration of hyperalgesia with two different animal pain models. In a thermal sensitivity (TS) pain test conducted on rat hindpaws to study the effects of spontaneous firing of C-fiber nociceptors, Djouhri and colleagues (2006) defined the representation of hyperalgesia to be a reduced response latency to a normally noxious heat stimulus. Pinheiro Machado and colleagues (1998) developed a radiant TS test used for

measuring the nociceptive threshold to morphine sulphate as tested on the forefoot of peri-parturient dairy cows. Nolan and colleagues (1987) also tested a ramped radiant TS on the pinna of ewes ear and found that this apparatus produced reliable nociceptive thresholds. The two previous experiments assessed testing devices that provided a ramped thermal stimuli which increased in temperature until a withdrawal response was detected. The TS test assessed in this thesis provided a constant temperature heated light source and the latency to this constant temperature was measured.

The effects of thermal nociception have also been tested on healthy and painful humans (Agostinho et al, 2009; Djouhri et al, 2006; Granot et al, 2003). Research from Agostinho and colleagues (2009) investigated habituation effects during thermal quantitative sensory testing using eight repetitive measurements for thermal detection and pain thresholds in healthy human subjects. This research tested repeatability by comparing measurements on two different days as well as within trial habituation over eight successive testing repetitions and found high correlations over two testing days with no significant differences of the heat pain threshold over two days. Results from this study suggest pronounced habituation for heat pain threshold over eight successive repetitions.

The TS assessment using a laser technique has been used successfully to measure nociceptive thresholds in humans (Arendt-Nielsen and Bjerring, 1988), laboratory rodents (Fan et al., 1995) and farm animals (Veissier et al., 2000; Herskin et al, 2003, 2004, 2009). Thermal nociception responses were assessed by Herskin and colleagues (2004) with a $CO_2$ laser to compare the effects of social and restraint challenges to contrast behavioral, nociception and adrenocortical responses in dairy cows housed in tie-stalls. Herskin and colleagues (2009) measured thermal nociception in group-housed swine using a $CO_2$ laser aimed at either the hind leg or the shoulder region of gilts. The Herskin and colleagues' study provided evidence that behavioral responses to a nociceptive $CO_2$ cutaneous laser stimulation are a valid measure of thermal nociception in group-housed gilts due to the increased behavioral responses and decreased latencies to behavioral responses with elevated laser power output. Our research is the first analyzing the objectiveness and repeatability of TS to focused radiant heat for lameness detection in sows and hyperalgesia associated with cautery disbudding in calves.

### 2.4.3 Physiological Indicators of Pain

Pain is commonly associated with changes in the autonomic nervous system function, leading to increased heart rate, respiratory rate, and biochemical or other factors. While these parameters are objective, they are rather unspecific and may be influenced by environment, stress, or anxiety (Driessen and Zarucco, 2007; Dobromylskyi et al., 2000). Most of the physiologic indicators are components of the fight or flight response, priming the animal for action. Physiologic factors may increase with excitement or handling and are not necessarily aversive or painful to the animal.

According to Harbuz and Lightman (1992), acute stress results in an immediate increase in corticotrophin releasing factor (CRF) which evokes the release of ACTH and a subsequent increase in circulating corticosterone and cortisol. Graf and Senn (1999) measured plasma concentration of vasopressin, ACTH, and cortisol, and found that each biomarker concentration increased following cautery disbudding in non-anesthetized calves. Presence of corticosteroids in the blood indicate that the hypothalamic-pituitary-adrenal (HPA) axis and the 'stress response' have been activated, but may not be necessarily caused by pain alone. Cortisol has been shown to spike immediately following dehorning and again at the end of the duration of action of local anesthetic, then declines to a plateau level for approximately seven to nine hours before returning to baseline (Stafford and Mellor, 2005). The hypothalamic-pituitary-adrenal (HPA) axis is very sensitive to mild stress, which according to Harbuz and Lightman (1992) prevents the use of circulating levels of hormone to differentiate between different stressors. Continued and prolonged stress may disturb the HPA axis; the adaptive responses of the HPA axis may become maladaptive with the general effect of these maladaptive changes leading to elevated basal glucocorticoid concentration, altered circadian rhythmicity of ACTH release, reduced negative feedback control, and adrenal hypertrophy (Blackburn-Munro and Blackburn-Munro, 2001). The degree of HPA maladaption is dependent on the nature, duration and mode of the stressor.

The first published recording of heart rates for detection of pain for dehorning of calves was by Grøndahl-Nielsen and colleagues (1999) with electrocardiogram (ECG) recording for the first 4 hours post dehorning who found increased heart rates for dehorned calves administered no anesthetic or sedation and decreased heart rates for calves administered sedatives for the first 213 minutes post dehorning. Stewart et al

(2009) provided evidence that calves dehorned without local anesthetics or NSAIDs had elevated heart rates for three hours post cautery disbudding.

Alvarez and colleagues (2009) measured heart rate and respiratory rate for the first four hours following disbudding of goats, and heart rate and respiratory rates did not differ from kids disbudded with local anesthetic compared to kids sham disbudded with a local block or kids disbudded with no block. Heinrich and colleagues (2009) used cautery to disbud dairy calves and showed elevated respiratory rates for 6 hours post disbudding, and elevated heart rates for 24 hours post-disbudding.

In a study conducted by Stewart and colleagues (2009), eye temperature was measured with infrared thermography (IRT) after bovine cautery disbudding treatment with and without local anesthetic. The results of this study provide evidence of a decrease in eye temperature as the local anesthetic was wearing off post-disbudding, suggesting that this technique may be a useful pain assessment tool. Stubjøen and colleagues (2009) also evaluated IRT as a non-invasive method to assess lameness pain in sheep, but did not detect differences in eye temperature in lame sheep.

Electroencephalogram (EEG) testing has been validated for assessment of noxious sensation caused by scoop dehorning in calves either with or without a lidocaine block by Gibson and colleagues (2007). Dehorning without the local anesthetic resulted in an increased cortical function frequency following dehorning with no change in calves administered the nerve block (Gibson et al., 2007).

#### 2.4.4 Growth and Production

Within animal pain research models, weight and growth can be measured, as well as feed and water intake. Cytokines released as a result of the inflammatory response can induce anorexia and lethargy (Johnson, 1997), negatively impacting animal health. Faulkner and Weary (2000) and McMeekan and colleagues (1999) measured a reduction in feed intake and body weight following calf dehorning. Production factors may indicate pain. In a study by Green and colleagues (2002), clinically lame dairy cows decreased milk yield up to 360 kg over the course of a lactation with reduced milk yields from 4 months before the clinical diagnosis of lameness to 5 months post diagnosis.

### 2.4.5 Behavioral Alterations and Activity

Many different behavioral changes can be seen across species in response to pain either from injury or as a result of routine management procedures. Types of behavior measured and analyzed are dependent on the animal pain model being studied and may include, but are not limited to changes in behavioral patterns, appearance, facial expressions, vocalization, posture, feeding, drinking, licking, shaking, restlessness, response to handling or fatigue (Anil et al., 2002; Bath, 1998; Fitzpatrick et al., 2006; Molony and Kent, 1997; Smulders et al., 2006; Stasiak et al., 2003). Definitions of specific behaviors and postures are determined within a detailed ethogram for each research experimental design.

There are many papers studying behavior associated with lameness in different animal species. The following are a few examples of the wide array of behaviors that can be measured associated with lameness. Behavioral measures for lameness in include gait scores, time spent lying down, frequency of steps, and weight distribution. Chapinal and colleagues (2010) measured gait scores, time spent lying down, frequency of steps and weight distribution between legs in lactating dairy cows after hoof trimming. In the Chapinal study, neither hoof trimming nor a combination of hoof trimming and administration of flunixin meglumine prior to hoof trimming affected gait score or weight distribution between legs, however cows spent more time lying in the two days following hoof trimming independent of the analgesic treatment. O'Callaghan and colleagues (2003) evaluated the daily activity levels as an indicator of pain and discomfort resulting from lameness in dairy cattle and significant reductions in daily activity levels were associated with the presence of foot lesions. The frequencies of vocalization and lip licking were assessed in sheep with moderate pain induced by a tourniquet attached to the forelimb, and a reduction in the frequencies were detected in both behaviors for the first three days following induced ischemic pain, as was a reduction in ischemic pain over the same timeline as tested with heart rate variability (HRV) (Stubjøen et al., 2009).

Behavior associated with calf disbudding and dehorning pain has been studied to measure the duration of pain as well as duration of pain mitigation agents. Graf and Senn (1998) measured behavior associated with cautery disbudding, specifically: tail wagging, head moving, head shaking, tripping, and rearing. Compared to a sham disbudding procedure, disbudded calves displayed increased frequencies of tail wagging, head moving, tripping, and rearing as well as abnormal backward-locomotion and head shaking with the behavioral reactions

eliminated or markedly reduced in calves administered a lidocaine local anesthetic (Graf and Senn, 1998). When comparing behavioral responses between disbudded calves with or without the analgesic ketoprofen, Faulkner and Weary (2000) measured the frequency of head shaking or ear flicking and found that ketoprofen mitigated pain after cautery disbudding by reducing the frequency of head shaking and ear flicking as well as head rubbing. Grøndahl-Nielsen and colleagues (1999) found that head and leg movements were reduced when a cornual nerve anesthetic was administered prior to disbudding in 4-6 week old calves. McMeekan and colleagues (1999) calculated the percentage of calves lying, grazing or ruminating for up to two days post disbudding in 3-4 month old calves and detected that calves dehorned without a local anesthetic (lidocaine) or with ketoprofen and no anesthetic spent more time lying and less time grazing or ruminating immediately following dehorning compared to control calves not dehorned. Heinrich and colleagues (2010) found that meloxicam-treated calves were less active than lidocaine treated controls during the first 5 hours following dehorning. Results from the Heinrich study also found the meloxicam-treated disbudded calves displayed less ear flicking during the +44 h following disbudding and less head shaking during the first +9 h following dehorning which suggest that meloxicam was effective for reducing post-surgical pain associated with cautery disbudding.

## 2.5 Animal Pain Models

For this thesis, two specific animal pain models were utilized to examine the ability of PA and TS pain assessment tests to detect differences in nociceptive thresholds. The following portion of the literature review discusses previous research pertaining to livestock lameness, the utilization of Amphotericin B for a transient lame-induced research model and bovine disbudding.

### 2.5.1 Lameness

According to a study by Anil and colleagues (2009), lame sows' are at 1.7 times greater risk of being removed from the breeding herd within 350 days after lameness assessment. According to the USDA (2007), lameness was the third main reason producers cull breeding-age females from breeding herd, third only to old age and reproductive failure in 2006. Johnson and colleagues (1997) reported that pro-inflammatory cytokines

may inhibit growth and may reduce appetite, and may affect metabolism. If left untreated, lameness, inflammation and pain negatively impact not only animal health and welfare, but also has an economic impact on producers. In order to minimize economic losses to producers, pain assessment tools need to be validated for detecting lameness and NSAIDs need to be tested for efficacy in treating pain associated with lameness.

O'Callaghan and colleagues (2003) reported that a trained observer rescoring the gait of 129 cows was consistent for only 56% of observations. This inconsistency in lameness assessment provides evidence for the need of objective pain assessment tools to detect lameness. Much of the published research concerning livestock lameness has validated lameness scoring assessments (Main et at., 2000; Rajkondawar et al., 2006), limb force distribution (de Carvalho et al., 2009; Neveux et al., 2006), and gait analysis (Flower et al., 2005; von Wachenfelt et al., 2009). When animals are lame, relief may be gained by reducing the weight loaded on the painful limb by transferring weight to the other limbs (Gahery and Nieoullon, 1978). Deviations in gait are thought to be due to the pain associated with injuries on the hooves and legs (Whay et al., 1998). Flower and colleagues (2005) analyzed the biomechanical differences in gait variables in dairy cattle and found that healthy non-lame cows walked faster, had shorter stride durations, and longer strides compared to cows with sole ulcers.

Piesla and colleagues (2009) compared different validated rodent models of inflammatory and neuropathic pain to compare alterations in gait, and to pharmacologically determine if changes in gait were a result of pain or other factors such as edema or motor nerve dysfunction. Results of the Piesla study provide evidence that both inflammation and nerve injury lead to abnormal gait and that changes in gait, as a result of inflammation, are driven by pain.

Rushen and colleagues (2007) injected lame dairy cows with the local anesthetic lidocaine into the affected lame hoof bulb. After administration of the local nerve block, lame cows showed improved gait scores and a more even weight distribution, as measured with force plates. Results of a study conducted by Flower and colleagues (2008) provide evidence of a modest improvement in the gait scores of lame dairy cows treated with the NSAID ketoprofen. Whay and colleagues (2005) reported that hyperalgesia associated with lameness was decreased in lame cows that received ketoprofen, as assessed using MNT testing. Welsh and colleagues (1995) tested sheep with foot-rot compared to control sheep and showed that flunixin meglumine ( i.v. 1.0 or 2.0

mg/kg) had no improved analgesic effect over 6 h compared to control sheep for either dosage. However, the repeated administration of flunixin meglumine (i.v. 1.0 mg/kg, q 24 h) over three days reduced their thresholds to noxious mechanical stimulation to within the same range as in matched healthy sheep. Although neither flunixin meglumine nor sodium salicylate are specifically FDA approved for analgesic use in swine lameness, a lack of available analgesics warrants testing for possible efficacy.

### 2.5.2 Amphotericin B

Amphotericin B was first used intra-articularly to treat coccidioidal synovitis, and the injection itself caused an acute localized inflammatory response (Aidem, 1968). The injection of Amphotericin B leads to the development of a temporary acute localized synovitis by inducing the synovial cells to produce and secrete cytokines, a process which triggers a local inflammatory response within the joint. Amphotericin B-induced lameness models provide benefit compared to naturally-occurring lameness, in that an inducible model produces a predictable, reliable, reproducible and moderate synovitis that is transient in duration, while naturally-occurring lameness models cannot control the severity or duration of pain. This lack of control limits the interpretation of the degree of pain and leads to difficulty in validating pain assessment tools. The chemical induction of lameness allows for a known and consistent degree of lameness, which is beneficial for evaluating pain assessment tools and effectiveness of non-steroidal anti-inflammatory drugs (NSAIDs) for mitigating lameness pain. Amphotericin B has been shown to induce acute transient lameness in cattle (Kotschwar et al, 2009) and horses (Hulten et al, 2002; Marttinen et al, 2006; Bussieres et al, 2008). Amphotericin B has been used extensively to induce lameness in horses at injection levels of 5-25 mg evaluating the effectiveness of analgesics (Pleasant et al., 1997; Marttinen et al., 2006; Suominen et al., 2001; Sysel et al., 1996), pain therapy (Crawford et al., 1991), and pain assessment (Bussieres et al., 2008). Karriker and colleagues (L. A. Karriker, Iowa State University, Ames, Iowa, personal communication) have shown that injection of Amphotericin B into the interdigital space of the claw causes transient lameness for 7 to 10 days in mature sows.

### 2.5.3 Disbudding

Dehorning is a common management practice on dairy farms throughout the world (Stafford and Mellor, 2005). Hot-iron disbudding is a routine husbandry practice performed on dairy calves that prevents horn development by destroying horn tissue using heat cauterization. Cautery disbudding typically occurs between 2 to 6 weeks of age, and should be done at an early age, because after the horn bud develops and become fixed to the skull, amputation dehorning is necessary. Amputation dehorning is considered more painful than cautery disbudding (Stafford and Mellor, 2005). Horn removal, either disbudding or dehorning, is performed because it reduces the risk of injury to herd mates and to human handlers. Herd mate injury contributes to carcass bruising and hide damage, both of which are of economic importance to producers.

According to the American Veterinary Medical Association (AVMA) (2010), disbudding and dehorning of cattle in the United States is not currently regulated. As of 2008, the AVMA (2008) recommends disbudding as the preferred method of dehorning calves, that dehorning be done at the earliest age practical and that local anesthetics should be considered. The Farm and Welfare Council (FAWC) of the United Kingdom recommends that disbudding should take place before calves are two months of age, and that under the Protection of Animals (Anesthetics) Act of 1954, it is an offense to disbud calves or dehorn any cattle without the use of an anesthetic other than when chemical cauterization is used (DEFRA, 2003). Recommendations pertaining to disbudding of calves from the Canadian Veterinary Medical Association (CVMA, 1996) are that this procedure be performed within the first week of life. Research from Petrie and colleagues (1996) support cautery disbudding as resulting in less distress than scoop dehorning based on the reduced levels of plasma cortisol, and fewer stress-related behaviors.

It is well established in published research that disbudding without a local anesthetic causes pain (Petrie et al., 1996; Graf and Senn, 1999). Faulkner and Weary (2000) found that pain caused by dehorning is considerable and may persist for at least 24 hours. In a study conducted by Heinrich and colleagues (2010), pain persisted for 44 hour post dehorning.

