2015

Transmammary-delivered meloxicam in piglets undergoing castration and tail docking: impact on pharmacokinetics and pain biomarkers

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Transmammary-delivered meloxicam in piglets undergoing castration and tail docking: Impact on pharmacokinetics and pain biomarkers

by

Jessica Lea Bates

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

MASTER OF SCIENCE

Major: Veterinary Preventative Medicine

Program of Study Committee:
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Anna K. Butters-Johnson

Iowa State University
Ames, Iowa
2015

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DEDICATION

This thesis is dedicated to my parents, Kent and Marla Bates. Without their unwavering support, guidance, and encouragement towards education, I wouldn’t be the person and the veterinarian that I am today.
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ACKNOWLEDGEMENTS

I would like to thank my graduate study committee members: Drs. Locke Karriker, Alejandro Ramirez, and Anna K. Butters-Johnson. Your support and instruction throughout this process was unfaltering and entirely positive. I especially am thankful that I was able to learn from your excellent examples of how to be an educator, something I consider a very important lesson. An enormous thanks goes to Dr. Karriker who often concurrently played the role of major professor, work supervisor, classroom instructor and mentor in my life. I am incredibly grateful for your guidance and the opportunity to work for the Swine Medicine Education Center (SMEC) for the past three years.

I would not have been able to complete the live animal work of this study without the help of the SMEC interns of 2013. Kelly Pertzborn was a dedicated summer scholar for the project, along with Dr. Kimberly Crawford, Joel Sparks, Luke Baldwin, Sarah Balik, and Lauren Scruggs. The Pharmacology Analytical Support Team at Iowa State University also played an integral role in sample analysis. Dr. Johann Coetzee provided the genesis for this proof of concept study and offered much wisdom and advice. Additionally as part of that team, Drs. Larry Wulf and Matthew Stock spent countless hours in the laboratory running assays. A significant amount of gratitude goes to Dr. Stock for his patient help navigating all things of academia and research when I first returned to Iowa State University. Your friendship is one of the best benefits from my time here.

Most of all, I thank my family for their love and unfailing support throughout my return to graduate school. Your encouragement from the start of my veterinary career has been a lifeline, as I often heard your “life lessons” echoing in my head in difficult situations. Thank you for helping me keep this in proper perspective and ground me in what is truly important. Also,
there is a much-deserved thank you to Justin, who was not here for the start of this project, but has seen the end of it. You have offered kindness, a listening ear and wisdom to see the future value of finishing this well (AKA giving me time and quiet to write). Finally, I thank God for seeing me through it all, for without Him none of this would be possible.
ABSTRACT

To investigate a novel route for providing analgesia to processed piglets via transmammary drug delivery, meloxicam was administered orally to sows after farrowing. The objectives of this study were to prove the transmammary delivery of meloxicam from sows to piglets, describe initial pharmacokinetic parameters and to characterize the analgesic effects in piglets after processing through assessment of pain biomarkers and infrared thermography (IRT). Ten sows received either meloxicam (30 mg/kg) (n=5) or whey protein (placebo) (n=5) in their daily feedings, starting four days after farrowing and continuing for three consecutive days. During this period, blood and milk samples from sows and piglets were collected at 12-hour intervals. On Day 5 after farrowing, three boars and three gilts from each litter were castrated or sham castrated, tail docked and administered an iron injection. Piglet blood samples were collected immediately before processing and at predetermined times over an 84-hour period. IRT images were captured at each piglet blood collection point. Plasma was tested to confirm meloxicam concentrations using a validated high-performance liquid chromatography-mass spectrometry method. Meloxicam was detected in all piglets nursing on medicated sows at each time point, and the mean (±standard error of the mean) meloxicam concentration at castration was 568.9 ±105.8 ng/mL. Sow plasma and milk, along with piglet plasma reached steady concentrations at 36 hours after the administration of meloxicam. Furthermore, ex-vivo prostaglandin E$_2$ (PGE$_2$) synthesis inhibition was greater in piglets from treated sows compared to controls (p=0.0059). However, button gastric ulcers and subacute gastritis were noted in 2/5 meloxicam-treated sows and their litters. Initial sow oral meloxicam pharmacokinetic parameters (±SD) were calculated including absorption rate constant (ka) of 0.13 (0.05) hr$^{-1}$, volume of distribution (V) 149.65 (8.94) mL/kg, and clearance (Cl) of 18.86 (0.45) mL/hr/kg.
There was a time-by-treatment interaction for plasma cortisol (p=0.0009), with meloxicam-treated piglets demonstrating lower cortisol concentrations than control piglets for 10 hours after castration. No differences in mean plasma substance P concentrations between treatment groups were observed (p=0.67). Lower cranial skin temperatures on IRT were observed in placebo compared to meloxicam-treated piglets (p=0.015).

Although there is great potential benefit to this method in the realms of analgesia, safety, and efficiency, dose refinement and a full understanding of pharmacokinetics of meloxicam by this route is needed before its application into swine.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

The swine industry is experiencing increasing concern from producers and consumers regarding the well-being of food producing animals [1]. Pain management during routine piglet husbandry practices (also known as processing), such as castration and tail docking, is of particular concern. Over 112 million pigs were marketed in the US in 2013 [2] and in 2014 over 2 million metric tons of pork were exported to other countries [3]. Practically speaking, half of those were castrated males and nearly all were tail-docked. Although piglets have appeared to adapt to these husbandry practices, there continues to be requests from those whom work directly and indirectly with animal agriculture to consider pharmacological interventions to modulate acute and chronic pain.

In the US, there are currently no Food and Drug Administration (FDA)-approved drug regimens for livestock pain relief in livestock and analgesia is not routinely provided at processing time. Analgesics such as nonsteroidal anti-inflammatory drugs (NSAIDs), specifically injectable meloxicam, are approved in other countries for the relief of post-operative pain in swine with minor soft tissue surgery such as castration [4]. Oral meloxicam has been shown to provide analgesia to bovines at the time of castration [5,6], which logically leads to the application of the same concept in swine.

Since piglets are castrated and tail-docked while still suckling milk from their dam, a novel manner of meloxicam administration would be through this route. Administering oral meloxicam to sows during lactation would potentially provide analgesia during processing procedures by allowing passive drug transfer through her plasma, into the milk that in turn would be consumed by her piglets. This route is safer for both the handler and the animal when
compared to injections because there is no needle usage or entry into the sows farrowing stall. It is also easily administered and allows a large number of animals to be medicated at once, thus eliminating the need for individual injections. Although there are no peer-reviewed studies demonstrating transmammary analgesia in swine, NSAIDs have been shown to transfer through milk in both cattle [7] and humans [8,9].

It is this void in the scientific knowledge that provided for this proof of concept study demonstrating the successful transfer of meloxicam from sows to piglets through milk at processing, with described effects of analgesia, pain biomarkers, and initial pharmacokinetic parameters.

**Thesis Organization**

This thesis is organized in journal paper format. Chapter 1 includes a general introduction followed by a review of the literature. Chapters 2 and 3 are the author’s research projects prepared in manuscript form for publication. Jessica L. Bates was the primary researcher and author of all manuscripts with assistance from co-authors. Chapter 4 is the general conclusions and implications of the conducted research.

**Literature Review**

**Surgical castration and tail docking**

Historically, the castration of pigs has been carried out for the following benefits: 1) prevention of unwanted breeding 2) behavioral modification, and 3) improvement in meat quality [10,11]. Most pigs in the US are marketed at the cusp of age and weight for puberty induction. The potential exists for exhibiting reproductive behavior, which is detrimental to
market growth efficiency [10]. Intact males are more aggressive and have increased mounting behavior, which can result in health problems and physical characteristic problems, such as leg injuries [10]. These leg injuries and possible subsequent lameness, along with skin lesions are also painful and can detrimentally affect animal well-being. Finally, consumers, especially in the US, are averse to eating pork with “boar taint”, an unpleasant odor and flavor due to androstenone and skatole produced in the testicles [10]. Androstenone is a testicular steroid that accumulates in fat tissue manifesting a urine-like odor and skatole is a product of tryptophan breakdown in the gut exhibiting a fecal-like odor [11].

Tail docking in piglets is also carried out to protect against tail biting. Tail biting is considered to have multiple etiologies and previous work has considered physical (i.e. floor type), environmental, nutritional, and feeding management, over-crowding, gender, genetics, length of tail and lack of substrates [12]. Kritas and Morrison found an increased association between severity of swine tail bite lesions and lung abscesses, pleuritic lesions, and prevalence of external carcass lesions and carcass trimming at the abattoir [13]. Practices used on commercial farms include surgical docking (using side cutters or a sharp knife to remove the tail) or using a cautery iron to sever the tail.

**General measurement of pain**

It is a generally accepted thought that piglet castration and tail docking causes pain and distress [14-16]. Evidence based pain management in domestic animals depends on two factors: first, an ability to assess pain effectively and accurately under clinical conditions and, second, having the tools with which to alleviate the identified pain [17]. Research to date on pain assessment in animals has tended to use one of three approaches: 1) measures of general body
functioning such as food and water intake or weight gain, 2) measures of physiological responses such as plasma cortisol concentrations, and 3) measures of behavior such as vocalizations [18].

**Pain biomarkers**

Several physiological parameters to castration pain have been measured, and these parameters are often termed pain biomarkers. A biomarker is officially defined by National Institutes of Health as “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [19]. Biomarkers contribute knowledge about clinical pharmacology and provide a basis for the designing of clinical trials. They expeditiously and definitely evaluate safety and efficacy while at the same time providing information for guidance in dosing and minimize inter-individual variation in response. They may be especially helpful in “proof of concept trials” [19]. A recent systematic review of pain management during routine piglet processing procedures highlighted the need for additional validation of pain biomarkers in peer-reviewed studies [20,21]. This thesis will describe the use of plasma cortisol, plasma substance P and infrared thermography in detail.

**Plasma Cortisol**

Glucocorticoids are secreted in response to a real or perceived stressor, such as castration, and are generally considered to be a stress indication [22]. Plasma cortisol has long been described in scientific literature as a standard physiological parameter to monitor at piglet processing [12,14,22-32]. Plasma cortisol concentrations are generally shown to peak at 30 minutes after processing [14,21,23,24,29,31]. In light of the short lasting increase, Hay suggested that their measurement may not be relevant for monitoring sub-chronic or chronic pain [33].
Piglet plasma cortisol does have limitations as an objective pain biomarker. For instance, stressors such as handling may cause an increase in plasma cortisol concentrations. However, research by Prunier et al. suggests that sham-castrated pigs have lower amplitudes and durations of plasma cortisol than castrated piglets [14], and these are likely to be connected to pain or tissue damage [31]. Until more pain biomarkers can be clearly described and validated, cortisol remains one of the most easily and accurately identifiable means of describing piglet pain.

Substance P

Substance P (SP) is a neuropeptide considered to function as a neurotransmitter or neuromodulator in both the central and peripheral nervous systems, as well as eliciting a variety of biological responses. These include stimulation of smooth muscle contraction, exocrine and endocrine gland secretion and plasma extravasation, as well as being involved in the regulation of immune and inflammatory responses [34]. Substance P’s effects are localized in the primary sensory neurons and neurons intrinsic to the GI, respiratory, and genitourinary tracts [35]. These effects are also involved in the integration of pain and stress [36,37].

There is a singular peer-reviewed study describing the use of SP as a pain biomarker at piglet castration. Sutherland et al. found no significant differences in SP levels between castrated and sham-castrated piglet [23]. However, in bovines, Coetzee et al. reported observed elevated SP levels in castrated calves compared to their non-castrated counterparts [6]. Although SP has the potential to accurately characterize physiological pain, further research is needed to determine its value in swine.
Other Plasma Pain Biomarkers

Several other studies describe a variety of physiological pain indicators. These include adrenocorticotropic hormone (ACTH)\cite{14,38-40}, serum amyloid A (SAA)\cite{41-43}, β-endorphin\cite{32,38,40}, C-reactive protein\cite{23,43}, epinephrine\cite{33} and lactate\cite{14,25}. However, there is a lack of consistent data to make a compelling argument for the use of any one of these as an objective pain biomarker.

Infrared thermography

Infrared thermography (IRT) involves the measurement of infrared radiation emitted by an object, which allows the surface temperature to be determined \cite{44}. Specialized cameras produce images that show temperature variation on a body surface by their representation on a colored scale. Infrared thermography examines many aspects of thermal physiology, including injury and disease diagnosis \cite{45}. Animals that are stressed or in pain can exhibit decreases in cutaneous temperature due to sympathetic nervous system activation, which causes vasoconstriction, shifting of the blood from the skin to the organs, and loss of heat in the periphery of the body \cite{45,46}.

In swine, IRT was also used by Hansson et al. to measure temperatures at 24 hours after piglet castration. Piglet ear temperature was observed to be significantly higher in control piglets versus those given either lidocaine or a lidocaine/meloxicam combination. In that same study, no significant differences were observed when measuring the skin around the castration site \cite{42}. This one-time measurement reflects the differences noted in this study up to 24 hours after castration but fails to provide a depiction of chronic piglet pain.
Schmidt et al. also investigated the use of IRT when determining disease states in sows and found the eye (near the medial and lateral canthus) and the back of the ear to show promise as locations for accurate measurement of sow temperature during disease states [47]. Wariss et al. also described the use of IRT to illustrate stress at the abattoir and potential to assess meat quality [48]. They found ear temperature to be positively correlated with creatine kinase (CK) and blood temperature to be positively correlated with cortisol. Higher temperature, CK, and cortisol values are all associated with higher levels of stress.

