Evaluation of Analgesia Efficacy in Piglets Undergoing Surgical Castration and Tail Docking

by

Abbie Victoria Viscardi

A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Doctor of Philosophy
in
Pathobiology

Guelph, Ontario, Canada

© Abbie V. Viscardi, May, 2018
Surgical castration and tail docking are performed routinely on commercial pig farms to minimize boar taint, aggression and tail biting among pigs. While these procedures are known to be painful, piglets are not always provided sufficient analgesia or anesthesia for pain relief. This thesis project aimed to determine which analgesic drug or drug combination sufficiently reduced surgical castration and tail docking pain in piglets using behavior, vocalization and facial grimace analysis. It also assessed whether topical anesthesia could be used as an adjunct to mitigate pain and whether male and female piglets respond differently to pain or pain treatments. Finally, it sought to validate the Piglet Grimace Scale (PGS) as a tool for pain assessment. Two nonsteroidal anti-inflammatory drugs, meloxicam (0.4 mg/kg or 1.0 mg/kg) or ketoprofen (6.0 mg/kg), administered intramuscularly (IM) 20 min prior to surgical castration were ineffective at reducing pain behaviors and facial grimacing of piglets up to 24 h post-procedure. Meloxicam (0.4 mg/kg) was also insufficient at alleviating behavioral indicators of tail docking pain. Buprenorphine (an opioid, 0.04mg/kg IM) significantly reduced piglet pain behaviors and facial grimacing post-castration and tail docking with no obvious side effects. A multimodal approach to pain management [0.4 mg/kg meloxicam, 0.04 mg/kg buprenorphine, and topical lidocaine (Maxilene®)] did not provide piglets with significantly more pain relief than administration of buprenorphine alone. At the time of castration and tail docking, none of the drug treatments were
able to reduce pain-related vocalizations. There was no difference in pain behavior or response to analgesia treatments between male and female piglets. The PGS corresponds well to piglet pain behaviors when experienced scorers are used, and this may become a useful tool for piglet pain assessment. These findings are important for improving piglet welfare in the swine industry and may be used to inform future recommendations concerned with piglet pain control.
DEDICATION

In loving memory of my Nana, Lois Andrews.

I know you would have been so proud.
    I miss you.
ACKNOWLEDGEMENTS

First and foremost, I’d like to thank my advisor, Dr. Patricia Turner, for giving me this incredible opportunity and trusting me with such an important project. I’ve learned and grown so much over the past five years and I owe much of it to you. To my committee members, Dr. Bob Friendship and Dr. Tina Widowski, thank you for sharing your valuable knowledge and expertise with me.

This work would not have been possible without many talented summer students: Taylor Braden, Lily Hu, Julia Whatley, Hailey Hoffman, Brianne Mercer, Stephanie Cervi, and Anna Maystrenko. I am eternally grateful for all your hard work and dedication to my project. Thank you to Glen Cassar, for taking the time to teach me how to castrate piglets. Thank you to Jackie Peterson and Dr. Hans Coetzee, for analysing our pig plasma samples. Thank you to Tim Thalen and personnel at Arkell Swine Research Station (notably Rhys Petersen) for your assistance. Finally, thank you to everyone who volunteered their time to the tedious task of scoring piglet facial expressions.

To my lab members and friends: Jessica Walsh, Wendy Xiao and Elein Hernández, thank you for your constant, overwhelming support. I’m going to miss our wine and Disney movie nights in the future. To my family and friends, thank you for your patience and understanding these past few years. To my dog Amira, thank you for putting up with me and all my late nights at the barn. Finally, to my boyfriend Brandon Reid, thank you for your unwavering love and support. I could not have done this without you.

A special thanks to Ontario Pork, National Pork and the Ontario Veterinary College for their financial support, without which this project would not have been possible.
DECLARATION OF WORK PERFORMED

All work presented in this thesis was completed by me, with the following exceptions:

- Taylor Braden, Lily Hu, Julia Whatley, Hailey Hoffman and Brianne Mercer assisted with data collection and behavior scoring for the NSAID castration trial (Chapter 2)
- Julia Whatley, Hailey Hoffman, and Brianne Mercer assisted with data collection for the opioid castration trial, multimodal castration trial, and tail docking trial (Chapters 3-5)
- Stephanie Cervi and Anna Maystrenko behavior scored the multimodal castration trial (Chapter 4) and took vocalization measurements from the spectrograms for all trials (Chapters 3-5)
- Dr. Patricia V. Turner instrumented piglets with jugular catheters and microscopically assessed piglet liver, stomach, and kidney samples for toxicity test (Appendix B)
- Meloxicam-plasma concentrations from piglets were analysed by Jackie Peterson at Iowa State University (Appendix B)
- Julia Whatley and Hailey Hoffman assisted with data collection for the infrared thermography trial (Appendix C)
# TABLE OF CONTENTS

ABSTRACT ........................................................................................................................................... ii  
Dedication ........................................................................................................................................... iv  
Acknowledgements ............................................................................................................................... v  
Declaration of Work Performed ......................................................................................................... vi  
TABLE OF CONTENTS ....................................................................................................................... vii  
List of Tables ......................................................................................................................................... xi  
List of Figures ......................................................................................................................................... xi  
List of Appendices ............................................................................................................................... xiii  
Abbreviations ......................................................................................................................................... xv  

CHAPTER 1: Literature Review ........................................................................................................ 1  

1.1 Introduction .................................................................................................................................. 1  

1.2 Pain .............................................................................................................................................. 1  

1.2.1 Identification and Assessment................................................................................................. 2  

Behavior ............................................................................................................................................. 3  

Physiology .......................................................................................................................................... 4  

Facial Grimace Analysis ....................................................................................................................... 5  

Vocalization ........................................................................................................................................ 6  

Infrared Thermography ...................................................................................................................... 7  

1.3 Processing Procedures for Neonatal Pigs .................................................................................. 7  

1.3.1 Surgical Castration .................................................................................................................... 8  

Procedure ........................................................................................................................................... 8  

Behavior ............................................................................................................................................. 9  

Physiology .......................................................................................................................................... 9  

Alternatives ......................................................................................................................................... 10  

1.3.2 Tail Docking ............................................................................................................................... 11  

Procedure .......................................................................................................................................... 11  

Behavior and Physiology .................................................................................................................. 12  

1.3.3 Other ......................................................................................................................................... 13  

1.4 Analgesia for Pain Management ................................................................................................ 14  

1.4.1 Nonsteroidal anti-inflammatory drugs .................................................................................... 14  

Meloxicam ........................................................................................................................................ 15  

Ketoprofen ......................................................................................................................................... 17  

Other .................................................................................................................................................. 17  

1.4.2 Opioids ...................................................................................................................................... 18  

Buprenorphine .................................................................................................................................. 18  

Butorphanol ....................................................................................................................................... 18  

1.5 Anesthesia for Pain Management ............................................................................................... 19  

Lidocaine ............................................................................................................................................ 20
### CHAPTER 2: Use of meloxicam or ketoprofen for piglet pain control following surgical castration

**Abstract**

**Introduction**

**Materials and Methods**

- Animals and Treatments
- Processing Procedures
- Behavioral Recording and Scoring
- Piglet Grimace Scale Recording and Scoring
- Data and Statistical Analysis

**Results**

- Behavioral Observations
  - Comparison between NSAID-treated and control piglets
  - Comparison between castrated and uncastrated piglets
- Piglet Grimace Scale
  - Comparison between NSAID-treated and control piglets
  - Comparison between castrated and uncastrated piglets

**Discussion**

**Literature Cited**

### CHAPTER 3: Efficacy of buprenorphine for management of surgical castration pain in piglets

**Abstract**

**Background**

**Methods**

- **Part I: Opioid Pilot Study**
  - Animals and Treatments
  - Processing Procedure
  - Behavioral Recording
- **Part II: Buprenorphine Definitive Study**
  - Animals and Treatments
  - Processing Procedure
  - Behavior Recording and Scoring
  - Piglet Grimace Scale and Scoring
  - Vocalizations
  - Data and Statistical Analysis

**Results**
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavioral Recording and Scoring</td>
<td>131</td>
</tr>
<tr>
<td>Piglet Grimace Scale Scoring</td>
<td>132</td>
</tr>
<tr>
<td>Vocalizations</td>
<td>132</td>
</tr>
<tr>
<td>Data and Statistical Analysis</td>
<td>133</td>
</tr>
<tr>
<td>Results</td>
<td>133</td>
</tr>
<tr>
<td>Behavioral Observations</td>
<td>133</td>
</tr>
<tr>
<td>Piglet Grimace Scale</td>
<td>134</td>
</tr>
<tr>
<td>Vocalization</td>
<td>135</td>
</tr>
<tr>
<td>Discussion</td>
<td>135</td>
</tr>
<tr>
<td>Conclusion</td>
<td>138</td>
</tr>
<tr>
<td>References</td>
<td>139</td>
</tr>
<tr>
<td>CHAPTER 6: General Discussion</td>
<td>151</td>
</tr>
<tr>
<td>6.1 Evaluation of nonsteroidal anti-inflammatory drugs to reduce surgical castration pain</td>
<td>152</td>
</tr>
<tr>
<td>6.2 Evaluation of opioids to reduce surgical castration pain</td>
<td>154</td>
</tr>
<tr>
<td>6.3 A multimodal approach to reduce surgical castration pain</td>
<td>155</td>
</tr>
<tr>
<td>6.4 A multimodal approach to reduce tail docking pain</td>
<td>156</td>
</tr>
<tr>
<td>6.5 Validation of the Piglet Grimace Scale</td>
<td>158</td>
</tr>
<tr>
<td>6.6 Pain assessment tool</td>
<td>159</td>
</tr>
<tr>
<td>6.7 Future directions</td>
<td>159</td>
</tr>
<tr>
<td>6.8 Conclusions</td>
<td>160</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>162</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>168</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 2.1........................................................................................................66
Ethogram used to score piglet behavior, grouped into feeding, locomotion, non-specific
behaviors, pain-related behaviors, posture, and social cohesion (adapted from Hay et al., 2003).

Table 2.2........................................................................................................67
Total number of piglet faces captured for Piglet Grimace Scale scoring; NSAID castration trial.

Table 2.3........................................................................................................68
Behavioral analysis of piglets across all litters, replicates and treatments. Values presented are
the proportional means ± SE; NSAID castration trial.

Table 2.4........................................................................................................69
Behavioral analysis of piglets across all litters, replicates and time points. Values presented are
the proportional means ± SE; NSAID castration trial.

Table 2.5........................................................................................................70
Behavioral analysis of piglets pre-treatment and post-treatment across all litters, replicates, and
time points. Values presented are the proportional means ± SE; NSAID castration trial.

Table 2.6........................................................................................................71
Piglet Grimace Scale scores of piglets within each treatment group, across all litters, replicates,
and time points. Values presented are the mean score ± SE; NSAID castration trial.

Table 3.1........................................................................................................99
Total number of piglet faces captured for Piglet Grimace Scale scoring; opioid castration trial.

Table 3.2.......................................................................................................100
Behavioral analysis of piglets pre-treatment and post-treatment across all litters and timepoints.
Values presented are the proportional means ± SE; opioid castration trial.

Table 4.1.......................................................................................................123
Total number of piglet faces captured for Piglet Grimace Scale scoring; multimodal castration
trial.

Table 4.2.......................................................................................................124
Behavioral analysis of piglets across all litters and time points. Values presented represent the
proportional mean ± SE; multimodal castration trial.

Table 4.3.......................................................................................................125
Behavioral analysis of piglets pre-treatment and post-treatment across all litters and time points.
Values represent the proportional mean ± SE; multimodal castration trial.
Table 5.1
Total number of piglet faces captured for Piglet Grimace Scale scoring. Black numbers are from male piglets and red numbers are from female piglets; tail docking trial.

Table 5.2
Behavioral analysis of piglets pre-treatment and post-treatment across all litters and time points. Values presented are the proportional means ± SE; tail docking trial.

Table 5.3
Behavioral analysis of piglets across all litters and time points. Values presented are the proportional means ± SE; tail docking trial.

Table 6.1
The risk of generating false positive and false negative results using each pain assessment tool in this research project.
LIST OF FIGURES

Figure 2.1........................................................................................................................................59
The Piglet Grimace Scale (PGS) is based on scoring three facial action units (FAUs): ear
position, cheek tightening/nose bulge, and orbital tightening.

Figure 2.2........................................................................................................................................60
The proportion of time (± SE) piglets demonstrated pain-related behaviors in each treatment
group; NSAID castration trial.

Figure 2.3........................................................................................................................................61
The proportion of time (± SE) piglets engaged in active behaviors throughout the observation
period; NSAID castration trial.

Figure 2.4........................................................................................................................................62
The proportion of time (± SE) castrated and uncastrated piglets demonstrated pain-related
behaviors in total; NSAID castration trial.

Figure 2.5........................................................................................................................................63
The proportion of time (± SE) castrated and uncastrated piglets demonstrated pain-related
behaviors throughout the observation period; NSAID castration trial.

Figure 2.6........................................................................................................................................64
Mean Piglet Grimace Scale scores (± SE) in each treatment group; NSAID castration trial.

Figure 2.7........................................................................................................................................65
Mean Piglet Grimace Scale scores (± SE) of castrated and uncastrated piglets; NSAID castration
trial.

Figure 3.1........................................................................................................................................92
Mean proportion of time (± SE) piglets engaged in active behaviors in each treatment group;
opioid castration trial.

Figure 3.2........................................................................................................................................93
Mean proportion of time (± SE) piglets displayed pain behaviors in each treatment group; opioid
castration trial.

Figure 3.3........................................................................................................................................94
Mean proportion of time (± SE) piglets engaged in active behaviors within each treatment group
across the observation period; opioid castration trial.

Figure 3.4........................................................................................................................................95
Mean proportion of time (± SE) piglets demonstrated pain behaviors within each treatment group
across the observation period; opioid castration trial.
Figure 3.5 Mean Piglet Grimace Scale scores (± SE) in each treatment group; opioid castration trial.

Figure 3.6 Vocalization (a) frequency, (b) amplitude, and (c) power (± SE) of all piglets undergoing each procedure; opioid castration trial.