Previous research has provided evidence that dehorned calves begin to feel pain once local anesthetics wear off (McMeekan et al., 1998a, 1998b; Faulkner and Weary, 2000). Local anesthetic effectively relieves pain and stress caused by the dehorning surgery itself and maintains relief for one to four hours, depending on the anesthetic agent and whether additives such as epinephrine are used. According to McMeekan and

colleagues (1998a), calves are insensitive to being pricked with needles in the area surrounding the horns throughout the duration of the nerve block.

Bupivicaine and lidocaine are the most common anesthetic blocks currently utilized in the pool of published research. Longer postoperative pain relief for hot-iron dehorning can be provided by longer-acting local blocks (bupivicaine) or by providing nonsteroidal antiinflammatories such as ketoprofen (Milligan et al., 2004). McMeekan and colleagues (1998a; 1998b) studied combinations of a local anesthetic, bupivicaine, and an injection of the NSAID ketoprofen, and found that when administering both bupivicaine and ketoprofen, the cortisol response did not differ from control calves that were not dehorned for the first nine hours post disbudding and that bupivicaine alone delayed the cortisol spike for four hours post scoop dehorning. Duffield and colleagues (2010) studied a combination of lidocaine as a nerve block plus ketoprofen and provided evidence that ketoprofen treated calves displayed less head movement behaviors, including ear flicks head shakes and head rubs, in the first seven hour post cautery disbudding but did not find a difference in cortisol concentrations between treatment groups. Doherty and colleagues (2007) compared the anesthetic effects of 2% lidocaine and a concentrated 5% lidocaine, and found that both provided the same level of anesthetic relief post-disbudding, but that 5% lidocaine treated calves displayed lower frequencies of kicking during disbudding suggesting a reduced level of discomfort at the time of disbudding.

Both concentrations of plasma cortisol (Boandl et al., 1989; Petrie et al., 1996; Sutherland et al., 2002a, 2002b; Stilwell et al., 2010) and behavioral assessments (Stilwell et al., 2010) and have been utilized to measure the duration of anesthetic effect with the use of local nerve block, as well as the duration of analgesic effect by use of NSAIDs. The most common behaviors measured around the time of dehorning associated with pain are: head movements, feeding, drinking, vocalizations, lying, grooming, rearing, and tail wagging (Graf and Senn, 1999; Faulkner and Weary, 2000; Duffield et al., 2010). Morrisse and colleagues (1995) studied the ratio of standing to lying in calves either chemically or cautery disbudded with or without anesthesia, and did not detect differences in the ratio of standing to lying between treatments. This study provided evidence that irrespective of treatment or anesthesia, the circadian activity was unchanged in the day prior and the day post disbudding. Pressure algometry (PA) is relatively new pain assessment tool for the disbudding research area and

has been previously validated as an objective pain assessment tool for disbudding pain by Heinrich and colleagues (2010).

**2.6 Summary**

Pain associated with injuries or routine management procedures can cause distress and affect animal welfare. Pain is the perception based on sensory input and emotional experience to tissue damage or a threat to tissue damage, and can be classified based on location, duration, or intensity of nociceptive signals. Nociception is the physiologic detection and transmission of noxious stimuli. Animal pain can be primarily mitigated pharmacologically through the use of local anesthetics, which inhibit the transmission of nociceptive signals, and or NSAIDS which inhibit COX enzymes from generating pro-inflammatory agents. Pain can be assessed using nociceptive threshold, either mechanical or thermal, physiologic parameters, production measures, or behavioral indicators, each of which have been studied in human and animal pain models. In order for novel anesthetics or analgesics to be approved for use in mitigating pain, objective pain tests must be validated for use in specific pain models such as lameness or disbudding.

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# CHAPTER 3: OBJECTIVE PAIN ASSESSMENT IN SOWS WITH INDUCED TRANSIENT LAMENESS

Modified from a paper to be submitted to the *Journal of Animal Science*

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**Abstract**

Sow lameness is an issue in the swine industry that can result in decreased animal health and productivity. The objectives of this study were to evaluate pressure algometry (PA) and thermal sensitivity (TS) as objective pain assessment tools for sow lameness and to evaluate analgesic drugs for mitigating lameness pain. Twelve mixed parity crossbred sows were anesthetized and injected with Amphotericin B in the distal interdigital joint space of both claws of one hind leg to induce transient lameness. Sows were randomly assigned to one of three analgesic treatment groups: 1. Sodium Salicylate (SS; 35mg/kg per os q.12.h + 0.04 ml/kg IM q.24.h sterile saline), 2. Flunixin meglumine (FM; 2.2 mg/kg IM q.24.h), or 3. Control (C; 0.04 ml/kg IM q.24.h sterile saline). All sows received each treatment over three trials, with a two-wk wash-out period between trials. Forty-eight h post-induction, analgesic treatments were administered daily for four consecutive d. Pain was assessed with PA and TS relative to a foot-lift response on each hind leg on d-1, d+1 and d+6 relative to induction (d0). Differences between sound (S) and lame (L) legs by trial d, with a simple effect comparison to analyze effect of treatment on d+6 were analyzed with GLIMMIX in SAS. Sows did not differ in response to PA on d-1 ($P>0.05$), and tolerated less pressure on L versus S on d+1 (d+1 PA Raw Means in kilograms of force: L 2.11±0.17; S 7.70±0.17 kgf; $P<0.01$). No treatment alleviated pain as tested with PA on d+6 (Raw Means (kgf): FM-L 5.39±0.34 FM-S 7.71±0.30; SS-L 4.61±0.37 SS-S 7.79±0.31; C-L 5.25±0.40 C-S 7.40±0.29; $P<0.05$ for all treatments). The TS latency difference from S to L differed on each trial d (d-1 $P=0.02$, d+1 $P<0.01$, d+6 $P<0.01$) regardless of treatment on d+6. (TS Raw means (s): d-1 L 7.34±0.55; d-1 S 9.09±0.64; d+1 L 3.26±0.21; d+1 S 6.80±0.64; d+6 L 5.99±0.56; d+6 S 8.41±0.73). In conclusion, these results support PA as an

objective non-invasive pain assessment tool for sows induced with transient lameness. The TS test was not valid for this induced lameness model due to high variability between hind leg latencies on d-1. Results from PA did not detect a benefit of SS or FM for mitigating lameness pain in this model of induced lameness.

**Keywords**: analgesia, lameness, pain, pressure algometry, swine, thermal sensitivity

## INTRODUCTION

Sow lameness negatively impacts animal health and welfare, as well as decreases productivity and sow productive lifetime, and is a major reason for culling sows from a breeding herd. (Stalder et al., 2004; Anil et al., 2008). In a producer survey, lameness was the reason for culling 9-15% of sows (Anil et al., 2005), but Knauer and colleagues (2007) reported that 23% of on-farm cullings were inaccurate, which may indicate that current estimated lameness-related culling rates are too conservative.

Swine producers and veterinarians lack accurate, non-invasive objective pain assessment tools. Pressure algometry (PA) measures the mechanical nociceptive threshold (MNT) relative to a reflexive withdrawal response, and has been tested in equines (Varcoe-Cocks et al., 2006; Haussler et al., 2006, 2007, 2008) and dairy cattle (Dyer et al., 2007). The thermal sensitivity (TS) test measures latency for a withdrawal response to precise, focused radiant heat and had been studied in laboratory animals (Hargreaves et al, 1988; Andrew and Greenspan, 1999; Chen et al, 1999), healthy and painful humans (Granot et al, 2003; Djouhri et al, 2006; Agostinho et al, 2009), and group-housed swine (Herskin et al., 2009). To date neither PA nor TS have been explored for assessing lameness pain in swine.

Currently, no Food and Drug Administration (FDA) approved non-steroidal anti-inflammatory drugs (NSAID) are available specifically for pain associated with lameness in swine. Sodium salicylate (SS) is commonly used as an analgesic, antipyretic, and anti-inflammatory drug (USP, 2004). Flunixin meglumine (FM) (Banamine®) is approved for use in swine for pyrexia associated with respiratory disease, but is not approved for pain. Although neither SS nor FM are approved for analgesic use in swine lameness, a lack of available analgesics warrants testing for possible efficacy.

Amphotericin B has been shown to induce acute transient lameness in cattle (Kotschwar et al., 2009) and horses (Hulten et al., 2002; Marttinen et al., 2006; Bussieres et al., 2008). Karriker and colleagues (L. A.

Karriker, Iowa State University, Ames, Iowa, personal communication) have shown that injection of Amphotericin B into the interdigital space of the claw induced a transient lameness for 7 to 10 days in mature sows.

The objectives of this study were to evaluate PA and TS as objective pain assessment tools for identifying lameness, and to assess SS and FM as treatments for pain associated with lameness. The prediction is sows will have a decreased tolerance for pain when lame compared to when sound and that NSAIDs will reduce sows' pain response.

## MATERIALS AND METHODS

The protocol for this experiment was approved by the Iowa State University Institutional Animal Care and Use Committee (4-09-6709-S).

*Experimental Design and NSAID Treatments*

A repeated measures design was used to compare responses by each sow during sound (D-1), most lame (D+1), and treatment day (D+6), relative to lameness induction (D0). Sows were randomly assigned to one of three analgesic treatment groups: 1. Sodium Salicylate (SS; 35mg/kg q.12.h PO + 0.04 ml/kg IM q.24.h sterile saline), 2. Flunixin meglumine (FM; 2.2 mg/kg IM q.24.h), or 3. Control (C; 0.04 ml/kg IM q.24.h sterile saline). All sows received each treatment over three trials, with a two-week wash-out period between trials. Forty-eight h post-induction, analgesic treatments were administered daily for four consecutive d. This lameness model facilitated data collection on the same sow when both sound and lame, thus reducing the total number of sows required. In addition, this model design allowed individual sows to be their own control by comparing lame hind leg to sound hind leg for each analgesic treatment. Sows were acclimated for seven d to both the pain tests and handling prior to the trial. Sows were randomly assigned to lameness induction in either the left or right hind leg; six sows' were assigned left-leg lame and six sows were right-leg lame with that designated lame leg remaining throughout the trial. Inter-observer variation was eliminated for the pain tests by having a single observer over all trials for both pain tests. Pain tests were performed at the same time of day to control for circadian patterns of pain sensitivity. The observer was blinded to the numeric output values during

the pain test assessment of the foot lift response. To control for observer bias, researchers were blinded to analgesic treatments, but could not be blinded to the trial d.

*Animals and Housing*

Twelve clinically normal mixed parity white crossbred sows were purchased from a private commercial producer in Iowa and were assessed for pain using PA and TS. The average weight of sows was 200.9±30.5 kg (441.9±67.1 lb).

To avoid confounding lameness associated with injury due to aggression, sows were individually housed in a research unit located at the Iowa State University College of Veterinary Medicine, Ames, IA. Each sow was housed in a concrete pen providing 5.1 $m^2$ and a 0.6 m deep concrete ledge along the rear wall of the pen where sows were fed. Pens contained a rubber mat that covered the entire floor area to minimize the abrasion of the concrete floor. Pens were set up in two rows with a central aisle and allowed for nose to nose contact with cohorts. Sows had *ad libitum* access to water and were fed six pounds per day of 14.8% CP TMR composed of ground corn, soybeans, and nutrients formulated according to Swine NRC guidelines with no antimicrobials. A commercially available, FDA approved estrus suppressant (0.22% Altrenogest, Matrix®, Intervet/Schering-Plough Animal Health, Millsboro, DE, USA) was orally top-dressed on the sows' daily feed at a dosage of 6.8 ml/d.

*Induction of Lameness*

Feed was withheld 18 h, and water withheld 1 h prior to anesthesia to reduce the possibility of vomiting and aspiration during the anesthesia and recovery periods. Sows were restrained with a wire hog snare and anesthetized (2.2 mg/kg Xylazine, 1.1 mg/kg Ketamine and 2.2 mg/kg Telazol injected intramuscularly). Following sedation, sows were placed in lateral recumbency and the assigned hind limb was washed with mild soap and water. The assigned leg was injected in the distal interdigital space on both lateral and medial claws with 10 mg amphotericin B (total volume of 1 ml in each claw) to induce lameness in that leg (Figure 3.1). Respiration rate and rectal temperature were monitored continuously until they returned to standing. Core temperature was regulated with heating mats and blankets as necessary. Pain and lameness was expected 24 h

post-induction, with full recovery expected in three to ten d post-induction. Although painful, previous experience with this model indicated that sows would be weight bearing, bright, alert and responsive upon recovery from the anesthetic (L. A. Karriker, Iowa State University, Ames, Iowa, personal communication).

*Pain Tests*

All pain tests were completed in a modified gestation stall (0.61 m x 2 m) outside of the sows' home pens. Sows were fed while in the gestation stall, during testing. Prior to pain tests, both hind legs were rinsed with water, dried and landmarks were marked with a permanent paint pen. Pain tests were administered on D-1, D+1, and D+6 relative to lameness induction (D0).

*Pressure Algometry (PA):* A hand-held pressure algometer (Wagner Force Ten™ FDX 50 Compact Digital Force Gage, Wagner Instruments, CT, USA) with a 1 $cm^2$ flat rubber tip was used to measure mechanical nociceptive thresholds (MNTs) in kilograms of force (kgf). The application rate for all sows on all landmarks was approximately 1 kgf/second. The maximum force applied was 10 kgf over a 10 s time-frame. Pressure was applied perpendicularly to three landmarks (Figure 3.2) in a randomized sequence for each sow: (1) middle of cannon (predicted to be non-painful control site; C), (2) 1 cm, above the coronary band on the lateral claw (Outer Hind Claw; O), and (3) 1 cm above the coronary band on the medial claw (Inner Hind Claw; I). The landmark sequence was repeated in triplicate on the right hind leg followed by the same sequence repeated in triplicate on the left hind leg. When a foot-lift response was observed, pressure was immediately removed, and the peak pressure representing the MNT was recorded (Figure 3.3). The observer remained blinded to the peak pressure output to prevent bias by having the screen of the algometer face away during the testing, and not viewing any output values until the trial was complete.

*Thermal Sensitivity:* Thermal sensitivity tests immediately followed the pressure algometry test and measured the latency for a sow to illicit a withdrawal movement in response to precise, focused radiant heat stimulation. The thermal sensitivity plantar equipment (IITC Plantar Analgesia Meter, IITC Life Science Inc., Woodland Hills, CA, USA) was used. The analgesia meter was set at a constant 80% beam intensity, emitting 200°C, with a cut-off time of 20 seconds to prevent tissue damage. Thermal measurements were taken in triplicate on the lateral side of each hind leg 1 cm above the coronary band on the right hind leg followed in triplicate on the left

hind leg (Figure 3.4). The light was focused perpendicularly to the landmark area, and the test head turned on. When a sow lifted her leg off the ground, the light beam was turned off, the equipment automatically recorded the latency, and the latency was manually documented. The researcher applying the thermal test was not allowed to view the latency output to prevent bias.

*Statistical Analysis*

Raw means were obtained by averaging the triplicate data points in Excel. Raw means were initially tested for normality using PROC Univariate. No transformations were required. The means were then analyzed with PROC MEANS in SAS Version 9.2. Due to the expected high degree of sow-to-sow variability, the difference in response between the sound and lame leg within sow was used as the statistically relevant value.

All statistical analyses used Proc Glimmix of SAS version 9.2 (SAS Inst. Inc., Cary, NC) to analyze the difference between sound leg and lame leg for both pressure algometry and thermal sensitivity. Main effects (treatment, landmark, trial day, trial) and their interactions were tested. This model assumed normal (Gaussian) random effects. For both pain test (PA and TS) models, the Kenward-Rogers method was used for computing the denominator degrees of freedom of the test. Correlations of the data were due to the repeated observations on the same sows over three repeated trials with multiple random effects of the sows, and sow within time groups. Least Square Means provided estimates, standard error, and p values for variable interactions and effect comparisons. Tukey's test for pair wise comparisons was used to examine the effect of day within each treatment. A $P$ value of $\leq 0.05$ was considered significant. Pressure algometry and thermal sensitivity data were analyzed separately.

## RESULTS

*Lameness*

By casual observation, Amphotericin B led to decreased speed in gait, and decreased willingness to bear weight on lame legs, with maximum lameness effect occurring 24-h post-induction. Sows were still able to walk and bear weight on lame legs, but had a decreased desire to do so. There was no highly noticeable

inflammation or reddening of the skin on lame versus sound limbs. Although not quantified, there seemed to be no decrease in appetite since all sows consumed their entire daily rations.

