Advancing techniques to promote the welfare of sows utilized in laboratory based lameness models

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Advancing techniques to promote the welfare of sows utilized in laboratory based lameness models

by

Monique Pairis-Garcia

A Dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Animal Physiology

Program of Study Committee:
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Iowa State University

Ames, Iowa

2014

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ABSTRACT

The first objective for this dissertation was to refine and enhance common techniques conducted in a laboratory setting including drug administration, anesthesia and behavioral modification for laboratory housed sows to improve the welfare of sows specifically by minimizing pain and distress. The second objective of this dissertation was to use an optimal dosing regimen for two non-steroidal anti-inflammatory drugs to determine their efficacy in pain management using nociceptive threshold tests when sows are induced lame through the use of a chemical synovitis model. The expected outcome was that in this lameness pain model, the pain mitigation agents will alleviate pain as assessed by two nociceptive tests.

For the first objective, I developed and refined a technique for catheter placement into the auricular vein of sows. This method was quick, effective and reliable, allowing a large drug volume to be administrated successfully without relying on prolonged restraint or general anesthesia of the sow. Research confirmed that Yohimbine is an effective anesthetic reversal agent in mature sows when anesthetized with xylazine, ketamine, and telazol. Yohimbine reduced overall recovery time and maintained physiological parameters closer to normal homeostatic ranges. Lastly, the results of a study assessing behavioral modification in an individual sow demonstrating oral and locomotor stereotypies suggests the promise of environmental enrichment as an effective treatment strategy for mitigating stereotypies performed in a laboratory setting.

The second objective of this dissertation was to use an optimal dosing regimen for two non-steroidal anti-inflammatory drugs (flunixin meglumine and meloxicam) to determine their efficacy in pain management using nociceptive threshold tests when lameness was induced through the use of a chemical synovitis model. Results from this study
indicate that flunixin meglumine and meloxicam administration mitigated pain sensitivity in lame sows post lameness induction when pain sensitivity was evaluated with pressure algometry and thermal sensitivity tests. These analgesic drugs may be a key tool to manage pain associated with lameness.
CHAPTER 1
GENERAL INTRODUCTION

1.1 Thesis Organization

This dissertation 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 laboratory techniques used on swine as research models, behavioral alterations in laboratory swine and objective pain assessment is found in Chapter 2. Chapters 3, 4, 5, 6 and 7 detail individual research projects refining laboratory techniques and utilizing animal pain models, with more specific introductory and background information included within the research chapters. Chapter 3 focuses on the development and refinement of a technique for short term intravascular auricular vein catheter placement and is formatted for Laboratory Animals journal. Chapter 4 focuses on Yohimbine and its effects on physiological recovery parameters in anesthetized sows and is formatted following Journal of Swine Health and Production guidelines. Chapter 5 is a case study identifying treatment of oral and locomotory stereotypic behaviors in a mature sow and is formatted following Journal of Veterinary Behavior guidelines. Chapter 6 focuses on measuring the efficacy of flunixin meglumine and meloxicam for lames sows using nociceptive threshold tests and is formatted for the Animal Welfare Journal. Chapter 7 is a summary of the research results and a general discussion of how the results apply to the use of sows a research model in a laboratory setting.
1.2 Study Objectives and Expected Outcomes

The first objective for this dissertation was to refine and enhance common techniques conducted in a laboratory setting. The expected outcome was that by developing and refining techniques for drug administration, anesthesia and behavioral modification for laboratory housed sows, the welfare for sows would be improved by minimizing pain and distress. The second objective of this dissertation was to use an optimal dosing regimen for two non-steroidal anti-inflammatory drugs to determine their efficacy in pain management using nociceptive threshold tests when lameness is induced through the use of a chemical synovitis model. The expected outcome was that in a lameness pain model, the pain mitigation agents will alleviate pain as assessed by the pressure algometer and thermal sensitivity test.
CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Swine are a common species utilized for biomedical research as well as research pertaining to agricultural needs. The United State Department of Agriculture (USDA) reported that in 2013 over 150 research projects using swine for both production driven research and biomedical research are active. In 2002, swine officially became the most common large laboratory animals species utilized with over 68,000 pigs registered for research (USDA, 2013). This is greater than the number of dogs utilized for biomedical research which has dropped 34% in the last 20 years. Recognizing the important role of swine as a research model, it is essential to assess their welfare and to ensure appropriate handling and treatment to minimize their pain and distress. The objectives of this literature review are to provide an overview of the pertinent literature regarding common laboratory techniques, behavioral modification and pain models utilizing swine as a research model. In addition, this literature review will provide a background on a chemical synovitis model used to evaluate pain associated with sow lameness.

2.2 Swine Utilized As Research Models

Swine are extensively used for biomedical research based on anatomical and physiological similarities to humans (Dopson, 1993). In the last two decades the use of swine for biomedical research has expanded and the pig is now used as a laboratory model in over 20 institutions in the United States and 2,500 National Institute of Health sponsored research
grants (Schook et al, 2011). In addition, there are over 66 million head of swine in the United States (USDA, 2013) accounting for 35,000 direct jobs and generating $34.5 billion to the United States gross national product (NPPC, 2011). Therefore the use of swine as a laboratory research model to improve productivity and efficiency of commercial swine is of importance.

2.3 Laboratory Techniques Performed on Swine

2.3.1 Intravenous Drug Administration

Intravenous (IV) access is important in veterinary medicine to administer drugs for treatment of clinical disease. However, IV drug administration is difficult to perform on swine due to inaccessible superficial veins and thick subcutaneous fat layers (Trim & Braun, 2011). For all swine ages and weights, the two most common methods for IV administration include: 1. Temporary catheterization (St. Jean & Anderson, 2006) or 2. Jugular vein injection using a “blind stick” approach (defined as drug administered without vein visualization; Brown et al, 1973; Muirhead, 1981). Blind-stick injection is unreliable as it is difficult to ensure that drug is not administered extravascularly. Thus, access to the vein using an indwelling catheter is the most appropriate method for intravenous drug administration. Catheters in swine have been successfully placed in the femoral artery and vein (Weirich et al, 1970; Jackson et al, 1972), subcutaneous abdominal vein, middle sacral artery (Ford & Maurer 1978; Witzel et al, 1973), aural vein, jugular vein (Brown et al, 1973; Wingfield et al, 1974) and the uterine vein (Rodriguez & Kunavongkrit 1983).
Although catheterization better ensures IV drug administration, catheter placement requires technical skill and relies on either prolonged physical restraint or general anesthesia for placement. Prolonged restraint may result in both physical and emotional distress for a compromised animal and general anesthesia may influence drug metabolism, absorption and efficacy and increase risk for post-anesthetic complications. Catheterization of the auricular vein is a potential alternative for IV drug administration as the auricular vein is easily accessible and does not require prolonged restraint or anesthesia. Two procedures for auricular vein catheterization for swine have been described in the literature (Phillips et al, 2012; Porter et al, 1992). Phillips and colleagues (2012) successfully placed auricular vein catheters for blood collection while sows were anesthetized. Porter and colleagues (1992) placed catheters in restrained sows with no local or general anesthesia. Although catheter placement was successful using this approach, a single catheter placement took on average 30 minutes and required prolonged sow restraint.

2.3.2 Blood Collection

Blood collection is a common diagnostic technique utilized in the laboratory to evaluate physiological parameter changes (Bauer et al, 2011; Broes et al, 2007; Deng et al, 2000). Blood collection techniques are chosen based on the animal species, laboratory attributes and the amount and/or frequency of blood collection (Arnold et al, 2008; Joslin, 2009; Lin & Chien, 1997).

Swine are challenging to restrain, due to their large size and can be easily stressed by physical restraint and handling (Trim & Braun, 2011). For all swine ages and weight, the most common and fastest method of blood collection on farm is venipuncture sampling from
the jugular vein or anterior vena cava (Brown, 1979; Muirhead, 1981) using a “blind stick” approach. Collection of blood from the jugular or anterior vein is reliable and quick. This method requires restraint of the animal using a snare. A steel cable is placed into the mouth behind the canine teeth of the animal, and then tightened around the maxilla and snout. Pain associated with pressure produces a withdrawal response preventing head or neck movements which facilitates venipuncture (French & Tully Jr., 2010). Snaring is a stressful event and can lead to conditioned (learned) avoidance. Aggressive responses to snaring and blood collection can pose safety risks to both animals and handlers. The major disadvantage of this technique is stress associated to handling during blood collection. Studies conducted using the snare as a temporary restraint device for venipuncture have demonstrated elevated levels of cortisol (Roozen et al, 1995), salivary amylase (Fuentes et al, 2011), epinephrine, norepinephrine, glucose, lactate, free glycerol, and ascorbic acid (Dubreuil et al, 1990; Dubreuil et al, 1993; Neubert et al, 1996; Rushen et al, 1993) as compared to blood collected from swine using a catheter.

In swine weighing less than 45 kg an additional blood sampling option includes collection of blood from the orbital sinus. Smaller pigs are restrained manually and a needle is placed in the medial canthus of the eye and advanced medially to puncture the venous sinus. Once the venous sinus has been punctured, blood slowly drips out of the needle and is collected (Huhn et al, 1969). Although this method does not require a snare, manual restraint of the animal is still required and leads to similar changes in physiological response. The major disadvantage of this technique includes its unaesthetic nature, slower collection time, post-collection orbital hemorrhage and pressure placed on the globe during collection (Swindle, 1998). For sows and boars, an additional blood collection technique using a
syringe and needle can be performed on ear veins, tail veins and the cephalic vein (Tumbleson et al, 1968; Sankari, 1983). However, regardless of technique chosen, physical restraint of animals and stress associated with restraint occurs.

Catheters can also be placed for serial blood collection as a means to mitigate or eliminate restraint. Placement of an indwelling catheter is more commonly utilized in laboratory settings for swine research, but can be used on farm. Advanced technology has now provided the ability to obtain automated blood samples from swine using indwelling catheters without restraint. The Pigtturn device is an automated sampling system used for individual swine placed in an automatic rotated holding pen. This technology allows pigs to be fitted with catheters whilst providing acclimation prior to serial blood collection without restraint (Marchant-Forde et al, 2011).

2.3.3 Anesthesia

Swine may be anesthetized in order to complete routine production procedures or surgical operations (Lu et al, 2011). Laboratory anesthesia examples include, but are not limited to, coronary angiography, ischemia and reperfusion models for human disease (Dominguez et al, 2011; Williams et al, 2012) tracheal culture and bronchoalveolar lavage for respiratory disease diagnosis (Solano & Pijoan, 1997; Jolie et al, 1999) and assistance with aggressive animals when performing reproductive procedures (Shipley, 1999) or euthanasia (Pelland, 2009). Anesthetizing sows represents a unique set of problems as physiological compromise (disease) and age can make anesthesia induction risky. According to the American Society of Anesthesiologists (ASA, 2011), age (geriatric), weight (Petry et al, 2009), disease status, and anatomical variation (Sinclair, 2011) contribute to a heightened
anesthetic risk and can lead to prolonged recovery times and increased post-anesthetic complications (Trim & Braun, 2011). In addition, studies evaluating natural on-farm sow deaths confirmed cardiovascular failure as one of the top three causes of mortality (Chagnon et al, 1991; D’Allaire et al, 1996; Engblom et al, 2008a). These factors increase anesthetic risk, as the cardiovascular system is a key system altered during anesthesia. In addition, direct observations in our laboratory revealed that anesthetized sows may exhibit prolonged recovery times between 5 and 10 hours. Acknowledging inherent sow risk factors, it is critical to design a protocol that minimizes risks associated with anesthesia.

Xylazine, ketamine, and telazol are a common combination of drugs used for anesthesia of swine both on farm and under research conditions (St-Jean & Anderson, 2006). The choice to use all three drugs in combination is based on a toxicologically wide margin of safety in swine and prolonged analgesic properties attained using all three drugs as compared to xylazine and ketamine administered together (Green et al, 1981) and telazol administered alone (Ko et al, 1993). To minimize overall recovery time and decrease complications post-anesthesia, reversal agents can be used. Yohimbine is an alpha-2 adrenoreceptor antagonist that has been reported to be effective in reversing xylazine effects in nursery-age swine and other food-producing animals (Kitzman et al, 1982; Mohammed et al, 1995; Kim et al, 2007). In cats, Yohimbine acts as a stimulant, shortening both ketamine-induced anesthesia and the effects of xylazine (Hsu & Lu, 1984) Additional alpha-2 adrenoreceptor antagonists include atipamezole which also work effectively as a reversal agent for xylazine in swine (Tendillo et al, 1996; JaeYeon & MyungCheol 2011). In addition to minimizing pain and distress during physical restraint and data collection, abnormal behaviors may develop in laboratory housed swine as a means to cope with their
environment. Assessing the pig’s behavior and identifying abnormal behaviors that may implicate poor welfare will be the next area focused on in this review.

2.4 Behavioral alterations in swine

2.4.1 Stereotypic Behavior

Oral-nasal stereotypies are the most commonly identified stereotypies in confined ungulates, comprising 70% of all stereotypic behavior. The most common stereotypies in swine include bar biting, sham chewing, tongue sucking and object licking (Sambrus, 1985; Whittaker et al, 1998; Horrell, 2000). Previous research estimates sows spend between 7%-55% over an 8 hour period dedicated to performing oral stereotypies on farm (Broom & Potter, 1984; Von Borell & Hurnik, 1990). Oral stereotypies have been associated with inadequate gut fill or thwarting of the appetitive and/or consummatory phases of rooting and foraging (Mason & Mendl, 1997). Locomotor stereotypies, like weaving, have been less commonly identified in ungulates, compromising only 10% of all stereotypic behaviors performed (Rushen and Mason, 2006). Locomotor stereotypies have been associated with an animal’s motivation to escape aversive stimuli and are often performed at the barrier preventing them from escape (Mason, 1993; Mayer-Holazpfel, 1968).

Previous research can provide important insights on proximate factors contributing to the causation and development of stereotypic behaviors in ungulates (Jensen, 1988; Bergeron & Gonyou, 1997; Clubb et al, 2006) as well as addressing ultimate questions on the evolutionary significance and function of these behaviors (Mayer-Holzapfel, 1968; Bergeron et al, 2006). However, there has been little empirical research conducted on evaluating
treatment options for stereotypic behaviors in swine. Previous studies have successfully mitigated oral stereotypies by satisfying gut fill by providing straw (Stewart et al., 2011), high fiber diets (Robert et al., 2002), and sugar beet pulp (Brouns et al., 1997), however no published studies to date have assessed treatment options for locomotor stereotypies in swine. Mitigation strategies for locomotor stereotypies have been performed on equines. Cooper and colleagues (2000) evaluated five different facility types for stabled horses performing weaving stereotypies. They found that providing a front and side panel open for the horse to view an adjacent stall decreased the occurrence and frequency of weaving behavior. In addition McAfee and colleagues (2002) found that modifying the visual environment of the horse’s stall using mirrors decreased the incidence of weaving behavior (McAfee et al., 2002). Such successful treatment options in horses may be applied to swine exhibiting locomotor stereotypies in the laboratory.