Figure 3.7 Vocalization (a) frequency, (b) power, and (c) energy (± SE) of piglets in each treatment group; opioid castration trial.

Figure 4.1 Mean proportion of time (± SE) piglets demonstrated pain-related behaviors in each treatment group; multimodal castration trial.

Figure 4.2 Mean proportion of time (± SE) piglets demonstrated pain-related behaviors within each treatment group and time point; multimodal castration trial.

Figure 4.3 Mean Piglet Grimace Scale scores (± SE) in each treatment group; multimodal castration trial.

Figure 4.4 Mean vocalization (a) frequency (Hz) and (b) power (dB) ± SE of piglets across all procedures in each treatment group; multimodal castration trial.

Figure 5.1 The mean proportion of time (± SE) piglets demonstrated pain-related behaviors after tail docking.

Figure 5.2 The mean proportion of time (± SE) piglets demonstrated pain-related behaviors after tail docking across time.

Figure 5.3 The mean proportion of time (± SE) piglets engaged in active behaviors after tail docking.

Figure 5.4 Mean Piglet Grimace Scale scores (± SE) within each treatment group after tail docking.

Figure 5.5 The (a) frequency (Hz), (b) power (dB), and (c) energy (dB) of vocalizations emitted by piglets during marking, injection and tail docking ± SE.
LIST OF APPENDICES

APPENDIX A: Development of a Piglet Grimace Scale to evaluate piglet pain using facial expressions following castration and tail docking: a pilot study

Abstract .................................................................................................................................................. 169
Introduction .......................................................................................................................................... 170
Animals and Methods .......................................................................................................................... 171
  Animals and Treatments .................................................................................................................... 171
  Processing Procedures ...................................................................................................................... 172
  Behavioral Recording and Scoring ................................................................................................... 173
  Piglet Grimace Scale (PGS) Recording and Scoring ....................................................................... 174
  Data and Statistical Analysis ........................................................................................................... 175
    Behavior Analysis ........................................................................................................................... 175
    Grimace Scale Analysis .................................................................................................................. 175
    Correlation between Behavior and Grimace Scores ...................................................................... 176
Results ..................................................................................................................................................... 176
  Behavioral Observations after Castration and Tail Docking ............................................................ 176
  Piglet Grimace Scale ......................................................................................................................... 176
  Correlation between Behavior and PGS .......................................................................................... 177
Discussion .............................................................................................................................................. 177
Animal Welfare Implications and Conclusion .................................................................................. 180
References ............................................................................................................................................ 182
Figures ................................................................................................................................................... 186
  A.1: The Piglet Grimace Scale ........................................................................................................... 186
  A.2: Comparison of piglet activity level and grimace scores .............................................................. 187
Tables ..................................................................................................................................................... 188
  A.1: Number of piglets per treatment group ...................................................................................... 188
  A.2: Total number of faces captured for Piglet Grimace Scale scoring ........................................... 189
  A.3: Behavioral analysis of castrated and tail docked piglets across all treatments and time points .................................................................................................................................................. 190

APPENDIX B: Pharmacokinetics of 1.0 mg/kg meloxicam in piglets

Introduction .............................................................................................................................................. 191
Materials and Methods ....................................................................................................................... 192
  Animals and Procedures .................................................................................................................. 192
  Pharmacokinetic Sampling ............................................................................................................. 193
  Gross and Microscopic Pathology Evaluations ............................................................................... 193
  Data Analysis ..................................................................................................................................... 194
Results ................................................................................................................................................... 194
Discussion ............................................................................................................................................ 195
References ............................................................................................................................................ 196
APPENDIX C: Utility of infrared cranial temperature measurements for assessing pain in piglets

Introduction .................................................................................................................. 202
Materials and Methods .............................................................................................. 203
Results ......................................................................................................................... 205
Discussion ................................................................................................................... 206
Conclusions .................................................................................................................. 207
References ..................................................................................................................... 209

Figures ......................................................................................................................... 212
C.1: Infrared thermography image capturing piglet cranial temperature .................. 212
C.2: Mean cranial temperature (°C ± SE) of piglets in each treatment group .......... 213
C.3: Mean weight (kg ± SE) of piglets in each treatment group .............................. 214
C.4: Mean cranial temperature (°C ± SE) of boar and gilt piglets combined at each
time point .................................................................................................................. 215

Tables ......................................................................................................................... 216
C.1: Number of piglets per sex assigned to each treatment group ....................... 216
# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUP</td>
<td>Buprenorphine</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>FAU</td>
<td>Facial action unit</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass correlation coefficient</td>
</tr>
<tr>
<td>IM</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>IRT</td>
<td>Infrared thermography</td>
</tr>
<tr>
<td>KET</td>
<td>Ketoprofen</td>
</tr>
<tr>
<td>LBW</td>
<td>Low body weight</td>
</tr>
<tr>
<td>MAX</td>
<td>Maxilene®</td>
</tr>
<tr>
<td>MEL</td>
<td>Meloxicam</td>
</tr>
<tr>
<td>NSAID</td>
<td>Nonsteroidal, anti-inflammatory drug</td>
</tr>
<tr>
<td>PGS</td>
<td>Piglet Grimace Scale</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetic</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>SNS</td>
<td>Sympathetic nervous system</td>
</tr>
</tbody>
</table>
CHAPTER 1

Literature Review

1.1 Introduction

Piglets raised on commercial pig farms in North America undergo several painful procedures, including surgical castration and tail docking, for which they are often not provided sufficient analgesia or anesthesia for pain relief (Sutherland, 2015). The Canadian Code of Practice for the Care and Handling of Pigs as well as animal care guidelines in the EU require analgesia administration prior to processing (EU Commission, 2010; NFACC, 2014). However, there is limited research regarding effective pain management strategies for piglets post-procedure, making recommendations difficult (O’Connor et al., 2014; Sutherland, 2015). This research aims to strengthen the literature regarding piglet pain control by assessing multiple analgesics from different classes, with or without topical anesthesia, for their ability to reduce surgical castration and tail docking pain in piglets. These results are important for veterinarians, producers and other groups to make accurate and specific recommendations, ensuring optimal piglet care and welfare.

1.2 Pain

Pain is an integral consideration when discussing animal care and welfare. It is defined as an “unpleasant sensory and emotional experience relating to actual or potential tissue damage or described in terms of such damage” (IASP, 1979). This definition implies that pain is a subjective state that can differ between individuals. As such, pain assessment relies heavily on patient self-reports, with the National Institute of Health reporting it as the most reliable indicator for pain presence and intensity (Bahreini et al., 2015). As animals are unable to verbally communicate the source and feelings of pain they are experiencing, a subjective approach to pain assessment would have limited utility (Livingston, 2002). As well, whether or not animals experience an emotional response to pain is controversial (Anil et al., 2005). Perhaps
a more relevant definition, focusing on physical pain in animals, would be that if analgesics improve the outcome of a situation, the animal was experiencing pain (Gibson, 1985).

The structures and pathways that are used to receive and send pain signals are well defined in animals, as are the pharmacologic methods available for pain mitigation (Weary et al., 2006). Drugs, such as analgesics, are used to prevent pain and are most beneficial when administered prior to a painful procedure to permit adequate blood and tissue drug levels to occur (Anil et al., 2005). However, when a procedure is performed on an animal that has the potential to cause pain, appropriate treatment does not always follow (Weary et al., 2006; Rault et al., 2011). In the case of food animals, this may be due to drug approval limitations, the added cost, time and effort involved with implementing analgesia into protocols or the extra training that would be needed (Rault et al., 2011; Aluwé et al., 2015). It has also long been supposed that neonates are incapable of experiencing pain because of their immature nervous system and lack of specific behavioral signs (Lee, 2002). For many years, this belief has led producers and veterinarians to provide no analgesics or anesthetics to animals if painful procedures were conducted at a young age. However, there is now evidence to suggest neonates experience pain more intensely than adults, making pain-relieving drugs even more important for young animals undergoing a surgical procedure (Mellor and Gregory, 2003).

1.2.1 Identification and Assessment

Identifying pain in animals is crucial for appropriate treatment and prevention. Difficulties in recognizing pain contribute to suboptimal analgesia use in veterinary practice (Reid et al., 2007). Objective means of pain assessment often involve measures of general physiologic function, such as body weight loss and plasma cortisol concentrations, and behavior analyses, such as vocalizations and activity-time budgets (Leidig et al., 2009; Sutherland et al., 2011; Kluivers-Poodt et al., 2012; Cassar et al., 2014). By using multiple measures of analysis for pain assessment, results will be more reliable and the risk of false positive or false negative outcomes will be reduced. However, for a method to be practical for use on-farm, it needs to be quick and inexpensive (Weary et al., 2006).
To validate pain indicators, measures such as behavior and physiology are taken of an animal in a non-painful (baseline) state. The animal will then undergo a procedure that causes tissue trauma and/or inflammation (an assumption can be made that this results in pain) and the same measures are taken again. A consistent and predictable change in an animal’s behavior and physiology when pain is induced has resulted in these measures becoming validated as reliable indicators of pain.

**Behavior**

The most important single indicator of pain is a change from normal behavior (Anil et al., 2005). Therefore, behavioral analysis is often regarded as the gold standard for pain evaluation in animals (Weary et al., 2006). Specific behavioral changes associated with many frequently studied pain conditions have been described (Keefe et al., 1991). Behavior scoring methods range from using a visual analogue scale (VAS) to a sophisticated software program, such as Observer XT. The VAS is simply a sliding scale from 1 (no pain) to 10 (severe pain) and is based on an individual’s assessment of an animal’s pain condition. Data collection using the VAS is quick and easy but highly subjective, as individuals vary in their ability to recognize pain symptoms, which may lead to inappropriate analgesia treatment of animals (Roughan and Flecknell, 2003; Hansen, 2003). The Observer XT program is used to collect, analyze, and present observational data. It can be used to record the frequency and duration of pain-related behaviors displayed by an animal, making it a more objective method of pain assessment than the VAS; however, data is collected manually, which can be time-consuming and impractical for use on a large-scale commercial farm (Hay et al., 2003). Automated measures to assess animal behavior are significantly less laborious to collect and limit the need for direct human contact. Prey species have been known to alter or mask their pain response in the presence of a perceived predator (e.g., human), which could confound pain assessments (Weary et al., 2009). An automated behavior scoring system used for mice (HomeCageScan) was unable to differentiate between surgical stress and post-surgical pain whereas manual scoring of pain behaviors specifically assessed post-surgical pain (Leach et al., 2012), suggesting a manual behavior scoring system is currently better suited to pain assessment. Automated scoring systems may be more appropriate to assess general changes in behavior after a painful event, such as posture,
feeding, drinking and locomotion (Jourdan et al., 2001; Richardson, 2015). Species-specific pain scales have also been developed, such as the Glasgow Composite Measure Pain Scale (CMPS) used to assess acute pain in dogs. The CMPS uses seven behavioral categories to assess pain: activity, posture, vocalization, attention to wound or painful area, mobility, demeanour, and response to touch (Reid et al., 2007). This scale was used to define an analgesic intervention score for painful dogs, making it practical for use in a clinical setting. Ideally, pain scoring systems should be applicable to a range of strains and ages of animal, and to different surgical procedures (Roughan and Flecknell, 2003).

In general, animals in pain often reduce their activity level (Berger and Eeg, 2008; Gebhart et al., 2009). Prey species are normally stoic when ill or in pain, to reduce their risk of predation (Weary et al., 2009). This is a major limitation to relying solely on behavioral assessment to detect pain and why there is often greater concern for the welfare of a naturally stoic animal who is outwardly displaying signs of pain (Mason and Mendl, 1993).

**Physiology**

Physiologic measures can be used to assess pain in animals. Stress triggers the sympathetic nervous system which releases adrenaline and causes physiologic changes, such as increased heart rate, blood pressure, and respiration rate. (Gregory et al., 2004; Weary et al., 2006). It also affects the hypothalamic-pituitary-adrenocortical (HPA) axis, leading to increased levels of circulating cortisol in the blood (Weary et al., 2006). In human medicine, efforts to substitute self-report with a clinically valid, physiology-based measure of pain without the use of fMRIs or evaluating brain activity have been unsuccessful (Brown et al., 2011). Collection of physiologic measures often require animals to be handled or restrained, eliciting a stress response, and may be invasive or painful (e.g., venipuncture for blood draw). This limits the applicability of using physiology-based measures on-farm and is a significant confound, as fear, anxiety and stress from the data collection alone can enhance responses to painful events (Gebhart et al., 2009). Stress, pain, and excitement also trigger a similar physiologic response (e.g., increased blood cortisol) which is a major limitation to using these as primary pain assessment tools without a behavioral context (Aronson et al., 1992).
Facial Grimace Analysis

Facial grimace scales are a novel, non-invasive tool for pain assessment, using quantifiable changes to facial features (e.g., orbital tightening, ear position, cheek tightening) to detect pain. They have been developed for many animals, including mice, rats, rabbits, horses, sheep, lambs and piglets, and non-verbal humans (Langford et al., 2010; Herr et al., 2011; Sotocinal et al., 2011; Keating et al., 2012; Costa et al., 2014; Guesgen et al., 2016; McLennan et al., 2016; Häger et al., 2017; Viscardi et al., 2017). Most of these grimace scales have demonstrated high interobserver reliability when they are used to score still-images of animal facial expressions; however, this method of scoring is retrospective, resulting in a delay in pain treatment should pain be detected. The Mouse Grimace Scale (MGS) and Rat Grimace Scale (RGS) have been used to detect pain in animals real-time, allowing for rapid analgesic intervention and making them practical for use in a research or clinical setting (Miller and Leach, 2015; Leung et al., 2016). This suggests pen-side detection of pain is possible on-farm using a validated grimace scale. To be validated as a pain assessment tool, grimace scales must correspond well to known indicators of pain, such as behavior (Miller et al., 2016).