Infrared thermography has been shown to be a valuable tool for pain assessment in beef and dairy calves by non-invasively measuring autonomic nervous system responses to stress [49] during dehorning and castration [45,50]. It is a promising tool for application to determine acute and chronic pain in piglets at the time of castration.

**General anesthesia**

Once pain is objectively described, methods for its alleviation are applied. Several methods of anesthesia and/or analgesia have been tested to determine the most effective manner of pain mitigation during piglet processing. Some of these have more potential for practical application than others. General anesthesia is defined by a loss of consciousness in addition to loss of sensation, induced by a drug that acts on the central nervous system [51]. It can involve the use of inhaled or injectable agents.

**Inhalation anesthesia**

Inhalation anesthesia has several potential benefits, including a relatively brief induction time, short-term effects with reversibility, and needle-free administration [10]. However, many of these anesthetic agents are controlled drugs and necessitate specialized equipment, both of
which are reasons why it would not be practical for on-farm use. Additionally, these inhalation anesthetics may intensify the duration of handling and behavioral distress during castration, increase costs, and involve certain expanded legal and human safety regulations [10].

Various inhalant anesthetic agents have been studied piglet anesthesia. Walker et al. demonstrated isoflurane to have mixed success as an anesthetic and analgesic agent. At castration, anesthetized piglets had less reaction measured by palpebral reflex compared to non-anesthetized piglets, but no differences in physiological markers of pain were noted, such as ACTH and β endorphin values [38]. However, Schulz et al. observed no significant changes in plasma cortisol concentrations and deemed isoflorane to be ineffective in controlling piglet pain at castration [52] [53]. Nitrous oxide was characterized as effective at inducing anesthesia during piglet castration, but its analgesic effects were insufficient in preventing pain at that time [10].

Sutherland et al. also applied CO₂ inhalation anesthesia in combination with flunixin meglumine. However, neither of these applications markedly reduced the pain-induced distress caused by castration in pigs [23]. Plasma cortisol response was not reduced, SP was increased along time spent lying after post-operatively and increased high-frequency stress vocalizations at castration. Advantages of CO₂ according to Sutherland are the speed at which CO₂ can be administered and then the piglet returns to consciousness, and the fact it is an unrestricted drug with no known drug residues [23]. Both Gerritzen et al. and Zimmerman et al. reported that CO₂ was not effective for castration, due to a significant increase in adrenaline and noradrenaline, and behavioral abnormalities indicative of stress such as open-mouth breathing and seizures [54,55]. Kohler et al. also reported piglets displayed violent struggling, increased vocalization, elevated ACTH and β endorphin levels when CO₂ was used [40].
**Injectable Anesthesia**

Some of the earliest studies of general anesthesia by McGlone et al. involved the IV administration of xylazine, ketamine hydrochloride and glyceryl guaiacolate. Twenty-eight percent of two-week old piglets died within 24 hours of administration. When the same protocol was repeated with 6 to 7 week old piglets, there was 0% mortality, although they nursed 1.5 less times in the 3-hour period post castration, compared with other piglets that did not receive general anesthesia [56].

Other early attempts at alleviating piglet pain at castration include butorphanol administered IV to 8-week-old piglets. Although this analgesia reduced feeding time and weight gain in castrated piglets compared to non-castrated counterpart, this research demonstrated no measurable behavior changes (in the form of reduced suckling and standing times, along with increased lying times) between those groups [16].

Azaperone, a sedative labeled for swine in Europe, has been used pre-operatively to diminish stress and painful reactions of piglets at castration whether alone [57] or combined with ketamine [58]. However, work by Driessens et al. reported that azaperone decreased the intensity of vocalizations (in decibels) during physical castration in one-week old piglets compared to their non-treated castrated counterparts, rather than providing analgesia. Schmidt et al. reported piglets castrated with azaperone exhibited increased a 200% increase in time away from the sow teat area [58].

**Local Anesthesia**

The use of local and topical analgesics during piglet castration has also been explored. Cutting and/or tearing of the spermatic cord has been shown to be the most painful part of
castration [15]. Several local anesthesia products have been tested. Lidocaine is a membrane stabilizer that abolishes the ability of an electrical stimulus to elicit an action potential and therefore prevents pain stimuli to reach the central nervous system [59]. Ranheim et al. demonstrated after an intratesticular injection of lidocaine, drug concentrations reached peak levels at three minutes post-injection [60]. Lidocaine decreased pain responses in piglet castration when administered pre-castration. Castrated piglets had a lower frequency of vocalizations (Hz) and less resistance movements compared to their non-lidocaine counterparts due to the significant decrease in vocalizations during castration [25,42,61] and plasma cortisol levels [25]. Lidocaine injected on each side of scrotum, then 10 minutes later intratesticularly, was found effective for decreasing pain-associated behaviors, such as lying, huddling, and eating, in 2-week-old piglets [56].

Topical anesthesia has the potential to reduce needle use and the need to handle piglets twice (once for local administration, then for the actual act of castration) [62]. Topical cryogen spray and local procaine hydrochloride with epinephrine were found to be ineffective at reducing plasma cortisol levels prior to castration in castrated piglets compared to non-castrated piglets [26] in castrated piglets compared to non-castrated piglets. Sutherland et al. used short-acting (cetacaine, benzaine, butamben, and tetracaine hydrochloride) and long-acting (lignocaine, bupivacaine, adrenaline, and cetrimide) topical anesthetics but both were ineffective in reducing cortisol levels and percentages of stress vocalization in castrated piglets compared to those who were sham castrated. Additionally, piglets receiving the short acting topical anesthetic had worse wound healing scores at the surgical site 9 and 14 days post-castration. [62].

A product called Barrier Wound Spray® has been marketed for use at piglet castration. It contains 2% of both lidocaine and povidone iodine [63], however, there is little peer-reviewed
data to support its use. In one study by Hawkins et al. no observed differences in behavioral pain were noted in piglets at castration, although there is potential for increased wound healing [64].

**Nonsteroidal Anti-Inflammatory Drugs**

Nonsteroidal anti-inflammatory drugs, or NSAIDs, by definition are a group of drugs that have analgesic, antipyretic, and anti-inflammatory properties, due to their ability to inhibit prostaglandin synthesis [65]. NSAIDs are advantageous as pain management tools, for example, these advantages include a longer duration of effect, anti-inflammatory and analgesic activity, minimal behavior effects, lack of cardiorespiratory effects, readily available oral formulations, non-controlled drug status, and a license for use in food animals in other countries [66].

The development of NSAIDs with analgesic properties in the last two decades has revolutionized analgesia [17]. Early attempts at alleviating piglet pain at castration include oral aspirin, which demonstrated no measurable effects on piglet time spent drinking, lying, and standing post-castration[16]. Paracetamol, administered as a rectal suppository, was also demonstrated to provide effective post-operative analgesia in piglets using physiological biomarkers such as temperature, heart rate, respiratory rate, and blood pressure. However, paracetamol’s half–life is relatively short at 62 minutes [67] compared to meloxicam (8 hours) [68].

Tolfenamic acid is another NSAID with potential for pre-emptive use in piglet castration. However, it has not been widely documented and when published, results are mixed. Zoels et al. showed a reduction in piglet cortisol after castration [29], however, Wavreille et al. demonstrated conflicting results with an increase in plasma cortisol post-castration, when compared to meloxicam and sham treated groups [69]. Finally, research by Zoels et al. showed both
tolefenamic acid and meloxicam reduced piglet plasma cortisol levels postsurgical after piglet castration with no difference between these two products [29].

Flunixin meglumine (FM) has also been examined as a route of analgesia in piglet processing. Marketed under the trade name of Banamine-S® (Merck Animal Health), it is labeled as an antipyretic agent in swine [70]. Sutherland et al. lists the advantages of FM as IM administration, its approved use in swine (Banamine-S®), and can be easily administered by trained staff with only one handling of the animal [23]. When administered both pre- and immediately post-castration, FM decreased the cortisol levels out to an hour post-castration [24,27,71] and stress calls of piglets [24] when compared to those receiving no analgesia. However, this same study also demonstrated that wound healing was significantly delayed in analgesic-treated groups out to four days post-castration [24]. In addition, it has been shown that FM has evidence of macroscopic and histopathological injection site lesions, causing much more significant damage than meloxicam [72].

Ketoprofen has also been investigated in piglet castration. It is labeled for use in swine in Canada for the treatment of fever and inflammation associated with respiratory infections [73]. Von Sonja et al. indicated that administration 10-30 minutes pre-castration reduced plasma cortisol and ACTH concentrations when compared to controls [39]. Cassar et al. supported these findings with a decrease in plasma cortisol at 90 minutes post-castration using ketoprofen [74].

**Meloxicam**

Meloxicam is a NSAID of the oxicam class[75]. It is approved for swine in the EU and Canada for several conditions, including non-infectious locomotor disorders to reduce the
symptoms of lameness and inflammation, the adjunctive therapy in the treatment of MMA (mastitis-metritis-agalactia syndrome), and the relief of post-operative pain associated with minor soft tissue surgery such as castration[4].

**Mechanism of Action**

Meloxicam’s mechanism of action involves a disruption in the inflammatory cascade, inhibiting the production of prostaglandins and other inflammatory mediators [67]. When cell membranes are damaged due to a trauma or painful stimulus, there is a release of arachidonic acid. Arachidonic acid is a substrate for prostaglandins, including PGE$_2$ and cyclooxygenase (COX).

PGE$_2$ is an endogenous pyrogen, leading to upward resetting of the temperature regulating center in the anterior hypothalamus [76]. PGE$_2$ can also synergize with primary inflammatory mediators such as histamine and bradykinin to cause hyperalgesia and allodynia. The net effect is a sensitization of pain receptors, thus lowering the pain tolerance threshold. In response to an effective analgesic, PGE$_2$ plasma concentrations are reduced, providing decreased nociocepetion following noxious stimulus.

Cyclooxygenase exists in isoforms. Cyclooxygenase-1(COX-1) is present constitutively in most types of cells and has numerous functions in homeostasis, including blood clotting, regulation of vascular integrity, and protection of the renal and gastric endothelium. Cyclooxygenase-2 (COX-2) is inducible at the cellular level and found at sites of inflammation, producing pro-inflammatory effects. Meloxicam is relatively specific for the COX-2 isoform, which is believed to play the major role in inflammation. A third isoform, cyclooxygenase-3 (COX-3), recently described in the central nervous system of the dog, is a spliced COX-1
variant and has been postulated to be responsible for some of the analgesia produced by NSAIDS [77].

Newer NSAIDS, such as meloxicam, with partial specificity for COX-2 inhibition, are termed COX-2 preferential and those with no significant effect on COX-1 are described as COX-2 selective[77]. Due to this specificity in meloxicam, the adverse effects due to inhibition of the housekeeping functions of COX-1, such as maintenance of renal and gastric mucosa and regulation of blood flow are avoided [67].

**Pharmacokinetics of Meloxicam**

NSAIDs, including meloxicam, are generally well absorbed after oral dosing due to their properties as weak organic acids and moderate-high lipid solubility. As a general rule, the volume of distribution is relatively low, particularly penetration of most NSAIDs into milk in the absence of mammary gland infection is poor, with milk concentrations being of the order of 1% or less of plasma total (protein-bound plus free) concentration. This results from the high degree of binding to plasma protein. Distribution into milk is also limited by the Henderson-Hasselbalch mechanism, as milk pH is less than that of plasma [76].

Due to their weakly acidic nature, the elimination of most NSAIDs in urine varies with urine pH. However, the overriding factor of this is the high degree of plasma protein binding of NSAIDs, which limits passage into glomerular ultrafiltrate [76]. Meloxicam is cleared almost exclusively metabolically, with only low levels of parent compound detected in bile, urine, and feces. Therefore, biotransformation governs the elimination of parent compound in all species. In a broad, species-comparative study, the main metabolites of meloxicam in humans, rats, mice, and mini-pigs was found to be a 5’-hydroxymethyl derivative and a 5’-carboxy derivative.
The excretion balance was similar to that in humans, with approximately half of the dose being eliminated in the urine and one half in the feces [68].

*Analgesic Effects of Meloxicam*

Research shows ambiguous results when meloxicam is administered at the time of castration with some studies indicating a reduction in piglet pain while others describe no differences observed using analgesia. Meloxicam had been reported to provide effective post-operative pain relief when compared with paracetamol in the manner of reducing a global pain score, consisting of piglet lameness, vocalization, and restlessness [67]. However, Reiner et al. found no observed differences between piglets treated with NSAIDs (meloxicam and FM) post castration [24]. Piglet plasma cortisol levels and vocalization of all groups were all higher than non-castrated controls, and piglets with analgesics had slower wound healing time than those that did not[24]. Despite these perioperative studies, when given preoperatively meloxicam was demonstrated to significantly reduce plasma cortisol concentrations compared to piglets not given analgesia [27-29,78].