2.4.2 Pain Behavior

Although scientists cannot directly observe an animal’s subjective feelings, based on similarities in neural anatomy, behavior and physiological responses, it is highly suggested that animals have the capacity to feel (Dawkins, 1980). By recognizing this concept, it is critical that we understand affective states such as pain as it relates to that animal’s welfare. 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, 2004). Routine farm animal production practices such as castration, teeth clipping and tail docking have been viewed as acute and chronic pain inducing procedures and movements towards analgesic requirements for these procedures is being established (Millman, 2013; Coetzee, 2013). Diagnosis of pain is a difficult process
due to individual’s unique experience with pain and personal reaction to a painful stimulus (Gaynor & Muir, 2009) Pain tolerance and reaction also varies tremendously between species, breeds, gender, age, severity of stimulus, and duration of pain (Matthew, 2000). When assessing pain in human subjects, the gold standard is implementing a visual analog scale of verbal self-reporting (Hadjistavropolous & Craig, 2002). A subject can rate his or her pain by using a numerical scale that uses descriptions and comparisons to describe the state of pain that the subject is feeling (Jensen & Karoly, 1992). These scales have been used in several disease processes including back pain, temporomandibular pain, and rheumatoid arthritis (Prkachin et al, 2002; Vevoort et al, 2009). This allows the subject to decide what level of pain they are experiencing at that moment in time. However, the inability for animals to self-report has led scientists to evaluate the animal’s behavior as an indicator of pain.

Pain-related behavior can be used as a tool for scientists to evaluate the presence and severity of pain indirectly, therefore the animal’s behavior or reaction is not influenced by observer manipulation. Behavioral parameters that are focused on when evaluating animal pain include: body posture, activity, abnormal gait or movement, social interaction, physiological parameters (heart rate, respiratory rate, dilated pupils, hypertension, increased serum cortisol and epinephrine), facial expressions, and/or vocalization (Fitzpatrick et al, 2006; Molony & Kent, 1997; Smulders et al, 2006; Gaynor & Muir, 2009).
2.5 Animal Pain Models

2.5.1 Lameness

Lameness associated with painful joint lesions has been identified as a welfare challenge for confined sows (Elmore et al, 2010). Lameness, or feet and leg problems, was ranked as the number three reason for culling sows, comprising 15% of cull sows marketed in the United States (Schenk et al, 2010). Feet and leg problems were identified as the most common involuntary reason for culling sows (Stalder et al, 2004) and are associated with several variables that result in poor performance including poor farrowing performance and decreased litter size (Engblom et al, 2008b; Anil et al, 2009). Knauer and colleagues (2007) noted that between 10.5-14.9% parity one through three sows is culled for lameness. Culling females prior to the third parity is as an economic loss, as pig producers are neither able to pay off individual sow cost nor capitalize on the benefits of higher sow retention rates (Stalder et al, 2000; Stalder et al, 2003). Lameness in breeding aged swine therefore has a large negative economic impact to livestock producers (Wells, 1984) as it decreases economic gain by increasing labor and medical costs (Pluym et al, 2013) and shortens total sow productive lifetime. Lameness has also been recognized as a welfare concern on farm and been identified as an animal-based measurement in European Welfare Quality® (2011) and Pork Quality Assurance Plus® (2013) programs. Science-based guidance for pig producers for proper on-farm management and treatment protocols for lame sows is deficient. Research to address the limited knowledge in this area is essential in formulating science-based recommendations to enhance on-farm management and individual pig welfare on farm.
2.5.2 Amphotericin B

Amphotericin B is an antifungal antibiotic that is used to treat serious systemic fungal infections. It is a member of the polyene antibiotic class and its effective mode of action causes fungal cell wall damage by binding to ergosterol leading to a loss of small molecules and ions and eventual destruction of the cytoplasmic membrane (James & Rawlings, 1996).

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. 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. Lameness induction using amphotericin B in both horses and cattle has demonstrated a tremendous amount of versatility to induce clinically diverse lameness models.

Amphotericin B has been injected in horses at levels between 5-25 mg to evaluate the effectiveness of analgesics (Marttinen et al, 2006; Suominen et al, 1999; Sysel et al, 1996), pain therapy (Crawford et al, 1991), and pain assessment (Bussieres et al, 2008). Bussieres and colleagues (2008) injected amphotericin B into the tarsocrural joint of 9 horses allocated to the experimental group (amphotericin-B induced lameness) and compared them to 9 control horses (sedation with analgesia). This group successfully utilized the amphotericin B lameness model and developed and validated a composite multifactorial pain scale (CPS) to establish the value of specific behavioral and physiological data in determining pain intensity. In the bovine study (Kotschwar et al. 2009) the distal interphalangeal joint was successfully injected with 10 mg resulting in a predictable acute synovitis model and
demonstrated that sodium salicylate was not effective in providing analgesia after lameness induction. Most recently the amphotericin model has been developed in swine (Karriker et al, 2013). Amphotericin B was injected into the distal interphalangeal joint and induced an acute lameness that resolved spontaneously within 10 days after injection as measured by kinematic tools.

2.5.3 Mechanical Nociceptive Thresholds

Nociception is the process by which the detection, transduction, and transmission of a noxious stimulus to higher centers of the central nervous system occurs (Livingston, 2006). Mechanical and thermal nociceptive thresholds (MNT and TNT) can be defined as the amount of pressure or heat stimulation necessary to produce a behavioral response indicative of pain sensitivity (Haussler et al, 2007).

Pressure algometry is a farm practical tool that could be used to evaluate individual sow lameness. Pressure algometry is a non-invasive tool used to determine sensitivity to pressure and indicates pain threshold of a subject. Pressure can be applied to the body part of interest and observed for a response. Once a response is observed, pressure is immediately removed, and the peak pressure representing the MNT is recorded. Nociceptive thresholds have been used to evaluate painful states in several species including broilers (Hothersall et al, 2011), dairy cattle (Veissier et al, 2000; Herskin et al, 2003, 2009; Dyer et al, 2007; Heinrich et al, 2010; Higginson-Cutler et al, 2013; Fitzpatrick et al, 2013) sheep (Nolan et al, 1987; Ley et al, 1989; Stubsjøen et al, 2009) and swine (Jarvis et al, 1997; Di Giminiani et al, 2012; Janczak et al, 2012; Tapper et al, 2013).
Tapper and colleagues (2013) utilized a chemical synovitis model to induce lameness in sows. A hand-held pressure algometer (Wagner Force Ten™ FDX 50 Compact Digital Force Gage, Wagner Instruments, CT, USA) with a one cm² rubber tip was used to quantify MNTs in kilograms of force (Kgf). To standardize the procedure and reduce variability associated with handler application of the device, the applicator practiced applying the force at a rate of approximately one Kgf/second on a static surface for 10 second periods prior to data collection. During data collection, pressure algometry was applied at the landmarks at approximately one Kgf/second. The maximum force applied was 10 Kgf, after which the recorder said “Stop” and pressure was removed. Pressure was applied perpendicularly to three landmarks in a randomized sequence for each sow. The results of this study concluded the support of the pressure algometer as an objective non-invasive method for measuring pain sensitivity in sows induced with transient lameness. This tool can be used to evaluate efficacy of analgesic drugs to reduce pain sensitivity.

2.5.4 Thermal Nociceptive Thresholds

Thermal nociceptive tests are an additional means to assess pain sensitivity using precise focused radiant heat stimulation. The thermal analgesia meter has been used extensively in rat and laboratory mice (Andrew and Greenspan, 1999; Chen et al, 1999; Hargreaves et al, 1988; Djouhri et al, 2006). Andrew and colleagues (1999), Chen and colleagues (1999) and Djouhri and colleagues (2006) demonstrated that the use of a thermal analgesia meter can objectively assess pain sensitivity, and that pain associated with inflammation models can be explained due to nociceptive sensitization and hyperalgesia.
For swine specifically, three studies have evaluated thermal nociceptive tests to assess pain sensitivity. Herskin and colleagues (2009) and DiGiminiani and colleagues (2012) measured thermal nociception in swine using a thermal laser. Both studies confirmed the thermal laser as an objective tool to detect and quantify pain sensitivity. DiGiminiani and colleagues (2012) qualified the pig to be used successfully as a cutaneous pain model in humans, exhibiting lower pain thresholds based on pig size, anatomical location and stimulation intensity. Herskin and colleagues (2009) concluded that nociceptive cutaneous laser stimulation is a valid measure of nociception in group-housed gilts and this type of data collection to assess pain is ideal as it results in minimal disturbance of daily routines.

Tapper and colleagues (2013) were the first to study the objectivity and repeatability of thermal nociceptive test utilizing a focused radiant heat that has been mainly used in mice and rats. These researchers found that pain sensitivity varied among non-lame sows on baseline day and therefore data between treatments for this study could not be compared. However, recent data from our laboratory group has demonstrated that by increasing the number of time points for data collection, the thermal nociceptive test can detect pain lameness in sows and detect differences in treatment groups between those sows provided with analgesics and those given saline (Pairis-Garcia et al, 2014).

2.6 Summary

In the last two decades, the utilization of swine as an animal research model has increased, resulting in a need to identify and assess welfare challenges associated with techniques and housing swine in laboratory settings. Identifying refinements and alterations in common techniques utilized in the laboratory to minimize pain and distress is important
from an ethical standpoint and a research standpoint as poor individual animal welfare may lead to alterations and outliers within data sets. In addition, there has been preliminary success in using sows to determine pain sensitivity when lames using a variety of tools, which in turn can help formulate science-based recommendations for pig producers on lameness pain mitigation on farm.
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CHAPTER 3

DEVELOPMENT AND REFINEMENT OF A TECHNIQUE FOR SHORT-TERM INTRAVASCULAR AURICULAR VEIN CATHETER PLACEMENT IN MATURE SOWS

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Abstract

Intravenous drug administration in adult swine is difficult to perform due to inaccessible superficial veins and thick subcutaneous fat layers. However, successful intravenous drug administration is critical for many biomedical applications including pharmacokinetic studies as extravascular drug administration can influence the drug’s absorption and elimination rate. The purpose of this study was to develop and refine an
effective technique for indwelling auricular vein catheter placement in the conscious mature sow. We developed a protocol using a topical anesthetic cream and minimal physical restraint to place indwelling catheters in the auricular vein of six multiparous sows. This method was quick (3 minutes 20 seconds ± 8 seconds (mean ± SE per catheter)), effective (11/12 catheters successfully placed) and reliable, allowing a large drug volume (20-22ml) to be administrated successfully during the trial without relying on prolonged restraint or general anesthesia of the sow.

**Keywords:** Catheterization, Auricular vein, Sow, Refinement

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Iowa State University (United States)

Intravenous access is important in veterinary medicine to administer drugs for treatment of clinical disease. However, intravenous drug administration is difficult to perform on swine due to inaccessible superficial veins and thick subcutaneous fat layers. For all swine ages and weights, the two most common methods for intravenous administration include: 1. Temporary catheterization or 2. Jugular vein injection using a “blind stick” approach (defined as drug administered without vein visualization). Blind-stick injection is unreliable as it is difficult to ensure that drug is not administered extravascularly. Thus, access to the vein using an indwelling catheter is the most appropriate method for intravenous drug administration.

To the authors’ knowledge three procedures for auricular vein catheterization for swine have been reported in the literature. Phillips and colleagues successfully placed auricular vein catheters for blood collection while sows were anesthetized. General anesthesia use can contribute to increased variation when conducting pharmacokinetic
studies because anesthetic agent systemic effects on clearance, absorption time and volume of distribution for the drug of interest are most often unknown. Porter and colleagues placed catheters in restrained sows with no local or general anesthesia used. Single catheter placement took on average 30 minutes requiring prolonged sow restraint. In clinical cases where an animal may have cardiovascular or respiratory complications, prolonged restraint is inappropriate as it may cause the animal to go into distress. Rushen and colleagues did not provide detail on the catheter placement process itself. Although catheterization of other veins including the jugular vein, subcutaneous abdominal vein, and uterine vein has been successfully conducted in swine, catheterization of these veins requires anesthesia or prolonged restraint of the patient. This indwelling ear vein catheter placement technique is novel because we developed and refined an effective access route to confidently administer drugs intravenously in adult swine without relying on prolonged restraint or general anesthesia. Prolonged restraint may result in both physical and emotional distress for a compromised animal and general anesthesia may influence drug metabolism, absorption and efficacy. Furthermore, this novel auricular vein catheter placement technique utilized local anesthetic to minimize pain sensitivity during placement. The combination of sow welfare during placement and the resultant success for intravenous access reinforces the novelty of this procedure.

This laboratory technique was carried out on healthy female sows (Sus scrofa domestica; age: 1-3 years, weight: 202-222 kg) concurrently enrolled in a pharmacokinetic study in which they received either an oral or intravenous meloxicam dose (Loxicam, Norbrook Inc. Lenexa, USA). Health status was determined by physical examination of each sow. Physical examination included lung and heart auscultation, rectal temperature and
reproductive tract ultrasonography. The animals were cared for in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals, 8th edition. This work was performed in an Association for Assessment and Accreditation of Laboratory Animal Care (AALAC) accredited facility at the Iowa State University College of Veterinary Medicine. Sows were housed individually in pens (3.7 m length x 1.4 m width x 1.2 m height) with a rubber mat (3.5 meter length x 1.3 m width). Lights were on a 12:12 light dark cycle (light hours [0600 and 1800]) and room temperature ranged between 12.5°C to 20.5°C. No other additional bedding material (straw, hay etc.) was provided but sows were provided with cotton rope for environmental enrichment. Animals had free access to drinking water and were hand-fed a custom mixed diet composed of corn, soybean meal and soy hulls. They were allowed a 14-day acclimatization period before the beginning of the study. Experimental procedures were approved by the Institutional Animal Care and Use Committee at Iowa State University (United States). Two sows were used to refine the catheterization technique prior to study commencement, and the remaining 6 sows were catheterized twice, once in each ear during the trial.

One hour prior to catheterization, a topical anesthetic cream (15g; EMLA cream; Hi-Tech Pharmacal Co Inc, Amityville, USA) was applied to each sows’ ear pinna covering the entire length of the ear from base to tip (Figure 3.1). After 1 hour, excess cream was wiped off using a paper towel. Once sows were manually restrained with a pig snare, the ear was cleaned with 70% isopropyl alcohol and 0.75% iodine scrub. An indwelling catheter (22 gauge, 2.54 cm long; manufacturing code: SMIT-9986, Smiths Medical, Dublin, USA) was inserted into the distal portion of the largest and most prominent auricular vein (per visual assessment) until blood was present in the catheter hub. Once placed, the stylet was removed
and transparent tape (Dermiclear Tape, Johnson & Johnson, New Brunswick, USA) was placed over the catheter to temporarily secure it. The catheter was capped with a threaded injection cap (Hospira Inc, Lake Forest, USA) and the skin surrounding the catheter was dried with gauze to absorb any blood that may prevent proper tape adherence to the skin.