Facial grimacing may not be consistent across all breeds or strains of the same species. Miller et al., (2015) found isoflurane anesthesia increased grimace scores in male DBA/2 mice, yet isoflurane anesthesia alone had no effect on facial grimacing in male CBA mice. As well, all facial action units within each grimace scale may not be equally useful in detecting pain. A Piglet Grimace Scale developed by Di Giminiani et al., (2016) and a Ferret Grimace Scale developed by Reijgwart et al., (2017) determined orbital tightening alone most strongly related to animal pain. With these considerations, grimace scale use has become much more prevalent in animal pain research. The MGS was found to be sensitive enough to detect low-level pain in mice 24 h after experimental myocardial infarction; this pain was not identified using a standard welfare scoring system, suggesting the MGS has benefits over other pain assessment tools (Faller et al., 2015). The MGS has also been used to assess the efficacy of recommended doses of four analgesics commonly used in mice: buprenorphine, carprofen, ketoprofen, and acetaminophen, to reduce facial grimacing after laparotomy (Matsumiya et al., 2012). Buprenorphine was found to be highly effective at reducing pain-induced facial grimacing at doses equal to or lower than
current recommendations, ketoprofen and carprofen were effective at doses higher than recommended and acetaminophen was ineffective at any dose studied, suggesting recommended doses for postoperative pain management in mice may need to be revised (Matsumiya et al., 2012). Using the RGS, Oliver et al., (2014) determined an analgesic intervention score; when facial grimace scores of rats move beyond an identified threshold, analgesic intervention should occur, thus improving the clinical application of the RGS. This novel tool for pain assessment has the potential to greatly improve animal welfare, as it has demonstrated success in rapidly and specifically detecting pain and has on-farm applicability due to the cost (free), ease of use and non-invasive nature.

**Vocalization**

Animals, such as mice, rats, dogs, cats, piglets, and non-human primates, as well as non-verbal humans have a vocal response to pain (Cooper and Vierck Jr., 1986; Noonan et al., 1996; Leidig et al., 2009; Mogil, 2009; Brondani et al., 2011; Lautenbacher et al., 2017). Compared to handling alone, piglets in pain often emit more vocalizations of higher frequency, amplitude, energy, and power that extend for a longer duration of time (Taylor and Weary, 2000; Marx et al., 2003; Leidig et al., 2009). Classification of these pain vocalizations have led to the development of both manual (e.g., Raven) and automated (e.g., STREMODO: Stress Monitor and Documentation unit for pigs) sound analysis software to detect when an animal is in pain or distress (Jourdan et al., 2001; Schön et al., 2004; Bovey et al., 2014). However, in a farm setting, other sounds (e.g., environment-based, such as automated feeders and fans) can interfere with vocalization monitoring (Vandermeulen et al., 2015).

All animals do not vocalize within the audible range. Rodents, for example, emit ultrasonic vocalizations unless they are subject to severe acute pain (Mogil, 2009). This requires specialized equipment for detection. As well, high frequency vocalizations are not always an indicator of pain. Piglets have a low threshold for producing these vocalizations, which may be mistaken for pain in the absence of proper analysis or a behavioral context within which the calls were emitted (Stafleu et al., 1992; Gigliuto et al., 2014). Therefore, vocalization as a single
indicator is not a sensitive pain measure but can be used as an adjunct to behavioral or physiologic analysis (Gigliuto et al., 2014).

**Infrared Thermography**

As previously mentioned, the sympathetic nervous system is activated when an animal is stressed or in pain, causing an increase in blood pressure, heart and respiration rate, and peripheral vasoconstriction (Macefield, 2010; Herborn et al., 2015). Vasoconstriction decreases blood flow to the skin, reducing cutaneous temperature and increasing core temperature (Oka et al., 2001). This physiologic response to pain has been measured in many species, including pigs, cattle, and chickens, using an infrared thermography camera (Stewart et al., 2005; Edgar et al., 2013; Bates et al., 2014; Lonardi et al., 2015). After surgical castration, piglets were found to have increased eye temperature and decreased cranial skin temperature (Bates et al., 2014; Lonardi et al., 2015). There was a positive association found between blood plasma meloxicam levels and cranial skin temperature; increased meloxicam in the blood correlated to an increase in cranial temperature after surgical castration (Bates et al., 2014). This suggests a physiologic benefit to meloxicam administration prior to a painful procedure. Further validation of infrared thermography as a pain assessment tool is needed, to determine how well it correlates with behavioral measures.

Infrared thermography is a novel, non-invasive tool to objectively assess pain; however, it has low specificity, as vasoconstriction can occur outside of a painful event (e.g., animals exposed to cold environmental conditions) (Young et al., 1996). As with vocalization measures, infrared thermography should be used in conjunction with other pain assessment methods and not as a stand-alone tool. Infrared thermography cameras are also very expensive, which may limit their on-farm practicality.

**1.3 Processing Procedures for Neonatal Pigs**

Piglets undergo many processing procedures during their first week of life, potentially including an iron injection, castration, tail docking, teeth clipping, and ear notching (Leslie et al., 2010; Sutherland, 2015). These procedures are known to cause pain, yet piglets are not always
provided analgesia or anesthesia for pain relief (Taylor et al., 2001; Van Beirendonck et al., 2011). Behavioral changes associated with pain tend to persist when piglets undergo multiple procedures in a relatively short amount of time, as is often the case for piglets when they are processed (Noonan et al., 1994). The focus of this review will be on surgical castration and tail docking of piglets.

1.3.1 Surgical Castration

Castration refers to the removal of the testicles or destruction of testicular function (Rault et al., 2011). Male piglets on commercial production farms are routinely castrated to prevent boar taint (an unpleasant smell or taste associated with the cooked meat from intact male pigs) and agonistic behaviors (Hay et al., 2003). In North America, castration is performed surgically when piglets are 3-7 days old (CVMA, 2010). When done by a skilled and trained operator, the procedure is rapid and takes between 21-71 seconds (Fredriksen et al., 2009). Different techniques may be used for surgical castration. The incision can be made as one horizontal cut (across both testicles) or, more commonly, as two vertical incisions (one down each testicle) (Rault et al., 2011). There is no research to date regarding which of these incision techniques causes the least amount of pain or distress to the piglet. Once the testicles are exposed, removal from the body involves severing the spermatic cord by cutting or tearing (Taylor and Weary, 2000). This has been identified as the most painful part of the castration procedure (Leidig et al., 2009). Both techniques elicit the same intensity of pain response from piglets, indicating one method need not be preferred over the other (Taylor and Weary, 2000). Weary et al. (1998) studied the variation in piglet handling during castration (e.g., suspension from legs, placement in a V-trough) and failed to identify any one handling technique that caused less pain and distress to the animal over another. There are also complications that can arise from this procedure, such as hemorrhage, infection, excessive swelling and intestinal herniation through the castration site (Taylor and Weary, 2000). Surgical castration can also cause an increase in pre-weaning mortality in low body weight piglets (partially attributable to surgical and post-surgical complications) and a decrease in growth rate, leading to production losses (Morales et al., 2017).
Studying the change from normal behavior in piglets has led to the identification of castration-related pain behaviors. These include an increase in awake inactive (or restless) behaviors, more time spent isolated from littermates, stiffness, trembling, scratching the rump, tail wagging, and defensive behaviors when handled (Hay et al., 2003; Leidig et al., 2009). As well, piglets produce distinct high-frequency vocalizations associated with the castration procedure (Weary et al., 1998; Leidig et al., 2009). Finally, piglets spent more time at the udder, engaging in suckling behaviors, which has been suggested to produce analgesic-like effects by promoting central endorphin release in neonates (Field and Goldson, 1984; Blass, 1994).

Piglets had increased heart and respiration rates associated with castration (White et al., 1995). They also had higher blood cortisol levels after castration, when compared to cortisol levels of piglets that were just handled and not castrated (Kluivers-Poodt et al., 2012). Physiologic measures should be approached cautiously or used in conjunction with behavioral measures when interpreting pain, as there may be other explanations for changes in physiology that are not pain-related, such as handling stress (Soltis et al., 2003).

Heinritzi et al. (2006) found that piglets castrated at 4 days vs. 28 days of age had wounds that healed more rapidly and there were less complications associated with the procedure. Concerning age, there has been debate over whether the age at which piglets are castrated affects their pain response. McGlone and Hellman (1988) found 2-week-old piglets were less affected by castration than 7-week-old piglets, based on behavioral measures. However, Taylor et al. (2001) studied the behavior of castrated pigs at 3, 10, and 17 days of age and found no difference in vocalizations or behavior, suggesting pain was similar irrespective of age. As well, McGlone et al. (1993) found castration produced a similar pain response in piglets castrated at 1, 5, 10, 15, and 20 days of age. Piglets are typically castrated during their first week of life under current management practices, to reduce complications observed in late-age castrations (CVMA, 2010).

Castration is known to cause acute pain to piglets but it may also induce chronic pain (Rault et al., 2011). This is an area that needs to be explored further. Hay et al. (2003) found that
behavioral alternations associated with castration persisted beyond 24 hours, with some abnormal behaviors present 4 days later. Moya et al. (2008) also found that pain and distress associated with surgical castration persisted for up to 4 days. Longer-term studies on castration pain persistence are needed to examine the existence or absence of chronic pain. This is important for animal welfare, as well as production, as ongoing pain negatively affects growth and immune function of pigs (Anil et al., 2005). As well, neonatal pain exposure in rats has been shown to alter the developing brain and lead to decreased pain thresholds, stress vulnerability and anxiety in adulthood (Anand et al., 1998). This increased stress response, if found in pigs, would be an animal welfare and economic concern, as stressed pigs tend to have poorer meat quality after slaughter (Van de Perre et al., 2010).

Anatomic issues limit potential alternative procedures that can be done in lieu of surgical castration. These include rubber ring and Burdizzo methods that are commonly used in cattle and sheep, which have more externalized scrotum (Molony et al., 1995; Kent et al., 2001). Immunocastration is a viable alternative to surgical castration. It involves giving two doses of a gonadotropin releasing factor (GnRF) vaccine that inhibits testicular function by down-regulating the hypothalamic-pituitary-gonadal axis, thereby reducing androgen levels (Baumgartner et al., 2010). When compared to surgically castrated pigs, immunocastrated pigs (i.e., piglets that received an injection) were more active post-procedure, suggesting that it is less painful (Baumgartner et al., 2010). As well, vaccination against GnRF effectively prevents boar taint. There have been mixed results on the impact it has on aggressive behaviors (Cronin et al., 2003; Baumgartner et al., 2010). A commercially available vaccine (Improvac®) has been approved for use in over 50 countries and may be a viable alternative to surgical castration, although public acceptance to date has been poor (Rault et al., 2011). Another solution to avoid surgical castration would be to sort semen according to sex and produce only female pigs (Fredriksen et al., 2009). This would remove the need to castrate entirely, although it would lead to an overall decrease in feed efficiency and conversion ratios, which are higher in boar pigs (Morales et al., 2011). This technique is not available for routine use in pigs at present (Fredriksen et al., 2009). Entire male production and lower slaughter weight is becoming more common in Europe; however, issues surrounding heightened levels of aggression on-farm and
boar-tainted carcasses at slaughter have prevented its widespread adoption (Bee et al., 2015). Consumers preferred all alternative methods to surgical castration without pain relief and cited animal welfare being an important factor (Rollins, 2004; Heid and Hamm, 2013). Producers appear to favor production of entire males as the best alternative to surgical castration, based on perceived improvement on performance, profitability, and reduced labor demands (Aluwé et al., 2015). Currently, the most practical solution to reducing the pain of surgical castration is to provide piglets with an analgesic or anesthetic agent pre-procedure. Analgesia protocols can be easily implemented into any commercial system and are now required to be administered to piglets in Canada prior to castration (NFACC, 2014).

1.3.2 Tail Docking

Tail docking refers to the removal of approximately one third of the piglet’s tail to reduce the likelihood of tail biting among pigs (Zonderland et al., 2010). Tail biting can be a serious issue in commercial pig production systems. There are obvious welfare concerns for the pig being bitten, including pain, risk of infection, abscesses, and even death, if the wounds are severe (Noonan et al., 1994; Schröder-Petersen and Simonsen, 2001). The pig biting the tail is also at risk of contracting disease, if they ingest infected flesh and blood (Cowen et al., 1990; Schröder-Petersen & Simonsen, 2001). Zonderland et al. (2010) found female pigs were more likely to tail bite and, when housed in mixed-sex groups, male pigs were more likely to have tail damage from being bitten. Further research is needed to confirm these sex differences and determine why they might occur. Age may also play a role in tail biting prevalence. For example, Van de Weerd et al. (2005) found pigs displayed less tail biting behavior as they got older. The most widely accepted conclusion for why pigs bite tails is because they are frustrated (Schröder-Petersen & Simonsen, 2001). This may be due to poor environmental enrichment, inappropriate housing temperatures or ventilation, high stocking densities, inappropriate sex ratios/pen and methods of feeding management (Hunter et al., 2001; Zonderland et al., 2010). Hunter et al. (2001) found that when pigs were provided with straw, they spent more time rooting and manipulating the straw and were less likely to tail bite. This group also found that artificial ventilation and floor feeding increased tail biting behaviors. Pigs housed in single-sex groups were more likely to bite tails, as are pigs subjected to high stocking densities (Kjell et al., 1982; Hunter et al., 2001). Tail biting
may still occur in docked pigs, but this is currently the most effective management procedure to reduce the incidence of tail biting (Noonan et al., 1994; Hunter et al., 2001).

Similar to surgical castration, tail docking produces pain-related behaviors as well as a physiologic response. Noonan et al. (1994) found an increase in tail wagging and tail jamming (tucking the tail stump between the hind limbs) behaviors in docked piglets. Tail jamming may serve a protective function, whereas tail wagging could be related to tail stump hyperalgesia (Hay et al, 2003). Piglets also emit increased stress vocalizations at the time of tail docking (Sutherland et al., 2011). Regarding physiology, docked piglets had increased cortisol levels when compared to piglets that were handled only and not tail docked (Sutherland et al., 2008).