*Pressure Algometry*

On D-1, there was no MNT difference (P = 0.55) between the baseline values of the sound and lame legs for any treatments over all trials (Raw Means: L 7.22±0.15; S 7.38±0.16 kgf). There was no MNT difference between landmarks on D-1 (Table 3.1 and Figure 3.5). There was a difference (P = 0.0023) between sound and lame legs on D+1 (L 2.11±0.17; S 7.70±0.17), at all landmarks (C P = 0.0025, I P = 0.0003, O P = 0.0004; Table 3.1). The MNT difference from sound to lame leg was significant from D-1 to D+1 for all landmarks and all treatment interactions for all three pairwise interactions of treatment landmark and day (P < 0.0001) (Table 3.1).

*Thermal Sensitivity*

The TS latency difference from sound to lame leg was different on all trial days (D-1 P = 0.0232, D+1 P < 0.0001, D+6 P = 0.0064) over all trials for the TS pain test (Table 3.1), with no difference between trial days (P > 0.05). Figure 3.6 graphs the raw means on sound and lame legs for D-1 and D+1 relative to induction (D0). When analyzing a trial day effect, there was no difference from D-1 to D+1 (Tukey-Kramer adjusted P = 0.14; Table 3.1).

*Analgesic Effect*

Increased MNT was observed in the PA test for the control treated sows from D+1 to D+6 (Table 3.2). A simple effect comparison of the interaction of treatment by day for PA revealed no differences between any treatment: FM vs. C (P = 0.90), FM vs. SS (P = 0.17) on Day +6 (Table 3.2 and Figure 3.7).

## DISCUSSION

*Transient Lameness Model*

To reduce the confounding effects of the high variability in pain tolerance between sows, this transient lameness model compared the pre- and post- lameness measurements from the same sow, as well as their recovery. This model allowed the same group of twelve sows to be repeatedly tested with different NSAID treatments over the entire trial with sufficient wash-out periods between each treatment. The two-wk wash-out period was determined to be sufficient based on the lack of differences between trials for baseline (D-1) PA MNT values. This transient-induced lameness model reduced the number of sows required and provided a consistent degree of moderate lameness. To further reduce confounding effects within each sow, the difference between the sound and lame leg on each trial day and landmark was the statistically relevant value.

Injecting Amphotericin B into the synovial joint produces a temporary acute localized synovitis by inducing the synovial cells to produce and secrete cytokines, which triggers a local inflammatory response within the joint. Amphotericin B-induced lameness models produce a predictable, reproducible and moderate-severity synovitis that is transient in duration. Naturally-occurring lameness research models cannot control the severity or duration of pain associated with lameness which limits the interpretation of the degree of pain and leads to difficulty in validating pain assessment tools. The severity of duration of lameness in this sow lameness model was similar to the results of Karriker and colleagues (L. A. Karriker, Iowa State University, Ames, Iowa, personal communication), in which the Amphotericin B-induced lameness was first documented in swine. The lameness severity and duration were similar to findings of Kotschwar and colleagues (2009) who first published an Amphotericin-B induced lameness in bovines.

*Pain Assessment Tests*

Pressure algometry (PA) was found to be an easy to apply, non-invasive, and relatively inexpensive ($400/PA kit) method to objectively quantify the pressure applied to anatomical landmarks. The mechanical nociceptive threshold (MNT) is defined as the amount of applied pressure necessary to produce pain (Fischer, 1987). Lower MNTs correlate with increased pain. The confounding issue of multiple observers and inter-rater reliability was eliminated from this research protocol by utilizing one observer for all trial days and both pain tests. Several published studies have researched the reliability and repeatability over consecutive days, and found that the pain threshold does not change in healthy subjects over consecutive testing days (Nussbaum and

Downes, 1998; Persson et al., 2004; Ylinen et al., 2007). Inter-examiner variability has also been tested using pressure algometry and results found good inter-rater reliability (Antonaci et al., 1998). Chesterton and colleagues (2007) concluded that inter-rater reliability has greater for observer mean values than their first rating, and suggests that means rather than single measurements should be used in multiple-observer studies of MNT.

This is the first research quantifying MNTs for sows with induced transient lameness. Dyer et al. (2007) quantified claw pain in dairy cattle and its relationship to limb locomotion using PA. The results of Dyer's study support PA as an objective measure of pain in dairy cows. This data from this study demonstrated that the magnitude of claw pain correlated to the number and severity of lesions and locomotor disturbances. Stubsjøen and colleagues (2009) measured MNTs to compare the level of pain induced by inflation of a tourniquet on lamb forelimbs with an electronic algometer, although was not able to detect differences due to the short duration of tourniquet administration. Varco-Cocks et al. (2006) quantified the intensity muscle pain in racehorses suspected to have sacroiliac dysfunction (SID) and found a significant correlation between the MNT and suspected SID grade and manual palpation response, confirming that PA is a repeatable test that can objectively measure muscle pain in horses. Pressure nociception has also been researched for evaluation of analgesia in cats (Dixon et al., 2007) and rats (Andrew and Greenspan, 1999; Chen et al., 1999).

One of the assumptions tested was to determine if the cannon landmark was an appropriate non-painful control site. The simple effect of the comparison between landmark and trial day showed that the difference between the sound and lame leg at the cannon landmark between D-1 and D+1 was significant. While the MNT for the cannon landmark on the lame leg was elevated compared to the inner or outer claw, the decreased force compared to its baseline, did not support this landmark as an appropriate non-painful control landmark as tested with PA. In future studies, other possible non-painful control landmarks could include the claws of the front legs, or other anatomical landmarks other than the lower hind limbs.

Rats and mice have been tested extensively with thermal nociceptive threshold tests (Hargreaves et al, 1988; Andrew and Greenspan., 1999; Chen et al., 1999). The effects of thermal nociception have also been tested on humans classified as healthy and in pain (Granot et al., 2003; Djouhri et al., 2006; Agostinho et al., 2009). Herskin and colleagues (2009) measured cutaneous thermal nociception in group-housed swine using a

$CO_2$ laser, and found negative correlations between the power output of the laser and the latency to respond, and positive correlations between the laser output and forcefulness of the withdrawal response on sow hind legs. The benefit of the $CO_2$ laser was that sows were tested in their home pens with the test equipment outside of the pen. Higher laser outputs resulted in a faster and more intense leg-lift or kick. Our research is the first analyzing the objectiveness and repeatability of TS to this type of focused radiant heat for lameness detection in swine. The results of our study found the TS tests to not be valid based on the high degree of variability between the sound and lame legs for baseline values. Given that the baselines latencies between the hind legs differed, the accuracy of the TS latency results cannot be ensured.

There is a possibility that a small amount of residual water, from cleaning each leg, may have altered the conduction during the TS testing. Evaporation of the water from the skin could have altered the heat transfer from the light source. Evaporation and level of skin wetness was not tested, nor was the temperature of the skin measured before or after the TS assessment. In future studies, landmarks may be tested without prior cleaning with water to test if latency differences are detected when landmarks are semi-wet versus dry. Several seconds passed between each measurement while the hoof was moved to within three inches of the test head. This extra time and handling was not standardized between measurements, which could have also altered the response latency, and should be accounted for in future studies.

One of the assumptions tested was that the sows fully recovered from each trial induction, and that the wash-out period between trials was appropriate. This assumption was based on the transient sow lameness model validated by Karriker and colleagues (L. A. Karriker, Iowa State University, Ames, Iowa, personal communication) stating that pain associated with transient lameness lasts 7 to 10 days. The lack of difference between baseline MNT values over all trials supports the assumption that the sound to lame difference is not different between trials. The results from this analysis provide evidence that the recovery period between inductions was sufficient. In future studies additional measurement could be taken throughout the wash-out period to test the duration of recovery compared to baseline values.

*NSAIDs*

Coetzee and colleagues (2007) showed that sodium salicylate can be used to moderate pain associated with castration in calves. More recently, Kotschwar and colleagues (2009) studied the effects of sodium salicylate on bovine lameness, but found no analgesic benefit. Neither NSAID treatment resulted in improved MNTs resulting in less pain on D+6 compared to the control. Results from the PA indicate improvement from D+1 to D+6 in the difference between the sound and lame leg for each treatment, but these differences were not different between treatments. These changes represent a degree of natural resolution because the control treated sows improve from D+1 to D+6. The short half-life of both NSAID tested in this trial could have been washed-out of the sows on D+6 because the final doses were administered on Day+5 either 24 (sodium salicylate) or 32 (flunixin) hours prior to D+6 pain assessments.

Coetzee and colleagues (2007) and Kotschwar and colleagues (2009) both found the half-life of sodium salicylate to be approximately 4 h in cattle. Research findings by Kotschwar and colleagues (2009) suggests that the lack of analgesia provided by sodium salicylate (i.v. 50 mg/kg) was most likely was due to the very short half-life of the drug in the Amphotericin B-induced bovine lameness model. Buur and colleagues (2006) found that the half-life for flunixin meglumine (i.v. 2.0 mg/kg) in pigs to be 7.76 h. Chapinal and colleagues (2010) found little to no effect of providing flunixin meglumine (i.m. 2.2 mg/kg) during dairy hoof trimming when measuring daily lying time and gait scoring. Welsh and Nolan (1995) tested sheep with foot-rot compared to control sheep and showed that flunixin meglumine (i.v. 1.0 or 2.0 mg/kg) had no improved analgesic effect over 6 hours compared to control sheep for either dosage. However, the repeated administration of flunixin meglumine (i.v. 1.0 mg/kg, q 24 hours) over three days reduced their thresholds to noxious mechanical stimulation to within the same range as in matched healthy sheep. These previous research results suggest that flunixin or sodium salicylate may be beneficial in some research models. Several of these previous studies had administered the analgesics prior to or immediately after lameness insult. Our study began administration of analgesics on D+2, 48 h after the induction of lameness, and 24 h after the detection of lameness, which is a more accurate representation of real-life production analgesic treatment regimens.

In conclusion, these results support PA as an objective noninvasive pain assessment tool for sows induced with transient lameness. The TS test was not a valid pain assessment tool for this induced lameness

model due to the high degree of baseline variability. Results from PA did not differentiate a positive effect of either NSAID treatment to mitigate pain on D+6.

## IMPLICATIONS

This study describes and evaluates two methods to measure pain sensitivity in sows. Pressure algometry proved to be a valid and sensitive objective pain assessment tool for lameness in mature culled sows. This method for assessing pain sensitivity might be useful for research into analgesics and anesthesia, as well as testing changes in pain sensitivity caused by chronic pain over time.

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## FIGURES AND TABLES

Table 3.1 Pressure algometry and thermal sensitivity test output for 12 commercial sows. No difference between sound and lame legs for PA on D-1, with significant differences observed between sound and lame at all PA landmarks on D+1. Differences seen from D-1 to D+1 at all PA landmarks (P <0.0001). No difference between outer or inner hind claw on D+1 (P=0.73).

| | Day -1 | Day +1 |
|---|---|---|
| ***Pressure Algometry (kgf)*** | | |
| **CANNON HIND CLAW** | | |
| LAME | 6.51 ± 0.26 | 3.61 ± 0.32 |
| SOUND | 6.93 ± 0.25 | 7.67 ± 0.27 |
| Difference | 0.42 ± 0.28[a] | 4.06 ± 0.31[b,**] |
| **INNER HIND CLAW** | | |
| LAME | 7.44 ± 0.26 | 1.57 ± 0.21 |
| SOUND | 7.78 ± 0.28 | 8.18 ± 0.28 |
| Difference | 0.34 ± 0.23[a] | 6.62 ± 0.33[c,**] |
| **OUTER HIND CLAW** | | |
| LAME | 7.70 ± 0.24 | 1.14 ± 0.14 |
| SOUND | 7.44 ± 0.30 | 7.23 ± 0.30 |
| Difference | -0.26 ± 0.31[a] | 6.09 ± 0.32[c,**] |
| ***Thermal Sensitivity (s)*** | | |
| LAME | 7.34 ± 0.55 | 3.26 ± 0.21 |
| SOUND | 9.09 ± 0.64 | 6.8 ± 0.64 |
| Difference | 1.75 ± 0.28* | 3.54 ± 0.59** |

Means ± SE and differences between sound and lame leg.
Statistical analysis conducted on the difference between the sound and lame leg output for both PA and TS. PA and TS analyzed separately.
[a-c] Within a row and column, PA means without a common superscript differ (P < 0.05)
[*, **] Sound and lame leg differs (P <0.05), (P <0.01) respectively

Table 3.2 Pressure algometry and thermal sensitivity pain test output for 12 commercial sows by analgesic treatment. No differences seen between treatments within trial day. Pressure algometry differences observed between trial days.

| | DAY -1 | D+1 | D+6 |
|---|---|---|---|
| *Pressure Algometry (kgf)* | | | |
| **FLUNIXIN MEGLUMINE** | | | |
| LAME | 7.27 ± 0.27 | 2.08 ± 0.32 | 5.39 ± 0.34 |
| SOUND | 7.47 ± 0.28 | 7.41 ± 0.35 | 7.71 ± 0.30 |
| Difference | 0.20 ± 0.34[a] | 5.33 ± 0.42[b,*] | 2.31 ± 0.38[c,*] |
| **SODIUM SALICYLATE** | | | |
| LAME | 6.98 ± 0.28 | 1.98 ± 0.26 | 4.61 ± 0.37 |
| SOUND | 6.77 ± 0.31 | 7.86 ± 0.25 | 7.79 ± 0.31 |
| Difference | -0.21 ± 0.24[a] | 5.88 ± 0.35[b,*] | 3.19 ± 0.40[c,**] |
| **CONTROL** | | | |
| LAME | 7.40 ± 0.24 | 2.27 ± 0.31 | 5.25 ± 0.40 |
| SOUND | 7.91 ± 0.21 | 7.82 ± 0.27 | 7.40 ± 0.29 |
| Difference | 0.50 ± 0.23[a] | 5.55 ± 0.32[b,*] | 2.15 ± 0.41[c,*] |
| *Thermal Sensitivity (s)* | | | |
| **FLUNIXIN MEGLUMINE** | | | |
| LAME | 7.72 ± 1.04 | 3.53 ± 0.37 | 7.33 ± 1.37 |
| SOUND | 8.59 ± 0.98 | 6.49 ± 0.76 | 9.77 ± 1.57 |
| Difference | 0.87 ± 1.02 | 2.95 ± 0.98** | 2.44 ± 1.49 |
| **SODIUM SALICYLATE** | | | |
| LAME | 6.59 ± 0.97 | 2.67 ± 0.33 | 5.82 ± 0.77 |
| SOUND | 10.27 ± 1.42 | 6.48 ± 1.19 | 7.68 ± 0.98 |
| Difference | 3.68 ± 1.44** | 3.81 ± 1.15** | 1.87 ± 1.25 |
| **CONTROL** | | | |
| LAME | 7.71 ± 0.86 | 3.58 ± 0.38 | 4.82 ± 0.52 |
| SOUND | 8.41 ± 0.86 | 7.43 ± 1.36 | 7.78 ± 1.18 |
| Difference | 0.70 ± 0.74 | 3.85 ± 1.19** | 2.96 ± 1.27* |

Means ± SE and differences between sound and lame leg.
Statistical analysis conducted on the difference between the sound and lame leg output for both PA and TS.
PA and TS analyzed separately.
[a-c] Within a row and column, PA means without a common superscript differ ($P < 0.05$)
[*, **] Sound and lame leg differs ($P < 0.05$) and ($P < 0.01$) respectively

Figure 3.1 Amphotericin B Induction Injection Site. Dots denote injection site for Amphotericin B.