The catheter hub was glued to the ear skin using surgical glue (3M Vetbond Tissue Adhesive, 3M Animal Care Products, St. Paul, USA) applied underneath the transparent tape for further security. Two transparent dressings (Tegaderm, 3M Animal Care Products, St. Paul, USA) were placed over the entire catheter hub and injection port. Special care was taken to orient the dressing where the distal edges could be folded over the ear for further security (Figure 3.2). A 30.5 cm butterfly catheter (SurFlo Plastics & Engineering Inc, Warren, USA) was inserted through the transparent dressing perforating the injection cap and flushed with 6 ml heparinized saline (Hospira Inc, Lake Forest, USA; 2 IU Heparin/ml saline) to ensure patency.

Three hours after drug administration, transparent dressing, tape and the catheter were removed from the auricular vein. Gauze was taped over the catheter site providing pressure to prevent bleeding. Gauze was removed 1 hour later and the ear was checked for bleeding. If excess surgical glue was difficult to remove, hydrogen peroxide was applied to the ear to assist in removal. Ears were cleaned with isopropyl alcohol and evaluated for inflammation or hematoma formation.

Prior to the study commencement, 2 sows were used to refine catheter placement and access technique. Catheters were placed as described above with the exception that sows were not physically restrained. For these efforts, sows were controlled by providing feed in a
small pan to limit excessive movement. Success rate for catheter placement was low (25%) resulting in multiple hematoma formation and failed patency. Therefore manual restraint with a snare was required.

The original goal for this project was to develop a technique for indwelling catheter placement without the use of physical restraint. However, during our pilot study using only 2 sows, catheter placement without restraint was difficult to perform and had a low success rate. Based on these difficulties, the 6 sows on-trial were restrained using a pig snare for catheter placement and drug administration. Although restraint may have caused temporary stress, success rate improved dramatically (92%); hematoma formation was rare and overall handling and interaction time per sow decreased. Placement time was also less compared to previous published techniques using physical restraint. Although the use of a sedative to restrain the sow may have caused less temporary stress compared to the snare, the sedative drug’s unknown systemic effects on clearance, absorption time and volume of distribution made its use problematic in the context of a pharmacokinetic study.

Intravenous catheters were successfully placed in both ears of 6 on-trial restrained sows for a total of 12 successful auricular vein catheter placements. Catheters were placed in the auricular vein within 3 min 20 sec ± 8 sec (mean ± SE per catheter) from the time sow restraint began. Catheter patency was evaluated with a sterile saline flush and all catheters with one exception remained patent for drug administration. Each sow was administrated 20-22 ml meloxicam over 90 seconds. Total drug volume was successfully administered to each sow while restrained using a pig snare.

No permanent physical alterations were noted with this technique. The most prominent change to the ear was temporary pinna discoloration at cream application site and
mild epithelial bruising around catheter placement site. Full recovery of ear occurred one week after placement.

The catheter that failed to maintain patency was removed by a neighboring sow through pen bars. After this incident, sows were moved before the trial start into housing that minimized sow to sow contact until after the drug was administered via the catheter. Although catheters could hypothetically be removed by physical force (rubbing, scratching), no such behavior was noted during the trial. No sows appeared to react to catheter presence and no sows attempted to remove their own catheter.

We found the described catheter placement technique to be an effective and quick way to administer drug intravenously. This technique provides secure intravenous access and can be utilized for studies or treatments requiring intravenous drug administration. Blood was not collected from the auricular vein catheter and further evaluation of the efficacy of this type of catheter for blood collection is needed. The application of this method contributes to the ‘refinement’ of experimental procedures, according to the principle of ‘3Rs’, by reducing unnecessary sow stress and pain associated with prolonged restraint and catheter placement.

**Conflict of interest:** Actual or potential conflicts of interest do not exist.
Literature Cited


Figure 3.1: Topical anesthetic cream (15g; EMLA cream; 2.5% lidocaine and 2.5% prilocaine) was applied to each sows’ ear pinna covering the entire length of the ear from base to tip.
Figure 3.2: Final layer of transparent dressing is placed of the catheter hub and injection port and prevented displacement of the catheter from the vein.
CHAPTER 4

Effects of yohimbine, an alpha 2-antagonistic reversal agent, on physiological recovery parameters of anesthetized sows

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4.1 Summary

**Objective:** To evaluate the efficacy of yohimbine as an anesthetic reversal agent for sows anesthetized with a combination of xylazine, ketamine, and telazol.

**Materials and methods:** Anesthesia was induced with xylazine, ketamine, and telazol in a single syringe, injected intramuscularly (IM). Following a 20-minute stabilization period, palpebral reflex was evaluated, and if absent, sows were injected IM with sterile saline (Control sows; n = 12) or yohimbine HCl (0.1 mg per kg; Yohimbine sows; n = 12). Data collected included insensibility measures (palpebral reflex, jaw tone, nose prick, alertness to human approach test, body posture) and physiologic measurements (heart rate, rectal temperature, respiratory rate, oxyhemoglobin saturation). Data was collected every 10 minutes until complete sensibility was attained.

**Results:** Yohimbine sows recovered from anesthesia 162 minutes earlier than Controls ($P < .01$). For all insensibility measures, Yohimbine sows regained a normal response more quickly than Controls ($P < .001$). In addition, Yohimbine sows maintained greater heart rate ($P < .05$) and rectal temperature ($P < .001$) between onset of anesthesia (the time anesthetic agents were injected) to completion of the trial (when sow attained complete return to sensibility). Respiratory capability (respiratory rate and oxyhemoglobin saturation) was maintained within normal physiological ranges throughout anesthesia, confirming that it was not compromised under this anesthetic protocol.

**Implications:** Yohimbine is an effective reversal agent in sows anesthetized with xylazine, ketamine, and telazol administered simultaneously. This agent can be used by veterinarians to ensure a quicker recovery from anesthesia with minimal complications.
**Keywords:** swine, anesthesia, yohimbine, reversal agent

4.2 Introduction

Sows represent a unique population in the breeding herd, as physiological compromise (disease) and age can make anesthesia induction risky. According to the American Society of Anesthesiologists,\(^1\) age (geriatric), weight,\(^2\) disease status, and anatomical variation\(^3\) contribute to a heightened anesthetic risk and can lead to prolonged recovery times and increase post-anesthetic complications.\(^4\) In addition, studies evaluating natural on-farm sow deaths confirmed cardiovascular failure as one of the top three causes of mortality.\(^5\)\(^-\)\(^7\) This increases sow anesthetic risk, as the cardiovascular system is a key system altered during anesthesia. Furthermore, direct observations in our laboratory revealed that anesthetized sows (anesthesia induced with xylazine, ketamine, and telazol injected simultaneously in a single syringe) exhibited prolonged recoveries, on average between 5 and 10 hours. Acknowledging inherent sow risk factors, it is critical to design a protocol that minimizes risks associated with anesthesia.

Swine may be anesthetized in order to complete routine production procedures or surgical operations.\(^8\) Laboratory and on-farm anesthesia examples include, but are not limited to, coronary angiography, ischemia and reperfusion models for human disease,\(^9\)\(^,\)\(^10\) tracheal culture and bronchoalveolar lavage for respiratory disease diagnosis,\(^11\)\(^,\)\(^12\) and assistance with aggressive animals when performing reproductive procedures\(^13\) or euthanasia.\(^14\) A major disadvantage with anesthesia use in swine is the unpredictable recovery time, which results in increased post-anesthetic risks and costs attributed to employee time spent monitoring the animal. Although utilization of on-farm anesthesia on a daily basis is not common, anesthesia combined with an effective reversal agent can provide an additional diagnostic tool for
veterinarians. Sows are difficult to restrain due to their large size and can be easily stressed by physical restraint and handling. Anesthetic administration routes are limited in adult swine due to inaccessible superficial veins and thick subcutaneous fat layers. In addition, their responses and reactions to anesthesia can vary, as noted by resistant responses to certain sedative drug combinations. Xylazine, ketamine, and telazol are a common combination of drugs used for anesthesia of swine both on farm and under research conditions. The choice to use all three drugs in combination in our laboratory was based on a toxicologically wide margin of safety in swine and prolonged analgesic properties attained using all three drugs as compared to xylazine and ketamine administered together and telazol administered alone. Yohimbine is an alpha-2 adrenoreceptor antagonist that has been reported to be effective in reversing xylazine effects in nursery-age swine and other food-producing animals. In cats, yohimbine acts as a stimulant, shortening both ketamine-induced anesthesia and the effects of xylazine. Providing a quicker recovery may decrease post-anesthetic complications in sows, providing a more efficient, cost-saving method for anesthesia to be applied on farms. Although yohimbine has proven effective in nursery-age swine, inherent anesthetic risk of sows makes it inappropriate to infer that sows will respond in the same manner as younger, healthier swine. The objective of this study was to determine yohimbine efficacy as an anesthetic reversal agent in sows anesthetized with xylazine, ketamine, and telazol injected simultaneously in a single syringe.
4.3 Materials and Methods

The protocol for this study was approved by the Iowa State University Animal Care and Use Committee.

4.3.1 Animals and housing

Twelve multiparous, non-pregnant, crossbred commercial maternal-line cull sows were used (mean bodyweight ± standard deviation = 233.6 ± 18.7 kg). All sows received a physical examination, which included lung and heart auscultation, rectal temperature, and reproductive tract ultrasonography. These sows were handled daily for research projects and were familiar with their environment and caretakers. The laboratory was located at Iowa State University, College of Veterinary Medicine, Ames, Iowa. To avoid confounding injury due to aggression, each sow was housed in an individual pen; however, sows could see, smell, hear, and have nose-to-nose contact with other cohorts. Sows were provided ad libitum access to water via one nipple drinker per pen (Model 65; Trojan Specialty Products, Dodge City, Kansas). Sows were fed twice daily on a single feed bunk with a diet designed to meet or exceed nutrient requirements for gestating sows.

4.3.2 Treatments

Sows were blocked by body weight and randomly allocated using a random number generator to two treatments. Treatments were as follows: Yohimbine, yohimbine HCl (0.1 mg per kg) administered intramuscularly (IM) into the neck muscle (n = 12); Control, sterile saline administered IM at an equivalent volume (n = 12).
4.3.3 Experimental design

All sows were acclimated to the laboratory environment for 7 days prior to study commencement. All 12 sows received both treatments in a cross-over design with a 10-day washout period. This experimental design provided robust control of intra- and inter-animal variation and reduced the animal number required to find significant differences. Investigators were blinded to treatments to reduce the possibility of observer bias.

4.3.4 Anesthesia protocol

Sows were fasted overnight (16 hours), but were provided ad libitum access to water until 1 hour prior to anesthesia administration. Sows were restrained by a common pig snare in their home pen and anesthetized. The injection combined the following anesthetic agents at the doses indicated: xylazine (4.4 mg per kg; Anased, Lloyd Laboratories, Shenandoah, Iowa); ketamine HCl (2.2 mg per kg; Ketaset, Wyeth, Madison, New Jersey); and tiletamine HCl and zolazepam HCl in combination (4.4 mg per kg; Telazol, Wyeth). Anesthesia onset began once anesthetic agents were injected. Ten minutes after anesthesia onset, sows were placed in lateral recumbency and postural adjustments were made if involuntary movements resulted in compromised respiratory or circulatory capability. Twenty minutes after anesthesia onset, sows were evaluated for a palpebral reflex. This was determined by placing a finger on the medial canthus of the accessible eye and gently running the finger along the eyelashes. The presence or absence of the palpebral reflex was determined by attempting to elicit a blink response with three successive attempts. If a palpebral reflex is absent, one of the two treatments was administered. If a palpebral reflex was present, the sow was monitored every 10 minutes until the palpebral reflex was absent, and treatment was then
administered. To prevent confounding effects of external stimuli such as human traffic and talking within and between the pens on anesthesia induction, these distractions were minimized. During anesthesia and recovery, electrical heating pads and blankets were utilized when the sow’s rectal temperature dropped below 36°C.

4.3.5 Measures

**Insensibility measures.** Insensibility measures collected included the human approach test, body posture, palpebral reflex, jaw tone, and nose prick test. Each measure was scored on a 0 to 2 scale, with score 0 representing a normal alert response, score 1 representing diminished response from normal, and score 2 representing no response (Table 4.1). Insensibility was measured immediately before anesthesia administration (Baseline), and every 10 minutes after anesthesia onset until sows reached a 0 score (Recovery). A sow was considered to have completed the trial once a 0 score for all measures was attained.

**Physiologic measures.** Physiologic measures were collected at the same time points as insensibility measures and included heart rate by auscultation (WLS5605-Cl Stethoscope; United Inc, India), rectal temperature (Jumbo Display Digital Thermometer; Graham Field Health Products Inc, Atlanta, Georgia), respiratory rate, and oxyhemoglobin saturation (SpO$_2$) collected from a pulse oximeter probe placed on the sow’s lip or tongue (OxiMax N-65 Quick Guide; NellCor, Boulder, Colorado).

4.3.6 Statistical analysis

Data were analyzed using SAS software version 9.3 (SAS Institute Inc, Cary, North Carolina). Data were analyzed for normality by plotting a predicted residual plot and a
quantile-quantile plot. Insensibility and physiologic measures were analyzed using a mixed model procedure utilizing polynomial regression in SAS. The insensibility statistical models included the fixed effect of treatment (Control versus Yohimbine), day (2 days), day-by-treatment interaction, and weight as a linear covariate. Sow was included as a random effect. The physiologic statistical models included the fixed effect of treatment (Control versus Yohimbine), day (2 days), day-by-treatment interaction, weight, and time (minutes) categories. Time categories were created starting at anesthesia onset. Sixty-minute time-point interval blocks were included. All interactions were included in the model. A $P$ value of $<.05$ was considered to be significant for the MIXED analysis of variance and when separating means. Fixed effect least squares (LS) means were separated using the PDIF option in SAS and data were expressed as LS means (95% confidence intervals) and mean ($\pm$ SD).

4.4 Results

4.4.1 Insensibility measures

No difference was observed between treatments (Control versus Yohimbine) for time to administration, with all sows receiving either Yohimbine or saline on average between 23.5 minutes (95% CI, 17.6-29.4) and 27.0 minutes (95% CI, 21.4-32.6) post anesthesia administration. Yohimbine sows recovered from anesthesia 162 minutes earlier than Control sows (290 minutes, 95% CI, 195.4-384.6 versus 452 minutes, 95% CI, 364.2-559.7; $P < .01$). Time to return to sensibility for all measures (score 0) was shorter for Yohimbine sows than for Control sows ($P < .01$; Figure 4.1).
4.4.2 Physiologic measures

**Heart rate.** There was a treatment-by-time interaction, with Yohimbine sows demonstrating a greater heart rate over the anesthesia course than Control sows ($P < .05$; Figure 4.2). Within 2 hours post anesthesia administration, heart rate did not differ between treatments. During the following 6 hours, Yohimbine sows maintained greater heart rates ($P < .01$). When a 0 score was attained, Yohimbine sows’ mean heart rate, 69.9 beats per minute (95% CI, 63.0-76.8), was greater than Control sows’ mean, 49.1 beats per minute (95% CI, 42.7-55.5).