Tail docking is performed surgically, when piglets are 1-3 days old (NFACC, 2014). The tail is removed using side-pliers, a scalpel blade, scissors, or electrical cautery iron (Sutherland et al., 2015). Sutherland et al. (2008) compared two methods of tail docking and found higher plasma cortisol levels in piglets docked using side-pliers than with a cautery iron; however, the behavioral response to both methods was similar. This suggests that the piglet’s acute physiologic stress response can be reduced using a hot cautery iron docking method.

Bovey et al. (2014) compared the responses of low birth weight (LBW) piglets and average birth weight (ABW) piglets to the tail docking procedure and found LBW piglets produced fewer calls with lower frequency than ABW piglets. This suggests LBW piglets had a less painful response to the procedure, although it was noted that piglets in this weight class spent more time isolated, missed nursing bouts, and were noticeably lethargic. These behaviors are suggestive of acute pain and discomfort. Bovey et al. (2014) then compared the tail docking pain response of piglets at 1 vs. 3 days of age and found ABW piglets were less vocally reactive to the procedure when it was performed at the younger age. It may be beneficial to perform tail docking procedures on younger piglets of average birth weight, but to delay procedures for low birth weight piglets as they are often weaker and have a lower chance of surviving to weaning (Van Beirendonck et al., 2012). This would reduce their unnecessary exposure to painful procedures.
The high prevalence of tail biting is a serious concern on commercial pig farms, with as many as 95% of piglets having tail damage as a result (Zonderland et al., 2010). The most effective, and seemingly practical, solution to this problem with current management systems is to dock the tail. While this procedure is known to be painful, alternatives are not readily available. As such, piglets should be provided with analgesia prior to being docked for pain management. As with surgical castration, piglets in Canada must now be provided analgesia to control post-procedural pain (NFACC, 2014).

1.3.3 Other

Piglets receive an intramuscular iron injection (often 200 mg iron dextran) in their first week of life to prevent anaemia (Yu et al., 2002; Sutherland, 2015). In outdoor systems or in the wild, piglets root in the soil and eat plants to acquire iron; however, this is not possible in indoor systems (Kleinbeck and McGlone, 1999). Piglets rely solely on the sow’s milk to meet their needs for essential minerals, which does not provide enough iron to maintain adequate haemoglobin levels in the piglet’s blood, hence the need for supplementation (Brown et al., 1996). The handling required to administer an iron injection is stressful to the piglet and poor injection technique may cause muscle trauma or abscesses (Brown et al., 1996; Loh et al., 2001). Alternatively, piglets could be provided iron orally or naturally (if given access to soil or organic matter); however, the iron available to the piglet through these routes of administration may not be sufficient to prevent anaemia (Sutherland, 2015). Under current management practices, the benefits provided by the iron injection likely outweigh the acute stress and pain experienced by the piglet.

Piglets are born with eight, sharp ‘needle teeth’ used to inflict damage to littermates when competing for teat position (Brown et al., 1996). These fights can cause piglets to suffer severe facial injuries and may cause painful lesions on the udder of the sow (Sutherland et al., 2015). Clipping of the needle teeth (removing the top third or the entire tooth) is done using an electric grinder or side-cutting pliers to prevent fighting injuries. Regardless of the tool used, this procedure can cause mouth lesions, abscesses or hemorrhaging (Hay et al., 2004). Using an electric grinder to teeth clip requires piglets to be handled longer than using side-pliers, which
increases their stress and cortisol response (Marchant-Forde et al., 2009). The use of side-pliers increases the incidence of pulp cavity openings, tooth fractures, and gum lesions, causing pain and putting piglets at risk of bacterial infection (Brown et al., 1996; Hay et al., 2004; Gallois et al., 2005). The literature does not conclusively support teeth clipping as a preventative to injuries caused by piglet fighting (Marchant-Forde et al., 2009). The only alternative to teeth clipping is to not perform the procedure and leave the needle teeth intact.

Ear notching or ear tagging piglets is done for identification purposes, to assist with on-farm pig management and to ensure traceability of animals at slaughter (for quality and public health assurance) (Leslie et al., 2010). Ear notching appears to cause short-term acute pain, based on vocalization and behavioral alterations (Torrey et al., 2009). Cryoanesthesia using a topical vapocoolant spray reduced the pain response of piglets to the ear notching procedure (Lomax et al., 2018). This type of anesthesia is unlikely to provide post-procedural pain management but is practical to administer on-farm and can provide piglets with pain relief at the time of ear notching.

1.4 Analgesics for Pain Mitigation

Analgesics are pharmaceutical substances that are used to treat and prevent pain and are most effective when given pre-emptively.

1.4.1 Nonsteroidal anti-inflammatory drugs

The most common class of analgesics given to food animals, such as pigs, are nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs produce analgesia and suppress inflammation by targeting cyclooxygenase-1 and -2 mediators (Papich, 2008). Both forms of cyclooxygenase catalyze arachidonic acid to create prostaglandins (Thun, 2008). COX-1 is expressed in nearly all cells of the body and is needed for normal physiologic function of the gastrointestinal tract, liver and kidneys whereas COX-2 is up-regulated by growth factors and inflammatory cytokines as part of an immune response (Papich, 2008; Thun, 2008). Inflammation, along with release of a cascade of mediators (such as bradykinins, leukotrienes and prostaglandins), are what evoke a pain response in the animal (Schweizer et al., 1984).
Pharmaceutical companies have attempted to develop NSAIDs that are COX-2 specific to avoid the gastrointestinal side effects of COX-1 inhibition; however, side effects still exist with COX-2 inhibitors, such as cardiovascular toxicity (Thun, 2008).

**Meloxicam**

Meloxicam is an NSAID that is used to treat pain in a range of animal species, with pharmacologic effects lasting for at least 4 hours (Keita et al., 2010). It has been shown to be efficacious in mitigating pain for procedures such as dehorning in calves as well as castration and tail docking in lambs (Heinrich et al., 2009; Small et al., 2014). It is also one of the few analgesics approved for use in swine in the EU and Canada (Bates et al., 2014; CPC 2016). It can be given orally, but more commonly, it is injected intramuscularly (Papich, 2008). Bates et al. (2014) found that oral meloxicam given to the sow is transferred to piglets through the sow’s milk. Transmammary drug delivery would eliminate the need to inject each piglet prior to a painful procedure, which would reduce piglet stress associated with prolonged handling and allow procedures to be carried out more rapidly. Unfortunately, gastrointestinal, hepatic and renal toxicity are significant concerns, as well as the economic cost, as sows had to be administered meloxicam at a dose 75x the current recommendation to achieve minimal concentrations in nursing piglets (Bates et al., 2014).

Meloxicam has had variable success in significantly reducing surgical castration pain in pigs. A previous study concluded it was ineffective at decreasing painful behaviors (Kluivers-Poodt et al., 2012), yet others have found meloxicam successfully reduced castration-related pain behaviors in piglets (Keita et al., 2010; Hansson et al., 2011; Kluivers-Poodt et al., 2013). However, these latter studies contain design flaws that must be addressed. Keita et al. (2010) only observed piglets for a few minutes at each time point (0.5, 1, 2, 4, and 24 h post-castration) and used a very simplistic ethogram, only scoring piglets on the presence (1) or absence (0) of prostration, tremors, tail movements and isolation. These presence and absence scores were tallied at the end of the study and used to determine whether or not piglets were in pain. Because this study design was oversimplistic, had a short observation period with a poorly developed ethogram, the conclusion that meloxicam reduced pain behaviors should be challenged. While
Kluivers-Poodt et al. (2013) used a more detailed ethogram and sophisticated scoring system (Observer software), they employed a scan sampling method of data collection which may have resulted in a large amount of pain behaviors being missed. Hansson et al. (2011) used a higher dose of meloxicam than what is currently being recommended (1.0 mg vs. 0.4 mg/kg). This group also castrated piglets between 1-7 days of age and all piglets received 1.0 mg of meloxicam regardless of their body weight. This higher dose of meloxicam was likely responsible for the decrease in pain behaviors that was reported. Cortisol concentrations were also lower in piglets that received meloxicam pre-procedure (Keita et al., 2010). Bates et al. (2014) used infrared thermography cameras and found that meloxicam caused an increase in piglet cranial skin temperature. When animals are stressed or in pain, the sympathetic nervous system is activated, vasoconstriction occurs and there is a decrease in cutaneous temperature (Bates et al., 2014). Both cortisol measures and cranial skin temperature are not specific to the painful experience and should not be used solely to assess drug efficacy. NSAID use has also been unsuccessful in reducing post-surgical pain behaviors caused by tail docking (Herskin et al., 2016).

The recommended dose of meloxicam for piglets is 0.4 mg/kg. This was determined from studies evaluating sows suffering mastitis-metritis-agalactia and lameness in adult pigs (Hirsch et al., 2003; Mustonen et al., 2011). The metabolism of meloxicam in neonatal pigs likely differs from that of grown pigs; therefore, the recommended dose for adults may not be adequate for piglets. Fosse et al. (2008) conducted a pharmacokinetic study using 0.4 mg/kg of meloxicam in piglets. They used an inflammation model in which a subcutaneous pouch was created and implanted with sponges soaked in 1% solution of carrageenan (an irritant) and found that 0.4 mg/kg was unsuccessful at reducing inflammation. Fosse et al. (2011) repeated the study using 0.6 mg/kg of meloxicam in piglets using a different inflammation model involving subcutaneous injection of kaolin into the metacarpus, and again, found limited anti-inflammatory and analgesic effects in piglets. Reyes et al. (2002) provided 1.0 mg/kg of meloxicam to piglets undergoing central arterial catheter implantation and found it effectively provided postoperative analgesia, without impairing kidney and liver function. A dose of 1.0 mg/kg meloxicam was also efficacious for treating lameness in breeding swine (Pairis-Garcia et al., 2015).
A group of swine experts reviewed the existing literature and determined the quality of evidence to support NSAID use was low, and they gave a weak recommendation for the use of NSAIDs to mitigate piglet surgical castration pain (O’Connor et al., 2014). Despite this, the Canadian Pork Council recommends NSAIDs, specifically 0.4 mg/kg meloxicam, to alleviate surgical castration and tail docking pain and to comply with the recent Codes of Practice (NFACC, 2014; CPC, 2016).

**Ketoprofen**

Ketoprofen is another NSAID that is used to treat pain in animals. It has demonstrated analgesic efficacy for food animals, such as calves undergoing castration (Stafford et al., 2002; Ting et al., 2003). There is some evidence to suggest that the route of drug administration impacts the effectiveness of ketoprofen. When given at 3 mg/kg IV, ketoprofen was found to greatly reduce the cortisol response of calves to the castration procedure (Stafford et al., 2002; Ting et al., 2003). However, when administered to calves at 3 mg/kg IM, ketoprofen had limited effects on reducing castration-associated pain (Moya et al., 2014). Ketoprofen has been approved for use in swine, but there is limited research on its efficacy after surgical castration or tail docking. It did not improve average daily gain of the piglets or survival (when given at 3 mg/kg) but it did reduce blood cortisol levels of piglets undergoing castration (Cassar et al., 2014). Ketoprofen is usually given as an intramuscular injection at a dose of 3 mg/kg (Flecknell, 2009). Fosse et al. (2010) found 6 mg/kg to be a more efficacious dose of ketoprofen for piglets, determined through a trial using the kaolin-induced inflammatory model. As with the inconsistency in effective pain relief using the label dose of meloxicam, it is important to determine the appropriate dose of ketoprofen to maximize piglet pain mitigation.

**Other NSAIDs**

There are many other NSAIDs available for pain management in humans and animals, however, they have either not been approved for use in food animals or they have been found to be ineffective. For example, flunixin meglumine was ineffective at relieving castration-associated pain in piglets as well as preventing changes in the cortisol response (Sutherland et al., 2012). It may be that a single dose of NSAID prior to a painful procedure may not be
sufficient. Additional analgesia may be necessary for pain that persists over time, such as that experienced by piglets having undergone castration (Van Beirendonck et al., 2011). However, this may not be realistic protocol in a production setting.

1.4.2 Opioids

Opioids are more potent analgesic drugs, binding to μ, δ, and κ opioid receptors in the brain, spinal cord, and peripherally to suppress central pain signal transmission (Chahl, 1996). There has been very limited research into opioid use to reduce pain in pigs, primarily due to them being a controlled substance, only to be administered by a veterinarian and not licensed for use in food or food-producing animals (FDA, 2014). As such, they have little on-farm practicality.

Buprenorphine

Buprenorphine is one of the most widely used opioid analgesics in veterinary clinical practice (Capner et al, 1999; Hunt et al, 2013; Sano et al, 2018). It has unique pharmacological properties, acting as an agonist at μ-opioid receptors and antagonist at κ- and δ-opioid receptors (Lutfy and Cowan, 2004), is approximately 25 to 100 times more potent than morphine in humans, mice and rats (Cowan et al, 1977; Khanna and Pillarisetti, 2015), and has a low risk of potentially dangerous side effects, such as respiratory depression and hyperalgesia (Davis, 2012). When administered IM, buprenorphine has a relative bioavailability of 70% with a duration of action of 8 h to 12 h (with < 0.1 mg/kg dose) (Thiede et al, 2014; Khanna and Pillarisetti, 2015).

Buprenorphine has demonstrated efficacy in providing post-operative pain relief for companion and laboratory animals, including mice, rats, dogs, cats and ponies (Roughan and Flecknell, 2002; Christoph et al, 2004; Taylor et al, 2010; Ko et al, 2011; Love et al, 2013). It has also effectively reduced pain and lameness in pigs (Hermansen et al, 1986; Rodriguez et al, 2001; Meijer et al, 2015).

Butorphanol

Butorphanol has agonist-antagonist properties, acting as a strong agonist at κ-receptors and antagonist at μ-receptors (Chen et al., 2016). Adverse effects are rare, but include sedation,
increased heart rate and increased post-operative feeding behavior in rats (Koyyalagunta, 2007; Mitra et al., 2012). There is a ‘ceiling effect’ observed on respiratory depression and analgesia (Talbert et al., 1988). Butorphanol administration (0.1-0.3 mg/kg IM) provides analgesia in pigs for approximately 4-6 h (Smith and Swindle, 2008). It is often used in combination with general anesthesia in a clinical setting to reduce the anesthetic induction requirement (e.g., of propofol), prolong patient sedation, and provide analgesia (Kaur et al., 2013). Butorphanol has been used in combination with anesthetics (xylazine-ketamine, medetomidine, azaperone-detomidine-ketamine, and midazolam-ketamine) in pigs to prolong sedation (Nishimura et al., 1992; Sakaguchi et al., 1992; Heinonen et al., 2009; Schöffmann et al., 2009). When administered alone, it was found to be ineffective at reducing pain of surgical castration in ponies or 8-week-old piglets (McGlone et al., 1993; Love et al., 2009).