Meloxicam also has an indirect effect on piglet well-being by directly affecting sow health and welfare. It is labeled in the EU and Canada for the adjunctive therapy of septicemia and toxemia associated with mastitis-metritis-agalactica syndrome (MMA) [4]. The pathogenesis of MMA involves bacterial endotoxins acting to release mediators of acute inflammation such as prostaglandins. As previously noted, meloxicam inhibits the production of such inflammatory mediators. Additional studies have demonstrated that meloxicam administered to the sow at farrowing decreases preweaning mortality [79-81], reducing the mortality rate by over 50% in litters of sows affected with MMA [81]. Most notable was the
30% reduction of piglet mortality rate in sows requiring farrowing assistance. This supports the theory that sows suffer from post-labor pain causing distress and decreased care for their piglets [79]. Nonsteroidal anti-inflammatory drug treatment such as meloxicam after birth could improve sow welfare and piglet survival [79].

When production parameters are analyzed, studies demonstrated no increases in average daily gain and piglet feed consumption [25,28] when meloxicam is given pre-or pericastration. However, in herds where MMA may be clinical or subclinical, meloxicam has been shown to significantly increase piglet average daily gain, with meloxicam being superior to both FM [82] and tolfenamic acid [80].

**Transmammary Drug Transfer**

**Normal Swine Lactational Physiology**

The transmammary route provides a unique opportunity for piglet medication during regular feeding. Piglets suckle approximately every 45-50 minutes and the mammary gland refills back to capacity within 35 minutes [83]. Piglets may stimulate milk let down for a period of minutes through nuzzling the mammary gland, but actual milk ejection from the gland is only about 15 seconds [83]. At the end of gestation and during the colostral phase, the junctions between epithelial cells surrounding the alveoli are not tight. This allows serum transudate to leak from the bloodstream into the mammary secretions and milk components from the mammary gland alveoli to leak back to the bloodstream [84]. Despite the fact that the colostral phase is ending at the latest by 24 hours after parturition, there is still potential for the mammary epithelium to have the ability to pass drugs through to the milk. Due to the continuous cycle of lactation, there is great potential to medicate baby piglets with an analgesic for several days.
Swine Transmammary Drug Transfer

Aside from the work reported in this thesis, no current peer-reviewed literature describes the transfer of any drug via the transmammary route after oral administration in swine. A recent National Pork Board report describes research that found that injectable meloxicam is transferred through the milk to piglets at processing [85]. However, this transfer utilized a one-time intramuscular dose of 1 mg/kg meloxicam to each sow, which resulted in 2.647 ng/mL of meloxicam in the piglet serum at 5 hours after administration [85]. Piglet plasma meloxicam levels in this report were only measured out to this time point. The combination of oral administration to sow with lactational transfer to piglets has to this point not been described.

Transmammary Drug Transfer in Other Species

In the absence of swine data, other species can be used to demonstrate the possible effects of transmammary NSAID transfer. Meloxicam has also been indicated to transfer through placenta and milk in rats. After oral dosing with a radioactively marked meloxicam (5 mg/kg/day), steady state conditions were achieved by the third dose. Radioactively-marked meloxicam was used to study elimination in rat milk nursing 9 to-11 day pups. Oral administration (5 mg/kg/day) resulted in higher concentration of radioactivity in milk than plasma at 5 hours and 24 hours post dosing. Sixty to 70% of radioactivity in the milk was associated with the unchanged parent compound [68]. There was a curious pharmacokinetic gender difference in that study. Although identical initially in distribution phase, female rats demonstrated considerably higher meloxicam concentrations compared to male rats. Female rat area under curve (AUC) was 217 mg*eq*hr/L and male rat AUC 70.9 mg*eq*hr/L, resulting
from a slower rate of elimination. Elimination half-life of meloxicam in females was more than double that of males (37 hours vs. 13 hours) [68].

Malreddy et al. established that meloxicam administered orally to dairy cows at 1 mg/kg did have discernible milk levels out to 80 hours after administration. The mean (±SD) plasma C\text{max} and T\text{max} for meloxicam were 2.89 ± 0.48 μg/mL and 11.33 ± 4.12 hours, respectively, while the mean (±SD) milk C\text{max} and T\text{max} for meloxicam were 0.41 ± 0.16 μg/mL and 9.33±3.11 hours, respectively.

Human lactation has the largest body of research observing the transfer of NSAIDs to breast milk. Since analgesics are often administered to women after childbirth, whether for a Caesarian section or other painful procedure, there is often a concern regarding the possible transfer of these medications to infants. Studies demonstrate mean milk doses of NSAIDs (weight adjusted with maternal doses) to be ranging from 0.16%-0.60% of the maternal doses [8,9,86,87]. These studies included the investigation of oral dosing of ketoprofen, celecoxib, and ketolorac tromethamine. All of these studies concluded these levels would be much below the toxic dose for infants.

Drug transfer into human milk is largely a function of lipophilicity, molecular weight, protein binding, negative logarithm of the acid dissociation constant (pKa), and maternal plasma concentration[8]. The amount of most NSAIDs in milk are expected to be low because they are weak acids, lipid-soluble, and mostly protein-bound in plasma [86]. Despite extensive literature searches, an accurate amount of the milk consumed by piglets is difficult to estimate, along with the pharmacokinetics of any NSAID in the lactating sow. However, much as in swine, during
the early human postnatal period, the tight junctions between lactocytes are underdeveloped. Thus, the potential elevations of milk/plasma ratios are suspected [8].

A thorough review of the scientific literature demonstrates there is a need for and great opportunity to explore further the area of piglet castration analgesia. Combining an established analgesic drug, such as meloxicam, in a novel route using exciting knowledge about pain measurement and lactation establishes the foundation for new research in the areas of pharmacokinetics and pain biomarkers.

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CHAPTER 2. SUCCESSFUL TRANSFER OF TRANSMAMMARY-DELIVERED MELOXICAM IN SWINE FOR USE IN ANALGESIA AFTER CASTRATION AND TAIL DOCKING

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Abstract

To investigate a novel route for providing analgesia to processed piglets via transmammary drug delivery, meloxicam was administered orally to sows after farrowing. The objectives of this study were to prove the transmammary delivery of meloxicam from sows to piglets and describe initial pharmacokinetic parameters. Ten sows received either meloxicam (30 mg/kg) (n=5) or whey protein (placebo) (n=5) in their daily feedings, starting four days after farrowing and continuing for three consecutive days. During this period, blood and milk samples from sows and piglets were collected at 12-hour intervals. On Day 5 after farrowing, three boars and three gilts from each litter were castrated or sham castrated, tail docked, and administered an iron injection. Piglet blood samples were collected immediately before processing and at
predetermined times over an 84-hour period. Plasma was tested to confirm meloxicam concentrations using a validated high-performance liquid chromatography-mass spectrometry method. Meloxicam was detected in all piglets nursing on medicated sows at each time point, and the mean (±standard error of the mean) meloxicam concentration at castration was 568.9 ±105.8 ng/mL. Sow plasma and milk, along with piglet plasma reached steady concentrations at 36 hours after the administration of meloxicam. Furthermore, ex-vivo prostaglandin E₂ (PGE₂) synthesis inhibition was greater in piglets from treated sows compared to controls (p=0.0059). However, button gastric ulcers and subacute gastritis were noted in 2/5 meloxicam-treated sows and their litters. Initial sow oral meloxicam pharmacokinetic parameters (±SD) were calculated including absorption rate constant (ka) of 0.13 (0.05) hr⁻¹, volume of distribution (V) 149.65 (8.94) mL/kg, and clearance (Cl) of 18.86 (0.45) mL/hr/kg. Although there is great potential benefit to this method in the realms of analgesia, safety, and efficiency, dose refinement and a full understanding of extra-label drug use regulatory issues is needed before its application into swine.

**Keywords:** meloxicam, swine, lactation, castration, analgesia

**Introduction**

Pork producers and consumers are increasingly concerned about livestock well-being. The management of pain during routine swine husbandry practices, such as castration and tail docking in piglets, is of particular concern. The European Union (EU) recently legislated to ensure that all piglets are castrated using analgesia/anesthesia. However, in the United States, there are currently no Food and Drug Administration (FDA)-approved drug regimens for livestock pain relief, and analgesia is not routinely provided at the time of processing.
Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID) that is approved for swine in the EU and Canada for several conditions, including the relief of post-operative pain with minor soft tissue surgery [1]. When injected before piglet castration, meloxicam reduces serum cortisol concentrations [2], [3], [4]. Meloxicam has also been shown to reduce behavioral signs that are associated with piglet distress at castration and is considered to be superior to other analgesics when assessing pain-related behavioral criteria [5].

Administering oral meloxicam to sows during lactation could potentially provide analgesia during processing procedures by allowing passive drug transfer through the milk to entire litters. This route is safer for both the handler and the animal when compared to injections with a needle. It is also easily administered and allows a large number of animals to be medicated, thus eliminating the need for individual injections. Prior to this study, there were no peer-reviewed studies demonstrating transmammary analgesia in swine, although NSAIDs can transfer through milk in both cattle [6] and humans [7], [8], [9]. The objectives of this study were to prove the transmammary delivery of meloxicam from sows to piglets and describe initial pharmacokinetic parameters. The findings demonstrate the successful transfer of meloxicam from sows to piglets through milk and associated physiologic proof of analgesia after processing, The effectiveness of this transfer is supported by a decrease in plasma PGE$_2$ levels. Preliminary sow pharmacokinetic parameters are comparable to those previously established [10].

**Materials and Methods**

Before the initiation of this study, all techniques regarding animal use, housing, handling, and sampling were approved by the Iowa State University Animal Care and Use Committee (IACUC # 8-12-7430-S).
**Animals**

Ten Yorkshire x Landrace sows at approximately one week prior to farrowing (average weight of 277.3 kg ±1.6 standard error of the mean [SEM]) were obtained from a commercial swine farm. Upon arrival, each sow was given a thorough physical exam and confirmed to be healthy and pregnant by a veterinarian, and a unique numerical ear tag (Allflex Global Ear Tags, Allflex USA, Inc., DFW Airport, TX) was applied to the right ear. Sows were housed at the Iowa State University Animal Resource Station in accordance with recommendations outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Use and Research and Teaching [11]. Sows were placed in Quad- or Euro-style farrowing stalls (Thorp Equipment, Thorp, WI), depending on availability. Both stall types were equally represented in both treatment groups. Regardless of stall type, each sow was housed in a farrowing stall area measuring 0.6 m × 2.1 m. Quad and Euro stalls had piglet creep areas of 7.0 m2 and 6.4 m2, respectively. After farrowing, a heat lamp was provided on each side of the creep area in each stall for the piglets.

**Feeding and treatment administration**

Prepartum sows were hand-fed 1.6 kg of an organic corn/soybean meal diet, which was confirmed to be free of meloxicam, twice daily. This diet was compatible with the National Research Council’s nutrient requirements for lactating sows [12]. Intake was gradually increased ad-libitum after farrowing. Sows had free access to water at all times through a nipple waterer in their stalls. On Day 4 after farrowing, sow treatments began and continued for six days. Sows assigned to the meloxicam-treatment group (n=5) received 30 mg/kg meloxicam (Meloxicam, Aurobindo Pharma, India, Batch X1513019-A, Expiration Date 2/2015), which was divided equally between two feedings at 0700h and 1600h. The meloxicam was ground from tablets into
a powdered consistency using a commercial grinder (Spice & Nut Grinder, Cuisinart, East Windsor, NJ), after which it was incorporated into each sow’s daily feed ration in a portable mixer (Kobalt Model #043206, Monarch Industries, Winnipeg, MB, Canada). Control sows (n=5) received 30 mg/kg of whey protein placebo (Health Watchers, Inc., Bohemia, NY), which is a pharmacologically inactive excipient used in the manufacturing of meloxicam tablets. The placebo was prepared in a separately marked bucket by thorough hand-mixing with gloved hands to prevent cross contamination.

**Animal phase study design**

The sows were allowed to farrow naturally without induction methods. They were then randomly assigned to two groups. The first sow to give birth was allocated to the meloxicam-treated group (MEL), and the second sow was allocated to the whey placebo group (CONT). The alternating pattern continued for the remaining sows, based on the farrowing date. The day of farrowing was designated as “Day 0” for each sow and litter. The overall time scheme of activities, including drug administration and sample collection, is detailed in Figure 1. On Day 3, post-farrowing piglets in the litter were weighed and ranked in a descending order. In each litter, the heaviest three boars and three gilts were selected and ear tagged (Allflex Global Ear Tags, Allflex USA, Inc., DFW Airport, TX). The next three heaviest piglets, regardless of sex, were selected, tagged and deemed as sentinels to specifically measure the inhibition of plasma prostaglandin E₂ (PGE₂) levels and demonstrate the pharmacodynamic effect of meloxicam. In total, nine piglets per litter were tagged. Two litters did not have three live boar piglets. In those instances, all available boars were used as test piglets. In another litter, a male test piglet was laid on and subsequently died after being identified but before blood sampling.
began. No other piglets were substituted into the test category. Cross-fostering was not performed at any phase of the study.

Piglet processing occurred on Day 5 after farrowing. After a pre-processing blood draw, the boars were immediately castrated and tail-docked. They then received 1 mL (100 mg) iron IM (Ferrodex 100, AgriLabs, St. Joseph, MO). Castration was performed in accordance with standard swine industry practices by making two vertical incisions approximately 2-3 cm long in the scrotum with a number-ten scalpel blade and scalpel handle, marsupializing the testicles, and finally providing manual pressure on the spermatic cord until it separated from the piglet’s body. Piglet tails were docked to an approximate length of 2 cm using a pair of side cutters. Immediately after each piglet castration and tail docking, the scalpel blade, handle and side cutters were immersed in a dilute chlorhexidine mixture for disinfection between each piglet procedure per typical swine industry practice. All castrations were performed by a single experienced swine veterinarian to minimize variation (JLB). Gilts were handled in a similar manner, and they also underwent tail docking and received iron.