**Rectal temperature.** The interaction between treatment and time ($P < .001$) demonstrated greater rectal temperatures throughout anesthesia for Yohimbine sows than for Control sows (Figure 4.3). Within 2 hours post anesthesia administration, rectal temperature did not differ between treatments. During the following 7 hours, Yohimbine sows maintained greater rectal temperatures ($P < .001$). When a 0 score was attained, mean rectal temperature was greater in Yohimbine sows (34.8°C; 95% CI, 34.2-35.3) than in Control sows (32.2°C; 95% CI, 31.7-32.8).

**Respiratory rate and SpO2.** Treatment, time, treatment-by-time interaction, and body weight had effects on respiratory rate between Yohimbine sows (21.0 breaths per minute; 95% CI, 18.6-23.3) and Control sows (21.9 breaths per minute; 95% CI, 20.0-23.7); $P < .01$ (treatment); $P < .001$ (time); $P < .001$ (treatment-by-time interaction); $P < .001$ (weight) (Figure 4.4). For the first 2 hours following anesthesia induction, Control sows had greater respiratory rates than did Yohimbine sows. For the remaining 7 hours, Control and Yohimbine sows had similar respiratory rates. There was no difference in respiratory rate between treatments once sows attained a 0 score.
There were no treatment differences for SpO$_2$, but for both Yohimbine and Control sows, SpO$_2$ percentage increased with time under anesthesia (Figure 4.5).

4.5 Discussion

The objective of this study was to determine yohimbine’s efficacy as an anesthetic reversal agent in sows. On the basis of previous work conducted on nursery pigs, $^{21,27,28}$ we expected treatment with yohimbine to decrease overall recovery time, decrease latency time to regain sensibility, and maintain physiologic parameters closer to normal homeostatic levels than treatment with saline.

When evaluating insensibility measures, yohimbine administration resulted in sows recovering sooner than Control sows, with Control sows taking over 7 hours to regain full sensibility. The results from this study are in agreement with previously published findings that yohimbine reduces overall time under anesthesia, but the anesthesia duration for mature sows was longer than for nursery-age swine. Kim and colleagues$^{21}$ reported that pigs receiving yohimbine 20 minutes after anesthesia induction regained sternal recumbency (52.2 ± 8.9 versus 76.2 ± 20.6 minutes) and the ability to stand (77.0 ± 9.8 versus 98.7 ± 15.8 minutes) and walk (81.3 ± 11.3 versus 110.8 ± 18.6 minutes) faster than did pigs that did not receive yohimbine. Overall, recovery from anesthesia was three to five times longer in sows receiving the same anesthetic protocol at the same dose without the administration of yohimbine. This may be explained by the difference in body composition and repartitioning of drug in mature animals. However, further studies should be conducted. Regardless of prolonged anesthesia duration, yohimbine effectively reduced the time under anesthesia and in turn decreased the risk of post-anesthetic complications.$^{24,29}$
During anesthesia recovery, Yohimbine sows maintained physiologic parameters more closely resembling a healthy sow at rest; however, Control sows had depressed physiological measures. In all species undergoing anesthesia, it is expected that the animals’ physiologic state (i.e., heart rate, temperature, respiratory rate, and oxygen exchange) will be altered from normal homeostatic levels. This is due to effects on receptors in the heart, lungs, and peripheral veins by selected anesthetic agents such as xylazine. Antagonistic agents like yohimbine may alter the impact that anesthetic agents like xylazine have on the sow’s physiologic status.

Yohimbine sows had faster heart rates compared to Control sows, with Yohimbine sows maintaining heart rates within a normal range throughout anesthesia (60 to 90 beats per minute). Bradycardia is a common side effect noted in animals anesthetized with xylazine. Xylazine is an alpha-2-adrenergic agonist that generates systemic vascular resistance by acting on the alpha-2 receptors located on peripheral veins. This in turn produces hypertension and a short transient tachycardia, followed by a “compensatory baroreceptor-mediated reflex” resulting in bradycardia and decreased cardiac output. This physiologic event can lead to inadequate oxygen-rich blood perfusion to vital organs and compromise basal metabolic requirements. This may cause complications during recovery and may lead to permanent organ-system damage. The Yohimbine treatment effectively antagonized xylazine effects on the sow cardiovascular system, as was demonstrated in Yohimbine sows that did not become bradycardic during the anesthesia procedure and maintained a normal heart rate once a 0 insensibility score was attained.

During the anesthesia course, Yohimbine and Control sows reached subnormal body temperatures, but Yohimbine sows maintained higher overall temperatures within the last
anesthetic hours. Rectal temperature in both treatment groups dropped approximately 1°C per hour within the first 2 hours after anesthesia. Between the third and fourth anesthesia hours, temperature dropped only 0.3°C per hour for Yohimbine sows, whereas the temperature from Control sows dropped 0.7°C per hour. Neither treatment group attained normal rectal temperature (38.0°C to 39.0°C) when the sows reached a 0 insensibility score. However, Kim and colleagues reported that nursery pigs had lower rectal temperatures when yohimbine was administered compared to control pigs. In their study, from the time pigs were anesthetized until recovery, rectal temperature dropped in total by 1°C. In the present study, rectal temperatures of both Yohimbine and Control sows dropped approximately 1°C within the first hour. However, temperature continued to drop in both treatment groups due to prolonged recovery times, which differs from previous work.

Hypothermia in anesthetized companion animals is often overlooked, but can have severe consequences. To date, to the authors’ knowledge, there are no published studies evaluating hypothermia in swine. In companion animals, hypothermia has been defined as core body temperature dropping below 36°C, and this was used in our study as a critical control point for thermal supplementation. Electrical heating pads and blankets were utilized when the sow’s rectal temperature dropped below 36°C. Yohimbine sows maintained greater rectal temperatures and exhibited a slower rate of decline in rectal temperature over time. This is an advantage to the animal, as consequences of heat loss under anesthesia increase the risk of impaired immune system function, impaired blood coagulation, cardiovascular depression, acidemia, and increased morbidity and mortality.

Baseline respiratory rate was different between treatment groups, with Control sows having greater mean respiratory rate than Yohimbine sows. It is unclear why Control sows had a
greater respiratory rate than Yohimbine sows and why both groups had baseline respiratory rates greater than reported normal respiratory rates in adult swine (10 to 20 breaths per minutes). Housing conditions were regulated to maintain a constant ambient temperature throughout study and is unlikely the source for elevated respiratory rate. One possible explanation for increased baseline respiratory rate may be behavioral excitability. Sows were not provided with a morning feed ration in order to decrease risk of aspiration or regurgitation while under anesthesia. It was noted that the sows exhibited increased vocalization and activity on trial day, compared to their typical behavior. The increased vocalization and activity may have resulted from the sows not being fed the trial morning, which in turn elevated their respiratory rates. In addition, differences in baseline respiratory rate between treatments (Yohimbine versus Control) may have also been influenced by the sows’ respiratory capability. Respiratory capability is influenced by lung development, maturation, and structural status. Previous sow history was not known, and therefore any previous respiratory disease or compromised lung function may have influenced respiration under anesthesia. It was noted that body weight had an effect on respiratory rate. However, because of the experimental design, weight or behavioral excitability would be unlikely to influence respiratory rate, as the same individual received both treatments.

Although Control sows did have greater mean respiratory rate during the first 2 hours, it should be noted that during the remaining time under anesthesia respiratory rate did not differ between treatment groups. A common side effect observed with xylazine administration includes respiratory depression. Kim et al reported that Yohimbine only reversed respiratory depression in younger pigs 5 minutes after administration. The results of our study and those of Kim et al suggest that either yohimbine does not play a substantial role
in controlling or regulating swine respiration under anesthesia, or the measurement methods (evaluating abdominal movements for 15 seconds) may not have been sufficiently sensitive to detect observed respiratory changes.

Treatment had no effect on sows’ SpO₂ concentrations in the present study, but all sows demonstrated a gradual increase in SpO₂ as time under anesthesia increased. No sows were provided with supplemental oxygen, therefore increased SpO₂ over time must be attributed to improved oxygen exchange by the sow. Gianotti and colleagues³⁸ determined normal SpO₂ levels in swine aged 60 to 90 days to be 96% (± 2.10%). On the basis of data from this study, SpO₂ averages were within normal levels and did not fall below 90% SpO₂. These data suggest that this anesthetic protocol did not compromise sow respiratory or oxygen exchange capability. However, caution should be taken when evaluating these results, as methods chosen for measurement may not be sensitive enough. In comparing pulse oximetry accuracy to capnography in dogs, cats, horses, and white tail deer, it has been demonstrated that the accuracy and consistency of pulse oximetry varies widely and does not provide readings as accurate as arterial blood gas analysis.³⁹,⁴⁰ Although capnography may be a more accurate method than pulse oximetry, additional expense and technical skills make it difficult to apply on farm and it was not chosen for this study. In addition, pulse oximetry results were difficult to collect once sows began regaining consciousness, as the probe needed to be clamped onto either a tongue or lip. Difficulty in placing the probe resulted in less data collection for Yohimbine sows.

In conclusion, on the basis of insensibility and physiologic measures, yohimbine was an effective reversal agent in sows anesthetized with xylazine, ketamine, and telazol. Overall anesthetic recovery time was shorter, and sows in an anesthetized state were able to maintain
physiological parameters closer to normal homeostatic values. However, the effects of yohimbine on physical and behavioral recovery remain unknown. Video data analysis may provide additional information regarding the degree of post-anesthesia ataxia or thrashing with and without yohimbine. Yohimbine could be used by veterinarians to provide a desired analgesic and anesthetic effect while surgical procedures are performed, with a shorter recovery time that may decrease physiologic complications associated with anesthesia.

4.6 Implications

- Yohimbine is an effective reversal agent in sows anesthetized with xylazine, ketamine, and telazol administered simultaneously in a single syringe.

- Sows treated with xylazine, ketamine and telazol recover sooner when yohimbine is administered as a reversal agent, and physiological parameters return to normal homeostatic ranges more quickly.

- Recovery time after administration of xylazine, ketamine and telazol may be longer in sows than in nursery pigs, and anesthesia protocols may need to be adjusted for mature sows.
Literature cited


* Non-refereed reference
**Table 4.1:** Criteria and scoring system* used to assess insensibility throughout anesthesia† and recovery in sows treated with yohimbine or saline

<table>
<thead>
<tr>
<th>Measure</th>
<th>Definition</th>
<th>Score</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palpebral reflex</td>
<td>Eye reaction to physical examination</td>
<td>2</td>
<td>No blink response when stimulated 3 times</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Blink reflex stimulated by ≤ 2 touches</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>Normal blink response with 1 touch</td>
</tr>
<tr>
<td>Jaw tone</td>
<td>Jaw manipulation</td>
<td>2</td>
<td>Flaccid jaw tone: observer able to open jaw with no resistance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Resistant jaw tone: observer able to open jaw, slight muscular resistance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>Normal jaw tone: sow does not allow jaw to be manipulated</td>
</tr>
<tr>
<td>Nose prick</td>
<td>Needle tip prick</td>
<td>2</td>
<td>No response: no movement associated with needle tip prick</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Diminished response: some movement associated with needle tip prick</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>Normal response: movement associated with needle tip prick, sow is evasive</td>
</tr>
<tr>
<td>Human approach test</td>
<td>Response of sow to human</td>
<td>2</td>
<td>No response: no orientation towards stimulus.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Diminished response: uncoordinated eye, ear, or head movement in response to stimulus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>Normal response: oriented eye, ear, or head movement toward and in response to stimulus</td>
</tr>
<tr>
<td>Sow body posture</td>
<td>Body posture</td>
<td>2</td>
<td>Lateral recumbency with no movement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>Lateral recumbency with spontaneous movement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>Standing on all four limbs</td>
</tr>
</tbody>
</table>

* Adapted from Kim et al and Heinonen et al.
Table 4.1 Continued

† Sows were anesthetized with xylazine (4.4 mg/kg), ketamine HCl (2.2 mg/kg), and telazol (4.4 mg/kg) administered simultaneously in a single intramuscular injection. Treatments administered following anesthesia onset were yohimbine (alpha-2 adrenoreceptor antagonist) administered intramuscularly at 0.1 mg/kg (Yohimbine sows) or an equivalent volume of saline (Control sows). Insensibility measures were assessed every 10 minutes from injection of anesthetic agents until sows reached a 0 score.
Figure 4.1: Latency to regain sensibility least squares means (± standard error) (minutes) for anesthetized sows administered yohimbine or saline to mitigate recovery effects ($P < .01$). Sows were anesthetized and treatments administered after anesthesia onset as described in Table 4.1. Statistical analysis was performed using a mixed model. Insensitivity measures included palpebral reflex, nose prick test, jaw tone, human approach test, and body posture.
Figure 4.2: Heart rate least squares means by time for anesthetized sows administered yohimbine. Sows were anesthetized and treatments administered after anesthesia onset as described in Table 4.1. Statistical analysis was performed using a mixed model. Best fit lines for each treatment were fitted using a polynomial function. Black vertical line represents time block when heart rate differed between Yohimbine and Control sows.
Figure 4.3: Rectal temperature least squares means by time for anesthetized sows administered Yohimbine or saline to mitigate recovery effects ($P < .001$). Sows were anesthetized and treatments administered after anesthesia onset as described in Table 4.1. Statistical analysis was performed using a mixed model. Best fit lines for each treatment were fitted using a polynomial function. Black vertical line represents time block when heart rate differed between Yohimbine and Control sows.
**Figure 4.4**: Respiratory rates least squares means by time for anesthetized sows administered yohimbine or saline to mitigate recovery effects* ($P < .01$). Sows were anesthetized and treatments administered after anesthesia onset as described in Table 4.1. Statistical analysis was performed using a mixed model. Best fit lines for each treatment were fitted using a polynomial function. Black vertical line represents time block when heart rate differed between Yohimbine and Control sows.
Figure 4.5: Oxyhemoglobin saturation least squares means by time for anesthetized sows administered yohimbine or saline to mitigate recovery effects ($P > .05$). Sows were anesthetized and treatments administered after anesthesia onset as described in Table 4.1. Statistical analysis was performed using a mixed model. Best fit lines for each treatment were fitted using a polynomial function. Data points ended earlier in Yohimbine treated sows due to difficulty in continual probe placement during recovery.
CHAPTER 5

CASE STUDY: TREATMENT OF ORAL AND LOCOMOTORY STEREOTYPIC BEHAVIORS IN A MATURE SOW

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5.1 Abstract

A 32-month old female 225-kg non-pregnant cross-bred Newsham sow presented a 6-week history of stereotypic behaviors when housed in a laboratory research facility. A behavioral examination over 12 daylight hours revealed three main stereotypic motor patterns: 1. oral-nasal gate manipulation defined as placement of the snout between the bars of the pen gate with repetitive, forceful up and down movement. 2. Head weaving defined as repetitive lateral head and snout movement towards the pen gates while rocking back and forth on her forequarters and 3. Body weaving defined as repetitive shifting of body weight from one side. The sow performed the oral-nasal gate manipulation, head and body weaving 4.0%, 12.4% and 6.8% of her total baseline time budget respectively. The presumptive diagnosis was oral-nasal and locomotory stereotypies. Three treatments were employed to mitigate the duration and frequency of these stereotypic behaviors. Treatment One: Change social stimuli by providing visual and nose-to-nose contact with different neighboring sows, Treatment Two: Change foraging substrates by providing peat moss as a rooting substrate, and Treatment Three: Change pen configuration by increasing space. Treatment One; The sow performed the oral-nasal gate manipulation, head and body weaving 0.9%, 15.3% and 11.3% of her total time budget. Treatment Two; The sow performed the oral-nasal gate manipulation, head and body weaving 0.5%, 28.0% and 15.5% of her total time budget. Treatment Three; The sow performed the oral-nasal gate manipulation, head and body weaving 0%, 0.4% and 0.1% of her total time budget. This study is one of the first reports to evaluate treatment of established stereotypies in a mature sow. Results suggests the promise of environmental enrichment as an effective treatment strategy. Further research is needed to
evaluate the persistence of these behavioral changes and relative importance of different environmental manipulations provided.