1.5 Anesthesia for Pain Mitigation

Anesthetics, like analgesics, are used to prevent pain, although some, like isoflurane, have no pain-relieving properties and only induce a reversible insensibility (Hodgson, 2006). Unlike analgesics, anesthetic agents suppress pain centrally or locally, depending on the drug and how it is administered, by blocking nerve transmission to the pain centers within the central nervous system (Hemmings and Greengard, 2010). They come in many formulations (e.g. sprays, creams, injectable agents, inhalants) and the duration of action is quite variable and depends on the drug used (Sutherland et al., 2010). For example, the anesthetic effects of lidocaine, when administered subcutaneously, last for approximately 1 hour (Ranheim et al., 2005). Others, such as bupivacaine, have a much longer duration of action (Vesal et al., 2013). Lidocaine and thiopental are the only anesthetics approved for use in pigs in Canada (NFACC, 2014); however, thiopental can only be used under veterinary supervision, as it causes unconsciousness and is delivered intravenously, making it less practical for use on-farm. To ensure an appropriate duration of pain mitigation, anesthetics are best used in conjunction with an analgesic for painful procedures; the anesthetic can provide rapid insensibility of the animal or desensitization of the surgical site before the incision and the analgesic can prevent inflammation and provide pain relief.
**Lidocaine**

The most common local anesthetic used in piglet castration studies is lidocaine, a Class 1B drug that works specifically by inhibiting sodium (Na\(^+\)) channels in the cell membrane of nerve cells (Stark et al., 1997). Lidocaine is injected into each testicle or subcutaneously into the scrotum (White et al., 1995; Hansson et al., 2011). This allows the anesthetic to diffuse into the spermatic cord, the severing of which is thought to be the most painful part of the castration procedure. Leidig et al. (2009) found this administration method increased piglet stress calls and defensive behavior, suggesting it is painful, although it is unknown how much restraint stress contributed to the overall results. Some studies have found that lidocaine injection decreased the pain response of piglets undergoing castration, resulting in reduced heart rate post-procedure and less intense vocalizations during the procedure (White et al., 1995; Hansson et al., 2011; Kluivers-Poodt et al., 2012). Marx et al. (2003) found that piglets that were not given lidocaine pre-emptively vocalized twice as often during castration. Others have found lidocaine to be ineffective in reducing castration pain (Kluivers-Poodt et al, 2013; Maršálek et al., 2015). It is likely that the increased handling time and the route of administration of this local anesthetic induces stress in the piglets and may negate the potential positive effects (Leidig et al., 2009). A less invasive method of anesthetic administration is preferred. In addition, a minimum of 10 minutes is required between injection and castration to allow the local anesthetic to take effect, and this is not always practical for use on-farm.

**Other Anesthetic Agents**

There are other local anesthetics that have limited efficacy in reducing piglet surgical pain. Procaine administered intratesticularly had similar concerns to those outlined for lidocaine (Leidig et al., 2009). Cetacaine\(^\circledR\) and Tri-Solfen\(^\circledR\) come in spray and gel form and neither significantly improved the physiologic or behavioral response of piglets being tail docked (Sutherland et al., 2011). Lidocaine injected subcutaneously at the base of the tail prior to docking was also ineffective in reducing blood cortisol levels in piglets (Sutherland et al., 2011). Lomax and Windsor (2013) found that spraying Tri-Solfen\(^\circledR\), a lignocaine-bupivacaine combination, onto the exposed spermatic cords before the testes were removed during castration
in beef calves significantly reduced pain-related behaviors and wound sensitivity. This method of anesthesia administration has not been studied in piglets.

Carbon dioxide (CO$_2$) has been used as a general anesthetic to render the piglet unconscious before surgical castration. There are several issues with this method. CO$_2$ has no nerve blocking properties; when piglets regain consciousness, they display the same degree of pain-related behaviors as control piglets that were not anesthetized (Van Beirendonck et al., 2011; Sutherland et al., 2012). More importantly, CO$_2$ is known to be aversive to pigs as it causes a sense of breathlessness as well as pain upon induction related to carbonic acid formation on mucosal surfaces (Danneman et al., 1997). Piglets display a significant stress response prior to the onset of unconsciousness when induced by CO$_2$ inhalation (Raj and Gregory, 1995; Sutherland et al., 2011). CO$_2$ is likely impractical for use on-farm because of the time and equipment that is necessary as well as the heightened risk of piglet mortality if they are exposed to CO$_2$ for extended periods.

Schmidt et al. (2011) studied the effects of a general anesthesia (an injectable combination of ketamine and azaperone) in piglets undergoing castration. While this drug combination did reduce pain behaviors in piglets, there is a long post-operative sleeping phase (approximately 3 hours) that requires the piglets be separated from the sow to avoid the risk of crushing. When they recovered from the anesthesia and were able to be placed back in their pen, there was a noticeable decrease in suckling order stability (Schmidt et al, 2011). Piglets given general anesthesia also missed more nursing bouts (McGlone and Hellman, 1988). The stress associated with having to re-establish a suckling order as well as the uncoordinated behaviors and prolonged periods of sleeping in which piglets require protected separation from the sow make most forms of general anesthesia inappropriate for commercial use.

McGlone and Hellman (1988) suggested that older piglets may be less responsive to anesthetics. The anesthesia this group used was a mixture of xylazine, ketamine hydrochloride and glyceryl guaiacolate injected into the anterior vena cava prior to piglet castration. As neither the anesthesia nor the route of administration is currently used for piglets, the conclusions drawn
may be isolated to this study as there has been no further evidence to support an age difference in piglet anesthesia response. Future work is needed to identify an appropriate, easy to apply anesthetic for short-term pain relief in piglets.

1.6 Study Rationale

Piglets are routinely subjected to procedures that cause them pain and distress, including surgical castration and tail docking. They are not routinely provided an analgesic or anesthetic agent for pain management, which is a significant animal welfare concern. While analgesia administration is required in Canada and EU guidelines, there is limited evidence in the literature that strongly supports drug efficacy for any of the drugs licensed for use in swine, making recommendations difficult. This thesis project examined the use of nonsteroidal anti-inflammatory drugs, opioids, and topical anesthesia (used alone and in combination) for their ability to reduce surgical castration and tail docking pain in piglets using behavioral measures, the Piglet Grimace Scale and vocalization. The results aim to strengthen the literature regarding piglet pain control and allow for the most current and comprehensive recommendation to be given on pain management in piglets undergoing surgical castration and tail docking.

Two nonsteroidal anti-inflammatory drugs were used in this research project: meloxicam (at 0.4 mg/kg or 1.0 mg/kg) and ketoprofen (6.0 mg/kg). Two opioids were also assessed: 0.2 mg/kg butorphanol and 0.04 mg/kg buprenorphine. Finally, a lidocaine-based topical anesthetic (Maxilene®) was used in combination with an NSAID and opioid, to assess a multimodal approach to reducing surgical castration and tail docking pain.

The Piglet Grimace Scale (PGS) is a novel pain assessment tool (Viscardi et al., 2017) that has utility for use on-farm. For it to become validated, we must demonstrate that it corresponds well to known indicators of pain, such as behavior, in large-scale trials.
1.7 Study Objectives and Hypotheses

This study has six main objectives:

1) To determine if a high dose of meloxicam (1.0 mg/kg) will be more effective at relieving piglet surgical castration pain than the current label dose (0.4 mg/kg)

2) To determine which analgesic agent (meloxicam, ketoprofen, buprenorphine) is most effective in mitigating piglet surgical castration and tail docking pain

3) To determine whether topical anesthesia (Maxilene®) can be used as an adjunct for mitigating piglet surgical castration and tail docking pain

4) To determine whether a multimodal analgesia approach is more effective at mitigating piglet surgical castration and tail docking pain over NSAID-only or opioid-only pain treatment

5) To assess potential sex differences in piglet pain and response to analgesia after tail docking

6) To validate the Piglet Grimace Scale as a pain assessment tool

We hypothesize:

1) A high dose of meloxicam (1.0 mg/kg) will be more effective at mitigating surgical castration pain in piglets than the current label dose (0.4 mg/kg)

2) Buprenorphine will be the most effective analgesic agent at mitigating surgical castration and tail docking pain in piglets

3) The label dose of meloxicam and ketoprofen, administered alone, will not be sufficient to provide significant pain relief after piglet surgical castration and tail docking
4) Maxilene® will provide procedural pain relief by desensitizing the surgical castration and tail docking site pre-procedure

5) An opioid, used in combination with an NSAID and topical Maxilene®, will be the most successful drug combination in alleviating piglet surgical castration and tail docking pain

6) Females will have a stronger pain reaction if sex differences in pain and analgesia response are observed in sexually immature piglets (Goffaux et al., 2011)

7) The Piglet Grimace Scale will correspond well to observed piglet pain behaviors and become a validated, pain assessment tool
1.8 References


CHAPTER 2

Use of meloxicam or ketoprofen for piglet pain control following surgical castration

This chapter has been submitted for publication as: Viscardi, A.V., and Turner, P.V. 2018. Use of meloxicam or ketoprofen for piglet pain control following surgical castration. J Anim Sci, in review.
Abstract

Surgical castration of piglets is performed routinely on commercial pig farms, to prevent boar taint and minimize aggression. While this procedure is known to be painful, piglets are often not provided any analgesic for pain relief, leading to welfare concerns. The objectives of this study were to assess the efficacy of two nonsteroidal anti-inflammatory drugs (NSAIDs), meloxicam (MEL) (0.4 mg/kg or 1.0 mg/kg) and ketoprofen (KET) (6.0 mg/kg) in reducing behavioral indicators of pain in castrated piglets. This study also sought to validate the Piglet Grimace Scale (PGS) as a pain assessment tool. Nineteen litters of 5-day-old male piglets (120 animals total, 15 per treatment group) were used and piglets within a litter were randomly assigned to one of eight possible treatments: 0.4 mg/kg MEL-castrated or uncastrated, 1.0 mg/kg MEL-castrated or uncastrated, 6.0 mg/kg KET-castrated or uncastrated, saline (castrated control) or sham (uncastrated control). Treatments were administered i.m. 20 min prior to surgical castration. Piglets were video recorded for 1 h pre-procedure, for 8 h immediately post-castration and for another hour, 24 h post-procedure. Twenty-one behaviors and postures were scored continuously for the first 15 min of each hour and 1156 still images of piglet faces were collected from the first 30 min of every hour and scored using the PGS, by individuals blinded to piglet treatment, litter, and time point. Within each treatment group post-castration, castrated piglets displayed significantly more pain-related behaviors than uncastrated piglets (0.4 mg/kg MEL: \( P = 0.0339 \), 1.0 mg/kg MEL: \( P = 0.0079 \), 6.0 mg/kg KET: \( P = 0.0034 \), Controls: \( P < 0.0001 \)). Castrated piglets also grimaced significantly more post-procedure than uncastrated piglets (\( P = 0.0061 \)). Compared to the castrated control, none of the NSAID treatments significantly reduced piglet pain behaviors (0.4 mg/kg MEL: \( P = 1.0000 \), 1.0 mg/kg MEL: \( P = 0.9995 \), 6.0 mg/kg KET: \( P = 0.4163 \)) or facial grimacing. Piglets demonstrated significantly more pain behaviors 24 h post-castration than at all other time points (\( P < 0.0001 \)). The PGS did not have as high a sensitivity for detecting acute pain as behavior analysis. Our findings indicate that the use of these NSAIDs were ineffective for alleviating castration-associated pain in piglets. The PGS has utility as a pain assessment tool in neonatal pigs.

Keywords: analgesia, animal welfare, castration, piglet, pain assessment, NSAID
Introduction

Piglets are surgically castrated on commercial farms in North America to prevent boar taint and reduce aggression (Sutherland, 2015). This is known to be a painful procedure, based on specific behavioral and physiologic measures, including rump scratching, increased blood cortisol levels, and high-frequency vocalizations, yet piglets are generally not provided any analgesia or anesthesia for the procedure (Hay et al., 2003; Prunier et al., 2005; Kluivers-Poodt et al., 2012). This has been recognized as a significant welfare concern in pig production, with guidelines in the EU and Canada now requiring analgesia administration prior to surgical castration (EU Commission, 2010; NFACC, 2014). Nonsteroidal anti-inflammatory drugs (NSAIDs), such as meloxicam and ketoprofen, are the most common type of analgesics given to food animals and are currently being recommended for use in piglets to alleviate pain. There is limited research on the use of ketoprofen for pain control in piglets following castration and the label dose of meloxicam (0.4 mg/kg) has had variable success in significantly reducing surgical castration pain (Keita et al., 2010; Kluivers-Poodt et al., 2012).

The Piglet Grimace Scale (PGS) is a recent tool developed by our research group to rapidly assess pain based on piglet facial expressions (Viscardi et al., 2017). Determining how well piglet grimacing corresponds to expression of pain behaviors is a necessary step towards validating this novel pain assessment tool.

The objectives of this study were to assess the effectiveness of meloxicam at the label dose (0.4 mg/kg) and a high dose (1.0 mg/kg), as well as ketoprofen (6.0 mg/kg) in reducing pain resulting from surgical castration of piglets. We hypothesized that piglets receiving 1.0 mg/kg meloxicam would show the greatest reduction in pain behaviors. This study also aimed to determine if the PGS could be used as a pain assessment tool by comparing it against castration-related pain behaviors.

Materials and Methods

All animal use and procedures were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #3350). The institution is registered under the Animals
for Research Act of Ontario and holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care.