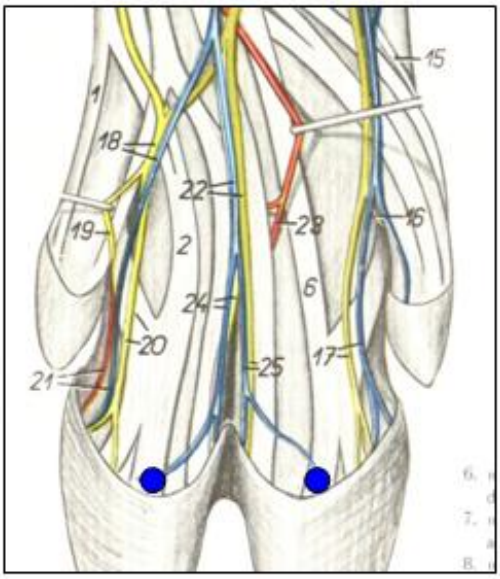


Figure 3.2 Pressure Algometer Landmark Schematic. C=Midpoint of Cannon; O = Outer Hind Claw; I = Inner Hind Claw

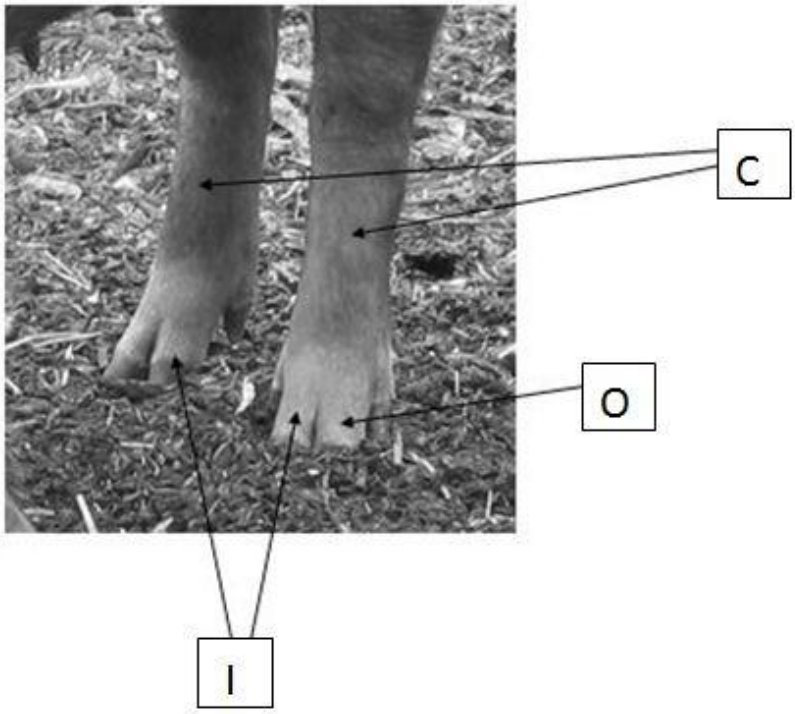


Figure 3.3 Pressure Algometry Pain Test. Algometer screen faced away from applicator during testing to prevent observer bias.

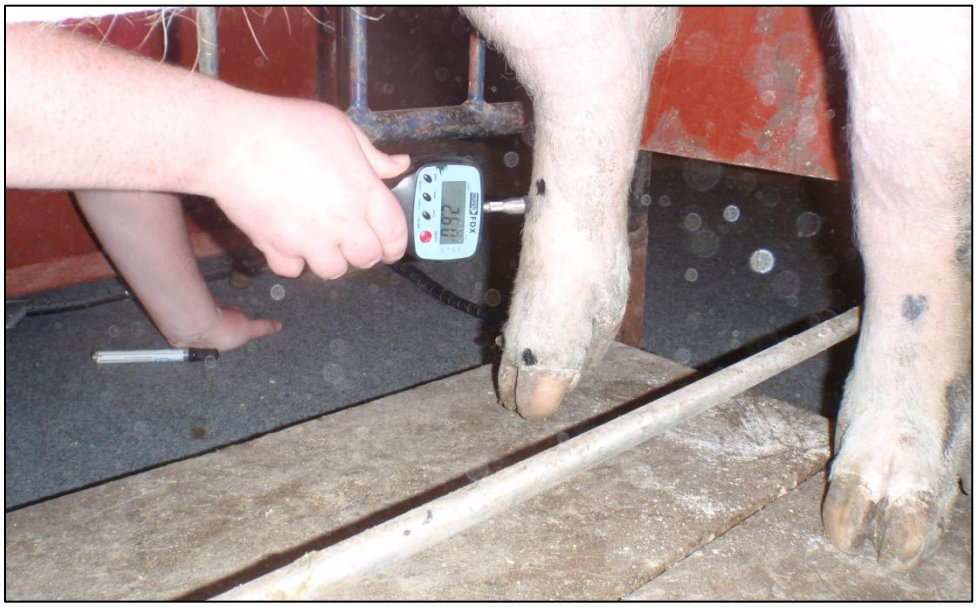


Figure 3.4 Thermal Sensitivity Pain Test. Two pen lasers intersected at the focal distance the light source required from the landmark (Approximately 7.5 cm). Thermal sensitivity test conducted at single landmark on medial side of hind limb 1 cm above coronary band.

Figure 3.5. Pressure algometry test output on baseline (D-1) and lame day (D+1) by landmark. No differences for baselines between any landmark. Differences observed on D+1 between sound and lame hind leg at all 3 landmarks. * P <0.01 ; ** P < 0.001.

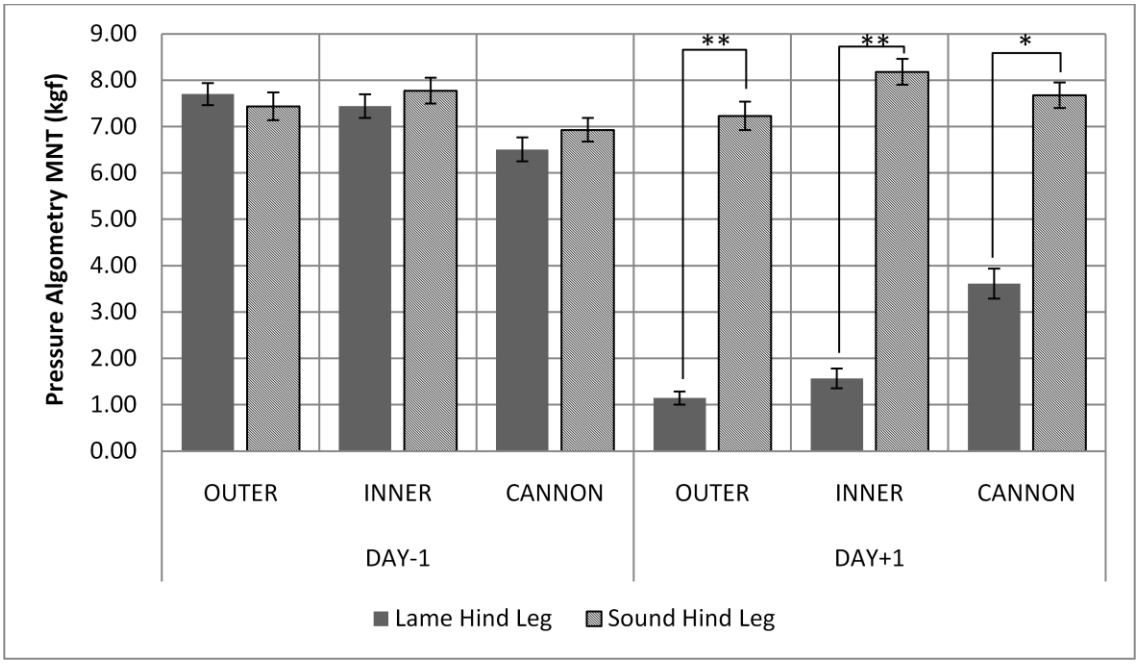


Figure 3.6 Thermal sensitivity test output on baseline (Day-1) and induced lame days (Day+1). Difference observed between lame and sound legs on baseline and on most lame day. (*) = P <0.05; (**) = P < 0.0001

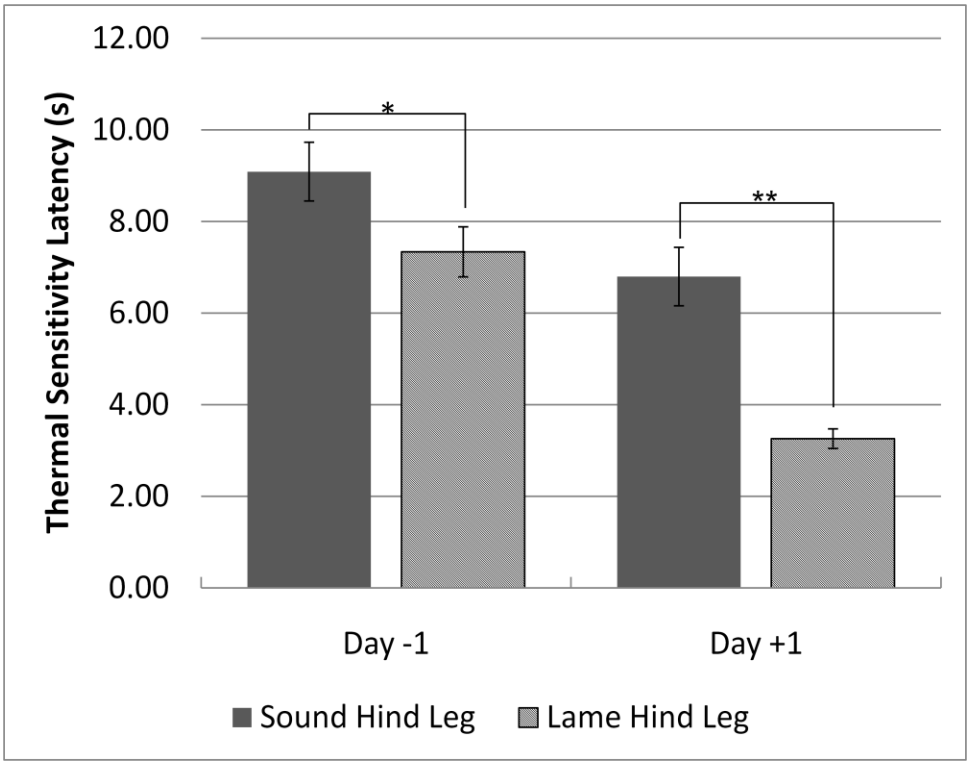
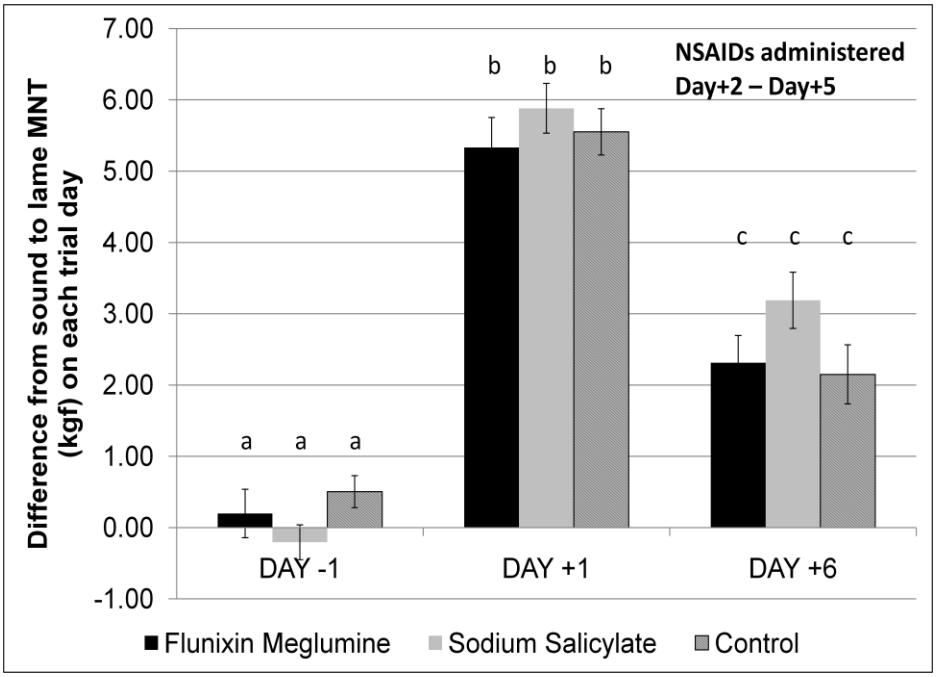


Figure 3.7 Pressure algometry MNT difference from sound to lame hind leg on each trial day. Difference observed between lame and sound legs on baseline (Day-1), induced-lame (Day+1) and recovery (Day+6) by NSAID treatment. a-c denote differences between treatments and trial days ($P < 0.05$).



# CHAPTER 4: NOVEL TECHNIQUES FOR LOCAL ANESTHESIA DURING CAUTERY DISBUDDING OF CALVES

Modified from a paper to be submitted to the *Journal of Animal Science*

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## ABSTRACT

The objectives of this study were to evaluate novel anesthetics to alleviate disbudding pain and to explore pressure algometry (PA), von Frey filaments (VF) and thermal sensitivity (TS) pain tests to measure duration of analgesia. Anesthetic efficacy was determined by latency to loss of sensation (LS), and duration of analgesia. Thirty calves were randomly assigned to one of three cornual anesthetic treatments: 100% ethanol (E), depot emulsion of 2% lidocaine in peanut oil (D), or control 2% lidocaine (C). On D0, 2 mL/horn of anesthetic was injected, latency to LS was measured using a horn bud pin prick and when LS was achieved calves were cautery disbudded. The PA and VF tests quantified mechanical nociceptive thresholds (MNTs) as kilograms of force (kgf), and TS quantified thermal nociceptive latency, relative to a head withdrawal at four sites around each horn bud and a control site. Pain tests and heart rates were were measured hourly on D-1 and D0, and at twelve hour increments through H+83. Plasma cortisol was collected at nine timepoints and an Actical® accelerometer continuously measured activity. Mean latency for LS and pain test data were analyzed using mixed models in SAS. Several calves displayed sensation at +10 min and required a second injection in one or both horns (E: 6/10 calves; D: 7/10 calves; Control: 2/10 calves, P<.0001), but there was no treatment effect for latency to LS (LS (min)±SE: E 26.40±6.12; D 25.60±4.78; C 13.50±4.47, P= 0.22). The VF and TS pain tests were not practical for this disbudding model and were discontinued during the first trial. Calves administered E did not differ from C at +1h, but displayed elevated pain thresholds through D+3 as tested with PA (PA Raw Means ± SE (kgf): D0 = E 4.4±0.1; C 3.6±0.1; D+3 = E 4.6±0.1; C 3.3±0.1; P<0.01). Response to PA did not differ from D to C (PA Raw Means ± SE (kgf): D0 = D 3.2±0.1; D+3 = D 3.0±0.1). Therefore only E and C plasma cortisol concentrations were analyzed with no treatment effect detected. No treatment

differences were observed for heart rate or activity. In conclusion, treatments did not differ in latency to LS administered as one or two injections. Ethanol provided superior analgesia compared to C, whereas D did not when measured using PA which was a useful and practical pain assessment tool. The VF and TS pain tests were not practical pain assessment tests in this disbudding pain model.

**Keywords:** anesthesia, disbudding, ethanol, pain, pressure algometry

## INTRODUCTION

Dehorning is a common management practice on dairy farms throughout the world (Stafford and Mellor, 2005). Horn removal reduces the risk of injury to human handlers or herd mates, which contributes to carcass bruising and hide damage, both of which are of economic importance. Previous research has provided evidence that dehorned calves begin to feel pain once local anesthetics wear off (McMeekan et al., 1998; Faulkner and Weary, 2000) and that dehorning pain can persist for at least 24 h (Faulkner and Weary, 2000) or 44 h (Heinrich et al., 2010). According to the American Veterinary Medical Association (AVMA), disbudding and dehorning of cattle in the U.S. is not regulated, but recommends that dehorning be done at the earliest age practicable, and that local anesthetic be considered (AVMA; 2008, 2010).

Pressure algometry (PA) and von Frey filaments (VF) are non-invasive tools that measure the mechanical nocicepetive threshold (MNT) relative to a withdrawal response. Heinrich and colleagues (2010) previously validated PA as an objective too for measuring disbudding pain. Haussler and colleagues (2006, 2007, 2008) have quantified pain-pressure thresholds in equines. Chaplan and colleagues (1994) quantified allodynia using VF on rats' paws to study neuropathic pain. The thermal sensitivity (TS) test measures latency to a head withdrawal in response to a thermal stimuli and has been tested on laboratory animals (Hargreaves et al, 1988; Andrew and Greenspan, 1999) and livestock (Nolan et al., 1987; Pinheiro Machado et al., 1998). No published research has tested TS or VF specifically for bovine disbudding.

The first objective of this study was to determine the effectiveness of two novel local anesthetic agents, ethanol and a depot formation of lidocaine, for extended analgesia during disbudding relative to a control lidocaine cornual nerve block with the hypothesis was either treatment will provide extended analgesia during

disbudding. The second objective was to evaluate VF and TS for assessing disbudding pain relative to PA, with the prediction that both pain tests would detect reduced pain thresholds post-disbudding.

## MATERIALS AND METHODS

The protocol for this experiment was approved by the Iowa State University Institutional Animal Care and Use Committee (Protocol #5-09-6744-B).

### *Experimental Design*

This experiment was conducted using trials of 3 to 6 calves for a total of 10 trials from July 2010 to October 2010. A repeated measures design compared duration of analgesic effect for calves on sham baseline trial day (D-1), and post-disbudding days (D+1-D+3) relative to disbudding (D0). On Day-2, Actical® accelerometers were attached to the right hind leg, and the hair immediately surrounding both horn buds were clipped to more accurately pinpoint landmarks and to decrease burning of hair during disbudding. On D0, calves were randomly assigned to and received one of three cornual anesthetic treatments: 1. 100% ethanol (E), 2. Depot emulsion of 2% lidocaine emulsified in peanut oil (D), or 3. Control 2% lidocaine (C).