Immediately prior to the administration of meloxicam, each sow had blood sampled not only to confirm a lack of meloxicam in the blood, but also for serum chemistry and complete blood counts (CBC) analyses. The serum chemistry and CBC testing was repeated on blood draws on both sows and piglets immediately prior to necropsy on Day 8. These samples were analyzed at the Iowa State University Clinical Pathology Laboratory in accordance with the specific standards of the lab.

Sow blood samples (8 mL/sample) were collected via the left or right jugular vein using a 25.4-mm, 16-gauge hypodermic needle (Air-Tite Products, Virginia Beach, VA) and 12-mL Luer lock syringe (TycoHealth Care, Mansfield, MA). During blood collection, sows were
manually restrained in their stalls using a pig snare. Piglet blood samples (2 mL/sample) were collected using the left or right jugular vein using a 3.8-cm, 22-gauge hypodermic needle (TycoHealth Care, Mansfield, MA) and 3-mL syringe (TycoHealth Care, Mansfield, MA). These samples were obtained using physical restraint by placing the piglet in a supine position.

On Day 8 after farrowing, sows were euthanized by a penetrating captive bolt, followed by exsanguination, and piglets were euthanized by blunt force trauma to the cranium, according to the American Veterinary Medical Association guidelines [13]. Necropsies were performed on the sows and processed piglets. The liver, kidney, gastric fundus, duodenum, semitendinosus/semimembranosus muscle, and fat were collected for analysis.

**Sample collection, processing, and analysis**

All drug concentrations in plasma were analyzed at the Iowa State University Veterinary Diagnostic Laboratory by the Iowa State University-Pharmacology Analytical Support Team (ISU-PhAST).

**Meloxicam analysis**

Blood for meloxicam analysis was placed in 10-mL and 3-mL heparinized blood collection tubes (BD Vacutainer, Franklin Lakes, NJ). Samples were centrifuged for 15 minutes at 1000 g at ambient temperature. The plasma was separated and placed into cryovials for storage at -80°C.

Plasma concentrations of meloxicam were determined using high-performance liquid chromatography (HPLC) (Accela Pump and Autosampler, Thermo Scientific, San Jose, CA, USA) with mass spectrometry (MS) detection (LTQ XL, Thermo Scientific, San Jose, CA, USA). Plasma samples, plasma spikes, and plasma quality control (QC) samples (200 µL each) were treated with 1 M trichloroacetic acid (100 µL) after the addition of the internal standard,
piroxicam (10 μL of 10 ng/μL). The samples were vortexed for 5 seconds and centrifuged for 20 minutes at 2000 g to sediment the precipitate. A portion of the supernatant (150 μL) was transferred to an injection vial that was fitted with a glass insert containing 100 μL of 1.9% ammonium hydroxide in 25% aqueous acetonitrile. The injection volume was set to 20 μL. The mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at a flow rate of 0.250 mL/min. The mobile phase began at 40% B with a linear gradient to 95% B at 4 minutes, which was maintained for 1.5 minutes, followed by re-equilibration to 40% B. Separation was achieved with a solid-core C18 column (KinetexXB-C18, 100 mm × 2.1 mm, 2.6-μm particles, Phenomenex, Torrance, CA, USA) that was maintained at 45°C. Piroxicam eluted at 2.6 minutes, and meloxicam eluted at 3.3 minutes. A full scan MS of the pseudomolecular ions of piroxicam (m/z 332) and meloxicam (m/z 352) was used for analyte detection. The sum of the intensities of ions at m/z of 115 and 141 were used for meloxicam quantitation. The internal standard, piroxicam, was quantitated with the sum of the ion intensities at m/z of 95, 121, and 164. Sequences consisting of plasma blanks, calibration spikes, QC samples, and porcine plasma samples were processed in batches with a processing method that was developed in the Xcalibur software (Thermo Scientific, San Jose, CA, USA). The processing method automatically identified and integrated each peak in each sample and calculated the calibration curve based on a weighted (1/X) linear fit. Plasma concentrations of meloxicam in unknown samples were calculated by the Xcalibur software based on the calibration curve. Results were then viewed in the Quan Browser portion of the Xcalibur software. Fourteen calibration spikes were prepared in porcine plasma covering the concentration range of 1–20,000 ng/mL. QC samples were prepared at concentrations of 15, 150, and 1500 ng/mL in duplicate with each set of samples. Calibration curves exhibited a correlation coefficient (R2) exceeding
0.997 across the entire concentration range. The QC samples at 150 and 1500 ng/mL were within 2–8% of their nominal values, and the low QC sample at 15 ng/mL differed from its nominal value by 10–15%.

**PGE$_2$ analysis**

PGE$_2$ concentrations were determined using methods that were previously described by Giorgi et al. [14]. Briefly, fresh piglet blood was collected into sterile tubes containing heparin. To stimulate ex-vivo PGE$_2$ production by monocytes, the heparinized whole blood was incubated for 24 hours at 37°C with 10 µg/ml lipopolysaccharide (LPS, derived from *Escherichia coli* 055:B5, Sigma Aldrich, St. Louis, MO), which was diluted in phosphate-buffered saline (PBS). The first blood collection occurred prior to treatments and was divided into two equal aliquots: one was incubated with LPS, and the other was incubated with an equivalent volume of PBS. These aliquots were used as positive and negative controls.

At the end of the incubation, all samples were centrifuged at 400 g for 10 minutes to obtain plasma: 200 µl of plasma were mixed with 800 µl of methanol (1:5 dilution) to permit protein precipitation. After a final centrifugation at 3000 g for 10 minutes, supernatants were collected and stored at -80°C.

The concentration of plasma PGE$_2$ was determined using an enzyme-linked immunosorbent assay kit (Cayman Chemical, Co, Ann Arbor, MI). The calculated coefficient of variation for intra-assay variability was 11.7%, and the inter-assay variability was 9.2%.

**Pharmacokinetic analysis**

Pharmacokinetic modeling used a population approach using Monolix (Lixoft, Version 4.3.3, Orsay, France) to fit a one compartment open model with oral absorption in the sows, using previously published data [88] to inform the model. Sow plasma meloxicam values were
analyzed and the parameters calculated include sow absorption rate constant (ka), volume of distribution (V), and clearance (Cl).

**Statistical analysis**

Data were analyzed using generalized linear mixed models fitted with the GLIMMIX procedure of SAS (SAS Institute Inc., Version 9.2). Treatment, procedure, time, and their interactions were used as fixed effects, whereas sow was a random effect, and piglet was the subject of repeated measures. A separate linear mixed model was run to study the effect of meloxicam concentrations on PGE\(_2\) by using Meloxicam_Levels as an explanatory variable. Baseline measurements were used as covariates in the above models. Model assumptions were considered to be appropriately met, based on diagnostics that were conducted on studentized residuals. Estimated least square means and corresponding standard errors, or 95% confidence intervals, are presented. A significant difference was considered to exist when p≤0.05, and a marginal difference was considered to exist when 0.05 < p≤0.10. Relevant pairwise comparisons were conducted when the significance of the interaction term was p≤0.10, using Tukey-Kramer adjustments as appropriate to avoid inflation of the Type I error rate due to multiple comparisons. Sow and piglet CBC and serum chemistry data was analyzed in a standard t-test using JMP (SAS Institute, Inc., Version 11.0, Cary, NC).

**Results and Discussion**

**Plasma meloxicam concentration**

The primary objective of this study was to confirm that the transmammary route of administration is achievable in piglets and describe initial pharmacokinetic parameters. Meloxicam was detected in the plasma of all piglets in the MEL group at every time point after treatment commenced (Figure 2). The mean ± standard error of the mean (SEM) meloxicam
concentration at castration was 568.9 ±105.8 µg/mL. No meloxicam was found in the CONT piglet plasma. In a recent National Pork Board (NPB) report [15], Brown reported that meloxicam administered IM to sows at processing time of the piglets was transferred through the sows’ milk to piglet. However, this transfer utilized a one-time intramuscular dose of 1 mg/kg meloxicam to each sow, which resulted in 2.647 ng/mL of meloxicam in the piglet serum at 5 hours after administration. Piglet serum meloxicam levels in this NPB study were only measured out to 5 hours. Due to differences in routes of administration and study design, further comparisons cannot be made.

Plasma meloxicam concentrations in both sow plasma, sow milk, and piglet plasma maintained steady-state concentrations for the duration of the treatment period. Plasma meloxicam concentrations began to decline after 72 hours, when the treatment was discontinued in the feed. It is important to note that sow plasma, sow milk, and piglet plasma meloxicam concentrations ran parallel to each other for the treatment period (Figure 2). Figure 3 presents the individual plasma profiles for meloxicam administered to the five sows in the current study at a dose of 30 mg/kg PO. In order to achieve the noted concentrations of meloxicam in piglet plasma, mean (±SEM) sow plasma concentrations at castration (24 hours after the start of administration) were 18,127.80 (2872.1) µg/mL. This emphasizes the need to formulate a sow dose large enough to achieve analgesic levels in piglets.

Table 2 describes the raw data relating meloxicam concentrations in piglet plasma, sow plasma, and milk. When considering the mean of all meloxicam concentrations, piglet plasma concentration is 1.1% of sow plasma and 16.5% of sow milk, while sow milk is 6.5% of sow plasma. To illustrate the extremes, the sow with the highest plasma meloxicam concentration was compared to the sow with the lowest plasma meloxicam concentration. In all three cases,
piglet plasma was represented as a percentage of sow milk and sow plasma, as well as sow milk as a percentage of sow plasma. Interestingly, the sow with the highest plasma meloxicam also transferred the largest amount to the milk, but its piglets had a lower plasma concentration that the sow with the lowest plasma meloxicam concentration. This illustrates the variability in transmammary drug transfer and possible piglet clearance factors that are yet to be elucidated.

Since this study was designed to prove the concept of transmammary meloxicam transfer, the appropriate sow dose was unknown. The dose for this study was extrapolated from estimates about swine lactation and data about transmammary transfer of meloxicam in dairy cattle [6]. Due to the nature of NSAIDs such as meloxicam and known pharmacokinetics in other species, this large sow dose and resulting sow plasma concentration for effective piglet plasma concentration is expected. Drug transfer into human milk is largely a function of lipophilicity, molecular weight, protein binding, negative logarithm of the acid dissociation constant (pKa), and maternal plasma concentration [8]. The amount of most NSAIDS in milk is expected to be low because they are weak acids, lipid-soluble, and mostly protein-bound in plasma [16]. However, as in swine, during the early human postnatal period, the tight junctions between lactocytes are underdeveloped. Thus, the potential elevations of milk/plasma ratios are suspected [8].

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mg/kg did have discernible milk levels [6]. These meloxicam concentrations in milk remained at low residue levels in the milk after plasma drug concentrations had fallen below effective levels [6].

**PGE$_2$**

PGE$_2$ was inhibited in MEL piglets as a result of treatment, demonstrating a treatment effect (p=0.0059) and differences (p<0.05) at each time point, with the exception of 24 hours after drug administration (p=0.0909) (Figure 4). However, using this analysis, there was no time-by-treatment interaction (p=0.1763) or effect of time (p=0.6064).

This inhibition of PGE$_2$ by meloxicam was anticipated, as a result of the blockage of the arachidonic acid pathway and cyclooxygenase-2. Prostaglandins contribute to the amplification of pain signaling by increasing nociception sensitization [18]. As such, reducing PGE$_2$ concentrations would provide decreased nociception following noxious stimuli, such as castration. Mean piglet plasma PGE$_2$ concentrations in all piglets sampled ranged from 66.2–719.9 pg/mL. These levels are much lower than those reported in equines (1.7 ng/mL), canines (329 ng/mL) and felines (0.7 ng/mL) [19]. No porcine comparisons are available in the literature. There are several potential explanations for the lower PGE$_2$ levels. First, the piglets were relatively young and blood from these animals may not have fully responded to LPS stimulation. Second, other studies have used different strains of LPS or different amounts of *E.coli* that are needed for LPS production. Finally, ex vivo stimulation of whole blood was used, which may contribute to lower levels. Despite these species-specific differences, decreases in plasma PGE$_2$ were observed at most time points in MEL piglets compared to CONT piglets. This suggests that meloxicam was successfully transferred through milk to piglets at concentrations that likely provided analgesia based on the demonstrated ex vivo inhibition of PGE$_2$ production.
**Side effects of meloxicam administration**

Health and wellbeing of sows and piglets was closely observed throughout the study. No adverse clinical effects were noted in any animal throughout the entire duration of the study. Pre-and post-treatment CBC and serum chemistry testing were all within the normal levels for swine. MEL sows had elevated blood urea nitrogen concentrations (p=0.03), compared to CONT sows (23.2 mg/dL and 13.8 mg/dL, respectively), yet this value was still within the normal range for swine (6-30 mg/dL). It is important to note that no kidney lesions as result of meloxicam administration were found on histopathology exam.