**Keywords:** Swine, stereotypies, environmental enrichment, behavior modification

### 5.2 Case presentation

A 32 month-old 225-kg (495-lb) non-pregnant cross-bred Newsham sow presented with abnormal behavior two days after arrival to a laboratory research facility at Iowa State University. The main complaint from the caretaker was abnormal head and body weaving directed toward the front or side metal gates of the pen. The sow was housed individually in a pen that measured 3.7 m length x 1.4 m width x 1.2 m height. A rubber mat (3.5 meter length x 1.3 m width) was provided for comfort but no other bedding material was provided. Metal gates were affixed at the end of each home pen and the sow was able to see outside the front and sides of the pen. Sows were housed in the adjacent right and left pens but there was no sow housed in the pen immediately across the 0.61-meter alley. The sow had *ad libitum* access to water via one nipple drinker and was hand-fed a custom mixed diet composed of corn, soybean meal and soy hulls. A daily total feed ration of 2.7 kg feed was split between morning and afternoon feedings. Matrix (Altrenogest; 6.8 ml; 15 mg) was added to one kg of feed daily to prevent estrus initiation.
5.3 History

Source farm history

According to the original source farm, no abnormal behaviors were noted in the sow’s history or records. On farm, the sow was housed individually in a 0.61 meter width x 2.1 meter length gestation stall. She was limit-fed a commercial diet composed of corn and soybean meal once a day. The sows’ reproductive history is as follows: 116 average days of gestation, 143-day farrowing interval, 14.6 average piglet number born alive and 24.8 piglets/litter/year. The sow was provided no access to enrichment material (straw, sawdust etc.) while on farm. One week prior to transport to the laboratory the sow was group housed in a 6.1 meter length X 2.4 meter width concrete pen with 11 other sows that were transported to the laboratory facility. Transportation time was approximately 50 minutes and no adverse events were noted during transportation.

Laboratory history

Two cohorts of 12 sows were transported from a local commercial sow unit and enrolled in a clinical lameness trial for seven weeks. Selection criteria for trial enrollment included multiparous, non-pregnant and non-lame sows with no clinical health abnormalities. Upon arrival, all sows underwent a seven day program where they were acclimated to laboratory facilities and equipment. This acclimation included handling, moving sows individually through the laboratory and restraint. All procedures associated with handling and restraint involved positive reinforcement through food rewards. Amongst all 24 sows enrolled in the study, only this sow demonstrated stereotypic behaviors whilst housed in her home pen.
5.4 Physical evaluation

An initial physical examination was performed on the sow upon arrival to the laboratory. The physical examination was unremarkable and included lung and heart auscultation, rectal temperature and reproductive tract ultrasonography. An 8 x 7 cm triangular alopecic area located on the dorsal aspect of the neck and a 3 cm soft tissue callus on the dorsal aspect of the nasal bone were noted during the physical. The sow had a body condition score three (defined as “ideal” on a one to five scale (PQA, 2013).

5.5 Behavioral evaluation

Approximately one week after arrival, a veterinarian and behavior consultant evaluated the sow’s behavior. In order to define abnormal behaviors video recording of the sow was conducted over a 12-hour period (6:00-1800) utilizing continuous behavior sampling methods (Altmann, 1974). Behaviors were evaluated using two 12 V color Close Circuit Television (CCTV) Panasonic cameras (Model WV-CP484, Matsushita Co. Ltd.), positioned centrally (2.9 m from the front of the pen) using an elbow bracket at a height of 2.8 m from the floor. Video was captured digitally utilizing a Noldus portable lab (Noldus Information Technology, Wageningen, NL). The cameras were fed into a multiplexer, which then allowed the image to be recorded onto a PC using HandiAvi at 30 frames per second. A computer screen was used to view the DVR output to ensure picture clarity and camera positioning prior to each behavioral recording. Behaviors of interest were identified and defined (Table 5.1). The duration of each behavior was quantified based on percent of time the behavior was conducted over the 12 hour video period and was considered the sow’s
baseline time budget (Figure 5.1). The sow’s abnormal behavior was categorized into three main behavioral motor patterns as described below.

*Oral-nasal gate manipulation*

The sow placed her mouth and/or snout in between the opening of the pen gates and forcefully pushed the gate up and down repetitively. During the baseline 12 hour video evaluation the sow performed this behavior for 4.0% of her total time budget, spending on average three seconds manipulating the gate per bout (bout defined as starting with visual movement of gate with head contact and ending when head is no longer in contact with fence), with a total of 607 bouts of gate manipulation over the 12 hour period.

*Head weaving*

The sow positioned her head 0.61 m from the ground and conducted repetitive lateral head and snout movement towards the pen gates while rocking back and forth on her forequarters. The sow did not perform oral manipulation such as bar chewing or object licking but she often touched the same bar with her snout or mouth (eighth gate bar from the floor). Head weaving bouts occurred in two pairs (pair defined as head movement to one side of the pen and then returned to original position). During the baseline 12 hour video evaluation the sow performed this behavior 12.4% of her time budget, spending on average four seconds per bout (bout defined as starting when head move from the resting position to either direction or pen and ending when head is stationary for more than 2 seconds) with a total of 1,154 bouts performed over the 12 hour period.
**Body weaving**

The sow repetitively shifted her body weight from one side to the other. Frequently including lifting the front foot off the ground and crossing over the opposite front foot. During the baseline 12 hour video evaluation the sow performed this behavior 6.8% of her time budget, spending on average two seconds per bout with a total of 1,104 bouts (bout defined as initiation of foot movement and body shifting to either direction of the pen and ending with body and feet remaining stationary for at least 1 second) performed during the 12 hour period.

5.6 Diagnosis

Based on behavioral evaluation our main diagnoses were locomotory and oral stereotypies utilizing the definition of stereotypy as a “*repetitive, unvarying, and apparently functionless behavior patterns*” (Mason, 1991). The locomotory stereotypy was expressed as repetitive shifting of body weight with intermittent lateral head movement. The oral stereotypy included oral-nasal manipulation of the gate. This was considered an established stereotypy as it was consistent with what was observed upon arrival to the laboratory and throughout the 6 week trial.

5.7 Treatment

The treatment plan was designed to address the oral and locomotor stereotypies. The following three treatments were designed to 1. Change social stimuli by providing visual and nose-to-nose contact with different neighboring sows 2. Change foraging substrates by providing peat moss as a rooting substrate and 3. Change pen configuration by increasing
space. All treatments were observed for a 12 hour period followed by a 24 hour recovery day where the sow was placed back into her original home pen.

**Treatment one: Change social stimuli.** We hypothesized that changing social contacts would decrease the frequency of head and body weaving behavior. We moved the sow to an identical pen that provided social contact visually with the sow across the alley and nose to nose contact with sows on both sides of the new pen.

**Treatment two: Change foraging substrates.** We hypothesized that peat moss would provide a robust stimulus to increase rooting and foraging behavior and decrease the frequency of the oral-nasal gate manipulation behavior. A rubber bowl filled with peat moss was placed into the home pen at the base of the feed area. This location was chosen because it was the farthest from the pen gates where the stereotypic behavior was performed.

**Treatment three: Change pen configuration.** We hypothesized that increased physical space and access to an area outside of her original home pen would increase exploratory opportunities and decrease frequency of head and body weaving. The home pen gates were opened up to provide access to the concrete alleyway and to an additional identical pen. This increased total pen dimensions to 8 m length x 1.4 m width x 1.2 m height.

**5.8 Follow-up**

**Treatment one: Change social stimuli:** Within the first hour of treatment one, the sow spent 46.9% of her time (Figure 5.2) exhibiting over 730 bouts of stereotypic behaviors (Figure 5.3). The sow performed the oral-nasal gate manipulation, head and body weaving 0.9%, 15.3% and 11.3% of her total time budget. After treatment administration the sow was placed back into her original home pen for a 24 hour recovery period (Recovery1). Stereotypic behavior did not return to baseline levels during Recovery1 day and both head
weaving and body weaving increased to 28.7 and 14.7% of total time budget. Oral-nasal gate manipulation remained lower than baseline levels at 0.1% of total time budget. The sow spent 87.9% of the first hour (Figure 5.2) back in her home pen exhibiting over 1200 stereotypic bouts (Figure 5.3).

**Treatment two: Change foraging substrate:** Overall, peat moss was manipulated for only 0.1% of the total daily budget. Within the first hour of the treatment being applied, the sow spent 77% of her time (Figure 5.2) exhibiting 975 stereotypic bouts (Figure 5.3). The sow performed the oral-nasal gate manipulation, head and body weaving 0.5%, 28.0% and 15.5% of her total time budget (Figure 5.1). After treatment administration peat moss was removed and the sow remained in her home pen for a 24 hour recovery period (Recovery2) while in her home pen. During this Recovery2 time, oral-nasal stereotypies did not change (0.5%) but head and body weaving behaviors decreased to levels more similar to baseline data (Head weaving: 10.4%; Body weaving: 3.1%; Figure 5.1). The sow also exhibited similar duration and frequency of stereotypic behaviors within the first hour compared to baseline day (Figure 5.2; 5.3).

**Treatment three: Change pen configuration:** Additional access to a pen and removal of gates was used to redirect the sow’s behavior using exploratory motivation and provide access to an area outside of her home pen. The sow performed the oral-nasal gate manipulation, head and body weaving 0%, 0.4% and 0.1% of her total time budget, exhibiting all stereotypic behaviors within the first hour of treatment 3 (Figure 5.1; Figure 5.2). After treatment administration pen gates were closed and the sow remained in her home pen for a 24 hour recovery period (Recovery 3). The sow exhibited 0.6%, 22.5% and 6.8% of oral-nasal gate manipulation, head weaving and body (Figure 5.1) with 748 of these
stereotypic bouts occurring within the first hour and 81.8% of the first hour dedicated to performing these behaviors (Figure 5.2; 5.3).

As this sow was enrolled in a research trial involving extra-label drug use, it was required that all sows were euthanized at the end of the study. A necropsy was performed and gross examination of the all major organs including the brain and cranial spinal cord was examined for lesions. No gross lesions were noted during necropsy.

5.9 Discussion

Research has been conducted evaluating stereotypic behaviors in swine. This previous research provides important insights on proximate factors contributing to the causation and development of stereotypic behaviors in ungulates (Jensen, 1988; Bergeron and Gonyou, 1997; Clubb et al. 2006) as well as addressing ultimate questions on the evolutionary significance and function of these behaviors (Mayer-Holzapfel, 1968; Bergeron et al., 2006). However, there has been little empirical research conducted on evaluating treatment options for stereotypic behaviors in swine. The objective of our study was to describe the efficacy of three treatments to mitigate stereotypic behaviors performed by a sow in a controlled laboratory environment.

Locomotor stereotypies, like weaving, have been less commonly identified in ungulates, compromising only 10% of all stereotypic behaviors performed (Rushen and Mason, 2006). Only two studies could be found describing similar head and body weaving behavior as observed in our laboratory in swine (Fraser 1975; Cronin, 1985), suggesting that these types of locomotor stereotypies may be relatively uncommon in this species. Locomotor stereotypies have been associated with an individual’s desire to reach
conspecifics housed nearby (Shepherdson, 1989; Carlstead 1998), thus treatment one was designed to change the social stimuli of the sow by placing her in a pen where she had visual access to a sow in the pen across from her, and nose-to-nose contact with two different but familiar sows. Providing a change to social stimuli did not drastically increase or decrease locomotor stereotypic behaviors, suggesting that the social environment of her home pen was not triggering this behavior performance. However, change to the expression of the motor pattern was noted in that the physical space in which she performed the behavior decreased in the presence an aggressive neighboring sow. Upon return to the home pen, the sow’s locomotor stereotypies doubled, performing stereotypies 87.9% of the first hour back. This intensity did not occur within the first hour when she placed in the new pen, therefore is not likely associated with novelty. The intensity and frequency of the locomotor stereotypies performed suggests that the behavioral triggers may be directly associated with the original home pen environment.

It was noted that the oral-nasal gate manipulation decreased to 0.9% of the total time budget during treatment one, reducing by over four-fold. Based on this information alone, it may suggest that a change to social stimuli mitigated the oral-nasal stereotypy. Aggressive interactions were noted between the sow and her neighboring sow and this may have limited her ability to perform the behavior. However, upon return to her home pen during recovery day, oral-nasal gate manipulation dropped again to 0.1% of her total time budget and remained at or within this level for the remainder of the trial. This suggests that the decrease in oral-nasal gate manipulation was not caused by changes to social stimuli. The oral-nasal gate manipulation tended to precede head and body weaving and is possible that this behavior was used as a transition behavior to head weaving. The decrease in oral-nasal gate
manipulation may be the indirect effect of changes to the behavioral pattern expressed by the sow over time.

Treatment two was designed to change the foraging substrate provided to the sow to increase rooting and foraging behavior. Oral-nasal stereotypies are the most commonly identified stereotypies in confined ungulates, comprising 70% of all stereotypic behavior, with the most common stereotypies in sows including bar biting, sham chewing, tongue sucking, stone chewing and object licking (Sambrus, 1985; Whittaker et al., 1998; Horrell, 2000). Previous research estimates sows spend between 7%-55% of an 8 hour period dedicated to performing oral stereotypies on farm (Stall-housed- Broom and Potter, 1984; Von Borell and Hurnik, 1990). These reference levels are high compared to the 4.0% of a 12 hour period our sow performed the oral-nasal gate manipulation. Oral stereotypies have been associated with inadequate gut fill or thwarting of the appetitive and/or consummatory phases of rooting and foraging (Mason and Mendl, 1997) with previous studies successfully mitigating these behaviors using straw (Stewart et al., 2011), high fiber diets (Robert et al., 2002), and sugar beet pulp (Brouns et al., 1997).

The sow spent only 0.1% of her total time budget manipulating the peat moss. Prior to peat moss addition, oral-nasal gate manipulation decreased to 0.1% of the sow’s total time budget, no longer a major contributor to her overall behavioral repertoire. The decrease in oral-nasal gate manipulation prior to peat moss presentation made it difficult to determine if the peat moss would have been a successful treatment for this stereotypy. However, the lack of overall interest in the peat moss and the gradual elimination of the oral-nasal gate manipulation suggests that this stereotypy may not have been a true oral-nasal stereotypy driven by the sow’s limit-fed concentrate diet, but may have been a transitional behavior
performed between head and body weaving. Access to a foraging substrate also did not affect head or body weaving stereotypies.