Anesthesia injections and cautery disbudding, as well as pain assessment tests were completed using a modified head restraint placed on the front gate of the calf's home pen. Within the head restraint, calves were able to move their head up and down (a range of 30-45 degrees each direction), but had limited ability to move their heads left or right, as well as forward or backward. During pin prick tests a hand covered the calf's eyes. During pain tests, calves were completely blindfolded. Heart rate and nociceptive tests were measured hourly for the first nine hours after sham or actual disbudding on Day -1 and Day 0, and at twelve hour increments on D+1 through D+3 post-disbudding (Hours relative to disbudding: -25, -23, -22, -21, -20, -19, -18, -17, -16, -15, -13, -1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 23, 35, 47, 59, 71, 83 (Figure 4.1)). Nociceptive tests, physiologic indicators, and activity were used to compare the duration of increased post-surgical pain sensitivity for all three anesthesia treatments.

### *Animals and Housing*

Thirty dairy calves, primarily Holstein with 2 Brown Swiss and 1 Jersey, were enrolled in this study at the Iowa State University Dairy Farm. Bulls and heifers ranged from 10-26 d of age. Calves were individually housed, with each calf's home pen used for data collection. Home nursery pens measured 1.2 m x 1.8 m, with straw bedding and a front metal gate. Calves were bottle-fed whole pasteurized milk twice daily. Calf starter and fresh water were available for consumption *ad libitum*.

*Cornual Nerve Block and Cautery Disbudding*

All calves were sham disbudded (Day -1) to collect baseline values, and disbudded the following day (Day 0). Calves had the cornual nerve blocked with the local anesthetic treatment injected subcutaneously (2 mL/horn) around the cornual nerve, located along the occiptical groove midway between the horn bud and eye with the same injection site for all treatments. On Day -1, all calves received 2% lidocaine HCl anesthesia. On Day 0, calves were randomly assigned to receive one of three cornual anesthetic treatments: 1. 100% ethanol (E), 2. Depot solution of 2% lidocaine suspended in peanut oil (D), or 3. Control 2% lidocaine (C). For both the sham and disbudding procedures, an electrically heated hot-iron disbudder was applied to each horn bud for approximately 15 s. During the sham procedure, the hot-iron was unplugged, with the tip at room temperature. On Day 0, the iron was preheated for at least 10 minutes to a temperature of approximately 600°C prior to the actual disbudding. Both procedures were carried out by the same person and at the same time of day, 0830, in the animals' home pens.

Efficacy of anesthesia was determined using latency for loss of sensation (LS), measured for all treatments at five minute increments from injection until complete LS using a needle prick test at four locations around the horn bud. According to McMeekan and colleagues (1998), calves are insensitive to being pricked with needles in the area surrounding the horns throughout the duration of the nerve block. A hypodermic needle was used to prick the skin immediately surrounding the horn bud to test for a withdrawal response (ear flick or head flinch). If a response from the pin prick test was seen at 10 minutes post injection, an additional 1 ML of the allocated anesthetic treatment was administered on the affected side at the same cornual nerve location. Once fully blocked, as determined by LS, sham or actual disbudding was performed.

*Pain Assessment*

For both mechanical and thermal nociception assessments, four landmark locations around each horn bud as well as a non-painful control landmark in the middle of the face were used (Figure 4.2). Landmark sequences were randomized per calf with that sequence remaining throughout the trial over all pain tests and on each horn. The order of horn testing was also randomized, with half of the calves tested on the right horn first, and half tested on the left horn first. Each landmark was tested in triplicate. Baseline measurements were collected one hour prior to cornual nerve blocking on D-1 and D0. Half of the data collections had the observer standing on the left side of the calf and testing both the left and right horn, and half of the data collections had the observer standing on the right side testing both horn buds. The observer was blind to the numerical output of both the mechanical and thermal nociceptive tests to prevent bias, by having a second observer write down the output values, both the force pressure for mechanical and latency in seconds for thermal nociceptive tests. For this experiment, a withdrawal response was defined as a jerk of the head away from the stimulus.

*Mechanical Nociceptive Threshold (MNT):* Presence and duration of analgesic effect were determined using pressure algometry (PA) and von Frey filaments (VF), which quantified mechanical nociceptive thresholds as kilograms of force (kgf) and grams of force (gf) respectively, relative to a head withdrawal response. The mechanical nociceptive threshold (MNT) is the peak applied force at which a withdrawal response is seen, or the minimum pressure that induces pain. Since PA was previously validated as a pain assessment tool for bovine disbuding, it was used as the pain assessment standard to the VF. A hand-held pressure algometer (Wagner Force Ten™ FDX 50 Compact Digital Force Gage, Wagner Instruments, CT, USA) with a 1 $cm^2$ flat rubber tip measured MNTs at each landmark (Figure 4.3). The VFs (IITC Life Science Electronic Von Frey Anesthesiometer, IITC Inc. Life Science, Woodland Hills, CA) measured MNTs with a 1000 maximum gram probe with a rigid tip if 0.8 mm diameter. Pressure was applied perpendicularly at a constant rate of approximately 1.0 kgf/second for the PA, and 100 grams/second for the VF. When a withdrawal response was detected, pressure was immediately removed and the peak pressure output was recorded for each test. The maximum time to apply pressure was eight seconds, which correlated to a maximum MNT of approximately 8 kgf for PA and 800 grams of force for VF. If no reaction was detected once time had elapsed, the peak pressure was recorded and a non-response was indicated on the collection form.

*Thermal Sensitivity (TS):* The TS was assessed with a plantar analgesia meter (IITC Life Science Plantar Test Analgesia Meter Model 390, IITC Inc. Life Science, Woodland Hills, CA) measuring the latency in seconds for a focused heated light to illicit a withdrawal response. The TS meter was set at a constant 80% beam intensity, emitting 200° C, with a cut-off time of 20 seconds to prevent tissue damage. When a withdrawal response was detected, the heat stimulus was immediately terminated and the latency was recorded by a second observer.

*Physiological Assessment*

Heart rates were taken before the initiation of pain tests at every data collection time point, approximately 5 minutes before pain tests began, before head restraint was placed in pen. Heart rates were determined manually by listening to the heart with a stethoscope, counting beats for 15 s and converting to beats per minute (bpm) with the calves standing calmly and unrestrained in the home pen.

*Endocrine Assessment*

Blood samples were collected at a total of nine timepoints during the trial to analyze plasma cortisol concentrations. A baseline sample was collected one hour prior to disbud or sham disbud on D-1 and D0. The second blood sample was collected thirty minutes post sham or actual disbudding and the final blood collection occurred 150 minutes after sham or actual disbudding. A single blood sample was also collected prior to the morning pain assessments on D+1 through D+3. Both the right and left jugular veins were used to collect samples, with 10 mL collected via a jugular syringe venipuncture which was immediately transferred to two chilled K2 EDTA vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ, USA) and stored on ice. Within 15 min of collection, samples were centrifuged for 15 min at 1500 g. Plasma was then pipetted to cryovials and frozen on-farm to -18°C for up to 6 hours then transferred to Iowa State University and stored at -80°C until analysis.

The concentration of cortisol in plasma was determined using radioimmunoassay kits (Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, CA). The kits were validated for use with bovine plasma (Protocol validated by D. Lay, Jr., Livestock Behavior Research, Agricultural Research Service, United States Department of Agriculture, West Lafayette, IN, personal communication). All samples were analyzed as

a single assay with the intra-assay coefficient of variation of 10.1%. Final sample concentrations were adjusted based on variation of the controls of known concentration. Samples were analyzed in duplicate, and the average of the duplicates was used as the final cortisol concentration for analysis

*Activity*

Total activity was measured using a 2D accelerometer (Acticals®, Mini Mitter®, Respironics, Bend, Oregon, USA), measuring occurrence of movement with a force of at least 0.01 *g* every 15 seconds continuously. On Day -2, Acticals® were placed on the right hind leg, and bandaged in place using gauze and vet-wrap. Acticals® were removed after +84h, and the data were downloaded and stored as the total activity per hour.

*Return of Sensation*

A final pin-prick test was administered after the final pain assessment on D+3 to determine if calf required additional testing for sensation around the horn bud. If there was full sensation in the area immediately surrounding both horn buds, the calf was removed from the trial. If there was partial or complete LS, calves were evaluated with a pin-prick test monthly until the calf returned to full sensation around each horn bud.

*Statistical analysis*

Latency to LS, nociceptive tests, heart rates, activity, and plasma samples were analyzed separately. A *P* value of $\leq 0.05$ was considered significant for each variable and nociceptive test.

*Latency to LS:* Latency times were initially tested for normality with Proc Univariate. No transformations were required. Latency to loss of sensation was analyzed with Proc Mixed, with a model that included the number of injections, treatment, calf sex and disbud age. The latency to loss of sensation had a random effect of sex within group.

*Pain Tests:* Raw means were obtained by averaging the triplicate data points in Excel. Raw means were initially tested for normality using PROC Univariate of SAS version 9.2 (SAS Inst. Inc., Cary, NC). No transformations were required. Mechanical nociceptive thresholds (MNTs) were analyzed using Proc Glimmix to analyze the

duration of analgesia between treatments. This model included trial hour, treatment, landmark, standing side, and the multiple interactions of these variables. Random effects included group, calf within group, and horn within calf within group. Least Square Means provided estimates, standard error, and P values for variable interactions and effect comparisons. Tukey's test for pair wise comparisons was used to examine the effect of time within each treatment.

*Heart Rate:* Heart rates were initially tested for normality with Proc Univariate. Average beats per minute, with no transformations, were analyzed by treatment and by trial day using a mixed model with main effects of treatment, trial hour, sex and age analyzed treatment differences in heart rate with a random effect of gender within group. A Kenward-Roger method for degrees of freedom was utilized to adjust for repeated measures.

*Blood Samples:* Proc Univariate was used to visually assess distribution of the data and a log transformation was applied to achieve normality. A mixed procedure with main effects of treatment, trial hour, sex and age with a random effect of gender within group analyzed differences between E and C treatments, and the difference in plasma cortisol concentration over time. Tukey's test for pair wise comparisons examined the effect of treatment by time.

*Activity:* The Univariate procedure was used to assess the distribution of the activity data. A log transformation was applied to achieve normality. A mixed model with a random effect of sex within group and main effects of treatment, trial hour, calf age, and sex was tested. Tukey's test for pair wise comparisons examined the effect of treatment by time.

## RESULTS

*Latency to Loss of Sensation (LS)*

There was a significant difference in LS at ten minutes, such that treatments differed for the number of calves that required an additional injection (E: 6/10 calves; D: 7/10 calves; Control: 2/10 calves, P <.0001). However, there was no difference between treatments for the number of injections required to reach LS (Latency to LS (min) ± SEM 1 injection: E 6.0±0.4; D 9.0±3.0; Control 8.0±0.7) or for latency to LS for two injections (Latency to LS (min) ± SEM 2 injections: E 40.0±4.5; D 33.0±4.4; Control 37.0±14.0) (P =0.5680)

(Figure 4.4) Figure 4.5 shows the number of calves per treatment that had achieved LS by chronological pin-prick testing and number of injections on D0.

By casual observation, no visual differences were seen in the calves treated with E compared to calves administered C. However, on Day+1, two of the calves administered D developed significant swelling at the site of anesthetic injection on each side of the skull (Figure 4.6). A veterinarian was consulted and diagnosed the swelling to be a reaction to the peanut oil. Calves were monitored for changes in respiration, heart rate, feeding and general behavior, and were allowed to remain in the trial pending no reduction in health. Calves did not display altered activity or health, and hence remained in the study. Swelling reduced daily with no veterinary assistance, and calves returned to normality after several days.

*Duration of analgesia*

*Pressure Algometry:* Results from the PA provided evidence of differences between treatment and trial day. When comparing sham (D-1) to disbud (D0) at each time point, differences were seen between treatments (Table 3.1 and Figure 3.8). For the lidocaine control treatment, D0 MNT values were decreased at each of the eleven timepoints compared to D-1 ($P < 0.0001$). The D treatment resulted in decreased MNT values on D0 compared to D-1 at each timepoint. Two of the timepoints were not different, but were numerically lower. Ethanol was the only treatment that resulted in the same or increased average MNT values at all timepoints comparing D0 to D-1. When compared to C, E resulted in increased MNT's from Hour+2 though the end of the trial at each data collection. Similarly, the MNTs were higher for the E at each data collection hour starting at Hour+4 compared to D ($P < 0.001$ at Hour+4 through Hour+83). Depot treated calves had MNTs that were the same or lower at each data collection hour compared to C (Figure 4.7).

*Von Frey Filaments:* During the initial pre-trial testing, the VF pain test took 11 minutes from start to completion when tested in triplicate on both horn buds for a single calf. There were also technical issues with the testing tips falling off of the equipment which prolonged the testing time. Due to technical issues and length of time required to test calves, the VF was not a practical pain test for this disbudding trial model. Therefore the assumption that the VF nociceptive test would detect decreased pain threshold after cautery disbudding could not be tested in this experimental design.

*Thermal Sensitivity:* During the first trial, E and D treated calves did not display a withdrawal response to the TS stimulus, and repeatedly reached the max time of 20 seconds. This repeated stimulation led to burning and blistering of the skin surrounding the landmarks around the horn bud of two calves. Calves with blistering were removed from the trial under the advisement of a veterinarian, and blister wounds were treated. For ethical reasons, the TS testing was discontinued. Data from the calves removed from the trial were not included into the study because it was an incomplete dataset. Therefore the assumption that the TS nociceptive test would detect decreased pain threshold after cautery disbudding is unknown in this experimental design.

*Heart Rate:* From D-1 to D0, the only treatment that showed a statistically significant difference was on the daily baseline (H-1 vs H-25) for the E treatment (Table 3.2 and Figure 4.9). When comparing the morning heart rate (H-25, H-1, H+23, H+47, H+71), treatment differences were found at H-1 and H+71 (Figure 4.10). Depot treated calves had elevated heart rates compared to E on D0. On D+3, D calves had decreased heart rates compared to C treated calves. Depot treated calves decreased heart rates from D-1 to D+3. Control treated calves did not show differences in heart rate throughout the trial. The E treated calves had elevated heart rates on D-1, but no differences throughout the rest of the trial (Figure 4.10).

*Cortisol:* Due to the lack of anesthetic relief provided by the D treatment, as assessed by PA, only the E and C treated calves' blood samples were assayed and analyzed. No differences were observed in the plasma cortisol concentration based on the adjusted P-value between E and C at each timepoint throughout the trial. There were also no differences within each treatment between timepoints based on the adjusted P-values. Figure 4.11 shows a graph of the plasma cortisol concentration over time between E and C.

*Activity:* No differences between treatments were observed at any timepoint from D-1 to D+3 (Figure 4.12). No differences were seen within treatment from D-1 (sham) to D0 (disbud) at any time.

*Final Pin Prick Test:* All C and D calves had full sensation around each horn bud when tested on D+3 after the final trial hour. Calves that had not returned to full sensation around both horn buds were pin-prick tested monthly until a full return to sensation was detected. Four of the ten E treated calves were bulls and were moved off farm and could not continually be pin-prick tested after the 5-d trial. Two E calves returned to full sensation around both horn buds within 100 d post-disbudding; two returned within 120 d post-disbudding, and the final two tested returned within 178 d post-disbudding.

## DISCUSSION

Because it is well established that disbudding without a local anesthetic causes pain (Petrie et al., 1996; Graf and Senn, 1999), all calves were given the local anesthetic treatment, with no negative control animals. Measuring the latency to LS provided evidence that there is a large variation in the amount of time it takes to completely block the tissue of individual calves within a given anesthetic treatment. Differences in the latency to LS could be due to inaccurate injection site or diffusion rates of anesthetic through tissue layers, but was not tested. This anecdotally confirms that sensation testing should always be administered prior to disbudding, and additional anesthetics should be provided if sensation occurs.

More than half of the ethanol or depot anesthetic treatments required a second injection to achieve complete loss of sensation in at least one or both horns. Other research protocols injected a larger single volume of 3 mL (Petrie et al., 1996) to 5 mL of lidocaine anesthetic at the cornual nerve (Stewart et al., 2009; Graf and Senn, 1999; Stilwell et al., 2010) with or without an additional ring block. The reason for the initial 2 mL injection was for consistency and the unknown action and pharmacokinetics of the depot and ethanol. All calves were completely blocked with a total of 2 or 3 mL of anesthetic treatment, but the latency to LS may have been reduced by a single larger injection of anesthetic treatment. Two calves originally in the trial were blocked with ethanol but failed to have a complete loss of sensation after two injections and were not included in the trial. This failure to block could have been a result of improper injection of the ethanol anesthetic.