Both gross and histopathologic changes were noted in the gastrointestinal systems of 2/5 MEL sow and their litters. The most prominent pathology in the sows was subacute, ulcerative to erosive, suppurative duodenitis. In contrast, the pathologist concluded that gross gastric sow lesions and mild lymphcytic-plasmacytic gastritis were likely age-related and not of clinical significance. Multifocal button gastric ulcers were observed in 10/11 piglets from these two MEL sows, along with multifocal, subacute suppurative gastritis with submucosal edema. The pathologist concluded the gastritis noted in 10/11 piglets in those litters and the duodenitis noted in the two sows was compatible with insult from high levels of NSAID treatment. No such lesions were noted in the control pigs.

The most frequent and clinically significant side effects of NSAIDs reported in the research literature are the irritant, ulcerogenic erosive effects on the gastrointestinal tract [20]. Other potential effects include renal toxicity, hepatotoxicity, blood dyscrasias and delayed soft tissue healing. It must be emphasized that clinical manifestations of toxicity do not occur in the great majority of animals receiving recommended dose rates of NSAIDs [20]. Modifications for
the protection of normal body function and safety must be balanced with an efficacious dose before application in the swine industry.

**Pharmacokinetic modeling**

Pharmacokinetic modeling was performed on the sow oral meloxicam dosing, describing absorption rate constant (ka), volume of distribution (V) and plasma clearance (Cl). Although there are important differences between study designs, the current study parameters were compared to the only other available published and peer-reviewed study describing meloxicam in sows [10]. The parameters (±SD) are described in Table 1. The ka 0.13 hr\(^{-1}\) (0.05) was numerically similar to that reported by Pairis-Garcia et al. with a ka of 0.10 (0.03) hr\(^{-1}\). The V was 149.65 (8.94) mL/kg and numerically less than Pairis-Garcia et al. at 425.0 (0.27) mL/kg. Plasma Cl was 18.86 (44.01) mL/hr/kg and slower than described by Pairis-Garcia et al. at 43.08 (0.45) mL/hr/kg. These comparisons are worth noting, however, due to several differences between the studies, further comparison would be misleading.

**Model limitations**

The construction of a pharmacokinetic model from this study has considerable limitations. First of all, the model using the same mathematical template as the only other peer reviewed study by Pairis-Garcia et al[10]. Several important differences exist between that study and the current one. The sows used in the Pairis-Garcia study were cull sows at the end of their reproductive lifecycle while the present study used farrowing and subsequently, lactating sows. The body condition and biological mechanisms noted in these types of sows are often quite divergent, which has potential to affect drug metabolism. The most common reasons for culling sows are reproductive failure and lameness [21], which are often chronic conditions affecting or
a result of, diminished body condition. Conversely, recently farrowed, lactating sows are generally in a much better body condition with more energy stores to feed a litter of pigs.

Lactation creates in sows a very physiologically dynamic state, balancing the synthesis of milk for piglets with the changing sow metabolism. Several biological mechanisms are causing the sow to repartition her fat stores to energy, all the while trying to maintain a consistent, upward feed intake after giving birth. This is all occurring in the face of an immune system in flux during the farrowing and post-farrowing period. In contrast, cull sows are generally assumed non-pregnant and non- or minimally-lactating. Although they may be experiencing some systemic catabolism, this is considered more of a chronic, static biological state in cull sows. These physiological differences make it is quite plausible that cull sows and lactating sows have potential to metabolize drugs differently.

Finally, the dosing methods were very distinct between studies both in amount, timing and administration. The current study incorporated the meloxicam tablets (30 mg/kg) ground into powder, mixed into the regular twice per day feeding, more accurately simulating the potential application of the practice on commercial sow farms. Uneaten feed from each sow was removed from the feeder and weighed back, and the approximate weight was 24% per MEL sow and 21% per WHEY sow. The exact drug concentration is known, but although mixing was performed the homogeneity of the feed is unknown. The Pairis-Garcia study utilized a one-time oral meloxicam dose (0.5 mg/kg), incorporating the meloxicam tablets into a ball of cookie dough, to which the sows had been trained using positive reinforcement. This ensured each sow ate the entire dosage in that mixture.

Several unknown factors preclude further pharmacokinetic analysis past the sow level. Although serial sampling characterized meloxicam levels in sow plasma, sow milk, and piglet
plasma, we are unable to fully describe piglet parameters. First of all, once meloxicam is in sow plasma, it can be transferred to several compartments for elimination, such as milk, kidney and liver circulation. It was beyond the scope of this study to accurately measure renal and hepatic elimination, therefore, the part they may play in overall pharmacokinetics is unknown.

Secondly, it is also very difficult to estimate the amount of milk the piglets drank. Piglets suckle approximately every 45-50 minutes and the mammary gland refills to back to capacity within about 35 minutes [22]. Piglets may stimulate milk let down for a period of minutes through nuzzling the mammary gland, but actual milk ejection from the gland is only about 15 seconds [22]. The average sow milk production varies greatly with parity, nutrition, and litter size. Bussiéres estimates that healthy sows with 11 piglets on a regular commercial ration can produce between 10.3 and 11.3 kg of milk per day [23]. However, this amount can vary greatly with parity, nutrition, health status and litter size. Additionally, there is very little data to estimate the exact amount each piglet drinks per suckling time, therefore making the piglet dose of any drug extremely challenging to describe.

**Regulatory issues**

The lack of approved analgesics in swine presents a conundrum. Veterinarians are charged in their oath [24] and the professional organizations of the American Veterinary Medical Association [25], and the American Association of Swine Veterinarians [26], to uphold animal welfare and reduce pain, yet there are no approved drugs available in the US. The Animal Medical Drug Use Clarification Act of 1994 and its regulations published at Title 21, Code of Federal Regulations, Part 530 (21 CFR 530) makes provision for veterinarians to employ extra label drug use (ELDU) under certain specific conditions within the context of a veterinary client patient relationship (VCPR) [27]. These provisions include a lack of an approved animal drug
that is labeled for the condition and that contains the same active ingredient in the required dosage form and concentration, a careful diagnosis of disease, a substantially extended, scientifically supported withdrawal time, and proper identification of the treated animal(s). Approved animal drugs, approved human drugs, and animal drugs compounded from approved animal or human drugs are eligible to be used in this manner. However, ELDU is limited to circumstances when the health of an animal is threatened, or suffering or death may result from failure to treat. This means that ELDU to enhance production is not permitted [28].

Caution must be exercised in the immediate application of transmammary meloxicam administration into commercial systems. There is no established meat withdrawal to enable meloxicam-treated swine to enter the food chain. Furthermore, ELDU in feed is expressly prohibited. Finally, although meloxicam is approved for IM use swine in Canada and the EU relief of post-operative pain associated with minor soft tissue surgery, such as castration, the dosage is much less (0.4mg/kg) than that of this study (30 mg/kg) Given these scientific and regulatory constraints, the transmammary method is not practical at the present time in commercial swine systems.

This pioneer study describes the first peer-reviewed report of the successful transmammary transfer of meloxicam in milk from sows to piglets. Efficacy was confirmed by a reduction in PGE$_2$, and initial sow pharmacokinetic parameters were described. The novel administration of analgesic drugs via transmammary transfer has significant potential benefits for the swine industry. As an entire litter can be medicated through the oral treatment of one sow, large numbers of piglets can receive pre-emptive analgesia without the need for additional handling and injections. This will also lead to reduced animal stress, improved safety for both the pig and handler, and a decreased potential for tissue lesions and drug residues when the
injections are removed. However, due to lack of meat withdrawal and ELDU regulations, this method is not recommended for present use in the swine industry. Overall, this study provides a foundation for future research investigations in meloxicam dose refinement, withdrawal periods, and other means of swine analgesia.

Acknowledgements

The authors thank the following individuals who provided invaluable support to the project: the 2013 Swine Medicine Education Center interns, Erica Voris, Jackie Peterson, and the ISU-PhAST.

References


TABLE 1. Individual sow pharmacokinetic parameter comparison between two sow oral meloxicam studies. Bates et al. administered 30 mg/kg meloxicam BID incorporated into the daily ration of five sows, while Pairis-Garcia et al. administered 0.5 mg/kg meloxicam in a one-time bolus incorporated into sugar cookie dough to six sows. Absorption rate constant (ka), volume of distribution (V), and plasma clearance (Cl) (±standard deviation (SD)) are described below.

<table>
<thead>
<tr>
<th></th>
<th>Mean ka (hr(^{-1})) (±SD)</th>
<th>V_mean (mL/kg) (±SD)</th>
<th>Cl_mean (mL/hr/kg) (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bates et al.</td>
<td>0.13 (0.05)</td>
<td>149.65 (8.94)</td>
<td>18.86 (44.01)</td>
</tr>
<tr>
<td>Pairis-Garcia et al.</td>
<td>0.10 (0.03)</td>
<td>425.00 (0.27)</td>
<td>43.08 (0.45)</td>
</tr>
</tbody>
</table>

TABLE 2. Mean meloxicam concentration at piglet processing in sow plasma, sow milk and piglet plasma. To illustrate the extremes, the sow with the highest plasma meloxicam concentration was compared to the sow with the lowest plasma meloxicam concentration. In all three cases, piglet plasma was represented as a percentage of sow milk and sow plasma, as well as sow milk as a percentage of sow plasma.

<table>
<thead>
<tr>
<th></th>
<th>Mean Plasma Meloxicam (n=5)</th>
<th>Highest Sow Plasma Meloxicam</th>
<th>Lowest Sow Plasma Meloxicam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piglet Plasma (ng/mL)</td>
<td>569.8</td>
<td>606.5</td>
<td>743.7</td>
</tr>
<tr>
<td>Sow Milk (ng/mL)</td>
<td>3459.3</td>
<td>3221.7</td>
<td>2012.8</td>
</tr>
<tr>
<td>Sow Plasma (ng/mL)</td>
<td>53,091.6</td>
<td>65,611.5</td>
<td>44,788.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Piglet Plasma/Sow Plasma</th>
<th>Piglet Plasma/Sow Milk</th>
<th>Sow Milk/Sow Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.1%</td>
<td>16.5%</td>
<td>6.5%</td>
</tr>
<tr>
<td>Piglet Plasma/Sow Milk</td>
<td>0.9%</td>
<td>18.8%</td>
<td>4.9%</td>
</tr>
<tr>
<td>Sow Milk/Sow Plasma</td>
<td>1.7%</td>
<td>36.9%</td>
<td>4.5%</td>
</tr>
</tbody>
</table>
FIGURE 1. Outline of study events for sows and their litters

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Sow farrows and is assigned to meloxicam (MEL) or whey placebo (WHEY) group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Sow and piglets monitored</td>
</tr>
<tr>
<td>Day 2</td>
<td>Sow and piglets monitored</td>
</tr>
<tr>
<td>Day 3</td>
<td>Sows and piglets weighed and identified</td>
</tr>
<tr>
<td></td>
<td><strong>SOWS</strong></td>
</tr>
<tr>
<td></td>
<td>Plasma Meloxicam</td>
</tr>
<tr>
<td>Day 4</td>
<td>Sow begins treatment in feed BID; Blood draws and milk samples at 0700h and 1900h</td>
</tr>
<tr>
<td>Day 5</td>
<td>Sow treatment in feed BID; Blood draws and milk samples at 0700h and 1900h</td>
</tr>
<tr>
<td>Day 6</td>
<td>Sow treatment in feed BID. Blood draws and milk samples at 0700h and 1900h</td>
</tr>
<tr>
<td>Day 7</td>
<td>Blood draw at 0700h</td>
</tr>
<tr>
<td>Day 8</td>
<td>Final blood draw at 0700h and necropsy</td>
</tr>
</tbody>
</table>
FIGURE 2. Comparison of plasma and milk meloxicam concentrations from sows and their piglets treated with 30 mg/kg meloxicam. The mean (±SEM) meloxicam levels at 24 hours (piglet processing) were 568.9 ±105.8 µg/mL. No meloxicam was found in CONT piglet plasma. Sow and piglet plasma and sow milk meloxicam concentrations maintained relatively constant levels for the duration of the treatment period, and they began to decline only after 72 hours when the treatment was discontinued in the feed.
FIGURE 3. Individual plasma profiles in six sows administered meloxicam per os at 30mg/kg.
FIGURE 4. Plasma PGE$_2$ ± SE levels from meloxicam (MEL) - and whey placebo (CONT) - treated piglets. MEL piglets had a greater amount of prostaglandin E$_2$ (PGE$_2$) inhibition compared to their CONT counterparts ($p=0.0059$). All time points that are marked with a and b were significantly different ($p<0.05$). The exception was 24 hours after administration ($p=0.0909$).
CHAPTER 3. IMPACT OF TRANSMAMMARY-DELIVERED MELOXICAM ON BIOMARKERS OF PAIN AND DISTRESS IN PIGLETS AFTER CASTRATION AND TAIL DOCKING


A manuscript published in PLOS ONE

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Abstract

To investigate a novel route for providing analgesia to processed piglets via transmammary drug delivery, meloxicam was administered orally to sows after farrowing. The objectives of the study were to demonstrate meloxicam transfer from sows to piglets via milk and to describe the analgesic effects in piglets after processing through assessment of pain biomarkers and infrared thermography (IRT). Ten sows received either meloxicam (30 mg/kg) (n=5) or whey protein (placebo) (n=5) in their daily feedings, starting four days after farrowing and continuing for three consecutive days. During this period, blood and milk samples were collected at 12-hour intervals. On Day 5 after farrowing, three boars and three gilts from each litter were castrated or sham castrated, tail docked, and administered an iron injection. Piglet blood samples were collected immediately before processing and at predetermined times over an
84-hour period. IRT images were captured at each piglet blood collection point. Plasma was tested to confirm meloxicam concentrations using a validated high-performance liquid chromatography-mass spectrometry method. Meloxicam was detected in all piglets nursing on medicated sows at each time point, and the mean (±standard error of the mean) meloxicam concentration at castration was 568.9 ±105.8 ng/mL. Furthermore, ex vivo prostaglandin E₂ (PGE₂) synthesis inhibition was greater in piglets from treated sows compared to controls (p=0.0059). There was a time-by-treatment interaction for plasma cortisol (p=0.0009), with meloxicam-treated piglets demonstrating lower cortisol concentrations than control piglets for 10 hours after castration. No differences in mean plasma substance P concentrations between treatment groups were observed (p=0.67). Lower cranial skin temperatures on IRT were observed in placebo compared to meloxicam-treated piglets (p=0.015). This study demonstrates the successful transfer of meloxicam from sows to piglets through milk and corresponding analgesia after processing, as evidenced by a decrease in cortisol and PGE₂ levels and maintenance of cranial skin temperature.