**Animals and Treatments**

A total of 120 Yorkshire-Landrace x Duroc male piglets (5 days-old, 1.15 to 2.95 kg BW) from 19 different litters were used in this study, divided into 4 batches of 4-5 litters, with approximately 30 animals in each batch and two months between batches. Cross-fostering of piglets did occur on-farm when necessary, but we specifically chose litters of piglets for this study that remained with their biological sow. Sows and piglets were housed in farrowing pens at the University of Guelph Arkell Swine Research Station. The floor space for each pen was 1.8 m x 2.4 m (6 ft x 8 ft) and the farrowing crate was 0.8 m x 2.3 m (2.5 ft x 7.5 ft). The farrowing rooms were maintained at ambient temperature (23ºC ± 0.5ºC) with lights on/off at 07:00/21:00, and natural light was provided by windows in each room. Sows were fed ad libitum beginning 4 days after farrowing. The creep areas for piglets were heated to approximately 30-35ºC by means of a heating pad or lamp.

Eight treatments were used, and each treatment group was identified by a unique symbol (a T, V, X, ∞, #, diamond, heart, or square) that was marked on the piglet’s forehead and back with a black permanent marker prior to castration. This was to ensure that individuals scoring post-castration behaviors and facial grimacing were blinded as to animal treatment. Numbers were also written on the back leg of piglets for individual identification. Fifteen piglets were assigned to each treatment group. Group size was based on a sample size estimate, using α = 0.05, population σ = 0.1 (determined from a pilot study) and 5% precision (Suresh and Chandrashekara, 2012; Viscardi et al., 2017). Within each litter, piglets were randomly assigned to one of the following treatments: 0.4 mg/kg meloxicam-castrated, 0.4 mg/kg meloxicam-uncastrated, 1.0 mg/kg meloxicam-castrated, 1.0 mg/kg meloxicam-uncastrated, 6.0 mg/kg ketoprofen-castrated, 6.0 mg/kg ketoprofen-uncastrated, saline (castrated control), or sham (uncastared control). Meloxicam (MEL) (Metacam 20 mg/mL; Boehringer Ingelheim Ltd., Burlington, ON, Canada) was administered i.m. at the label dose (0.4 mg/kg) and a higher, semi-log increment dose (1.0 mg/kg). Metacam was diluted to 4 mg/mL for administration of 0.4
mg/kg and 10 mg/mL for administration of 1.0 mg/kg, to give an average volume of approximately 0.2 mL to all piglets (range: 0.1-0.3 mL/piglet). Ketoprofen (KET) (Anafen 100mg/mL; Merial Canada Inc., Baie-D’Urfé, QC, Canada; extra-label use) was administered i.m. and diluted to 80 mg/mL to administer approximately 0.2 mL to all piglets (range: 0.1-0.2 mL/piglet). Saline was given i.m. at 0.2 mL/piglet. The sham treatment group was only handled and did not receive an injection.

**Processing Procedures**

Twenty-four hours prior to the trial, piglets were weighed and marked with the symbol that corresponded to their treatment group (treatments were not piglet weight-balanced; mean piglet weight in each treatment group is presented in Table 2.2). On the day of castration, male piglets within a pen were separated from their littermates, placed in a transport cart, and administered their assigned treatments 20 min prior to castration. Piglets were surgically castrated using 2 vertical incisions and tearing of the spermatic cord (Rault et al., 2011) and then returned to their pen. Separation from the sow and littermates lasted approximately 30-40 min. All castrations occurred between 08:00 and 10:00 and were conducted by one individual. The sham treatment group were the only non-castrated piglets that underwent a simulated castration.

**Behavioral Recording and Scoring**

Piglets were video recorded pre-procedure for 1 h using a high definition video camera (JVC GZ-E200 full HD Everio Camcorder, Yokohama, Japan) mounted on a tripod outside of the farrowing pens. Immediately post-castration, piglets were video recorded continuously for 8 h, and again for 1 h at 24 h post-procedure (i.e., 10 h of video data were collected in total for each litter of pigs). The behavior of each piglet was scored continuously by four trained observers for the first 15 min of every hour of data collected using the Observer XT program (Version 12.0: Noldus Information Technology, Wageningen, The Netherlands) according to a detailed ethogram adapted from Hay et al., 2003 (Table 2.1). The observers were blinded as to time point, litter, and piglet treatment; however, they could observe which piglets had been castrated and which had not. Two observers scored 2 pens each, one observer scored 6 pens, and one observer scored 9 pens. The scoring reliability between the 4 observers was assessed at three
points during the behavior scoring period, by having all participants score the same piglet in a video. The intraclass correlation coefficient (ICC) was calculated to ensure behaviors were being scored consistently (all interobserver reliability tests produced an ICC above 0.9, indicating excellent agreement between scorers). A total of 18,000 min (300 h) of behavior recordings were scored and analyzed for this study.

Piglet behaviors were analyzed individually and then grouped into “active”, “inactive” and “pain” categories, to assess the activity level of piglets across the observation period and the total proportion of pain behaviors displayed. Active behaviors included playing, walking, suckling, nosing, chewing, and running. Inactive behaviors included awake inactive and sleeping. Postures were used for this behavioral analysis; piglets that were sitting or standing were scored as demonstrating an “active” behavior and piglets that were lying were scored as exhibiting an “inactive” behavior. Sitting was placed in the active category because most piglets assumed this posture when suckling or scratching the rump and these were considered active behaviors. Pain behaviors included trembling, stiffness, spasms, tail wagging, and rump scratching (Hay et al., 2003).

**Piglet Grimace Scale (PGS) Recording and Scoring**

Still images of piglet faces were captured from the first 30 min of every hour of video data collected by an individual blinded as to animal treatment and time point. Videos were uploaded to the Everio MediaBrowser 4 program (Pixela Corporation, Osaka, Japan) and whenever a piglet face was in view, the video was paused, and the still image was captured (excluding times when piglets were lying with their head down or sleeping). We attempted to take at least one facial image of each piglet per time point in this study. A total of 1156 facial images were captured (Table 2.2). Prior to scoring, the images were uploaded to Photoshop (Adobe Systems Incorporated, San Jose, CA) and the symbol marked on each piglet’s forehead was blurred to ensure volunteer scorers were blind to treatment. Faces were then randomized into files using a random number generator (random.org).
The preliminary PGS (Viscardi et al., 2017) was modified for this study (Fig. 2.1). Ear position, which was originally placed on a 3-point scale (0-2), was expanded to a 4-point scale (0-3). Images of piglets with upright and floppy ears were included to make scoring ear position easier. Both front-facing piglets and profile images were added to the cheek tightening/nose bulge category and descriptive text was provided to explain the facial feature changes in detail. The maximum pain score using the revised PGS was 6. These changes were made to make the PGS more sensitive to pain expression, allowing for better reliability and to make the scale easier to use.

Eight individuals blinded as to piglet treatment, litter, and time point used the PGS to score each image. If an image could not be scored reliably, for example, due to poor image quality, the volunteers were instructed to exclude it from scoring (15 images were removed in total because of reported quality issues). The PGS score for each image was calculated by summing the scores given to the facial action units (ear position, cheek tightening/nose bulge and orbital tightening). If more than one image was pulled for a piglet at the same time point, the PGS scores were averaged across images prior to analysis, to prevent pseudo-replication.

**Data and Statistical Analysis**

The total duration of behaviors was converted into proportion of time a piglet engaged in each behavior prior to analysis (to account for periods of time when the piglet was not in view and could not be scored). Normality was evaluated using the univariate procedure in SAS (Statistical Analysis System 9.4, SAS Institute Inc., NC). Data were analyzed using a GLIMMIX procedure with a beta distribution, including treatment, time, litter, and time x treatment interaction. Litter was included as a random effect and time was a repeated measure with piglet as the experimental unit. Post hoc tests were conducted using the Tukey-Kramer adjustment, to control the false-positive rate (i.e., incidence of Type I error) for multiple comparisons (Ranganathan et al., 2016). Statistical significance was set at $P < 0.05$.

The grimace scale scores were analyzed using a mixed procedure, including litter, time, treatment, and time x treatment interaction. Litter was included as a random effect and time was
a repeated measure with piglet as the experimental unit. A post hoc Tukey’s test was conducted for significant outcomes.

The treatment variable was first set as each NSAID and control treatment administered to the piglets. When no significant effect of treatment and treatment*time interaction were found on any behavioral variable between 0.4 mg/kg MEL-castrated, 1.0 mg/kg MEL-castrated, 6.0 mg/kg KET-castrated, and saline piglets, data was pooled into a “castrated” group for further analysis. Similarly, when no significant effect of treatment and time*treatment interaction were found on any behavioral variable between 0.4 mg/kg MEL-unciastrated, 1.0 mg/kg MEL-unciastrated, 6.0 mg/kg KET-unciastrated, and sham piglets, data was pooled into a “unciastrated” group. These castrated and uncastrated groups were assessed for treatment and time*treatment effects. A final analysis was conducted on NSAID-castrated (0.4 mg/kg MEL-castrated, 1.0 mg/kg MEL-castrated, and 6.0 mg/kg KET-castrated) and NSAID-unciastrated (0.4 mg/kg MEL-unciastrated, 1.0 mg/kg MEL-unciastrated, and 6.0 mg/kg KET-unciastrated) piglet groups. Both behavioral and PGS data were used to assess the effectiveness of NSAID treatment in reducing surgical castration pain.

**Results**

**Behavioral Observations**

**Comparison between NSAID-treated and control piglets**

Nine individual behaviors were significantly affected by time across the observation period: awake inactive ($P < 0.0001$), lying ($P < 0.0001$), nosing ($P < 0.0001$), sleeping ($P < 0.0001$), standing ($P < 0.0001$), suckling ($P < 0.0001$), tail wagging ($P < 0.0001$), walking ($P < 0.0001$), and chewing ($P = 0.0129$) (Table 2.3). Across all treatment groups, piglets spent significantly more time walking and standing and less time lying at 0 h and 24 h post-castration compared to all other time points ($P < 0.05$). At 24 h post-castration, they spent significantly more time nosing and wagging their tails ($P < 0.05$). At 0 h post-castration, piglets spent significantly less time sleeping and were more awake inactive than at all other time points, except at 24 h ($P < 0.05$). Compared to pre-castration and 24 h post-castration, piglets slept
significantly more at 7 h ($P < 0.05$). Piglets spent significantly more time suckling 5 h post-castration than at all other time points, except at 3 h and 7 h ($P < 0.05$). Piglets demonstrated more chewing behaviors at 5 h post-castration than pre-castration ($P = 0.0186$). There were no significant behavioral differences between any of the treatment groups pre-castration ($P > 0.05$).

Only three individual behaviors, tail wagging ($P < 0.0001$), walking ($P = 0.0042$) and kneeling ($P = 0.0261$), were affected by treatment across all time points (Table 2.4). Within each treatment group, castrated piglets wagged their tails significantly more than uncastrated piglets (0.4 mg/kg MEL: $P = 0.0432$, 1.0 mg/kg MEL: $P = 0.0228$, 6.0 mg/kg KET: $P = 0.0098$, Controls: $P < 0.0001$). Piglets in the 0.4 mg/kg MEL-castrated group walked significantly less than piglets in the 1.0 mg/kg MEL-castrated group ($P = 0.0095$). Piglets in the 0.4 mg/kg MEL-unastrated group spent significantly more time kneeling than piglets in the 1.0 mg/kg MEL-unastrated group ($P = 0.0235$). Castrated piglets also displayed significantly more pain behaviors than uncastrated piglets within each treatment group (0.4 mg/kg MEL: $P = 0.0339$, 1.0 mg/kg MEL: $P = 0.0079$, 6.0 mg/kg KET: $P = 0.0034$, Controls: $P < 0.0001$) (Fig. 2.2). None of the NSAID treatment groups significantly reduced piglet pain behaviors post-castration (0.4 mg/kg MEL: $P = 1.0000$, 1.0 mg/kg MEL: $P = 0.9995$, 6.0 mg/kg KET: $P = 0.4163$).

There was no significant effect of treatment on piglet activity level ($P = 0.8557$) but there was a significant time effect, with piglets being more active at 0 h and 24 h post-castration compared to all other time points ($P < 0.0001$) (Fig. 2.3).

**Comparison between castrated and uncastrated piglets**

After analyzing the effect of each NSAID treatment on behavior and identifying no significant treatment-related effects, data were pooled into two groups: piglets that were castrated and those that were uncastrated. Only two behaviors, tail wagging ($P < 0.0001$) and pain ($P < 0.0001$), were significant across the entire observation period, with castrated piglets displaying significantly more tail wagging and pain-related behaviors than uncastrated piglets (Fig. 2.4). There were also time*treatment interactions found for lying ($P = 0.0027$), sleeping ($P = 0.0037$), standing ($P = 0.0024$), tail wagging ($P < 0.0001$), walking ($P < 0.0001$), isolated ($P = 0.0023$)
0.0018), activity ($P = 0.0045$), and pain ($P < 0.0001$). At 0 h, castrated piglets spent significantly less time lying and more time standing, walking, and engaged in active behaviors than castrated piglets at 3 h, 4 h, and 7 h, and uncastrated piglets at 4 h ($P < 0.05$). At 0 h, uncastrated piglets were also significantly more active, spending more time standing and walking and less time lying and sleeping than both castrated and uncastrated piglets from 1 h to 7 h post-castration ($P < 0.05$). At 24 h post-procedure, uncastrated piglets were significantly more active, spending less time lying and more time standing and walking than castrated piglets at 3 h, 4 h, and 7 h, and uncastrated piglets at 4 h and 6 h ($P < 0.05$). Castrated piglets spent significantly more time isolated from their littermates at 0 h than castrated pigs from 2 h to 5 h, 7 h, and 24 h and uncastrated piglets from 0 h, 1 h, 3 h to 24 h post-procedure ($P < 0.05$). Castrated piglets at 0 h to 3 h, 6 h and 7 h demonstrated significantly more tail wagging and pain-related behaviors than uncastrated piglets at 2 h and 5 h ($P < 0.05$). At 24 h post-castration, castrated piglets were observed engaging in significantly more tail wagging and pain-related behaviors than both castrated and uncastrated piglets from 0 h to 7 h ($P < 0.0001$) (Fig. 2.5).