There is some difficulty comparing the latency to LS because one observer anesthesized, pin-prick-tested, and disbudded all calves over all treatments, which impacted the exact timeframe for pin-prick testing. In future studies, when comparing the latency to LS, it may be more appropriate to test fewer calves or have more technicians to standardize pin prick testing times.

Pinheiro Machado and colleagues (1998) developed a radiant TS test used for measuring the nociceptive threshold to morphine sulphate as tested on the forefoot of peri-parturient dairy cows. Nolan and colleagues (1987) also tested a ramped radiant TS on the pinna of ewes ear and found that this apparatus produced reliable nociceptive thresholds. The radiant TS pain test utilized in this experiment differed from those used in both the Pinheiro Machado and Nolan experiments in that those testing devices provided a ramped

thermal stimuli which increased in temperature until a withdrawal response was detected. The TS test used in this current experiment provided a constant temperature heated light source and the latency to this constant temperature was measured.

Heinrich and colleagues (2010) first published research validating pressure algometry as a pain assessment tool for use in disbudding dairy calves. This experiment expands upon this previous research model by: comparing PA to other pain assessment tools, using PA to compare duration of anesthesia with several anesthetic treatments, and expanding the testing duration out to +84h instead of only +4h post-sham and +4h post-disbudding. Mean MNTs, as measured by PA by Heinrich and colleagues (2010), averaged around 2.5 kgf +4h following sham disbudding and dropped to approximately 1.6 kgf +4h post- actual disbudding with lidocaine only, which was relatively reduced compared to the MNT values of this current trial. The average over all treatments on D-1, over all landmarks and D-1 trial hours was 4.2 kgf, which reduced to an average of 3.5 kgf for only control-treated calves for the first +9h post-disbudding. Differences in MNT values between treatments were first detected at H+2 relative to disbudding. This was the first hour post disbudding in which decreased MNT values were detected with C and D treatments. Results of this experiment are in agreement with previous research that states that lidocaine wears off by two hours post disbudding, which was detected by decreased MNT values. Ethanol anesthetized calves withstood elevated MNTs relative to the control calves indicating elevated pain tolerance for the duration of the trial. The depot-treated calves tolerated reduced MNTs relative to the controls at several timepoints post disbudding indicating that depot may have actually caused irritation rather than alleviating pain. This potential irritation, as seen with decreased MNTs, is in accord with the calves that showed an inflammatory reaction to the depot. Both results indicate that this depot formation of lidocaine was not a suitable anesthetic treatment for disbudding calves. In future studies studying novel anesthetics, another PA landmark near the cornual nerve block injection site may be tested to compare a possible reduced pain response to the treatment injection.

A reduction in MNTs was seen throughout D-1 for each treatment. According to Garry and colleagues (2004), neurons in the dorsal spinal cord that are involved in processing mechanical and thermal sensory inputs can become functionally sensitized when subjected to persistent afferent activity. The multiple testing time points could have resulted in the linear reduction in MNTs. It has been well documented that 2% lidocaine

wears off at approximately 2 hours, but the half-life of depot is not known, leading to the experimental design for hourly data collections on D-1 and D0.

In this experimental design heart rates were collected prior to the hourly pain assessment tests. In future studies, pre- and post- heart rates could be taken at each pain assessment trial hour to test changes in heart rates for the actual pain assessment. By measuring pre- and post- heart rates for each pain test, this could indicate the extent of the noxious stimuli for each pain test. To account for confounding effects of handling, heart rates were taken before the equipment and head restraint was brought near the home pens usually by the recording observer while the testing observer set up equipment. Heart rates were manually collected by one observer standing in the calf's home pen with the calf standing calmly and unrestrained. There is a risk of observer error, with different observers throughout each trial at different timepoints. Automated heart rate monitors could be also attached to calves on trial to record a more accurate assessment of heart rates throughout the trial as opposed to only testing prior to each trial hour. The first published recording of heart rates for detection of pain for dehorning of calves was by Grøndahl-Nielsen and colleagues (1999) with electrocardiogram (ECG) recording for the first 4 h post-dehorning who found increased heart rates for dehorned calves administered no anesthetic or sedation and decreased heart rates for calves administered sedatives for the first 213 minutes post dehorning. In the Grøndahl-Nielsen study, no differences in heart rate were seen between calves that were sham disbudded compared to disbudded calves when both groups were administered a cornual nerve block. Stewart et al (2009) found evidence that calves dehorned without local anesthetics or NSAIDs had elevated heart rates for three hours post cautery disbudding. Results from this current study was consistent with heart rate differences from D-1 to Day0 observed by Heinrich and colleagues (2010), which further evidences that heart rates are elevated once the lidocaine anesthetic has worn off.

Cortisol has been shown to spike immediately following dehorning and again at the end of the duration of action of local anesthetic, then declines to a plateau level for approximately seven to nine hours before returning to baseline (Stafford and Mellor, 2005). This cortisol curve was not detected in this experiment and is in contrast with previous research measuring cortisol concentrations relative to disbudding. Heinrich and colleagues (2009) measured cortisol immediately after and at every half hour for the first 2 hours post-sham and post-actual disbudding and at +4, +6, and +24 post-disbudding in 6 to 12 wk old Holstein heifer calves. Results

from the Heinrich study indicated that cortisol was elevated in control and meloxicam-treated groups after disbudding compared to sham disbudding, with cortisol remaining elevated at +24h post-disbudding. Meloxicam-treated calves had a decreased elevation in cortisol compared to the control-treated calves only administered a local lidocaine nerve block and no meloxicam (Heinrich et al., 2009). Grøndahl-Nielsen and colleagues (1999) measured the cortisol response in 4 to 6 wk old Friesian calves cautery disbudded from -25 min to +3h post disbudding. For the first hour post-disbudding, blood samples were collected at +10 min increments, and at +30 min increments until +3 h post-disbudding. Results from the Grøndahl-Nielsen study indicate that calves disbudded without sedation or analgesia was elevated during the first +30 min post-disbudding compared to treatments groups with sedation and/or analgesia which is in contrast to Petrie and colleagues (1996) who cautery disbudded 6 to 8 wk old Friesian calves and collected blood for +10 h post-disbudding. Results from the Petrie study found that cautery disbudded calves had an initial transient rise in plasma cortisol which returned to baseline values by +1 h post-disbudding with this same curve and less dramatic peak seen in calves cautery disbudded with a local anesthetic. The largest cortisol levels for both E and C treated calves were seen at the very first blood collection on D-1 which likely indicates handling stress for this first blood collection. In future studies, a preliminary blood collection could be taken prior to the trial on D-2 to account for this confounding effect. For consistency, all calves were haltered and heads were tied outside the front pen gate to expose the jugular vein for a venipuncture collection with a syringe. Handling and restraint could have increased the overall plasma cortisol levels through activity and anxiety and masked any potential treatment differences. No differences were detected between treatments and within treatments on sham (D-1) baselines or from D0 through D+3. No spike or plateau cortisol concentration was seen throughout the trial. This lack of difference between treatments or within treatments over time could indicate an overall level of discomfort and stress of handling during each blood collection, or a lack of sensitivity or robustness in experimental design.

No differences were detected between treatments for the accelerometer measuring activity. When comparing Day-1 to Day0, no differences were observed within treatments. The activity results are in contrast Heinrich and colleagues (2010) who found that meloxicam-treated calves were less active than lidocaine treated controls during the first 5 hours following disbudding, although the differences between treatment groups were

small. The lack of differences seen between treatments are in agreement with McMeekan and colleagues (1999) who found no differences in the amount of lying behavior between calves that received lidocaine only and calves that received lidocaine plus ketoprofen. The results of this study are not surprising because all calves, regardless of treatment, were handled in the same manner at each trial hour. Handling and restraint would have impacted the activity count for all of the calves, resulting in a lack of differences between treatments, and decreased sensitivity as a pain measure especially on D-1 and D0 in which hourly data were collected.

In conclusion, ethanol provided extended anesthetic relief over several days compared to the control treated calves administered lidocaine as tested with PA. Ethanol anesthetized calves withstood elevated pressure thresholds relative to the control calves for three days after disbudding, indicating higher pain tolerance for the duration of the trial. The depot formation of lidocaine was not an effective anesthetic treatment for use in this experiment due to the efficacy of anesthesia, as tested with decreased MNTs after disbudding, and allergic reactions were seen in two calves to the peanut oil. In this experiment, neither VF nor TS pain tests were practical pain assessment tools for technical and ethical issues. No differences between treatments were detected as tested with heart rate, cortisol or activity, suggesting that the methods were not sensitive or robust enough in this experimental design.

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## TABLES AND FIGURES

Table 4.1: Pressure Algometry average MNT in kilograms of force for D-1 and D0, and the difference between D-1 and D0 at each timepoint, to compare baseline to disbudded MNT values for each treatment (Depot; ETOH = Ethanol; CONT = Control). A-d subscript values denote significant differences between sham and disbud day ($P<0.05$), ($P<0.01$), ($P<0.001$), ($P<0.0001$) respectively.

| | | 7:30 | 8:30 | 9:30 | 10:30 | 11:30 | 12:30 | 13:30 | 14:30 | 15:30 | 16:30 | 17:30 | 19:30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | H-1 | DISBUD | H+1 | H+2 | H+3 | H+4 | H+5 | H+6 | H+7 | H+8 | H+9 | H+11 |
| DEPOT | D-1 | 4.40 | SHAM | 4.53 | 4.31 | 4.03 | 3.87 | 3.87 | 3.58 | 3.58 | 3.85 | 3.78 | 3.45 |
| | D0 | 3.62 | DISBUD | 3.78 | 3.70 | 3.52 | 3.34 | 2.79 | 2.69 | 2.74 | 2.62 | 2.81 | 2.59 |
| | | 0.79 ±0.15$_d$ | | 0.75 ±0.15$_c$ | 0.61 ±0.15$_a$ | 0.51 ±0.15 | 0.53 ±0.15 | 1.08 ±0.15$_d$ | 0.89 ±0.15$_d$ | 0.83 ±0.15$_d$ | 1.23 ±0.15$_d$ | 0.97 ±0.15$_d$ | 0.86 ±0.15$_d$ |
| ETOH | D-1 | 4.72 | SHAM | 4.67 | 4.79 | 4.48 | 4.11 | 4.09 | 3.80 | 3.93 | 3.77 | 3.79 | 3.99 |
| | D0 | 3.56 | DISBUD | 4.27 | 4.37 | 4.58 | 4.62 | 4.17 | 4.49 | 4.01 | 4.12 | 4.14 | 3.75 |
| | | 1.15 ±0.15$_d$ | | 0.4 ±0.15 | 0.42 ±0.15 | -0.1 ±0.15 | -0.51 ±0.15 | -0.08 ±0.15 | -0.70 ±0.15$_b$ | -0.08 ±0.15 | -0.35 ±0.15 | -0.36 ±0.15 | 0.24 ±0.15 |
| CONT | D-1 | 4.86 | SHAM | 5.20 | 4.98 | 4.82 | 4.40 | 4.35 | 4.23 | 4.26 | 4.36 | 4.46 | 3.68 |
| | D0 | 4.06 | DISBUD | 4.32 | 3.63 | 3.34 | 3.59 | 3.48 | 3.49 | 3.40 | 3.26 | 3.17 | 3.03 |
| | | 0.8 ±0.15$_d$ | | 0.88 ±0.15$_d$ | 1.35 ±0.15$_d$ | 1.48 ±0.15$_d$ | 0.82 ±0.15$_d$ | 0.87 ±0.15$_d$ | 0.74 ±0.15$_d$ | 0.86 ±0.15$_d$ | 1.10 ±0.15$_d$ | 1.29 ±0.15$_d$ | 0.65 ±0.15$_d$ |

Table 4.2: Heart Rates. Average heartbeats per minute within treatments for D-1 and D0, and the difference between D-1 and D0 at each timepoint, to compare the effects of local anesthetic treatments on heartrate. (a) subscript values denote differences between sham and disbud day ($P<0.05$).

| | | 7:30 | 8:30 | 9:30 | 10:30 | 11:30 | 12:30 | 13:30 | 14:30 | 15:30 | 16:30 | 17:30 | 19:30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | H-1 | DISBUD | H+1 | H+2 | H+3 | H+4 | H+5 | H+6 | H+7 | H+8 | H+9 | H+11 |
| DEPOT | D-1 | 118.0 | SHAM | 104.0 | 103.2 | 100.8 | 95.6 | 100.0 | 100.0 | 104.8 | 102.8 | 106.8 | 103.2 |
| DEPOT | D0 | 115.2 | DISBUD | 112.8 | 98.8 | 104.8 | 101.2 | 97.2 | 102.8 | 102.4 | 100.8 | 106.4 | 107.2 |
| | | -2.80 ±6.43 | | 8.80 ±6.43 | -4.40 ±6.43 | 4.00 ±6.43 | 5.60 ±6.43 | -2.80 ±6.43 | 2.80 ±6.43 | -2.40 ±6.43 | -2.00 ±6.43 | -0.40 ±6.43 | 4.00 ±6.43 |
| ETHANOL | D-1 | 117.6 | SHAM | 100.8 | 100.0 | 102.0 | 97.6 | 98.0 | 100.4 | 104.8 | 101.2 | 97.6 | 104.4 |
| ETHANOL | D0 | 102.0 | DISBUD | 101.6 | 102.8 | 100.0 | 96.0 | 96.0 | 99.2 | 98.0 | 95.6 | 108.0 | 100.4 |
| | | -15.6 $\pm 6.43_a$ | | 0.80 ±6.43 | 2.80 ±6.43 | -2.00 ±6.43 | -1.60 ±6.43 | -2.00 ±6.43 | -1.20 ±6.43 | -6.80 ±6.43 | -5.60 ±6.43 | 10.40 ±6.43 | -4.00 ±6.43 |
| CONTROL | D-1 | 110.0 | SHAM | 101.6 | 96.0 | 95.6 | 94.4 | 99.2 | 100.0 | 108.4 | 101.6 | 106.0 | 110.0 |
| CONTROL | D0 | 106.8 | DISBUD | 104.8 | 106.0 | 106.8 | 100.8 | 96.4 | 103.6 | 100.0 | 102.8 | 102.4 | 101.6 |
| | | -3.20 ±6.43 | | 3.20 ±6.43 | 10.00± 6.43 | 11.20 ±6.43 | 6.40 ±6.43 | -2.80 ±6.43 | 3.60 ±6.43 | -8.40 ±6.43 | 1.20 ±6.43 | -3.60 ±6.43 | -8.40 ±6.43 |

Figure 4.1: Pain assessment and heart rate trial hours. Trial hours relative to disbudding. Heart rates manually collected prior to nociceptive tests on unrestrained calves. Nociceptive tests conducted while calves were blindfolded in the head restraint in each calf's individual home pen.

| | Day-1 | Day 0 | Day +1 | Day +2 | Day +3 |
|---|---|---|---|---|---|
| 0730 | Hour -25 | Hour -1 | Hour 23 | Hour 47 | Hour 71 |
| 0830 | **SHAM** | **DISBUD** | | | |
| 0930 | Hour -23 | Hour 1 | | | |
| 1030 | Hour -22 | Hour 2 | | | |
| 1130 | Hour -21 | Hour 3 | | | |
| 1230 | Hour -20 | Hour 4 | | | |
| 1330 | Hour -19 | Hour 5 | | | |
| 1430 | Hour -18 | Hour 6 | | | |
| 1530 | Hour -17 | Hour 7 | | | |
| 1630 | Hour -16 | Hour 8 | | | |
| 1730 | Hour -15 | Hour 9 | | | |
| 1830 | | | | | |
| 1930 | Hour -13 | Hour 11 | Hour 35 | Hour 59 | Hour 83 |

Figure 4.2: Pain Assessment Landmarks (Modified from Heinrich et al., 2010). Landmark sequence randomized per calf, with the same landmark sequence used on each horn and all pain tests per calf.

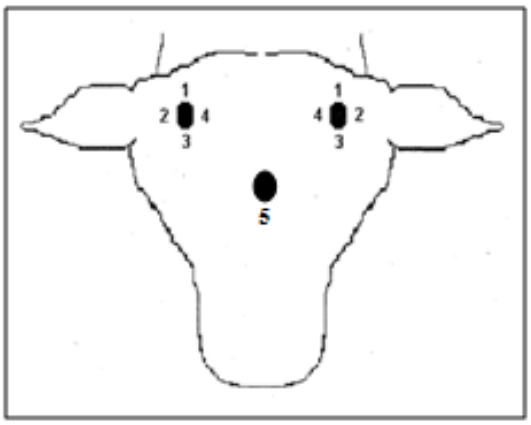


Figure 4.3: Pressure Algometry (PA) Pain Assessment. Calf is blindfolded throughout testing. Digital face of hand-held algometer is facing away from observer to prevent observer bias.