**Keywords:** castration, lactation, meloxicam, pain, swine, welfare

**Introduction**

Pork producers and consumers are increasingly concerned about the well-being of food producing animals. The management of pain during routine swine husbandry practices, such as castration and tail docking in piglets, is of particular significance. The European Union (EU) recently moved to ensure that all piglets are castrated using analgesia/anesthesia [1]. However, in the United States, there are currently no Food and Drug Administration (FDA)-approved drug regimens for pain relief in livestock, and analgesia is not routinely provided at the time of processing.
Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) that is approved for swine in the EU and Canada for several conditions, including the relief of post-operative pain with minor soft tissue surgery. When injected before piglet castration, meloxicam reduces serum cortisol concentrations [2], [3], [4]. Meloxicam has also been shown to reduce behavioral signs that are associated with piglet distress at castration and is considered to be superior to other analgesics when assessing pain-related behavioral criteria [5].

Administering oral meloxicam to sows during lactation would potentially provide analgesia during processing procedures by allowing passive drug transfer through the milk to entire litters. This route is safer for both the handler and the animal when compared to injections. It is also easily administered and allows a large number of animals to be medicated, thus eliminating the need for individual injections. Although there are no peer-reviewed studies demonstrating transmammary analgesia in swine, NSAIDs can transfer through milk in both cattle [6] and humans[7], [8], [9]. The objectives of this study were to demonstrate the transmammary delivery of meloxicam from sows to piglets and to assess the pharmacodynamics and analgesic effects in piglets after castration. The findings of this study demonstrate the successful transfer of meloxicam from sows to piglets through milk and associated analgesia after processing, as evidenced by a decrease in cortisol and PGE2 levels and maintenance of cranial skin temperature.

**Materials and Methods**

Before the initiation of this study, all techniques regarding animal use, housing, handling, and sampling were approved by the Iowa State University Animal Care and Use Committee (IACUC # 8-12-7430-S).
Animals

Ten Yorkshire x Landrace sows at approximately one week prior to farrowing (average weight of 277.3 kg) were obtained from a commercial swine farm. Upon arrival, each sow was confirmed to be healthy and pregnant by a veterinarian, and a unique numerical ear tag (Allflex Global Ear Tags, Allflex USA, Inc., DFW Airport, TX) was applied to the right ear. Sows were housed at the Iowa State University Animal Resource Station in accordance with recommendations outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Use and Research and Teaching[10]. Sows were placed in Quad- or Euro-style farrowing stalls (Thorp Equipment, Thorp, WI), depending on availability. Both stall types were equally represented in both treatment groups. Regardless of stall type, each sow was housed in a farrowing stall area measuring 0.6 m × 2.1 m. Quad and Euro stalls had piglet creep areas of 7.0 m² and 6.4 m², respectively. After farrowing, a heat lamp was provided on each side of the creep area in each stall for the piglets.

Feeding and treatment administration

Prepartum sows were hand-fed 1.6 kg of an organic corn/soybean meal diet, which was confirmed to be free of meloxicam, twice daily. This diet was compatible with the National Research Council’s nutrient requirements for lactating sows[11]. Intake was gradually increased ad-libitum after farrowing. Sows had free access to water at all times through a nipple waterer in their stalls. On Day 4 after farrowing, sow treatments began and continued for six days. Sows assigned to the meloxicam-treatment group (n=5) received 30 mg/kg meloxicam (Meloxicam, Aurobindo Pharma, India, Batch X1513019-A, Expiration Date 2/2015), which was divided between 2 feedings at 0700h and 1600h. The meloxicam was ground from tablets into a powdered consistency using a commercial grinder (Spice & Nut Grinder, Cuisinart, East
Windsor, NJ), after which it was incorporated into each sow’s daily feed ration in a portable mixer (Kobalt Model #043206, Monarch Industries, Winnipeg, MB, Canada). Control sows (n=5) received 30 mg/kg of whey protein placebo (Health Watchers, Inc., Bohemia, NY), which is a pharmacologically inactive excipient used in the manufacturing of meloxicam tablets. The placebo was prepared in a separately marked bucket by thorough hand-mixing with gloved hands to prevent cross contamination.

**Animal phase study design**

The sows were allowed to farrow naturally without induction methods. They were then randomly assigned to two groups. The first sow to give birth was randomly allocated to the meloxicam-treated group (MEL), and the second sow was allocated to the whey placebo group (CONT). The alternating pattern continued for the remaining sows, based on the farrowing date. The day of farrowing was designated as “Day 0” for each sow and litter. The overall time scheme of activities, including drug administration and sample collection, is detailed in Figure 1. On Day 3, post-farrowing piglets in the litter were weighed and ranked in a descending order. In each litter, the heaviest three boars and three gilts were selected and ear tagged (Allflex Global Ear Tags, Allflex USA, Inc., DFW Airport, TX). The next three heaviest piglets, regardless of sex, were selected and tagged as sentinels to specifically measure the inhibition of plasma prostaglandin E2 (PGE2) levels and demonstrate the pharmacodynamic effect of meloxicam. In total, nine piglets per litter were tagged. Two litters did not have three live boar piglets. In those instances, all available boars were used as test piglets. In another litter, a male test piglet was laid on and subsequently died after being identified but before blood sampling began. No other piglets were substituted into the test category. Cross-fostering was not performed at any phase of the study.
Piglet processing occurred on Day 5 after farrowing. After a pre-processing blood draw, the boars were immediately castrated and tail-docked. They then received 1 mL (100 mg) iron IM (Ferrodex 100, AgriLabs, St. Joseph, MO). Castration was performed in accordance with standard swine industry practices by making two vertical incisions approximately 2-3 cm long in the scrotum with a number-ten scalpel blade and scalpel handle, marsupializing the testicles, and finally providing manual pressure on the spermatic cord until it separated from the piglet’s body. Immediately after each piglet castration, the scalpel blade and handle were immersed in a dilute chlorhexidine mixture for disinfection between each piglet procedure per typical swine industry practice. All castrations were performed by a single experienced veterinarian to minimize variation (JLB). Gilts were handled in a similar manner, and they also underwent tail docking and received iron.

Sow blood samples (8 mL/sample) were collected via the left or right jugular vein using a 25.4-mm, 16-gauge hypodermic needle (Air-Tite Products, Virginia Beach, VA) and 12-mL Luer lock syringe (TycoHealth Care, Mansfield, MA). During blood collection, sows were manually restrained in their crates using a pig snare. Piglet blood samples (2 mL/sample) were collected using the left or right jugular vein using a 3.8-cm, 22-gauge hypodermic needle (TycoHealth Care, Mansfield, MA) and 3-mL syringe (TycoHealth Care, Mansfield, MA). These samples were obtained using physical restraint by placing the piglet in a supine position.

On Day 8 after farrowing, sows were euthanized by a penetrating captive bolt, followed by exsanguination, and piglets were euthanized by blunt force trauma to the cranium, according to American Veterinary Medical Association guidelines [12]. Necropsies were performed on the sows and processed piglets. The liver, kidney, gastric fundus, duodenum, semitendinosus/semimembranosus muscle, and fat were collected for analysis.
Following processing and each blood-sampling time point, changes in piglet skin temperature were measured using a commercially available infrared thermography (IRT) camera (FLIR SC660, Systems, Wilsonville, OR). Prior to each use, the camera was allowed to self-calibrate with the ambient temperature and relative humidity in the barn. Piglets were placed in a non-restrictive plastic tub measuring 50.8 cm in diameter and 43.2 cm tall for approximately ten seconds while thermographic images of the cranium, right and left ears, and snout were obtained (Figure 2).

**Sample collection, processing and analysis**

All drug concentrations in plasma were analyzed at the Iowa State University Veterinary Diagnostic Laboratory by the Iowa State University-Pharmacology Analytical Support Team (ISU-PhAST).

**Meloxicam analysis**

Blood for meloxicam analysis was placed in 10-mL and 3-mL heparinized blood collection tubes (BD Vacutainer, Franklin Lakes, NJ). Samples were centrifuged for 15 minutes at 1000 g at ambient temperature. The plasma was separated and placed into cryovials for storage at -80°C.

Plasma concentrations of meloxicam were determined using high-performance liquid chromatography (HPLC) (Accela Pump and Autosampler, Thermo Scientific, San Jose, CA, USA) with mass spectrometry (MS) detection (LTQ XL, Thermo Scientific, San Jose, CA, USA). Plasma samples, plasma spikes, and plasma quality control (QC) samples (200 µL each) were treated with 1 M trichloroacetic acid (100 µL) after the addition of the internal standard, piroxicam (10 µL of 10 ng/µL). The samples were vortexed for 5 seconds and centrifuged for 20 minutes at 2000 g to sediment the precipitate. A portion of the supernatant (150 µL) was
transferred to an injection vial that was fitted with a glass insert containing 100 μL of 1.9% ammonium hydroxide in 25% aqueous acetonitrile. The injection volume was set to 20 μL. The mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at a flow rate of 0.250 mL/min. The mobile phase began at 40% B with a linear gradient to 95% B at 4 minutes, which was maintained for 1.5 minutes, followed by re-equilibration to 40% B. Separation was achieved with a solid-core C18 column (KinetexXB-C18, 100 mm × 2.1 mm, 2.6-μm particles, Phenomenex, Torrance, CA, USA) that was maintained at 45°C. Piroxicam eluted at 2.6 minutes, and meloxicam eluted at 3.3 minutes. A full scan MS of the pseudomolecular ions of piroxicam (m/z 332) and meloxicam (m/z 352) was used for analyte detection. The sum of the intensities of ions at m/z of 115 and 141 were used for meloxicam quantitation. The internal standard, piroxicam, was quantitated with the sum of the ion intensities at m/z of 95, 121, and 164. Sequences consisting of plasma blanks, calibration spikes, QC samples, and porcine plasma samples were processed in batches with a processing method that was developed in the Xcalibur software (Thermo Scientific, San Jose, CA, USA). The processing method automatically identified and integrated each peak in each sample and calculated the calibration curve based on a weighted (1/X) linear fit. Plasma concentrations of meloxicam in unknown samples were calculated by the Xcalibur software based on the calibration curve. Results were then viewed in the Quan Browser portion of the Xcalibur software. Fourteen calibration spikes were prepared in porcine plasma covering the concentration range of 1–20,000 ng/mL. QC samples were prepared at concentrations of 15, 150, and 1500 ng/mL in duplicate with each set of samples. Calibration curves exhibited a correlation coefficient (R2) exceeding 0.997 across the entire concentration range. The QC samples at 150 and 1500 ng/mL were within
2–8% of their nominal values, and the low QC sample at 15 ng/mL differed from its nominal value by 10–15%.

**PGE_2 analysis**

PGE_2 concentrations were determined using methods that were previously described[13]. Briefly, fresh piglet blood was collected into sterile tubes containing heparin. To stimulate ex-vivo PGE_2 production by monocytes, the heparinized whole blood was incubated for 24 hours at 37°C with 10 µg/ml lipopolysaccharide (LPS, derived from Escherichia coli 055:B5, Sigma Aldrich, St. Louis, MO), which was diluted in phosphate-buffered saline (PBS). The first blood collection occurred prior to treatments and was divided into two equal aliquots: one was incubated with LPS, and the other was incubated with an equivalent volume of PBS. These aliquots were used as positive and negative controls.

At the end of the incubation, all samples were centrifuged at 400 g for 10 minutes to obtain plasma: 250 µl of plasma were mixed with 1000 µl of methanol (1:5 dilution) to permit protein precipitation. After a final centrifugation at 3000 g for 10 minutes, supernatants were collected and stored at -80°C.

The concentration of plasma PGE_2 was determined using an enzyme-linked immunosorbent assay kit (Cayman Chemical, Co, Ann Arbor, MI). The calculated coefficient of variation for intra-assay variability was 11.7%, and the inter-assay variability was 9.2%.