Treatment three was designed to change the pen configuration by increasing the space for the sow to explore and eliminating the visual barrier of the pen gates. Locomotor stereotypies have also been associated with an animal’s motivation to escape aversive stimuli and are often performed at the barrier preventing them from escape (Mason, 1993; Mayer-Holazpfel, 1968). This coincides with what was noted in this study as the sow directed all head and body weaving toward the gates of the home pen.

Providing a change to pen configuration decreased the performance of all stereotypic behaviors to less than 0.5%. Interestingly, it was noted that when the stereotypic behaviors were performed, the sow directed the head and body weaving to the pen gates. In addition, the sow dedicated more time performing natural behaviors such as walking, standing, rooting, and lying compared to her baseline day. Identifying a single causal factor for this mitigation is impossible as multiple factors were changed when the pen doors were opened. However, some possibilities that may have contributed to the change in the sow’s behavior include removal of the pen gate as a visual trigger, access to additional physical space for exploration, changes in olfactory and visual stimuli, and ability to escape from sows housed in adjacent pens. Cooper and colleagues (2000) evaluated five different facility types for stabled horses performing weaving stereotypies. They found that providing a front and side panel open for the horse to view an adjacent stall decreased the occurrence and frequency of weaving behavior. The authors of the publication suggested this may be due to increased environmental interaction, expression of new activities, and social interaction. Similarly
weaving behavior by the stabled horse was decreased when the visual environment was modified by the use of mirrors (McAfee et al., 2002).

Oral and locomotor stereotypies were identified in an individual sow housed in a research facility over a 6 week period. The association of stereotypies with poor animal welfare encouraged our group to assess, treat and manage these behaviors being performed in the context of a laboratory setting during a trial. The greatest success occurred when the sow was provided access to additional space, mitigating all stereotypic behaviors to 0.5% of the total time budget performed by the sow. This research suggests the promise of environmental enrichment as an effective treatment strategy for locomotor stereotypies in swine. However, as this was only one case study, further research is needed to evaluate several variables involved in the mitigation of these stereotypies including the persistence of behavioral changes over time, time or day effects and the relative importance of different environmental factors provided.

Acknowledgements

We would like to thank Gracey Alexander for video data collection and Rebecca Parsons for her help with lab management and care of sows.
Literature Cited


Shepherdson, D., 1989. Stereotypic behaviour: What is it and how can it be eliminated or prevented. Ratel 16, 100-106


**Table 5.1:** Behavioral ethogram of normal and abnormal sow behaviors.

<table>
<thead>
<tr>
<th>Measures</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head weaving</td>
<td>Includes when sow is positioned at pen gates with and rocks back and forth on her forequarters while pivoting her head and snout side to side.</td>
</tr>
<tr>
<td>Body weaving</td>
<td>Includes when sow is positioned at pen gates and takes three consecutive steps to the right or left direction and includes crossing of the forelimbs.</td>
</tr>
<tr>
<td>Oral-nasal gate</td>
<td>Includes when sow inserts mouth and/or snout in between the opening of the pen doors and forcefully pushes gate up and down.</td>
</tr>
<tr>
<td>Inactive</td>
<td>Includes sitting with front limbs extended and bearing weight and rear legs on the ground or lying down with all four limbs and body in contact with floor.</td>
</tr>
<tr>
<td>Active</td>
<td>Includes standing with all limbs extended and bearing weight on the ground or walking with limbs in both extension and flexion and moving through the pen</td>
</tr>
<tr>
<td>Maintenance</td>
<td>Includes foraging (defined as rooting, licking, exploratory behaviors directed at feed trough or ground), urinating and/or defecating.</td>
</tr>
</tbody>
</table>
Table 5.1 Continued. Behavioral ethogram of normal and abnormal sow behaviors.

| Unknown | Includes anytime the sow is out of pen or camera malfunctions and behaviors of sow cannot be identified |

---
Figure 5.1: Time budget (%) for an individual sow exhibiting stereotypic behaviors during a 12 hour observation period on baseline, treatment and recovery days.

1 Unknown behaviors include sow out of pen or camera visual and/or camera malfunction; Maintenance behaviors (Maintenance) includes foraging, urinating and/or defecating; Body weaving behavior (Body); Head weaving behavior (Head); Oral-nasal gate manipulation (Gate)
Figure 5.2: Stereotypic behavior time budget (%) for the first hour of baseline, treatment and recovery days for an individual sow during a 12 hour observation period

1 Body weaving behavior (Body); Head weaving behavior (Head); Oral-nasal gate manipulation (Gate)
Figure 5.3: Bouts of stereotypic behavior performed for the first hour of baseline, treatment and recovery days by an individual sow during a 12 hour observation period.

Body weaving behavior (Body); Head weaving behavior (Head); Oral-nasal gate manipulation (Gate)
CHAPTER 6
MEASURING THE EFFICACY OF FLUNIXIN MEGLUMINE AND MELOXICAM FOR LAME SOWS USING NOCICEPTIVE THRESHOLD TESTS

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6.1 Abstract

Lameness in breeding swine can cause severe pain leading to on-farm welfare issues and significant economic impacts. Non-steroidal anti-inflammatory drugs including meloxicam and flunixin meglumine are commonly used in veterinary medicine for their analgesic and anti-inflammatory properties. Pressure algometry and thermal sensitivity tests are non-invasive methods to quantify pain sensitivity using nociceptive thresholds to provoke withdrawal responses on lame and sound legs. The objective of this work was to determine the effects of these drugs on nociceptive thresholds in sows induced lame using pressure algometry and thermal sensitivity tests. Lameness was induced in 24 mature, mixed-parity sows using a chemical synovitis model and three treatments were compared: meloxicam (1.0 mg kg$^{-1}$ PO), flunixin meglumine (2.2 mg kg$^{-1}$ IM) and sterile saline (IM). Pressure algometry was measured on sound and lame rear legs with three replicates at three landmarks. Thermal sensitivity tests were done on sound and lame rear legs with three replicates using a thermal stimulus at one landmark. From 37 to 72 h after lameness induction, meloxicam- and flunixin meglumine-treated sows tolerated higher pressure algometer nociceptive thresholds compared to saline-treated sows. Changes in thermal nociceptive thresholds were evident at the Tmax time-points for meloxicam administration and 72 and 168 h post lameness induction for flunixin meglumine-treated sows. In conclusion, flunixin meglumine and meloxicam administration mitigated pain sensitivity in lame sows post lameness induction when pain sensitivity was evaluated with pressure algometry. These analgesic drugs may be a key tool to manage pain associated with lameness.
Keywords: animal welfare, flunixin meglumine, lameness, meloxicam, nociceptive threshold, swine

6.2 Introduction

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 2004). Lameness associated with painful joint lesions has been identified as a welfare challenge for confined sows (Elmore et al 2010) with lameness ranked as the third most common reason for culling sows, comprising 15% of cull sows marketed in the United States (Schenk et al 2010). Culling sows prior to completion of the third parity has been identified as an economic loss as pig producers are neither able to pay off individual sow costs nor capitalize on the benefits of higher sow retention rates (Stalder et al 2000, 2003).

Diagnosis of pain associated with lameness is a difficult process due to unique individual experiences with pain (Gaynor & Muir 2009) and differences noted in pain tolerance and reaction between species, breeds, sex, age, pain duration and stimulus severity (Matthew 2000). Danish animal welfare scientists and veterinarians reported that fractures, osteochondrosis dissecans (OCD), and infectious arthritis were ranked highest for pain severity for lameness in swine (Jensen et al 2012). Nociceptive thresholds’ testing, such as pressure algometry and thermal sensitivity tests, can be used for clinical evaluation of painful conditions and analgesic efficacy.

Nociception is the process by which the detection, transduction, and transmission of a noxious stimulus to higher centers of the central nervous system occurs (Livingston 2006).
Mechanical and thermal nociceptive thresholds (MNT and TNT) can be defined as the amount of pressure or heat stimulation necessary to produce a behavioral response indicative of pain sensitivity (Haussler et al 2007). Mechanical and thermal nociceptive threshold tests have been used as objective pain assessment tools in a variety of livestock animals including broilers (Hothersall et al 2011), dairy cattle (Veissier et al 2000; Herskin et al 2003, 2009; Dyer et al 2007; Heinrich et al 2010; Fitzpatrick et al 2013; Higginson-Cutler et al 2013), sheep (Nolan et al 1987; Ley et al 1989; Stubsjøen et al 2009) and swine (Jarvis et al 1997; Sandercock et al 2009; Di Giminiani et al 2012; Janczak et al 2012; Tapper et al 2013).

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most common categories of drugs used to manage pain in animals based on their anti-inflammatory, antipyretic and analgesic properties (Gaynor & Muir 2009). Flunixin meglumine is a common NSAID used in veterinary medicine and is currently labelled for pyrexia control associated with swine respiratory disease at 2.2 mg kg⁻¹ dose administered intramuscularly (Intervet Schering Plough 2013). Meloxicam is a member of the oxicam family and is labelled in swine for the treatment of non-infectious locomotor disorders and mastitis-metritis-agalactia syndrome in some European countries at 0.4 mg kg⁻¹ dose administered intramuscularly (Friton et al 2003). Neither drug is specifically labelled for swine pain management in the United States; any potential application must be considered and guided by a veterinarian in the context of the Animal Medicinal Drug Use Clarification Act (AMDUCA).

The objectives of this study were to determine the efficacy of meloxicam and flunixin meglumine for pain mitigation in lame sows using pressure algometer and thermal sensitivity nociceptive threshold tests.
6.3 Materials and Methods

The protocol for this study was approved by the Iowa State University Animal Care and Use Committee. The animals were cared for in accordance with the United States Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals, 8th Edition. This work was performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) at the Iowa State University College of Veterinary Medicine. As lameness induction resulted in transient states of pain, the experiment was designed to allow each sow to serve as her own control thus reducing the total number of sows required whilst maintaining population sizes large enough to achieve statistical power. Investigators established humane end-point criteria in which any sow that was unable to access water for 12 h, access food for 24 h or progressed to non-weight bearing lameness for 48 h was removed from the study and humanely euthanized. No sows met these criteria during this study. All sows were acclimated to housing and handling for seven days prior to trial initiation.

6.3.1 Animals and housing

Twenty-four multiparous (mean parity 6; range 2–9), non-pregnant, crossbred Newsham maternal cull sows were obtained from a commercial farm in Iowa (bodyweight 241.4 [± 15.5] kg). All sows underwent a physical examination and a lameness evaluation prior to selection by a trained veterinarian in charge with expertise in sow lameness. Lameness was evaluated using the following criteria: i) sow not moving freely using all four legs while walking; ii) weight-shifting during walking or standing; or iii) non-weight-bearing
on any leg. Sows selected for the project were categorized as non-lame. Physical examination and lameness evaluation were also conducted between each round during the trial to confirm no observable residual lameness was present.

To avoid confounding injury due to aggression, each sow was housed in an individual pen; however, sows could see, smell, hear and have nose-to-nose contact with other sows. Each pen measured 3.7 × 1.4 × 1.2 m (length × width × height) and had a solid concrete floor with a rubber mat (2.4 × 1.4 × 0.02 m). Metal fences (1.2 × 0.76 m; height × width) were affixed to the end of each home pen. Each pen was provided with a form of environmental enrichment, including chains and/or plastic toys attached to the pen gates. Sows were provided ad libitum access to water via one nipple drinker and hand-fed 2.7 kg of a custom-mixed diet of 14.8% CP TMR composed of ground corn, soybeans, and nutrients formulated according to Swine NRC guidelines (2012) with no antimicrobials. FDA approved Matrix® (0.22% Altrenogest; Intervet/Schering-Plough, Milsboro, USA; DE-Dose: 6.8 ml–15 mg) was added to 1 kg of feed daily to prevent estrus initiation.

6.3.2 Experimental design

Lameness was induced by injecting amphotericin B into the distal interphalangeal joint according to the methods previously described (Karriker et al 2013) and a repeated measures design compared responses up to seven days following lameness induction. The following treatments were administered twice during each round, 24 h apart: meloxicam (1.0 mg kg−1 per os), flunixin meglumine (2.2 mg kg−1 intramuscular) and sterile saline (equivalent volume administered intramuscular). Two trials consisting of 12 sows per trial were conducted for a total of 24 sows and each trial consisted of three rounds of treatment,
with a different treatment administered for each round. Sows were assigned to three blocks (four sows per block) by bodyweight and each block was randomly allocated to one of three treatments for round one. A ten-day wash-out period was provided between rounds to avoid previous treatment carry-over effects. In round one, sows were randomly assigned to one of three treatments and lameness induction was assigned to either the left or right rear leg so that leg assignment and treatment were balanced. In round two, sows were randomly assigned to one of the remaining two treatments and lameness was induced in the rear leg that was sound in the previous round. By the last round all sows received all three treatments. Prior to subsequent treatment round, pressure algometry and thermal sensitivity tests were conducted, sows were gait-scored and blood was collected to determine any lameness and residual drug carry-over.

6.3.3 Treatments

Three treatments were administered: i) meloxicam (1.0 mg kg⁻¹ per os administered in 8 g cookie dough with additional sterile saline injected intramuscularly; n = 24); ii) flunixin meglumine (2.2 mg kg⁻¹ administered intramuscularly with 8 g cookie dough; n = 24); or iii) sterile saline (administered intramuscularly at an equivalent volume to flunixin meglumine with 8 g cookie dough; n = 24). Flunixin meglumine treatments were administered 27.5 and 51.5 h post induction and meloxicam administered 28.5 and 52.5 h after lameness induction. Half of the saline-treated sows had treatment administered at 27.5 and 51.5 h post lameness induction to match sows receiving flunixin meglumine. The remaining half of the saline-treated sows received their treatments at 28.5 and 52.5 h after lameness induction to match sows receiving meloxicam. To control for observer bias, researchers were blinded to analgesic treatments, but could not be blinded to the trial day.
6.3.4 Pain sensitivity tests

Pain sensitivity tests were performed while sows were confined in a modified gestation stall (2.0 × 0.61 m; length × width) outside of the home pen, using methods previously described by Tapper and colleagues (2013). Sows were provided ad libitum access to feed by sprinkling feed into the stall feeder (≤ 1 kg feed per collection time) during testing to facilitate a relaxed standing posture. During acclimation, sows were trained to enter and stand in the testing stall where they received a portion of their standard feed to reinforce the behavior. Acclimation was assessed by the sow’s willingness to enter the stall feeder without human intervention, stand quietly and consume ration during data collection; at the end of acclimatization all sows met this criteria. Both rear legs were rinsed with water and dried using paper towels to completely remove any dirt and dried faecal matter that might have been present. Scrubbing was not performed on the leg and if excessive dirt or feces was present, the leg was soaked as to not cause a localized painful response. Pain sensitivity tests were performed at the same time of day to control for possible circadian behavior and pain sensitivity patterns (Hastings 2010). The observer was blinded to the numeric output values during the pain sensitivity test assessment.