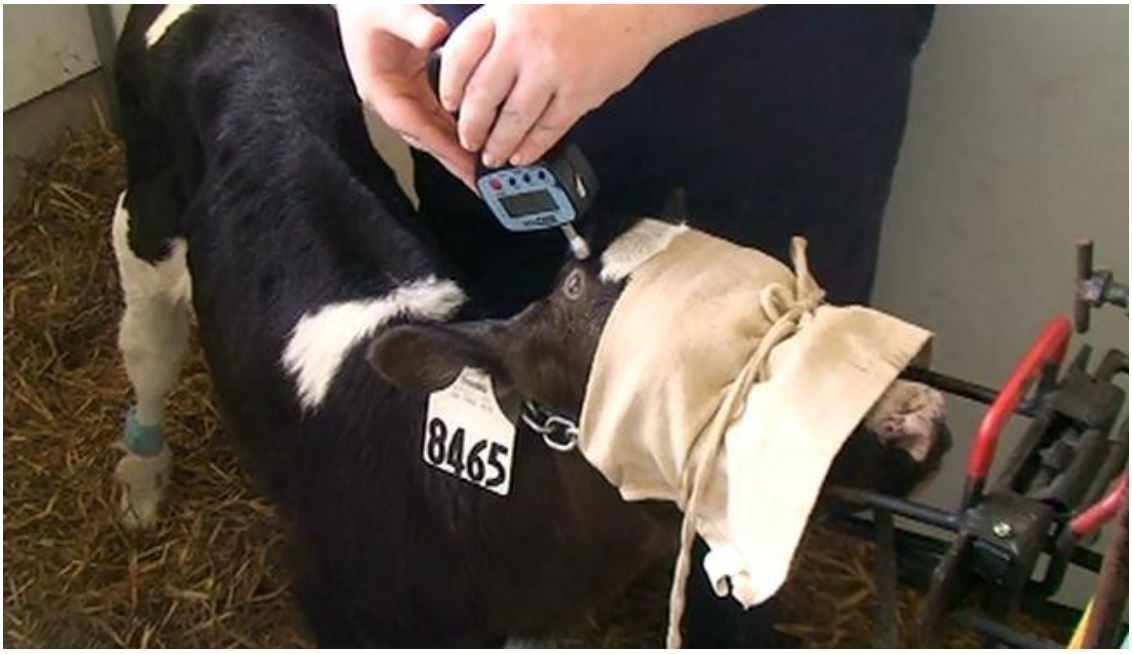


Figure 4.4: Latency to Loss of Sensation (Mean ± SEM; minutes) by treatment and the number (1 or 2 injections) of required cornual nerve block injections. No difference between treatments in the latency to LS for either one or two injections required to fully block, but as predicted, a difference between the mean LS for one injection versus two injections (P <0.0001). n=Number of calves per treatment and injections required. Values with different superscript letters indicate differences in latency to LS (P <0.05).

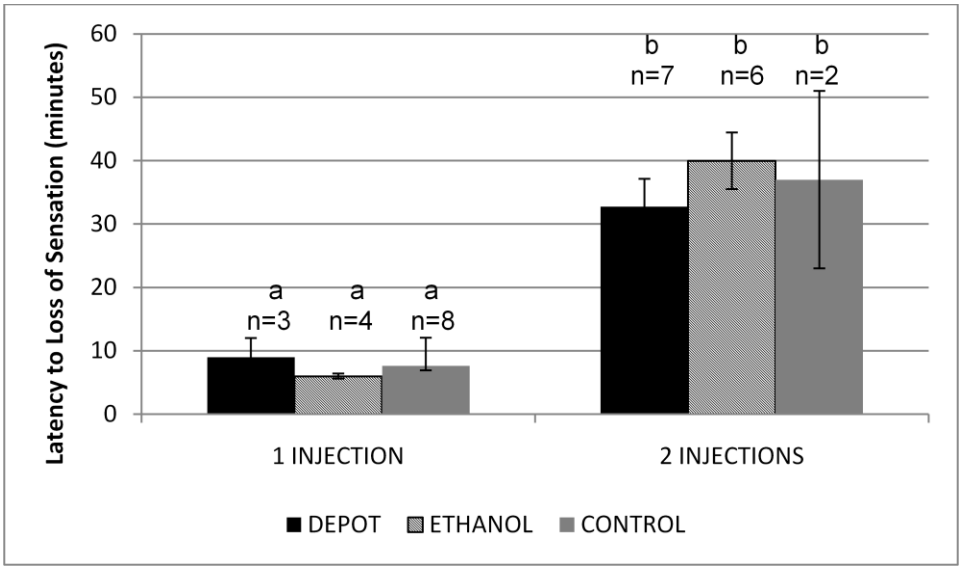


Figure 4.5: Pin prick testing to determine loss of sensation (LS). Results by treatment at each pin prick test post-nerve block to compare the number of calves fully blocked.

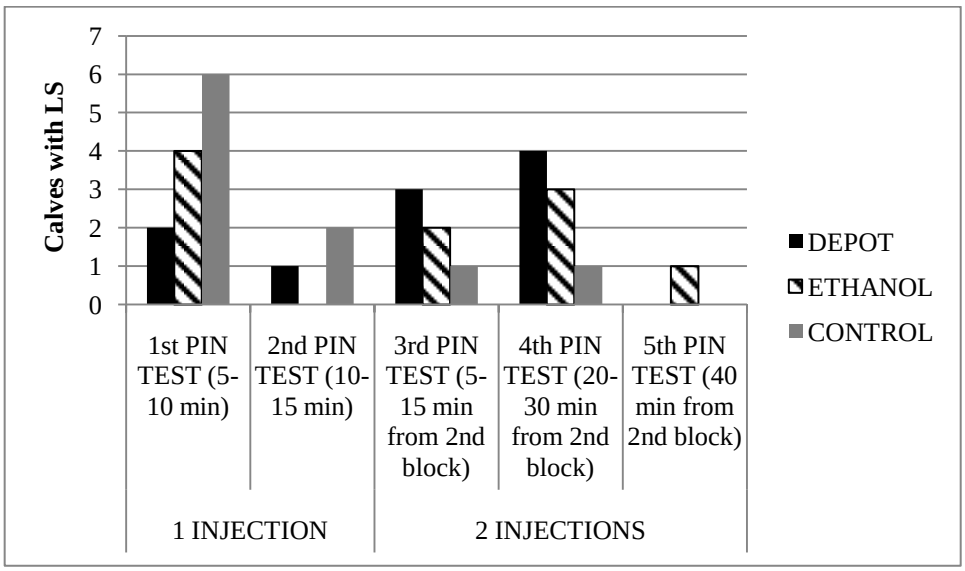


Figure 4.6: Localized inflammation due to depot formulation of lidocaine as a reaction to the peanut oil. Swelling observed at and around cornual injection site from eyebrow to horn bud area. Highest degree of swelling observed on D+1 and reduced daily with no veterinary assistance. Calf fully returned to normality after several days.

Figure 4.7: Mean (±SEM) Mechanical Nociceptive Thresholds (MNT) for pressure algometry in kilograms of force by treatment. On D-1, all calves were sham disbudded and were blocked with 2% lidocaine. Actual disbudding occurred on Hour0 on D0 with randomly assigned anesthetic treatment. a and b superscript values denote differences between treatments at trial hours ($P < 0.05$) and ($P < 0.01$) respectively.

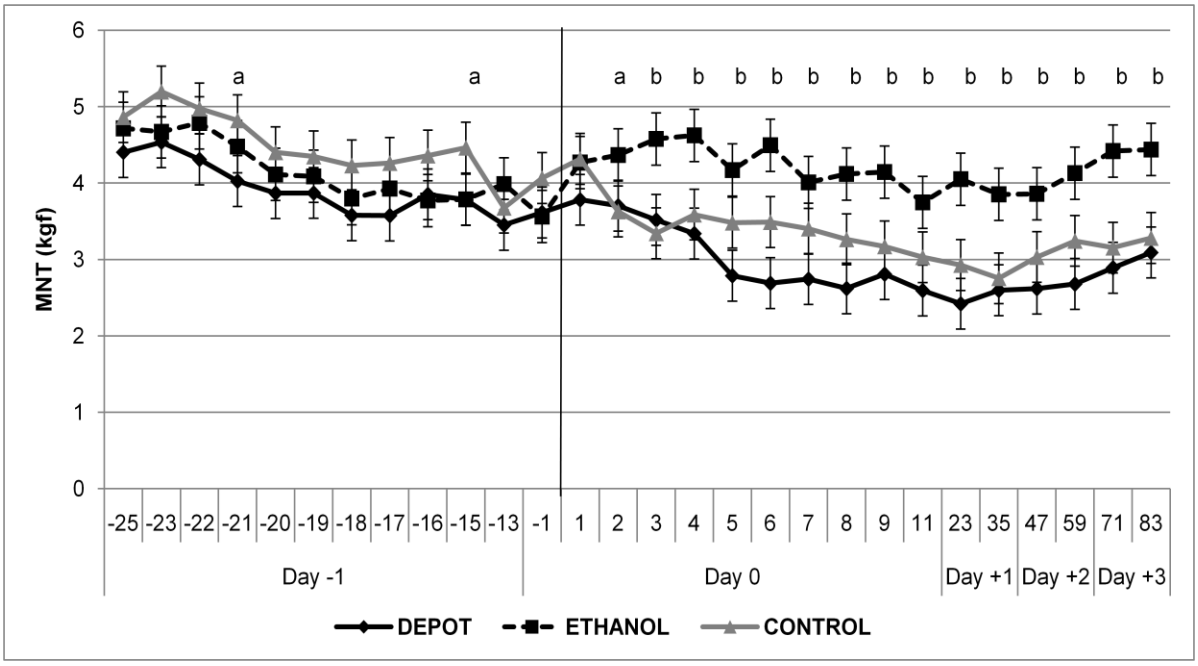


Figure 4.8: Mean±SEM change in pressure algometry MNT from Day-1 to Day0 by treatment. NS = No difference from Day-1 to Day0 (P > 0.05); All other timepoints P < 0.05.

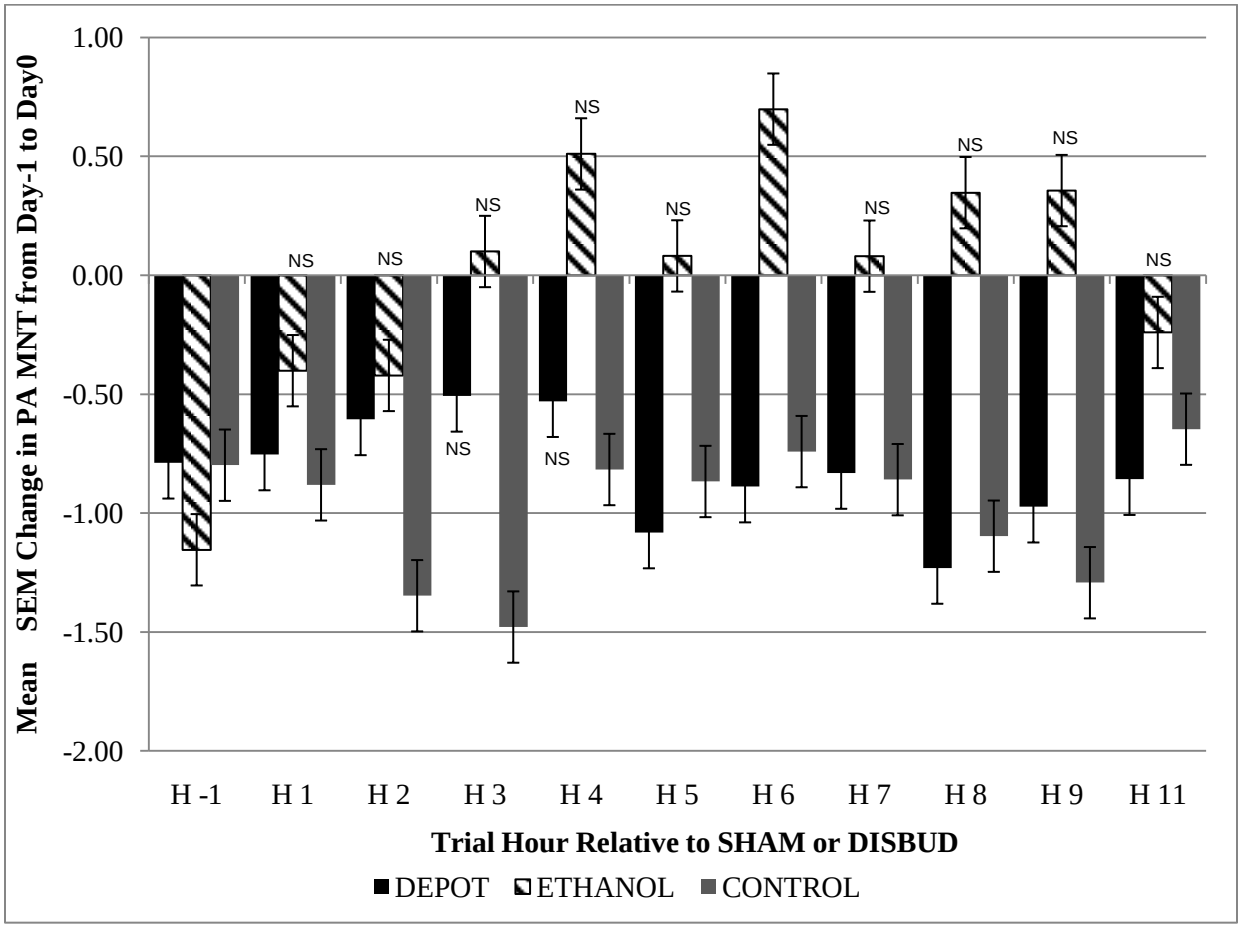


Figure 4.9: Mean±SEM change in heart rate from Day-1 to Day0 by treatment. The only difference in heart rate observed from D-1 to D0 was for ethanol treated calves at Hour-1, prior to disbudding. This difference is most likely a result of handling, and cannot be a result of treatment because this reduction was detected prior to treatment administration. * = P <0.05.

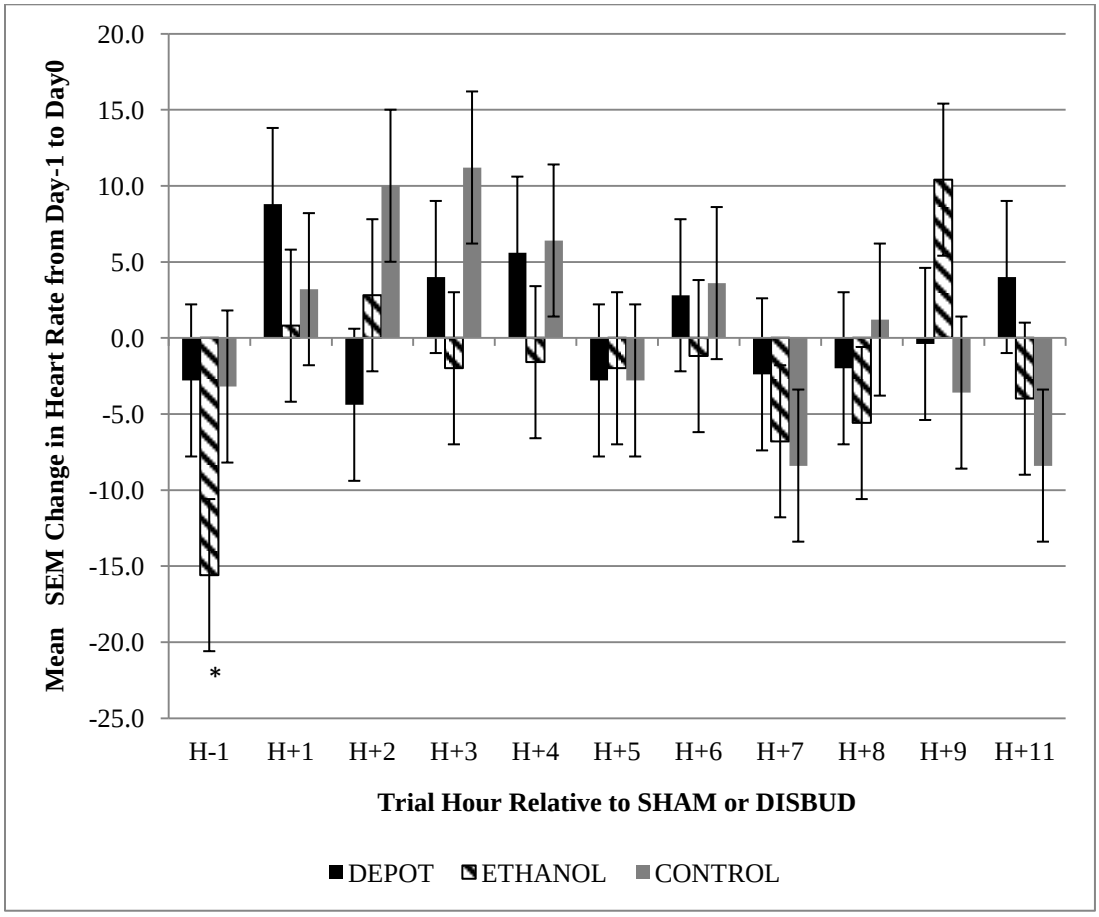


Figure 4.10: Average morning heart rates (beats per minute) per treatment over trial. (*) indicates differences between treatments ($p<0.05$). Depot treatment decreased in heart rate from Day-1 to Day+3. Standard treatment did not change over the trial. Ethanol treatment had elevated heart rate on Day-1, but no differences throughout the rest of the trial. Depot treated calves had elevated heart rates compared to ethanol on Day0. On Day+3, Depot treated calves had decreased heart rates compared to control calves. a-b denotes differences within treatments over all trial days.