**Cortisol analysis**

Blood for cortisol analysis was collected in a 3-mL heparinized blood collection tube (BD Vacutainer, Franklin Lakes, NJ) and then centrifuged for 10 minutes at 1500 g. The plasma was collected, immediately frozen, and stored at -80°C. Plasma samples were analyzed for
cortisol within 60 days after sample collection and within 10 consecutive days once analysis commenced.

Plasma cortisol concentrations were determined using a commercial radioimmunoassay (RIA) kit (Coat-A-Count Cortisol, Siemens Medical Solutions Diagnostics [formally Diagnostic Products Corp.], Los Angeles, CA). Samples were incubated at 4°C for 2 hours to improve assay sensitivity. Samples were assayed in duplicate with the reported concentration equaling the average cortisol concentration between duplicates. The calculated coefficient of variation for intra-assay variability was 9.2%, and the inter-assay variability was 9.3%.

**Substance P Analysis**

Blood (1 mL) for substance P (SP) analysis was collected in a 4-mL potassium ethylenediaminetetraacetic acid (EDTA) purple-top blood collection tube (BD Vacutainer, Franklin Lakes, NJ) that was previously spiked with 50 µL benzamidine. This blood was promptly centrifuged for 15 minutes at 1000 g. The plasma was immediately frozen and stored at -80°C.

The SP assay was performed as described by Liu et al.[14] with slight modifications using non-extracted plasma. Method validation using non-extracted plasma consisted of the complete recovery (±15%) of a known concentration of SP that was added to pooled baseline sample plasma. Samples were analyzed in duplicate with a double-antibody RIA using a primary antibody (polyclonal rabbit anti-SP; 1:20,000) from Phoenix Pharmaceutical, Inc. (Burlingame, CA, USA). EDTA (13 mM) and benzamidine (1 mM) were added as protease inhibitors. SP was assayed using the 125I-[Tyr8]-SP tracer (approximately 18000 cpm) (PerkinElmer, Inc., Waltham, MA, USA). Samples were assayed in duplicate with the reported concentration
equaling the average substance P concentration between duplicates. The intra- and inter-assay coefficients of variation were 7.6% and 14.9%, respectively.

**Infrared Thermography Analysis**

Standardized anatomical locations on the pig were identified by a technician in IRT digital images that were obtained of study piglets. IRT images were converted to temperature readings by proprietary software that was calibrated internally by the machine and designed to interface specifically with the camera (Thermacam Researcher Pro 2.8 SR-1, FLIR Systems). Data were analyzed for changes in temperature by comparing temperature values obtained at consistent anatomical locations on the pig over the range of sample time points. Four anatomical locations in each image were initially converted to temperature readings, but variations in piglet position and orientation to the camera effectively reduced the sample size for ear and snout readings. Consequently, these were discarded, and the more accessible cranium location was forwarded to the statistical analysis phase of the study.

**Statistical analysis**

Data were analyzed using generalized linear mixed models fitted with the GLIMMIX procedure of SAS (SAS Institute Inc., Version 9.2). Treatment, procedure, time, and their interactions were used as fixed effects, whereas sow was a random effect, and piglet was the subject of repeated measures. A separate linear mixed model was run to study the effect of meloxicam concentrations on PGE₂, substance P and IRT by using Meloxicam_Levels as an explanatory variable. Baseline measurements were used as covariates in the above models. Model assumptions were considered to be appropriately met, based on diagnostics that were conducted on studentized residuals. Estimated least square means and corresponding standard errors, or 95% confidence intervals, are presented. A significant difference was considered to
exist when $p \leq 0.05$, and a marginal difference was considered to exist when $0.05 < p \leq 0.10$.

Relevant pairwise comparisons were conducted when the significance of the interaction term was $p \leq 0.10$, using Tukey-Kramer adjustments as appropriate to avoid inflation of the Type I error rate due to multiple comparisons.

**Results and Discussion**

**Plasma Meloxicam Concentration**

Meloxicam was detected in the plasma of all piglets in the MEL group at every time point after treatment commenced (Figure 3). The mean (±standard error of the mean [SEM]) meloxicam concentration at castration was $568.9 \pm 105.8 \mu g/mL$. No meloxicam was found in the CONT piglet plasma. Plasma meloxicam concentrations in both sows and piglets maintained steady-state concentrations for the duration of the treatment period, and they began to decline only after 72 hours when the treatment was discontinued in the feed (Figure 3).

The pharmacokinetics of meloxicam after oral administration to mature swine has recently been described [15]. However, this is the first peer-reviewed report of a study documenting the transfer of an NSAID from the sow to the piglet via the transmammary route. In a recent National Pork Board (NPB) report [16], Brown found that injectable meloxicam is transferred through the milk to piglets at processing. However, this transfer utilized a one-time intramuscular dose of 1 mg/kg meloxicam to each sow, which resulted in 2.647 ng/mL of meloxicam in the piglet serum at 5 hours after administration. Piglet plasma meloxicam levels in the NPB report were only measured out to this time point. Due to differences in routes of administration and study design, further comparisons cannot be made. Other species, such as cattle [6] and humans [7], [8], [9], [17], have demonstrated NSAID transfer through milk. The importance of this study was to confirm that the transmammary route of administration is
feasible in piglets. Further pharmacokinetic modeling and dose-titration studies are needed to apply this information for the benefit of commercial swine production.

Meloxicam is an NSAID that is approved for swine in Canada and the EU. It is labeled for use in swine to treat non-infectious locomotor disorders by reducing the signs of lameness and inflammation. It is also used for adjunctive therapy in the treatment of puerperal septicemia and toxemia with appropriate antibiotic therapy and for the relief of post-operative pain associated with minor soft tissue surgery, such as castration [18]. Because no analgesic drugs are approved to provide pain relief to swine in the United States, the administration of meloxicam to swine constitutes extra-label drug use (ELDU). Under the Animal Medicinal Drug Use Clarification Act (AMDUCA), ELDU is permitted under veterinary supervision for the relief of suffering in swine when specific conditions are met[19]. In the absence of FDA-approved analgesic compounds in food animals, the use of oral meloxicam tablets for the alleviation of pain or stress in swine can be considered under AMDUCA. It is imperative to remember that the dose of oral meloxicam in this study was extrapolated from data from other species for proof of transfer. Pharmacokinetic analyses are pending and will assist in making further conclusions about the effective dose. However, at this time, the dose used in this study cannot be recommended for use in commercial swine operations due to lack of tissue residue data.

**PGE$_2$**

PGE$_2$ demonstrated a treatment effect (p=0.0059) with significant differences (p<0.05) at each time point, with the exception of 24 hours after drug administration commenced (p=0.0909) (Figure 4). However, using this analysis, there was no time-by-treatment interaction (p=0.1763) or effect of time (p=0.6064). Meloxicam concentration also had evidence of a negative association with plasma PGE$_2$ concentrations (p=0.0048).
This inhibition of PGE₂ by meloxicam was anticipated, as a result of the blockage of the arachidonic acid pathway and cyclooxygenase-2. Prostaglandins contribute to the amplification of pain signaling by increasing nociception sensitization [20]. As such, reducing PGE₂ concentrations would provide decreased nociception following noxious stimuli, such as castration. Mean piglet plasma PGE₂ concentrations ranged from 66.2–719.9 pg/mL. These levels are much lower than those reported in equines (1.7 ng/mL), canines (329 ng/mL), and felines (0.7 ng/mL) [21]. No porcine comparisons are available in the literature. There are several potential explanations for the lower PGE₂ levels. First, the piglets were relatively young and blood from these animals may not have fully responded to LPS stimulation. Second, other studies have used different strains or concentrations of LPS. Finally, we used ex vivo stimulation of whole blood, which may contribute to lower levels. Despite these species-specific differences, decreases in plasma PGE₂ were observed at most time points in MEL piglets compared to CONT piglets. This suggests that meloxicam was successfully transferred through milk to piglets at concentrations that likely provided analgesia based on the demonstrated ex-vivo inhibition of PGE₂ production.

**Pain Biomarker Analysis**

**Cortisol Analysis**

There was a time-by-treatment interaction for piglet plasma cortisol (p=0.0009) (Figure 5). MEL piglets had lower plasma cortisol than CONT piglets for the first 10 hours after processing. Although no individual time points demonstrated significant differences, p values with marginal significance were observed at 1 and 6 hours after processing (p=0.10 and p=0.12, respectively) (Table 1).
These study findings are in agreement with several other studies that associate a decrease in piglet plasma cortisol with pain mitigation. This decrease was noted with various analgesics at castration, such as meloxicam [22], [3], both meloxicam and flunixin administered separately [23],[24], and both meloxicam and tolfenamic acid administered separately [4].

The highest cortisol levels in both groups were observed at 60 minutes after castration. This peak is shown in Figure 5 and is consistent with previous studies showing that the highest cortisol levels are detected 30–90 minutes after processing [22], [25], [4], [23], [26], [27], [28]. Glucocorticoids are secreted in response to a stressor, such as castration, and are generally considered to be indicative of stress and, thereby, pain. [27]. However, piglet plasma cortisol does have some limitations as an objective pain biomarker. For instance, stressors such as handling may cause an increase in plasma cortisol concentrations [29]. However, research by Prunier et al. [28] suggests that sham-castrated pigs have lower amplitudes and durations of cortisol than castrated piglets, and these are likely to be connected to pain or tissue damage [22]. A systematic review of pain management during routine management procedures highlighted the need for additional validation of pain biomarkers in peer-reviewed studies [30,31]. Until more pain biomarkers can be clearly described and validated, cortisol remains to be one of the most easily identifiable means of describing piglet pain.

**Substance P**

Measurements of SP indicated no differences between MEL and CONT piglets at processing (p=0.6733). There was a significant change in SP levels over time (p=0.0024). However, there were no significant interactions in procedure by time (p=0.66) or treatment by procedure by time (p=0.33) (Table 2). There was also no association between meloxicam and SP levels (p=0.1444).
SP is an 11-amino acid neuropeptide that regulates nocireceptive neurons, which are involved in the integration of pain, stress, and anxiety [32], [33]. It has proinflammatory effects in immune and epithelial cells and participates in inflammatory diseases of the respiratory, gastrointestinal, and musculoskeletal systems [34].

These results are in agreement with a recent study by Sutherland et al. [25], in which no significant differences in SP levels between castrated and sham-castrated piglets were found. However, in bovines, Coetzee et al. [33] demonstrated that castrated calves have significantly elevated SP levels compared to their non-castrated counterparts. Although SP has the potential to accurately describe physiological pain, further research is needed to determine its value in swine.

**Infrared Thermography**

Example IRT images from a meloxicam-treated and placebo-treated control piglet after castration is presented in Figure 6 a-b. IRT demonstrated a significant time-by-treatment interaction in cranial temperature between MEL and CONT piglets (p=0.0148; Figure 7). The interaction was significant at all timepoints after castration (p<0.0001; Figure 7). After baseline measurements, CONT piglets had lower skin temperature than MEL piglets. There was a positive association between plasma meloxicam levels and cranial skin temperature (p=0.0345). Animals that are stressed or in pain can exhibit decreases in cutaneous temperature due to sympathetic nervous system activation, which causes vasoconstriction, shifting of the blood from the skin to the organs, and loss of heat in the periphery of the body [35], [36]. IRT has been shown to be a valuable tool for pain assessment in beef and dairy calves by non-invasively measuring autonomic nervous system responses at the time of dehorning and castration [36], [35], [37]. IRT was also used by Hansson et al. [24] to measure temperatures at 24 hours after piglet castration. Piglet ear temperature was found to be significantly higher in control piglets
versus those given either lidocaine or a lidocaine/meloxicam combination. In that same study, no significant differences were found when measuring the skin around the castration site. This one-time measurement reflects the differences noted in this study up to 24 hours after castration (Figure 6) but fails to provide a longer duration depiction of piglet pain.

Differences in IRT measurements in anatomical sites were noted. Cranial temperature was lower in CONT piglets. This significant time-by-treatment interaction (p=0.0148) suggests that this would be an effective anatomical site for assessing the effect of pain on cutaneous perfusion. However, there were no significant differences in temperature between treatment groups over time in the left ear (p=0.9744), right ear (p=0.7989), and snout tip (p=0.0936). There was also no association between plasma meloxicam concentrations and IRT measurements in the snout (p=0.8683), left ear (p=0.9141), and right ear (p=0.2029) (Table 2).

Analysis of the ears and snout areas proved to be difficult, due to the image capture method. Thermography images were taken by placing each piglet in a small plastic tub to reduce any confounding stress that was associated with further handling after castration and blood sampling. It was challenging to obtain consistent images, due to the anatomical configuration of the folded-over, floppy ears on a relatively mobile piglet. Also, the snout may have been too sensitive to ambient temperature to provide a meaningful assessment of individual piglet pain. Therefore, for the purposes of this study, it was determined that cranial skin temperature was the most accurate anatomical location for assessing piglet pain responses after castration.

The temperature measurement sites that were found to be useful in this study are in conflict with other studies using IRT. Schmidt et al.[38] found the eye and the back of the ear to be the most useful for assessing fever in sows. Additional sites in the literature include the mammary gland and vulva [39], [40]. However, these studies detected either fever response or
estrus onset in adult animals, which are likely different than pain-related thermoregulation processes in baby piglets.

Temperature differences in the treatment groups are further accentuated in a circadian rhythm. Peak temperatures in both MEL and CONT piglets were noted in the evening measurements at 12, 36, and 60 hours after castration. Trough temperatures were seen in morning measurements at 24, 48, and 96 hours after castration (Figure 6). Similar temperature circadian trends have long been noted in livestock, and they were recently demonstrated in dairy cows [41].