6.3.5 Pressure algometry

A hand-held pressure algometer (Wagner Force Ten™ FDX 50 Compact Digital Force Gage, Wagner Instruments, CT, USA) with a 1 cm2 flat rubber tip was used to quantify MNTs in kilograms of force (Kgf) as calculated by the instrument. In an attempt to standardize the procedure and reduce variability associated with handler application of the device, the technicians trained in the application of the pressure algometer practiced applying
the force at a rate of approximately one Kgf s\(^{-1}\) on a static surface for 10-s periods during
the seven-day acclimation period and immediately prior to data collection daily.
Furthermore, the technician was blinded to the numeric output values during the pain
sensitivity tests. An additional technician served as the recorder and was assigned to collect
the output data. During data collection, pressure algometry was applied at the landmarks at
approximately one Kgf s\(^{-1}\). The maximum force applied was 10 Kgf, after which the
recorder said ‘Stop’ and pressure was removed. Pressure was applied perpendicularly to three
landmarks in a randomized sequence for each sow: i) middle of cannon on the rear leg (C);
ii) 1 cm above the coronary band on the lateral rear claw (O); and iii) 1 cm above the
coronary band on the medial rear claw (I; Figure 6.1). The outer and inner landmark
represented where the drug was injected to induce lameness and was included as pain
landmarks. The cannon landmark was included as a control landmark. The landmark
sequences were repeated in triplicate on the right rear leg followed by the same sequence
repeated in triplicate on the left rear leg. When a foot-lift response was observed, pressure
was immediately removed, and the peak pressure representing the MNT recorded.

6.3.6 Thermal sensitivity

For consistent data collection across all treatments and trial days, the thermal
sensitivity test immediately followed the pressure algometer test and measured the latency
for a sow to withdraw her rear leg in response to precise, focused radiant heat stimulation.
The analgesia meter (IITC Plantar Analgesia Meter, IITC Life Science Inc, Woodland Hills,
CA, USA) was set at a constant 80% beam intensity; emitting 200°C. Prior research by the
authors (Tapper et al 2013) determined that tissue damage did not occur when using a 20-s
maximum duration. Thermal measurements were taken in triplicate 1 cm above the coronary
band on the lateral side of the right rear leg, followed by the left rear leg. The latency for the sow to withdraw her leg in response to the stimulus was recorded.

Data time-point collection schedule is described in Table 6.1. Data for the pressure algometer and thermal sensitivity test were collected at the following time-points: –24 h (baseline), 24 h (Day 1 pre-treatment), 28.5 and 30.5 h (Tmax for day 1), 36 h (Half-life for day 1), 48 h (Day 2 pretreatment), 52.5 and 54.5 h (Tmax2 for day 2), 60 h (Half-life2 for day 2), 72 h (Day 3), 168 h (Recovery) and 312 h (Baseline for next round). The Tmax is defined as the time in which the drug reaches its maximum concentration and half-life is defined as the amount of time it takes for the drug concentration to be reduced by one half. These values for flunixin meglumine were calculated in a previous pharmacokinetic experiment (Pairis-Garcia et al 2013). The Tmax and half-life for meloxicam were calculated using data from a previous pharmacokinetic experiment conducted in our laboratory (unpublished data). The Tmax for flunixin meglumine and meloxicam were 1 and 2 h after drug administration, respectively. As the Tmax for meloxicam and flunixin meglumine were different, measurements collected at this time could not be directly compared. For both NSAIDs, half-life was 8 h after drug administration.

Statistical analysis

Data were analyzed using SAS software version 9.3 (SAS Institute Inc. 2011). Data were analyzed for normality by plotting a predicted residual plot and a quantile-quantile plot using Proc-Univariate. PROC MIXED procedures of SAS was used to analyze response differences between sound and lame legs (Response). The pressure algometer and thermal sensitivity test statistical models included the fixed effect of treatment, round, time-point, leg
injected, treatment by time point interaction, and treatment by time-point by leg injected interaction. Sow within group by trial interaction was included as a random effect and replicate within round by time-point by leg injected interaction was included as a repeated statement. An auto-regressive correlation was used for the repeated statement. A $P$-value of $< 0.05$ was considered to be significant for the MIXED analysis of variance and when separating means. Fixed effect least square means were separated using the PDIF option in SAS and data were expressed as LS means ($\pm$ SEM).

6.4 Results

6.4.1 Transient synovitis model

Prior to anesthesia lameness induction and at the start of each subsequent round, all sows were clinically sound, defined as the ability to move freely using all four legs, showing no evidence of weight-shifting activities, non-weight-bearing, or reluctance to walk or stand on any leg. Peak lameness was observed on day one pre-treatment after lameness induction and all sows developed clinical signs of lameness including weight-shifting and reluctance to walk or stand on injected leg. No sows became non-weight-bearing during the trial. No differences were observed between baseline day and baseline for next round for pressure algometry and thermal sensitivity test responses (Figure 6.2[a], 6.3[a]). Blood analysis (data not shown; Pairis-Garcia et al 2013) confirmed systemic drug levels were below the limit of detection in between rounds suggesting that ten days was a sufficient wash-out period for systemic drug clearance.
6.4.2 Pressure algometry

Throughout the study, 4.6% of the response values were above the maximum pressure applied to 10 Kgf. Pressure tolerated on the sound and lame leg for saline-treated sows across all landmarks over the round can be found descriptively in Table 6.2. No differences were observed between sound and lame leg responses on baseline days between treatments ($P > 0.05$; Figure 6.2[a]). When comparing the pressure tolerated when sows were most lame (day 1 pretreatment) to baseline, sound and lame leg responses differed at all landmarks ($P < 0.0001$). However, there were no treatment differences between sound and lame leg responses on day 1 pre-treatment ($P > 0.05$; Figure 6.2[a]). Thirty-seven hours after lameness induction (Half-life) and up to Day 3 after lameness induction, both flunixin meglumine and meloxicam sows tolerated greater pressure compared to saline sows ($P < 0.01$). When comparing flunixin meglumine- to saline-treated sows at Tmax and Tmax2, no differences were observed (Figure 6.2[b]). However, when comparing meloxicam- to saline-treated sows, differences were observed at Tmax2 (Figure 6.2[b]). Leg injected (Left leg: 2.12 [± 0.17]; Right leg: 1.60 [± 0.17] KgF) and round (Round 1: 2.36 [± 0.18]; Round 2: 1.99 [± 0.17]; Round 3: 1.79 [± 0.17] KgF) had an effect on MNT ($P < 0.001$).

6.4.3 Thermal sensitivity

Throughout the study, 9.5% of the response values were above the maximum 20 s the thermal test was applied. Time that thermal heat was tolerated on the sound and lame leg for saline-treated sows can be found descriptively in Table 6.3. There was no difference between sound and lame leg latency responses on baseline days between treatments ($P > 0.05$; Figure 6.3[a]). When comparing time in which heat stimulation was tolerated when sows were most
lame (day 1 pre-treatment) to baseline, sound and lame leg latency responses differed \((P < 0.001)\). However, there were no treatment differences between sound and lame leg latency responses on day 1 pre-treatment \((P > 0.05; \text{Figure 6.3[a]})\). Sows administered flunixin meglumine tolerated heat stimulation longer compared to saline sows at 72 and 168 h after treatment administration \((P < 0.01; \text{Figure 6.3[a]})\); however this did not differ from meloxicam-treated sows. When comparing flunixin meglumine- to saline-treated sows at Tmax and Tmax2, no differences were observed \((\text{Figure 6.3[b]})\). However, when comparing meloxicam- to saline-treated sows, differences were observed at Tmax and Tmax2 time points \((\text{Figure 6.3[b]}; \ P < 0.001)\). Leg injected (Left leg: 2.22 [± 0.50]; Right leg: 0.77 [± 0.48] s) and round (Round one: 2.88 [± 0.5]; Round two: 1.20 [± 0.49]; Round three: 2.66 [± 0.49] s) had an effect on TNT \((P < 0.001)\).

6.5 Discussion

6.5.1 Transient synovitis model

This amphotericin B-induced lameness model produced a transient and reproducible synovitis of the distal interphalangeal joint for all sows. All sows were clinically sound prior to lameness induction for each round, showing no evidence of weight-shifting activities, non-weight bearing, or reluctance to walk or stand on any leg. Peak lameness was observed 24 h after induction with all sows demonstrating clinical lameness including weight-shifting and reluctance to walk. This coincides with results from previously published work assessing validity of amphotericin B-induced lameness model in swine (Karriker et al 2013; Tapper et al 2013) and cattle (Kotschwar et al 2009). Responses to pressure algometry and thermal sensitivity tests did not differ between baseline days at the start of each round confirming no
lameness carry-over from previous rounds. In addition, blood collection tests on baseline days confirmed systemic drug levels were below the limit of detection.

6.5.2 Pain sensitivity tests

Both the MNT and TNT were easily applied and successfully demonstrated differences in pain sensitivity between baseline and all time-points up to recovery (168 h) regardless of treatment. This suggests both the pressure algometer and thermal sensitivity test are objective tools to assess pain sensitivity in this lameness induction model. The pressure algometry and thermal sensitivity results coincide with results by Tapper and colleagues (2013) demonstrating similar although slightly lower thresholds for both the sound and the lame leg. For the pressure algometer, 4.6% of all data for the pressure algometer reached the maximum pressure of 10 Kgf, as compared to Tapper and colleagues with 13.5%. However, 9.5% of all thermal sensitivity data resulted in the maximum 20 s duration as compared to 4.6% in Tapper and colleagues’ work (2013). One possible explanation may be that Tapper and colleagues (2013) used a different veterinarian to perform the lameness induction protocol and different induction technique may have played a role.

In our study, the pressure algometer was able to detect changes in pain sensitivity using a lameness induction model. In addition, this tool was also able to detect differences between treated and non-treated animals, confirming the sensitivity of pressure algometry as a tool to evaluate analgesic efficacy in laboratory settings. Our study contributes to the growing body of knowledge across livestock species supporting the use of pressure algometry to quantify pain sensitivity (Dyer et al 2007; Sandercock et al 2009; Stubsjøen et al 2009; Heinrich et al 2010; Hothersall et al 2011; Nalon et al 2013; Tapper et al 2013).
Unlike Tapper and colleagues (2013), our data demonstrate that the thermal sensitivity test is also an objective tool to assess pain sensitivity. No differences were noted between treatments at baseline. However, differences were observed from baseline through to recovery, suggesting that the thermal sensitivity test detected pain sensitivity associated with lameness. Improvement in methodologies, such as completely drying both legs prior to thermal heat application, may have been a reason why differences were seen between days in our study. Although this test detected differences from baseline through recovery, it was not sensitive enough to detect drug effects.

Differences were only noted between saline- and meloxicam-treated sows when measurements were taken at the time meloxicam likely attained maximum drug concentration. Differences between the left and right rear legs were observed for both thermal and pressure algometry; the right leg consistently tolerated less pressure or thermal stimulation compared to the left leg. These differences may be due to the order in which the measurements were applied with the right leg always being measured first. Anticipatory or pain-related fear behavior has been acknowledged in human medicine as a cause for changes in pain sensitivity, often resulting in pain enhancement (Crombez et al 1999a, b). It is likely that the decrease in nociceptive thresholds on the right leg is due to the sow being startled by initial manipulation of the leg and anticipating or fearing pain onset. Nalon and colleagues (2013) found that when comparing nociceptive thresholds using a hand-held probe compared to a limb-mounted actuator, MNT were significantly lower with the hand-held probe. This may be due to the sow’s reaction towards the operator approaching the limb, anticipating that stimulus and therefore reacting faster. It is possible that by the time that data were collected on the right leg, the sow may have become desensitized to this manipulation and presence of
the observer resulting in higher nociceptive thresholds for the left leg. Further research is needed to determine if there is a true leg sensitivity difference or if this is a methodology artifact.

Round had an effect on the thermal sensitivity and pressure algometry tests. The TNT decreased during round two while the MNT decreased with each subsequent round. Janczak and colleagues (2012) evaluated the stability and repeatability of measuring MNT using a hand-held algometer in piglets not in pain. The authors of this study found several factors influencing MNT including habituation time, pig weight, testing week, days within test week and replication within the week. This study concluded that repeated measures can be used to evaluate changes in pain threshold in pigs; however habituation for at least several days is required to gain higher correlations among MNT responses (Janczak et al 2012). The sows used in this present study were acclimated to both tests for seven days prior to round one, but were not handled during the wash-out period prior to rounds 2 and 3. This may explain changes in response between rounds. There were no differences found between rounds when comparing baseline day, indicating that the difference in habituation does not explain the difference in response over rounds to these tests. It is possible that sows became sensitized to the lameness induction over rounds which resulted in increased responses during lameness. Further studies are needed to evaluate if there are changes to sow sensitivity and responses to lameness induction or amphotericin B.

6.5.3 Analgesic efficacy

Meloxicam and flunixin meglumine mitigated pain sensitivity between 36 and 72 h after lameness induction compared to saline-treated sows when using the pressure algometer
test. Previous studies have also demonstrated flunixin meglumine and meloxicam efficacy for acute and chronic pain mitigation in cattle (Currah et al 2009; Heinrich et al 2010; Schulz et al 2011; Fitzpatrick et al 2013 Huber et al 2013), sheep (Welsh & Nolan 1995) and swine (Friton et al 2003; Reiner et al 2012; Kluviers-Poodt et al 2013). Our results agree with Schulz and colleagues (2011) who found flunixin meglumine to be efficacious in providing analgesia for steers induced lame using an amphotericin B model. However, our results differ from previously published data evaluating flunixin meglumine using the same transient lameness model in sows (Tapper et al 2013).

Unlike Tapper and colleagues (2013), our experiment evaluated several additional time-points to assess pain sensitivity. The additional time-points and deliberate choice to collect data during the drug’s Tmax and half-life was based on the goal to collect data in a window of time in which the drug may be most effective. However, no studies, to date, have determined at what time either drug is maximally effective in sows. It is unknown if these additional time-points resulted in our ability to detect differences in pain sensitivity because: i) during these time-points the drug reached its maximum analgesic efficacy; or ii) additional time-points and increased enrolled sow numbers contributed to greater statistical power associated with a larger data set.

The analgesic effects of flunixin meglumine and meloxicam did not differ in the period between 36 and 72 h post lameness induction, although treatments could not be compared at Tmax as Tmax was different between meloxicam and flunixin meglumine. Meloxicam administered orally has several advantages over flunixin meglumine for use in the field including: i) cost (oral meloxicam administration costs approximately US$0.004 per kg bodyweight to administer at 1.0 mg kg\textsuperscript{–1}); ii) reduced stress (oral meloxicam does not
require physical restraint for administration); iii) decreased macroscopic lesions of the muscle and fibrous tissue at drug injection sites (Magyan & Glavits 2007); and iv) decreased public health risk associated with accidental needle breakage into muscle (Chase et al 2008).

As neither drug is specifically labelled for pain management for swine in the United States, administration of either product would be considered extra-label drug use (ELDU). This practice, which is regulated under the Animal Medicinal Drug Use Clarification Act (AMDUCA), requires that drugs be administered under the supervision or by a veterinarian with an established veterinary-client patient relationship (Coetzee 2011).