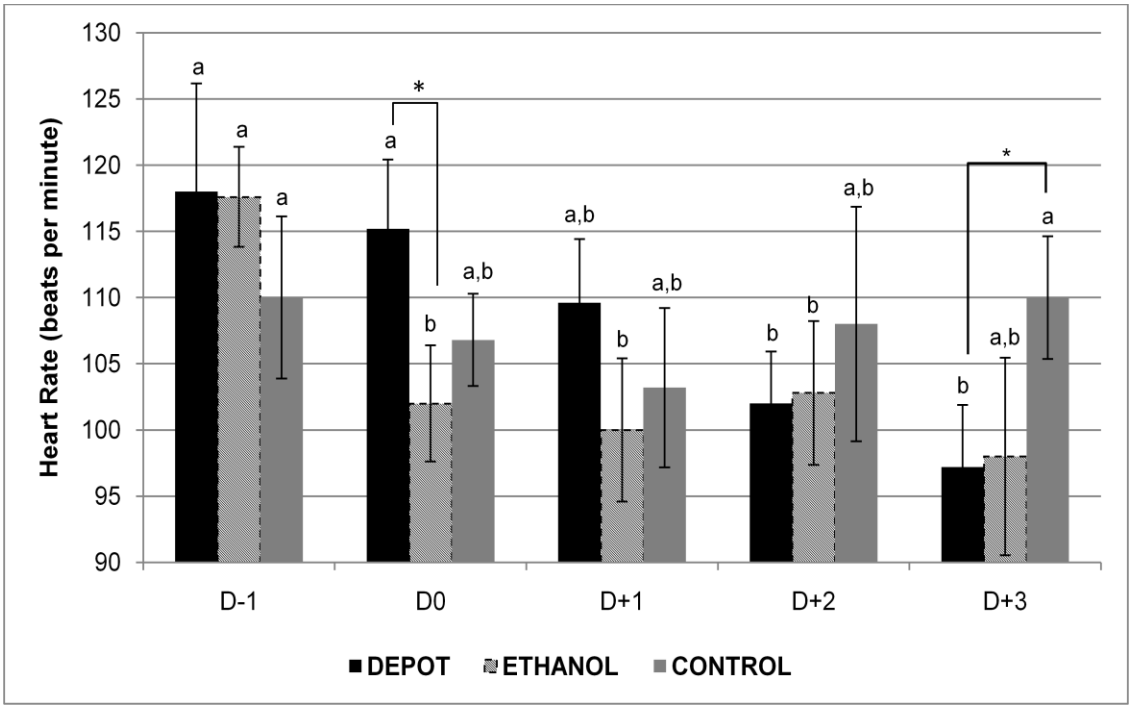


Figure 4.11: Average (± SEM) plasma cortisol concentration at each blood collection timepoint. No differences observed between ethanol and control lidocaine based on adjusted Tukey-Kramer pairwise comparisons over any timepoint. No differences were observed within either treatment over time (Adj. $P > 0.05$ for all timepoints comparisons and both treatments).

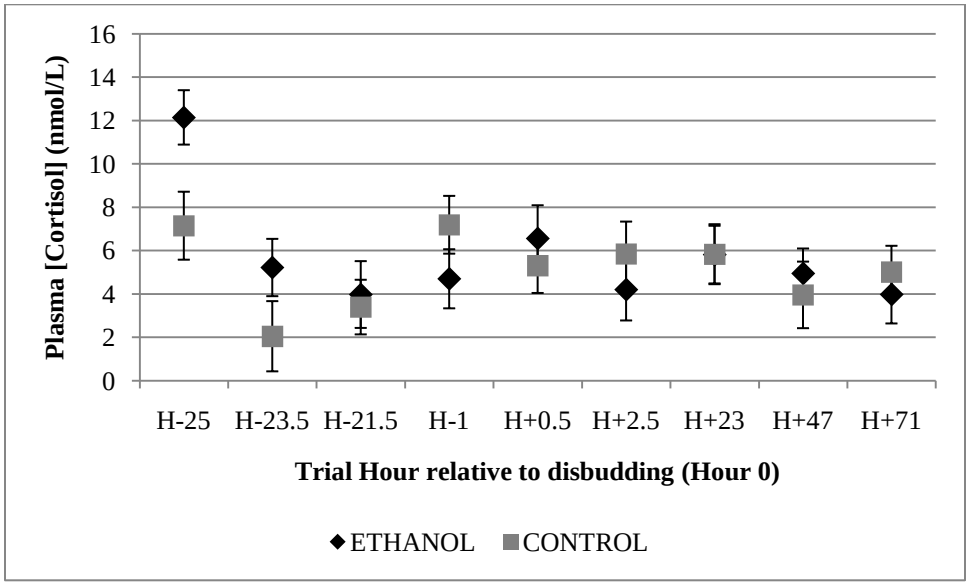
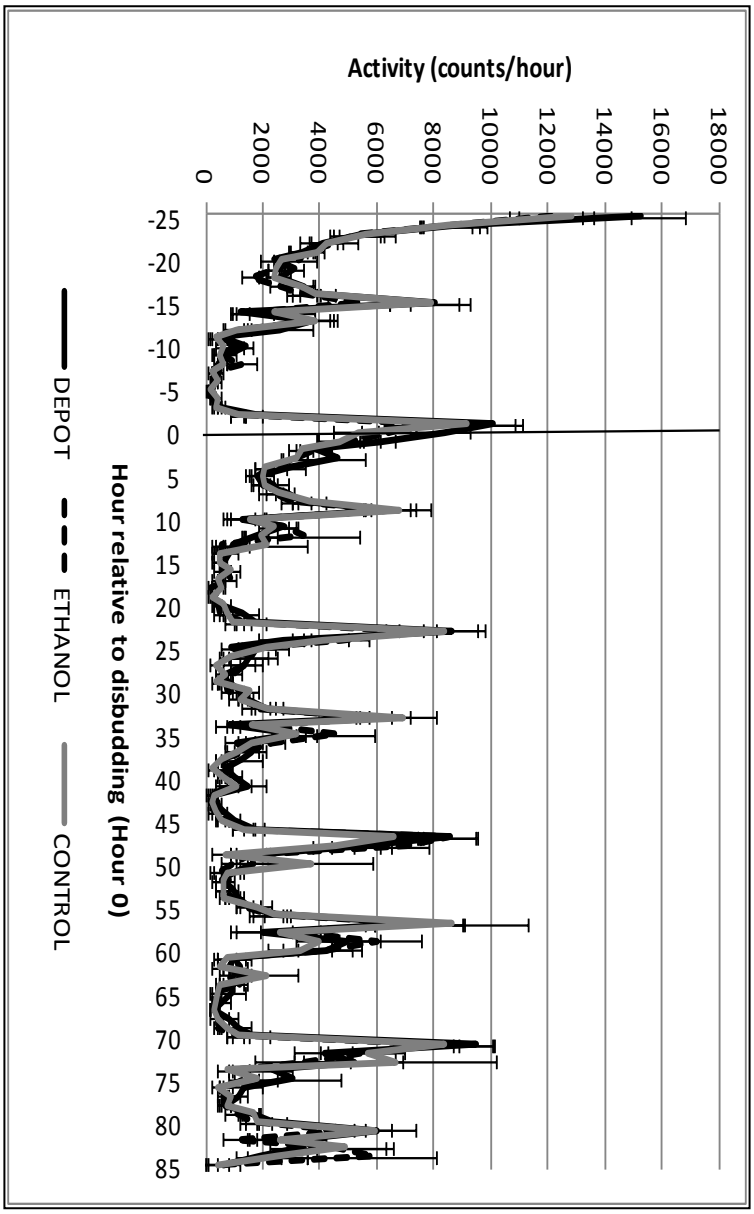


Figure 4.12: Activity (counts per hour ±SE) by treatment. Vertical line shows time of disbudding. No differences between treatments at all timepoints throughout the trial (P > 0.05).



## CHAPTER 5: GENERAL SUMMARY AND CONCLUSIONS

The first objective for this thesis was to examine mechanical nociceptive threshold pain tests, including pressure algometry (PA) and von Frey filaments (VF), and thermal nociceptive threshold as tested with thermal sensitivity (TS), as objective non-invasive measures of pain in cattle and swine. The second objective of this thesis was to examine novel pain mitigation agents, either local anesthetics or non-steroidal anti-inflammatory drugs (NSAIDs), for alleviating pain in cattle and swine. The objective of this final chapter is to review the results of the entire project, as well as to outline some of the limitations of this work that future research can modify when designing future experiments.

The third chapter of the thesis was an assessment of the objectivity of PA and TS to identify lameness in sows with induced transient lameness. This study also assessed sodium salicylate and flunixin meglumine (Banamine®) as treatments for pain associated with sow lameness. The Amphotericin B-induced lameness model provided a predictable and reproducible transient synovitis. The severity and duration of lameness in this sow lameness model were similar to the results of Karriker and colleagues (L. A. Karriker, Iowa State University, Ames, Iowa, personal communication), in which the Amphotericin B-induced lameness was first documented in swine. Similarily, severity and duration of lameness were consistent with findings by Kotschwar and colleagues (2009) who induced lameness in bovines using Amphotericin-B.

The PA tests was found to be an easy to apply, noninvasive method to objectively quantify the mechanical nociceptive threshold (MNT) prior to lameness induction, as well as when sows were lame. Reduction in MNTs from baseline to induced-lame provided evidence that lame sows withstood less pressure, which is consistent to previous MNT research in other livestock species (Dyer et al., 2007; Varco-Cocks et al., 2006).

One of the limitations of this study was the lack of an appropriate non-painful control anatomical landmark. While the MNT for the cannon landmark on the lame leg was elevated compared to the inner or outer claw, the decreased MNT compared to its baseline, did not support this landmark as an appropriate non-painful control site. In future studies, a new control landmark on the front legs, or hind quarters could be assessed for a lack of a pain response when sow is lame.

This research is the first analyzing the objectiveness and repeatability of TS for lameness detection in swine. This exact equipment has been researched in laboratory animals, especially rats and mice, as an immobile unit where the research animals were brought to the light instead of the light brought to the animal. This equipment was modified by adding lasers for appropriate light beam placement, but could not be stationary due to the short focal distance of the heated light beam. Radiant thermal sensitivity has been tested in sheep (Nolan et al., 1987) and peri-parturient dairy cows (Pinheiro Machado et al., 1998), although in each of these experiments, tests of TS used a ramped thermal stimuli which measured the temperature at the withdrawal response. The temperature of the heated stimulus was increased until a withdrawal response was detected, as opposed to measuring the latency to a constant heated stimuli temperature. Other TS tests in livestock have been more successful when analyzing $CO_2$ lasers which do not have the confounding issue of a short focal distance of the heated light beam. Several thermal nociceptive tests have also conducted data collection within the animals' home pen, virtually eliminating the effects of handling. In future studies, the TS test will require more standardization for this lameness model primarily a specified time between repetitions.

Neither NSAID treatments resulted in less pain on D+6 compared to the control. Results from the PA indicate improvement from D+1 to D+6 in the difference between the sound and lame leg for each treatment, but these differences were not significant between treatments. In future studies, different analgesic treatments could be tested for efficacy, and an additional assessment day post-induction could be added during the administration of the analgesic treatments to test the analgesic recovery pattern.

The fourth chapter of the thesis summarized research evaluating nociceptive tests, TS and VF, to assess disbudding pain in calves relative to PA. This study was also conducted to determine the effectiveness of ethanol (E) or a depot formation of lidocaine (D) for extended analgesia during disbudding relative to a control lidocaine cornual nerve block (C).

Measuring the latency to loss of sensation (LS) for novel anesthetic treatments provided evidence that there is a large variation in the amount of time required to completely block nociception within a given anesthetic treatment. This anecdotally confirms that sensation testing should always be administered prior to disbudding, and additional anesthetics should be provided if sensation occurs.

Generally, E or D anesthetic treatments required a second injection in at least one or both horns to achieve complete LS. All calves were completely blocked with a total of 2 or 3 mL of anesthetic treatment, but the latency to LS could likely have been reduced by an initial single larger injection of anesthetic treatment. There is some difficulty comparing the latency to LS because one observer blocked, pin-prick-tested, and disbudded all calves over all treatments, altering the exact timeframe for pin-prick testing. In future studies, one additional technician could administer treatments, allowing the testing observer to remain blind to the anesthetic treatment given. Two of the D treated calves developed a localized inflammatory reaction due to the peanut oil emulsion. Due to the negative reaction, this D treatment is not recommended for providing anesthesia.

Heinrich and colleagues (2010) first published research validating PA as a pain assessment tool for use in disbudding dairy calves. In the current experiment, a reduction in MNTs was seen throughout the sham disbudding day across all treatment groups. The effects of this reduction may be lessened in future studies by having fewer trial hours. This current research study expands upon Heinrich's previous research model by comparing PA to other pain assessment tools. In this current experimental design, neither VF nor TS pain tests were useful pain assessment tools. Due to time constraints when attempting to test all three pain assessment tests, future studies could compare either TS or VF to PA.

The duration of anesthesia was measured with nociceptive threshold tests, as well as physiological parameters including heart rate, plasma cortisol concentrations and activity. Calves anesthetized with E withstood elevated MNTs relative to the C-treated calves, and D-treated calves tolerated reduced MNTs compared to C. Results from the PA test indicate that E provides extended anesthetic relief, and that this depot formation of lidocaine was not a suitable anesthetic treatment for cautery disbudding calves. No differences were detected between anesthetic treatments as assessed with heart rate, plasma cortisol concentration or overall activity as measured with Acticals®.

In this experimental design, heart rates were collected prior to the hourly pain assessment tests. In future studies, a pre- and post- heart rate could be taken at each pain assessment trial hour to test changes in heart rates for the actual pain assessment. By measuring pre- and post- heart rates for each pain test, this could indicate the extent of the noxious stimuli for each pain test. Automated heart rate monitors could be also

attached to calves on trial to record a more accurate assessment of heart rates throughout the trial as opposed to only testing prior to each trial hour.

The largest cortisol levels for both E and C were seen at the very first blood collection on Day-1, with no differences between treatments or within treatments. This dramatic cortisol spike likely indicates high handling stress for this first blood collection. In future studies, a preliminary blood collection could be taken prior to the trial to account for this likely confounding effect of handling. The lack of difference between treatments could indicate an overall level of handling stress during each blood collection, which may be decreased in future studies by use of jugular catheters.

The E provided extended anesthetic relief over several days compared to the control treated calves administered lidocaine by withstanding elevated MNTs relative to C indicating higher pain tolerance for the duration of the trial. The depot formation of lidocaine was not an effective anesthetic treatment for use in this experiment due to the efficacy of anesthesia, as tested with decreased MNTs after disbudding, and allergic reactions were seen in two calves to the peanut oil. In this experiment, neither VF nor TS pain tests were practical pain assessment tools for technical and ethical issues. No differences between treatments were detected as tested with heart rates, cortisol or activity, suggesting that the methods were not sensitive or robust enough in this experimental design.

In conclusion, this research has validated the use of PA as an objective pain assessment tool for a transient-induced sow lameness model, as well as a cautery disbudding research model. The PA test was effective at discriminating between lame and sound legs in the sow lameness model, and was also an effective test to compare the duration of local anesthetics, as tested with the disbudding experiment. These results provide promising evidence as to the range of research capabilities offered by PA. In both experimental models, TS was not an appropriate measure of nociception due to the high variability of baseline latencies in the lameness research and the ethical implications of burning in the disbudding research. In the sow lameness research, PA did not differentiate a positive effect of either NSAID treatment for mitigating pain associated with lameness. As tested with PA, E provided extended local anesthetic relief over a period of several days whereas the D treatment was not an effective anesthetic for cautery disbudding compared to the control lidocaine treatment.

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