Data were also collapsed into two groups: NSAID-castrated piglets and NSAID-uncastrated piglets (after no behavior variables were found to be significant). The pre-treatment time point and the control piglets were removed from this analysis. The results were very similar to the above comparison between castrated and uncastrated piglets (Table 2.5).

**Piglet Grimace Scale**

**Comparison between NSAID-treated and control piglets**

There was a significant treatment effect on PGS score ($P = 0.0019$) (Table 2.6 and Fig. 2.6). Piglets in the sham treatment group grimaced significantly less than those in the 1.0 mg/kg MEL-castrated and 6.0 mg/kg KET-castrated piglets ($P = 0.0101$ and $P = 0.0491$, respectively), with a trend towards significance found between sham and 0.4 mg/kg MEL-castrated piglets ($P = 0.0724$). Castrated piglets treated with 1.0 mg/kg MEL grimaced significantly more than 1.0 mg/kg MEL-uncastrated and 0.4 mg/kg MEL-uncastrated ($P = 0.0366$ and $P = 0.0256$, respectively). None of the NSAID treatments significantly reduced facial grimacing in castrated piglets.
Comparison between castrated and uncastrated piglets

Collapsing data into castrated and uncastrated groups found, across the entire observation period, castrated piglets displayed significantly more facial grimacing than uncastrated piglets ($P = 0.0061$) (Fig. 2.7).

Discussion

Meloxicam and ketoprofen are commonly used analgesics in food animals. Both NSAIDs have demonstrated efficacy in treating lameness in sows (Hirsch et al., 2003; Mustonen et al., 2011; Pairis-Garcia et al., 2015) and in reducing blood cortisol levels, heart and respiration rate in dehorned calves (Stafford et al., 2002; Heinrich et al., 2009). Meloxicam has previously been suggested to provide at least some analgesic effects after surgical castration in piglets (Keita et al., 2010; Hansson et al., 2011; Kluivers-Poodt et al., 2013); yet other studies have also found that it had no beneficial effect (Kluivers-Poodt et al., 2012; Viscardi et al., 2017). The contradictory results following NSAID use for castration have made recommendations for piglet pain control difficult (O’Connor et al., 2014). Two studies that found meloxicam effectively reduced behavioral indices of castration pain had significant study design limitations. Keita et al. (2010) observed piglet behavior “for a few minutes” at each time point in their study (0.5, 1, 2, 4, and 24 h post-castration) and used a very simplistic ethogram, only scoring piglets on the presence (1) or absence (0) of prostration, tremors, tail movements, and isolation. Because that study had a very short observation period and used a poorly developed ethogram, the conclusion that meloxicam reduced castration-associated stress and pain should be challenged. While Kluivers-Poodt et al. (2013) used a more detailed ethogram and sophisticated scoring system (Observer software), they employed a scan sampling method of data collection at two periods in the day (once in the morning and once in the afternoon) which may have resulted in a large amount of pain behaviors being missed. Both studies were sufficiently blinded to treatment but made behavioral assessments through direct observation in the farrowing rooms, with no indication as to whether sows and piglets were habituated to the presence of an observer (Keita et al., 2010; Kluivers-Poodt et al., 2013). This may have impacted the behaviors observed. To address these limitations, we employed a much more comprehensive behavior scoring system (continuous observation of each piglet for 15 min per hour, at 10 time points, for a total of 2.5 h
scored per piglet), using video cameras to reduce the observer effect on animal behavior (Iredale et al., 2010). We also used female researchers for all handling and technical procedures, to eliminate any potential risk of increased stress and an altered pain response in animals exposed to male researchers, as has been shown in mice (Sorge et al., 2014). Our results determined that meloxicam (at either dose) and ketoprofen were ineffective in preventing or alleviating castration-associated pain in piglets. The treatment controls (piglets that were given an NSAID but were not castrated) confirmed that there are no negative behavioral side effects associated with either meloxicam or ketoprofen administration.

NSAIDs do not address the acute painful aspects of the castration procedure, such as the scrotal incision and tearing of the spermatic cord (Taylor and Weary, 2000). They primarily provide analgesia by suppressing synthesis of prostaglandins responsible for inflammation post-procedure (Papich, 2008). Lidocaine, a local anesthetic, has been shown to decrease the pain response of piglets during castration when injected directly into the testicle (White et al., 1995; Marx et al., 2003; Hansson et al., 2011; Kluivers-Poodt et al., 2012); however, this route of administration may be painful, and it provides minimal peri-operative analgesia (Leidig et al., 2009). A multi-modal approach to pain management (meloxicam and lidocaine) is effective in reducing castration pain in piglets and calves (Hansson et al., 2011; Meléndez et al., 2017), but would greatly increase the castration time for each piglet and thus, may have limited practicality on-farm. A more potent drug class, such as opioids, may be required to sufficiently reduce pain.

Castrated piglets demonstrated significantly more pain behaviors at 0 h to 3 h, 6 h and 7 h post-castration compared to uncastrated piglets, with castrated piglets exhibiting significantly more pain behaviors at 24 h post-castration compared to all other time points observed. This may be due to progression of the inflammatory processes, causing an increase in pain (Kumar et al., 2015). Previous work found behaviors indicative of castration pain can persist in piglets beyond 24 h and some were still present 4 days after the procedure (Hay et al., 2003). This suggests appropriate pain management for piglets may involve more than one dose of analgesic drug following castration.
Increased activity (or restlessness) has been observed in animals experiencing pain (Hay et al., 2003; Mellor et al., 2000). This was evident in our study, with piglets at 24 h post-castration having both a significant increase in activity level and pain behaviors. The significant increase in activity 0 h post-castration may be attributed to pain from the castration procedure itself or, for piglets in the uncastrated groups, the repeated handling, i.m. injection or prolonged separation from the sow prior to the observation. It is possible that working in the farrowing room and castrating piglets in nearby pens may have also caused piglets already castrated (with cameras recording their behavior for scoring at 0 h) to be more alert and alter their natural behavior. Castrated piglets immediately post-procedure spent significantly more time isolated from their littermates. This behavior has previously been observed in piglets after castration as a response to pain (Hay et al., 2003; Gottardo et al., 2016). An increase in tail wagging after castration has been reported in lambs, calves and piglets (Robertson et al., 1994; Hay et al., 2003; Rault and Lay, 2014; Jongman et al., 2016). It has been speculated that tail wagging may signal nociceptive pain from the rear part of the body (Prunier et al., 2013); however, tail wagging has also been shown to increase after dehorning calves (Graf and Senn, 1999). Tail swishing is thought to be involved in horse pain expression (Haskell et al., 2014). This suggests that it may serve as a less localized pain signal. It is worth noting that piglets in this study were not previously tail docked. An increase in tail wagging has been observed in piglets long after the tail docking procedure, which is thought to be attributed to tail stump hyperalgesia (Di Giminiani et al., 2017). This would not have been a cause for the significant increase in tail wagging noted here. Future work should examine tail wagging behavior as a potential indicator of pain.

Facial analysis is a novel approach to assessing pain in animals and humans. Species-specific grimace scales have been developed for many species, including mice, rats, rabbits, horses, sheep, and lambs (Langford et al., 2010; Sotocinal et al., 2011; Keating et al., 2012; Costa et al., 2014; Guesgen et al., 2016; McLennan et al., 2016), and involve characterizing and quantifying facial features that change in response to pain. Previous research has also described changes to piglet facial expression after a painful event (Di Giminiani et al., 2016). A cow pain face has also been described (Gleerup et al., 2015); however, this has not been associated with a
grimace scale to date. This type of pain assessment tool is non-invasive and has the potential to permit rapid detection of pain, leading to improved animal welfare if an appropriate intervention occurs promptly (Miller and Leach, 2015). For facial grimace scales to be validated, they must correspond well to known indicators of pain, such as behavior. In this study, we compared the Piglet Grimace Scale to pain behaviors of piglets. There was excellent agreement between the PGS and pain behaviors when assessing castrated and uncastrated pigs. When castrated and uncastrated piglets were separated into their initial treatment groups (0.4 mg/kg MEL-castrated, 0.4 mg/kg MEL-unastrated, 1.0 mg/kg MEL-castrated, 1.0 mg/kg MEL-unastrated, 6.0 mg/kg KET-castrated, 6.0 mg/kg KET-unastrated, saline, and sham), the relationship between these two pain assessment tools decreased; however, every significant treatment effect on PGS was supported by the pain behavior results. The 8 volunteers who used the PGS to score piglet faces came from various backgrounds and most had little animal experience. A more robust training session prior to having volunteers score could have been beneficial and may have resulted in stronger PGS results. Future work will focus on better training and include volunteers with greater animal experience. Overall, an increase in facial grimacing in castrated piglets corresponded to an increase in pain behaviors. This is a significant first step towards validation of the PGS as a pain assessment tool.

In conclusion, meloxicam at the recommended dose (0.4 mg/kg) and at a higher dose (1.0 mg/kg) and ketoprofen (6.0 mg/kg) were ineffective at alleviating surgical castration pain in piglets. Post-operative pain persists after castration of piglets and significantly increases at 24 h. Future work should assess a more potent drug class or drug combination to treat pain. The Piglet Grimace Scale did not detect pain as strongly as behavioral assessment, but it did correspond well with castration pain behaviors and may become a useful tool to assess piglet pain.
Literature Cited


The Piglet Grimace Scale

**Ear Position**

<table>
<thead>
<tr>
<th>Absent (0)</th>
<th>Somewhat present (1)</th>
<th>Moderately present (2)</th>
<th>Obviously present (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Ear Position" /></td>
<td><img src="image2" alt="Ear Position" /></td>
<td><img src="image3" alt="Ear Position" /></td>
<td><img src="image4" alt="Ear Position" /></td>
</tr>
</tbody>
</table>

- Determine ear position by looking at the base of the ear (point that connects to the piglet’s head)

**Cheek Tightening/ Nose Bulge**

<table>
<thead>
<tr>
<th>Absent (0)</th>
<th>Moderately present (1)</th>
<th>Obviously present (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5" alt="Cheek Tightening" /></td>
<td><img src="image6" alt="Cheek Tightening" /></td>
<td><img src="image7" alt="Cheek Tightening" /></td>
</tr>
</tbody>
</table>

- Determine nose bulge when the piglet’s face is in profile view by looking above the lip line

**Orbital Tightening**

<table>
<thead>
<tr>
<th>Absent (0)</th>
<th>Present (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image8" alt="Orbital Tightening" /></td>
<td><img src="image9" alt="Orbital Tightening" /></td>
</tr>
</tbody>
</table>

**Figure 2.1:** The Piglet Grimace Scale (PGS) is based on scoring three facial action units (FAUs): ear position, cheek tightening/nose bulge, and orbital tightening.
Figure 2.2: The proportion of time (± SE) piglets demonstrated pain-related behaviors (trembling, stiffness, spasms, tail wagging and rump scratching) in each treatment group. The control groups were saline-castrated and sham-uncastrated piglets ($n = 15$ piglets/treatment group). Observers ($n = 4$) were unaware of piglet treatment, litter, and time point when scoring. Different letters (a, b) indicate significant differences between treatments ($P < 0.05$).
Figure 2.3: The proportion of time (± SE) piglets engaged in active behaviors (playing, walking, suckling, nosing, chewing and running) throughout the observation period (n = 120 piglets/time point). Observers (n = 4) were unaware of piglet treatment, litter, and time point when scoring. Different letters (a, b) indicate significant differences between time points (P < 0.05).
Figure 2.4: The proportion of time (± SE) castrated and uncastrated piglets demonstrated pain-related behaviors (trembling, stiffness, spasms, tail wagging and rump scratching) in total ($n = 60$ piglets/group). Observers ($n = 4$) were unaware of piglet treatment, litter, and time point when scoring. Different letters (a, b) indicate significance ($P < 0.05$).
Figure 2.5: The proportion of time (± SE) castrated and uncastrated piglets demonstrated pain-related behaviors (trembling, stiffness, spasms, tail wagging and rump scratching) throughout the observation period (n = 60 piglets/group). Observers (n = 4) were unaware of piglet treatment and time point when scoring. Different letters (a, b) indicate significant differences between groups within each time point (P < 0.05).
**Figure 2.6:** Mean Piglet Grimace Scale (PGS) scores (± SE) in each treatment group. Higher PGS scores indicate increased pain expression. Volunteers (*n* = 8) were unaware of piglet treatment, litter, and time point when scoring. Different letters (a, b, c) indicate significant differences between treatments (*P* < 0.05).
Figure 2.7: Mean Piglet Grimace Scale (PGS) scores (± SE) of castrated and uncastrated piglets. Higher PGS scores indicate increased pain expression. Volunteers \((n = 8)\) were unaware of piglet treatment, litter, and time point when scoring. Different letters \((a, b)\) indicate significance \((P < 0.05)\).
Table 2.1. Ethogram used to score piglet behavior, grouped into feeding, locomotion, non-specific behaviors, pain-related behaviors, posture, and social cohesion (adapted from Hay et al., 2003)