6.6 Animal Welfare Implications and Conclusions

Meloxicam and flunixin meglumine were effective in modifying pain sensitivity in lame sows evaluated using pressure algometry. Thermal sensitivity tests were also applied during this time but were only sensitive enough to detect changes in pain sensitivity immediately after drug administration. Our research suggests that meloxicam and flunixin meglumine are effective pharmaceutical interventions for pain mitigation associated with a chemically induced synovitis model. Further research evaluating the efficacy and optimizing the dose regimen of these drugs in chronic or naturally occurring lameness on-farm should be investigated.

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Table 6.1: Pressure algometer and thermal nociceptive threshold data sampling time points

<table>
<thead>
<tr>
<th>Day</th>
<th>Baseline (-24h)</th>
<th>Induction (0h)</th>
<th>Lame (24h)</th>
<th>Lame (48h)</th>
<th>Lame (72h)</th>
<th>Recovery (168h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>Treatment²</td>
<td></td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmax³</td>
<td></td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-life⁴</td>
<td></td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Lameness induced using a chemical synovitis model (Karriker et al., 2013)

²Treatments; 1. Meloxicam (M; 1.0mg/kg per os in cookie dough n= 24) 2. Flunixin meglumine (FM; 2.2 mg/kg intramuscular (IM) n=24), or 3. Saline (S; equivalent volume to FM administered IM n= 24). Flunixin treatments administered 3.5 h after pre-treatment data collection. Meloxicam treatments administered 4.5 hours after pre-treatment data collection. Saline treatments randomly administered either 3.5 or 4.5 hours after morning data collection

³Tmax defined as the time in which drug reaches it maximum concentration. Tmax for meloxicam treated sows was 2 hours after drug administration (Unpublished data). Tmax for flunixin treated sows was 1 hour after drug administration (Pairis-Garcia et al 2013). Sows treated with saline had data collected randomly at either 1 or 2 hours after drug administration

⁴Half-life defined as the time in which the drug reaches half of its maximum concentration. Half-life for all three treatments was 8 hours after drug/saline administration (37 and 60 hours post induction for flunixin and meloxicam respectively) (Unpublished data, Pairis-Garcia et al 2013)
Table 6.2: Descriptive mean nociceptive threshold ± SE for a foot lift response to a pressure algometry test\(^1\) applied to saline treated sows using a lameness induction model

<table>
<thead>
<tr>
<th>Landmark(^3)</th>
<th>Leg(^4)</th>
<th>Baseline</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-</td>
<td>Pre-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>treatment</td>
<td>treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cannon</td>
<td>Lame</td>
<td>5.93 ± 0.34</td>
<td>2.93 ±</td>
<td>2.97 ±</td>
<td>3.66 ±</td>
<td>5.22 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Sound</td>
<td>5.42 ± 0.43</td>
<td>4.35 ±</td>
<td>5.26 ±</td>
<td>5.82 ±</td>
<td>5.46 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
</tr>
<tr>
<td>Outer</td>
<td>Lame</td>
<td>5.70 ± 0.34</td>
<td>1.12 ±</td>
<td>0.94 ±</td>
<td>1.07 ±</td>
<td>3.01 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Sound</td>
<td>4.30 ± 0.43</td>
<td>4.43 ±</td>
<td>4.04 ±</td>
<td>4.29 ±</td>
<td>4.90 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
</tr>
<tr>
<td>Inner</td>
<td>Lame</td>
<td>5.62 ± 0.34</td>
<td>1.03 ±</td>
<td>1.04 ±</td>
<td>0.93 ±</td>
<td>2.97 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Sound</td>
<td>4.47 ± 0.43</td>
<td>4.35 ±</td>
<td>4.61 ±</td>
<td>4.11 ±</td>
<td>4.64 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
</tr>
</tbody>
</table>

\(^1\)A foot lift response was used to determine the mechanical nociceptive thresholds (MNTs in kilograms of force, kgf) in response to a pressure algometry test.

\(^2\)This test was administered in triplicate to 24 sows on baseline (-24 h prior to lameness induction), Day 1 (24 h post lameness induction; pre-treatment), Day 2 (48 h post lameness induction) and recovery.
induction; pre-treatment), Recovery (72 h post lameness induction) and Resolution (168 h post lameness induction).

3Pressure was applied perpendicularly to 3 landmarks in a randomized sequence for each sow: 1. Middle of cannon on the rear leg (Cannon), 2. 1 cm above the coronary band on the lateral rear claw (Outer) and 3. 1 cm above the coronary band on the medial rear claw (Inner)

4Lameness was induced using a chemical synovitis model (Karriker et al 2013) and lameness was induced on either the left or right rear leg
Table 6.3: Descriptive mean nociceptive threshold ± SE for a foot lift response to a thermal sensitivity test\(^1\) applied to saline treated sows using a lameness induction model

<table>
<thead>
<tr>
<th>Trial Time point</th>
<th>Leg(^4)</th>
<th>Baseline</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lame</td>
<td>Pre-treatment</td>
<td>10.5 ± 0.70</td>
<td>5.18 ± 0.70</td>
<td>5.09 ± 0.70</td>
<td>4.88 ± 0.70</td>
<td>8.49 ± 0.70</td>
</tr>
<tr>
<td>Sound</td>
<td>Pre-treatment</td>
<td>9.43 ± 0.93</td>
<td>8.18 ± 0.93</td>
<td>5.90 ± 0.93</td>
<td>7.63 ± 0.93</td>
<td>10.7 ± 0.93</td>
</tr>
</tbody>
</table>

\(^1\)A foot lift response was used to determine the thermal nociceptive thresholds (TNTs in seconds) in response to a pressure algometry test.

\(^2\)This test was administered in triplicate to 24 sows on baseline (24 h prior to lameness induction), Day 1 (24 h post lameness induction; pre-treatment), Day 2 (48 h post lameness induction; pre-treatment), Recovery (72 h post lameness induction) and Resolution (168 h post lameness induction).

\(^3\)Thermal measurements were taken in triplicate 1 cm above the coronary band on the lateral side of the right rear leg, followed by the left rear leg using a laser set at 80% intensity, emitting 200°C.

\(^4\)Lameness was induced using a chemical synovitis model (Karriker et al 2013) and lameness was induced on either the left or right rear leg.
**Figure 6.1:** Pressure algometer landmark schematic. C = Middle of the cannon on the hind limb; O = 1 cm above the coronary band on the lateral hind claw; I = 1 cm above the coronary band on the medial hind claw.
Figure 6.2a: Mechanical nociceptive thresholds LS Means differences (sound limb- lame limb) KgF ±SE for a foot lift response to a pressure algometry test\(^1\) between sows treated\(^2\) when induced lame using a chemical synovitis model

\[\text{Mechanical Nociceptive Thresholds (MNTs) in kg of force tolerated, kgF} \]

\(^1\)A foot lift response was used to determine the mechanical nociceptive thresholds (MNTs in kg of force tolerated, kgF) in response to a pressure algometer. This test was administered in triplicate for all time points relative to lameness induction (0 h).

\(^2\)Treatments; 1. Meloxicam (M; 1.0mg/kg per os in cookie dough n= 24) 2. Flunixin meglumine (FM; 2.2 mg/kg intramuscular (IM) n=24), or 3. Saline (S; equivalent volume to FM administered IM n= 24).

* Denotes difference between saline and meloxicam treated sows (P <0.05)

\(\downarrow\) Denotes difference between saline and flunixin meglumine treated sows (P <0.05)

\(\times\) Denotes difference between meloxicam and flunixin meglumine treated sows (P <0.05)
**Figure 6.2b:** Mechanical nociceptive thresholds LS Means differences (sound limb- lame limb) KgF ±SE for a foot lift response to a pressure algometry test\(^1\) at Tmax and Tmax\(^2\) between sows treated\(^3\) when induced lame using a chemical synovitis model.

A foot lift response was used to determine the mechanical nociceptive thresholds (MNTs in kg of force tolerated, kgF) in response to a pressure algometer. This test was administered in triplicate for all time points relative to lameness induction (0 h).

The Tmax is defined as the time in which the drug reaches its maximum concentration after administration. The Tmax and Tmax\(^2\) for Flunixin and half of saline treated sows was at 28.5 and 52.5 hours after lameness induction. The Tmax and Tmax\(^2\) for Meloxicam and half of the saline treated sows was 30.5 hours and 54.5 hours after lameness induction.

Treatments: 1. Meloxicam (M; 1.0mg/kg per os in cookie dough n= 24) 2. Flunixin meglumine (FM; 2.2 mg/kg intramuscular (IM) n=24), or 3. Saline (S; equivalent volume to FM administered IM n= 24).

* Denotes difference between saline and meloxicam treated sows (P <0.05)
**Figure 6.3a:** Thermal nociceptive thresholds LS Means differences (sound limb- lame limb) s ±SE for a foot lift response to a thermal sensitivity test\(^1\) between sows treated\(^2\) when induced lame using a chemical synovitis model

\(^1\)A foot lift response was used to determine the thermal nociceptive thresholds (TNTs in seconds of thermal stimulation tolerated) in response to a thermal sensitivity test. This test was administered in triplicate for all time points relative to lameness induction (0 h).

\(^2\)Treatments; 1. Meloxicam (M; 1.0mg/kg per os in cookie dough n= 24) 2. Flunixin meglumine (FM; 2.2 mg/kg intramuscular (IM) n=24), or 3. Saline (S; equivalent volume to FM administered IM n= 24).

\(^¥\) Denotes difference between saline and flunixin meglumine treated sows (P <0.05)
**Figure 6.3b:** Thermal nociceptive thresholds LS Means differences (sound limb- lame limb) ±SE for a foot lift response to a to a thermal sensitivity test\(^1\) at Tmax and Tmax2\(^2\) between sows treated\(^3\) when induced lame using a chemical synovitis model

\(^1\) A foot lift response was used to determine the thermal nociceptive thresholds (TNTs in seconds of thermal stimulation tolerated, s) in response to a thermal sensitivity test. This test was administered in triplicate for all time points relative to lameness induction (0 h).

\(^2\) The Tmax is defined as the time in which the drug reaches its maximum concentration after administration. The Tmax and Tmax2 for Flunixin and half of saline treated sows was at 28.5 52.5 hours after lameness induction. The Tmax and Tmax2 for Meloxicam and half of the saline treated sows was 30.5 hours and 54.5 hours after lameness induction.

\(^3\) Treatments; 1. Meloxicam (M; 1.0mg/kg per os in cookie dough n= 24) 2. Flunixin meglumine (FM; 2.2 mg/kg intramuscular (IM) n=24), or 3. Saline (S; equivalent volume to FM administered IM n= 24).

* Denotes difference between saline and meloxicam treated sows (P <0.05)
Swine are a common species utilized for biomedical research as well as research pertaining to industry needs. The USDA reported that over 150 research projects using swine for both animal research and biomedical research are active. In 2002, swine officially became the most common large laboratory animals species utilized with over 68,000 pigs registered for research (USDA, 2013). Recognizing the important role of swine as a research model, it is essential to assess their welfare and to ensure appropriate handling and treatment to minimize their pain and distress. The first objective of this dissertation was to refine and enhance common techniques conducted in a laboratory setting.

In chapter three, I developed and refined a technique for intravenous auricular catheter placement in the sow. Intravenous drug administration in adult swine is difficult to perform due to inaccessible superficial veins and thick subcutaneous fat layers. However, successful intravenous drug administration is critical for many biomedical applications including pharmacokinetic studies as extravascular drug administration can influence the drug’s absorption and elimination rate. This protocol utilized a topical anesthetic cream and relied on minimal physical restraint to place indwelling catheters in the auricular vein of six multiparous sows. This method was quick (3 minutes 20 seconds ± 8 seconds (mean ± SE per catheter), effective (11/12 catheters successfully placed) and reliable, allowing a large drug volume (20-22ml) to be administered successfully during the trial without relying on prolonged restraint or general anesthesia of the sow.
In the fourth chapter of this dissertation, I evaluated the efficacy of yohimbine as an anesthetic reversal agent for sows anesthetized with a combination of xylazine, ketamine, and telazol. Sows represent a unique population in the breeding herd, as physiological compromise associated with disease and age can make anesthesia induction risky. According to the American Society of Anesthesiologists, age (geriatric), weight, disease status, and anatomical variation contribute to a heightened anesthetic risk and can lead to prolonged recovery times and increase post-anesthetic complications. Yohimbine is an alpha-2 adrenoreceptor antagonist that has been reported to be effective in reversing xylazine effects in nursery-age swine and other food-producing. This study confirmed that sows administered Yohimbine during anesthesia recovery occurred 172 minutes earlier than control sows. For all insensibility measures, Yohimbine sows regained a normal response more quickly and when evaluating physiological parameters such as heart rate and rectal temperatures, sows administered Yohimbine maintained physiological parameters closer to expected ranges.

In Chapter five, behavioral modification treatments were provided to a sow demonstrating both oral and locomotor stereotypies in a laboratory setting. The sow performed the oral-nasal gate manipulation, head and body weaving 4.0%, 12.4% and 6.8% of her total baseline time budget respectively. Three treatments were employed to mitigate the duration and frequency of these stereotypic behaviors. By changing the pen configuration and increasing space the sow’s stereotypic behavior decreases to less than .5% of her total time budget. This study is one of the first reports to evaluate treatment of established stereotypies in a mature sow and results suggest the promise of environmental enrichment as an effective treatment strategy.
Chapters three through five focused specifically on refining and enhancing common techniques conducted in a laboratory setting to reduce pain and distress and improve welfare in laboratory housed swine. These trials successfully identified new techniques to administer drugs intravenously, improve sow recovery during anesthesia and provide treatments to sows demonstrating stereotypic behavior. These three trials helped to minimize pain and distress associated with either laboratory techniques or by-products of housing sows in a confined setting, thus improving the welfare of laboratory housed sows.

The second objective of this dissertation was to use an optimal dosing regimen for two non-steroidal anti-inflammatory drugs (flunixin meglumine and meloxicam) to determine their efficacy in pain management using nociceptive threshold tests when sows are induced lame through the use of a chemical synovitis model.

Lameness in breeding swine can cause severe pain leading to on-farm welfare issues and significant economic impacts. Non-steroidal anti-inflammatory drugs including meloxicam and flunixin meglumine are commonly used in veterinary medicine for their analgesic and anti-inflammatory properties. Pressure algometry and thermal sensitivity tests are non-invasive methods to quantify pain sensitivity using nociceptive thresholds to provoke withdrawal responses on lame and sound legs. Meloxicam and flunixin meglumine were effective in modifying pain sensitivity in lame sows evaluated using pressure algometry. Thermal sensitivity tests were also applied during this time but were only sensitive enough to detect changes in pain sensitivity immediately after drug administration.

My research suggests that meloxicam and flunixin meglumine are effective pharmaceutical interventions for pain mitigation associated with a chemically induced
synovitis model. Further research evaluating the efficacy and optimizing the dose regimen of these drugs in chronic or naturally occurring lameness on-farm should be investigated.