Measurement of piglet body temperature using IRT shows promise as a piglet pain biomarker by demonstrating differences in cranial temperature. This non-invasive method allows pain to be assessed for up to 72 hours after castration.

This study is the first peer-reviewed report of the successful transmammary transfer of meloxicam in milk from sows to piglets. Piglet plasma cortisol levels and cranial IRT measurements demonstrated significant changes as a result of analgesic treatment with meloxicam. The novel administration of analgesic drugs via transmammary transfer has significant potential benefits for the swine industry. As one litter is medicated through the oral treatment of one sow, large numbers of piglets can receive pre-emptive analgesia without the need for additional handling and injections. This will also lead to reduced animal stress, improved safety for both the pig and handler, and a reduced potential for tissue lesions and drug residues when the injections are removed. Future research investigations can focus on providing data for meloxicam dose refinement and validating physiological pain indicators.
Acknowledgements

The authors thank the following individuals who provided invaluable support to the project: the 2013 Swine Medicine Education Center interns, Erica Voris, Jackie Peterson, and the ISU-PhAST.

References

1. (2010) European Declaration on alternatives to surgical castration of pigs


TABLE 1. Mean plasma cortisol concentrations (± SEM) after processing in piglets treated with meloxicam (MEL) or whey (WHEY) placebo.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>MEL Plasma Cortisol (ng/mL)</th>
<th>WHEY Plasma Cortisol (ng/mL)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91.18 ± 9.43</td>
<td>108.15 ± 8.96</td>
<td>0.12</td>
</tr>
<tr>
<td>6</td>
<td>56.46 ± 6.07</td>
<td>64.36 ± 4.63</td>
<td>0.10</td>
</tr>
<tr>
<td>12</td>
<td>45.45 ± 5.29</td>
<td>41.30 ± 3.65</td>
<td>0.74</td>
</tr>
<tr>
<td>24</td>
<td>35.38 ± 3.65</td>
<td>26.86 ± 2.79</td>
<td>0.37</td>
</tr>
<tr>
<td>36</td>
<td>35.86 ± 3.90</td>
<td>42.49 ± 4.85</td>
<td>0.15</td>
</tr>
<tr>
<td>48</td>
<td>34.83 ± 3.68</td>
<td>30.99 ± 3.75</td>
<td>0.78</td>
</tr>
<tr>
<td>60</td>
<td>40.86 ± 5.15</td>
<td>29.36 ± 3.27</td>
<td>0.45</td>
</tr>
<tr>
<td>72</td>
<td>30.98 ± 2.92</td>
<td>32.66 ± 4.80</td>
<td>0.73</td>
</tr>
</tbody>
</table>
TABLE 2. Comparison between the least squares (LS) means ± standard error (SE) of piglet serum chemistry biomarkers and infrared thermography (IRT) temperatures, as classified by the procedure (Proc) of castrated (CAST) and sham castrated (SHAM) and treatment (Trt) with 30 mg/kg PO meloxicam (MEL) or whey placebo (CONT) to sows on Days 4–6 after farrowing.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Proc</th>
<th>Experimental Group</th>
<th>Trt</th>
<th>Calculated Means (± SEM)</th>
<th>P VALUES (model adjusted)</th>
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<tr>
<td></td>
<td>CAST</td>
<td>CAST</td>
<td>Sham</td>
<td></td>
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<tr>
<td>Proc</td>
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<td>CAST</td>
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<tr>
<td>SHAM</td>
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<tr>
<td>Trt</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CONT</td>
<td></td>
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<td>MEL</td>
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<td>LS Means</td>
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<td>Trt</td>
<td>TrtXProc</td>
<td>ProcXTime</td>
<td>TrtXProcXTime</td>
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<tr>
<td>± SE</td>
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<tr>
<td>Average Cortisol, ng/mL</td>
<td></td>
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<tr>
<td>48.9 ± 3.49</td>
<td>&lt;0.0001</td>
<td>0.65</td>
<td>0.0009</td>
<td>0.14</td>
<td>0.39</td>
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<td>50.41 ± 3.82</td>
<td>0.65</td>
<td>0.0009</td>
<td>0.14</td>
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<tr>
<td>43.71 ± 2.96</td>
<td>0.0009</td>
<td>0.65</td>
<td>0.14</td>
<td>0.39</td>
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<tr>
<td>44.53 ± 2.73</td>
<td>0.14</td>
<td>0.39</td>
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<tr>
<td>Average Substance P, pg/mL</td>
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<td>0.0024</td>
<td>0.67</td>
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<td>89.24 ± 4.34</td>
<td>0.67</td>
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<td>95.59 ± 2.54</td>
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<td>81.13 ± 3.52</td>
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<td>96.60 ± 3.48</td>
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<td>0.33</td>
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<tr>
<td>Left Ear Temp, °C</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>32.06 ± 0.30</td>
<td>&lt;0.0001</td>
<td>0.85</td>
<td>0.97</td>
<td>0.73</td>
<td>0.96</td>
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<tr>
<td>32.42 ± 0.26</td>
<td>0.85</td>
<td>0.97</td>
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<tr>
<td>32.56 ± 0.30</td>
<td>0.97</td>
<td>0.73</td>
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<tr>
<td>32.35 ± 0.26</td>
<td>0.73</td>
<td>0.96</td>
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<tr>
<td>Right Ear Temp, °C</td>
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<tr>
<td>34.37 ± 0.22</td>
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<td>0.69</td>
<td>0.80</td>
<td>0.58</td>
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<tr>
<td>33.80 ± 0.23</td>
<td>0.69</td>
<td>0.80</td>
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<td>34.07 ± 0.22</td>
<td>0.80</td>
<td>0.58</td>
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<td>33.85 ± 0.21</td>
<td>0.58</td>
<td>0.74</td>
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<td>Snout Temp, °C</td>
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<td>31.56 ± 0.25</td>
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<td>32.40 ± 0.23</td>
<td>0.79</td>
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<td>32.10 ± 0.25</td>
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<td>31.93 ± 0.25</td>
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<td>Cranium Temp, °C</td>
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<td>37.35 ± 0.10</td>
<td>&lt;0.0001</td>
<td>0.32</td>
<td>0.01</td>
<td>0.87</td>
<td>0.99</td>
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<td>37.55 ± 0.08</td>
<td>0.32</td>
<td>0.01</td>
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<tr>
<td>37.35 ± 0.09</td>
<td>0.01</td>
<td>0.87</td>
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<tr>
<td>37.47 ± 0.08</td>
<td>0.87</td>
<td>0.99</td>
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<tr>
<td>Day 0</td>
<td>Sow fawors and is assigned to meloxicam (MEL) or whey placebo (WHEY) group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>Sow and piglets monitored</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Day 2</td>
<td>Sow and piglets monitored</td>
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<tr>
<td>Day 3</td>
<td>Sows and piglets weighed and identified</td>
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<td></td>
<td></td>
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<tr>
<td>SOWS</td>
<td>PROCESSED PIGLETS</td>
<td>SENTINEL PIGLETS</td>
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<td>Measurement Parameters</td>
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<td>Plasma Meloxicam, Cortisol, and Substance P; IRT</td>
<td>Plasma Meloxicam and PGE₂</td>
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<td>Day 4</td>
<td>Sow begins treatment in feed BID; Blood draws and milk samples at 0700h and 1900h</td>
<td>Piglets monitored; no procedures</td>
<td>Blood draws at 0700h and 1900h</td>
<td></td>
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<tr>
<td>Day 5</td>
<td>Sow treatment in feed BID; Blood draws and milk samples at 0700h and 1900h</td>
<td>Blood draws at 0800h and immediately followed by castration, tail docking, iron injection, infrared thermography. Blood draws at 0900h, 1400h, and 2000h.</td>
<td>Blood draws at 0800h and immediately followed by castration, tail docking, iron injection, infrared thermography. Blood draws at 0900h, 1400h, and 2000h.</td>
<td>Blood draws at 0700h and 1900h</td>
<td></td>
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<tr>
<td>Day 6</td>
<td>Sow treatment in feed BID. Blood draws and milk samples at 0700h and 1900h.</td>
<td>Blood draws at 0800h and 2000h</td>
<td>Blood draws at 0800h and 2000h</td>
<td>Blood draws at 0700h and 1900h</td>
<td></td>
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<tr>
<td>Day 7</td>
<td>Blood draw at 0700h</td>
<td>Blood draws at 0800h and 2000h</td>
<td>Blood draws at 0800h and 2000h.</td>
<td>Blood draws at 0700h</td>
<td></td>
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<tr>
<td>Day 8</td>
<td>Final blood draw at 0700h and necropsy</td>
<td>Final blood draws at 0800h and necropsies</td>
<td>Final blood draws at 0800h and necropsies</td>
<td>Final blood draws at 0700h and necropsies</td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 1. Outline of study events for sows and their litters.
FIGURE 2. Example of a digital image of infrared thermography (IRT) measurement. Each processed piglet was measured for temperature in °C at the top of the cranium (circled), right and left ears, and snout (cross marks).
FIGURE 3. Comparison of plasma meloxicam concentrations from sows and their piglets treated with 30 mg/kg meloxicam. The mean (±SEM) meloxicam levels at 24 hours (piglet processing) were 568.9 ±105.8 µg/mL. No meloxicam was found in CONT piglet plasma. Both sow and piglet plasma meloxicam concentrations maintained relatively constant levels for the duration of the treatment period, and they began to decline only after 72 hours when the treatment was discontinued in the feed.
FIGURE 4. Plasma PGE$_2$ ± SE levels from meloxicam (MEL) - and whey placebo (CONT) - treated piglets. MEL piglets had a significantly greater amount of prostaglandin E$_2$ (PGE$_2$) inhibition compared to their CONT counterparts (p=0.0059). All time points that are marked with a and b were significantly different (p<0.05). The exception was 24 hours after administration (p=0.0909).
FIGURE 5. Processed plasma cortisol concentrations after the treatment of sows with 30 mg/kg meloxicam (MEL) or whey placebo (CONT). Means ± SE are depicted.
FIGURE 6 a- b. Example IRT images from a meloxicam-treated (a) and placebo-treated control piglet (b) after castration. Color differences reflect activation of the sympathetic nervous system leading to peripheral vasoconstriction and a localized decrease in skin temperature. Figure 6a demonstrates a meloxicam-treated piglet with a higher (red) cranial skin temperature than the cranial skin temperature (yellow) of the whey-treated piglet.
FIGURE 7. Cranial infrared thermography (IRT) from meloxicam (MEL)- and whey placebo (CONT)- treated piglets. Means ± SE are depicted. There is a significant time-by-treatment interaction between MEL and CONT piglets (p=0.0148). The interaction was significant at all timepoints (p<0.0001). There was an association between plasma meloxicam levels and cranial IRT measures (p=0.0345).
CHAPTER 4. GENERAL CONCLUSIONS

This study marks the first successful proof of concept of transmammary drug transfer between the sow to her piglets after an oral administration. Not only was the concept of administering an oral medication to sows for it to effectively transfer to piglets through milk proven, novel physiology information as it relates to piglet pain has been created.

When sows were administered meloxicam in their daily rations, meloxicam was transferred into their plasma at mean concentrations (±SEM) of 568.9 (105.8) µg/mL. These levels significantly inhibited PGE$_2$ in MEL piglets when compared with CONT piglets. Both the established parameter of plasma cortisol, along with the novel method of IRT described the degree of pain alleviation in castrated piglets with the administration of meloxicam. Finally, sow pharmacokinetic parameters were described with the oral administration.

It is clear the United States swine industry must become increasingly proactive in the area of pain control. Swine veterinarians are professionally and ethically responsible the wellbeing and alleviation of pain reduction in their patients, yet there are no approved analgesics are available in this country. This study demonstrates great potential for the novel method of transmammary analgesia. As one litter is medicated through the oral treatment of one sow, large numbers of piglets can receive pre-emptive analgesia without the need for additional handling and injections. This will also lead to reduced animal stress, improved safety for both the pig and handler, and a decreased potential for tissue lesions and drug residues when the injections are removed.

However, the results of this study do offer some cautionary points that preclude any immediate application of the transmammary method. The sow oral dose must be amended to reduce the gastrointestinal pathology noted both sows and piglets. Additionally, the lack of
approved analgesics in swine presents a conundrum. Provisions for ELDU are made through AMDUCA under certain specific conditions within the context of a VCPR. However, ELDU is limited to circumstances using approved drugs when the health of an animal is threatened, or suffering or death may result from failure to treat. This means that ELDU to enhance production is not permitted. Debate exists in the industry whether castration should be described as a welfare practice or production practice. Both sides have valid arguments and there has been no clear resolution from regulatory authorities. Furthermore, ELDU in feed is expressly prohibited in the United States. Finally, there is no established meat withdrawal to enable swine treated with oral meloxicam to enter the food chain. Given these scientific and regulatory constraints, the transmammary method is not allowable at the present time in commercial swine systems.

Despite several yet undiscovered pharmacologic aspects and regulatory issues of transmammary analgesia, the swine industry’s charge to maintain high standards of animal welfare remains constant. This study provides a robust foundation for further research in the field of pain alleviation, which will potentially lead to the approval of swine analgesics in the United States.