<table>
<thead>
<tr>
<th>Behaviors</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suckling</td>
<td>Teat in mouth and suckling movements</td>
</tr>
<tr>
<td>Nosing udder/looking for teat</td>
<td>Nose in contact with udder, up and down head movements</td>
</tr>
<tr>
<td>Playing</td>
<td>Springing, bouncy movements with littermates</td>
</tr>
<tr>
<td>Agonistic</td>
<td>Biting or fighting other littermates</td>
</tr>
<tr>
<td>Walking</td>
<td>Moving forward at a normal pace</td>
</tr>
<tr>
<td>Running</td>
<td>Trot or gallop</td>
</tr>
<tr>
<td>Awake inactive</td>
<td>No special activity, but awake</td>
</tr>
<tr>
<td>Sleeping</td>
<td>Lying down, eyes closed</td>
</tr>
<tr>
<td>Nosing</td>
<td>Snout in contact with a substrate</td>
</tr>
<tr>
<td>Chewing</td>
<td>Nibbling at littermates or substrates</td>
</tr>
<tr>
<td>Trembling</td>
<td>Shivering, as with cold</td>
</tr>
<tr>
<td>Spasms</td>
<td>Quick and involuntary contractions of the muscles</td>
</tr>
<tr>
<td>Scratching</td>
<td>Rubbing the rump against the floor, pen walls, or littermates</td>
</tr>
<tr>
<td>Tail wagging</td>
<td>Tail’s movements from side to side (or up and down)</td>
</tr>
<tr>
<td>Stiffness</td>
<td>Lying with extended and tensed legs</td>
</tr>
<tr>
<td>Lying</td>
<td>Body weight supported by side or belly</td>
</tr>
<tr>
<td>Sitting</td>
<td>Body weight supported by hindquarters and front legs</td>
</tr>
<tr>
<td>Standing</td>
<td>Body weight supported by four legs</td>
</tr>
<tr>
<td>Kneeling</td>
<td>Body weight supported by front carpal joints and hind legs</td>
</tr>
<tr>
<td>Isolated</td>
<td>Alone or with one littermate at most, distance of 40cm separates the animal(s) from the closest group of littermates</td>
</tr>
<tr>
<td>Desynchronized</td>
<td>Activity different from that of most littermates (at least 75%)</td>
</tr>
</tbody>
</table>
Table 2.2. Total number of piglet faces captured for Piglet Grimace Scale scoring

<table>
<thead>
<tr>
<th>Time point (h)</th>
<th>0.4mg/kg MEL cast (2.28±0.2 kg)</th>
<th>0.4mg/kg MEL uncast (2.36±0.1 kg)</th>
<th>1.0mg/kg MEL cast (2.28±0.2 kg)</th>
<th>1.0mg/kg MEL uncast (2.22±0.1 kg)</th>
<th>6.0mg/kg KET cast (2.31±0.1 kg)</th>
<th>6.0mg/kg KET uncast (2.28±0.1 kg)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre</td>
<td>18</td>
<td>15</td>
<td>22</td>
<td>15</td>
<td>20</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>0</td>
<td>28</td>
<td>14</td>
<td>13</td>
<td>17</td>
<td>23</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>12</td>
<td>21</td>
<td>24</td>
<td>14</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>10</td>
<td>15</td>
<td>8</td>
<td>11</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>17</td>
<td>12</td>
<td>10</td>
<td>11</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>9</td>
<td>13</td>
<td>11</td>
<td>15</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>10</td>
<td>12</td>
<td>7</td>
<td>15</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>16</td>
<td>8</td>
<td>7</td>
<td>16</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>12</td>
<td>13</td>
<td>15</td>
<td>4</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>24</td>
<td>29</td>
<td>16</td>
<td>16</td>
<td>21</td>
<td>15</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>213</td>
<td>131</td>
<td>145</td>
<td>135</td>
<td>144</td>
<td>116</td>
<td>157</td>
</tr>
</tbody>
</table>

1Mean weight of piglets ± SE (n=15) in each treatment group
Table 2.3. Behavioral analysis of piglets (n = 120) across all litters, replicates and treatments. Values presented are the proportional means ± SE

<table>
<thead>
<tr>
<th>Behavior</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake inactive</td>
<td>7.09</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Lying</td>
<td>11.79</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Nosing</td>
<td>6.59</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Nosing udder</td>
<td>1.04</td>
<td>0.4077</td>
</tr>
<tr>
<td>Sleeping</td>
<td>6.56</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Standing</td>
<td>13.15</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Suckling</td>
<td>5.96</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Tail wagging</td>
<td>14.93</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Walking</td>
<td>24.25</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Sitting</td>
<td>1.08</td>
<td>0.3767</td>
</tr>
<tr>
<td>Spasms</td>
<td>0.72</td>
<td>0.6927</td>
</tr>
<tr>
<td>Kneeling</td>
<td>1.17</td>
<td>0.3175</td>
</tr>
<tr>
<td>Playing</td>
<td>1.50</td>
<td>0.1540</td>
</tr>
<tr>
<td>Scratching</td>
<td>2.53</td>
<td>0.0103</td>
</tr>
<tr>
<td>Isolated</td>
<td>1.40</td>
<td>0.1952</td>
</tr>
<tr>
<td>Desynchronized</td>
<td>1.73</td>
<td>0.0840</td>
</tr>
<tr>
<td>Stiffness</td>
<td>1.54</td>
<td>0.1462</td>
</tr>
<tr>
<td>Chewing</td>
<td>2.46</td>
<td>0.0129</td>
</tr>
<tr>
<td>Trembling</td>
<td>0.06</td>
<td>0.9998</td>
</tr>
<tr>
<td>Running</td>
<td>0.05</td>
<td>1.0000</td>
</tr>
<tr>
<td>Agonistic</td>
<td>1.41</td>
<td>0.1999</td>
</tr>
<tr>
<td>Active</td>
<td>11.24</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Pain</td>
<td>8.51</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (post-castration)</th>
<th>pre (115)</th>
<th>0 h (120)</th>
<th>1 h (117)</th>
<th>2 h (120)</th>
<th>3 h (120)</th>
<th>4 h (119)</th>
<th>5 h (119)</th>
<th>6 h (118)</th>
<th>7 h (119)</th>
<th>24 h (118)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake inactive</td>
<td>0.54±0.03</td>
<td>0.62±0.03</td>
<td>0.47±0.03</td>
<td>0.47±0.03</td>
<td>0.50±0.03</td>
<td>0.47±0.03</td>
<td>0.60±0.03</td>
<td>0.45±0.03</td>
<td>0.46±0.03</td>
<td>0.53±0.03</td>
</tr>
<tr>
<td>Lying</td>
<td>0.66±0.03</td>
<td>0.45±0.03</td>
<td>0.68±0.03</td>
<td>0.67±0.03</td>
<td>0.70±0.03</td>
<td>0.75±0.03</td>
<td>0.67±0.03</td>
<td>0.71±0.03</td>
<td>0.71±0.03</td>
<td>0.55±0.03</td>
</tr>
<tr>
<td>Nosing</td>
<td>0.05±0.01</td>
<td>0.04±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>Nosing udder</td>
<td>0.16±0.05</td>
<td>0.27±0.05</td>
<td>0.20±0.05</td>
<td>0.21±0.05</td>
<td>0.21±0.05</td>
<td>0.17±0.04</td>
<td>0.27±0.06</td>
<td>0.15±0.04</td>
<td>0.16±0.04</td>
<td>0.14±0.05</td>
</tr>
<tr>
<td>Sleeping</td>
<td>0.4±0.04</td>
<td>0.3±0.04</td>
<td>0.5±0.04</td>
<td>0.52±0.04</td>
<td>0.50±0.04</td>
<td>0.2±0.04</td>
<td>0.5±0.04</td>
<td>0.6±0.04</td>
<td>0.3±0.04</td>
<td>0.6±0.04</td>
</tr>
<tr>
<td>Standing</td>
<td>0.29±0.03</td>
<td>0.52±0.04</td>
<td>0.28±0.03</td>
<td>0.30±0.03</td>
<td>0.27±0.03</td>
<td>0.22±0.03</td>
<td>0.27±0.03</td>
<td>0.25±0.03</td>
<td>0.25±0.03</td>
<td>0.41±0.04</td>
</tr>
<tr>
<td>Suckling</td>
<td>0.16±0.02</td>
<td>0.18±0.02</td>
<td>0.16±0.02</td>
<td>0.14±0.02</td>
<td>0.23±0.03</td>
<td>0.18±0.03</td>
<td>0.28±0.03</td>
<td>0.18±0.03</td>
<td>0.21±0.03</td>
<td>0.11±0.02</td>
</tr>
<tr>
<td>Tail wagging</td>
<td>0.02±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
<td>0.02±0.00</td>
<td>0.02±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>Walking</td>
<td>0.09±0.01</td>
<td>0.17±0.02</td>
<td>0.08±0.01</td>
<td>0.10±0.01</td>
<td>0.07±0.01</td>
<td>0.05±0.00</td>
<td>0.04±0.00</td>
<td>0.06±0.01</td>
<td>0.06±0.00</td>
<td>0.16±0.02</td>
</tr>
<tr>
<td>Sitting</td>
<td>0.05±0.00</td>
<td>0.04±0.00</td>
<td>0.06±0.01</td>
<td>0.05±0.00</td>
<td>0.05±0.00</td>
<td>0.06±0.01</td>
<td>0.07±0.01</td>
<td>0.06±0.01</td>
<td>0.06±0.01</td>
<td>0.06±0.00</td>
</tr>
<tr>
<td>Isolated</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Desynchronized</td>
<td>0.12±0.04</td>
<td>0.15±0.05</td>
<td>0.12±0.04</td>
<td>0.05±0.02</td>
<td>0.06±0.03</td>
<td>0.06±0.03</td>
<td>0.10±0.04</td>
<td>0.13±0.05</td>
<td>0.12±0.05</td>
<td>0.11±0.05</td>
</tr>
<tr>
<td>Stiffness</td>
<td>0.00±0.00</td>
<td>-</td>
<td>0.00±0.00</td>
<td>0.01±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Chewing</td>
<td>0.00±0.00</td>
<td>0.01±0.00</td>
<td>0.00±0.00</td>
<td>0.01±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Trembling</td>
<td>0.01±0.00</td>
<td>0.02±0.04</td>
<td>0.07±0.36</td>
<td>0.01±0.03</td>
<td>0.03±0.04</td>
<td>0.06±0.73</td>
<td>0.00±0.02</td>
<td>0.03±0.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Running</td>
<td>0.01±0.04</td>
<td>0.00±0.02</td>
<td>0.00±0.00</td>
<td>0.01±0.04</td>
<td>0.00±0.02</td>
<td>0.00±0.00</td>
<td>0.02±0.05</td>
<td>0.03±0.09</td>
<td>0.00±0.00</td>
<td>-</td>
</tr>
<tr>
<td>Agonistic</td>
<td>0.03±0.00</td>
<td>0.05±0.00</td>
<td>0.05±0.00</td>
<td>0.06±0.01</td>
<td>0.05±0.00</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
<td>0.05±0.00</td>
<td>0.11±0.01</td>
</tr>
</tbody>
</table>

\(^a\)Means with different superscripts in the same row differ significantly (P < 0.05)

\(^b\)Total number of observations for each treatment group

\(^c\)Significant effects are indicated in bold

\(^d\)Not significant after Tukey-Kramer adjustment

\(^e\)Active behaviors include: nosing, suckling, walking, chewing, playing, and running

\(^f\)Pain behaviors include: stiffness, trembling, spasms, tail wagging and scratching

68
Table 2.4. Behavioral analysis of piglets (n = 120) across all litters, replicates and time points. Values presented are the proportional means ± SE

<table>
<thead>
<tr>
<th>Behavior</th>
<th>F value</th>
<th>Pr &gt; F</th>
<th>0.4mg/kg MEL cast (155)</th>
<th>0.4mg/kg MEL uncast (150)</th>
<th>1.0mg/kg MEL cast (145)</th>
<th>1.0mg/kg MEL uncast (150)</th>
<th>6.0mg/kg KET cast (146)</th>
<th>6.0mg/kg KET uncast (150)</th>
<th>Saline (142)</th>
<th>Sham (147)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake inactive</td>
<td>0.29</td>
<td>0.9589</td>
<td>0.48±0.05</td>
<td>0.53±0.04</td>
<td>0.49±0.05</td>
<td>0.50±0.04</td>
<td>0.51±0.04</td>
<td>0.53±0.04</td>
<td>0.53±0.03</td>
<td>0.53±0.04</td>
</tr>
<tr>
<td>Lying</td>
<td>0.38</td>
<td>0.9129</td>
<td>0.59±0.06</td>
<td>0.68±0.04</td>
<td>0.63±0.06</td>
<td>0.66±0.04</td>
<td>0.66±0.04</td>
<td>0.67±0.04</td>
<td>0.68±0.03</td>
<td>0.69±0.04</td>
</tr>
<tr>
<td>Nosings</td>
<td>1.96</td>
<td>0.0585</td>
<td>0.02±0.00</td>
<td>0.05±0.01</td>
<td>0.03±0.00</td>
<td>0.04±0.01</td>
<td>0.04±0.01</td>
<td>0.04±0.01</td>
<td>0.04±0.00</td>
<td>0.03±0.00</td>
</tr>
<tr>
<td>Nosings udder</td>
<td>0.13</td>
<td>0.9964</td>
<td>0.27±0.12</td>
<td>0.16±0.06</td>
<td>0.25±0.12</td>
<td>0.15±0.05</td>
<td>0.19±0.06</td>
<td>0.16±0.06</td>
<td>0.19±0.05</td>
<td>0.19±0.06</td>
</tr>
<tr>
<td>Sleeping</td>
<td>0.83</td>
<td>0.5661</td>
<td>0.56±0.06</td>
<td>0.41±0.04</td>
<td>0.55±0.06</td>
<td>0.44±0.05</td>
<td>0.49±0.04</td>
<td>0.44±0.05</td>
<td>0.46±0.04</td>
<td>0.45±0.04</td>
</tr>
<tr>
<td>Standing</td>
<td>0.38</td>
<td>0.9119</td>
<td>0.36±0.06</td>
<td>0.29±0.04</td>
<td>0.36±0.06</td>
<td>0.28±0.04</td>
<td>0.30±0.04</td>
<td>0.28±0.04</td>
<td>0.28±0.03</td>
<td>0.26±0.04</td>
</tr>
<tr>
<td>Suckling</td>
<td>0.89</td>
<td>0.5127</td>
<td>0.18±0.04</td>
<td>0.17±0.03</td>
<td>0.21±0.04</td>
<td>0.16±0.03</td>
<td>0.20±0.03</td>
<td>0.19±0.03</td>
<td>0.16±0.02</td>
<td>0.17±0.03</td>
</tr>
<tr>
<td>Tail wagging</td>
<td>7.15</td>
<td>&lt;.0001</td>
<td>0.06±0.01^a</td>
<td>0.02±0.00^bc</td>
<td>0.07±0.01^a</td>
<td>0.02±0.00^bc</td>
<td>0.04±0.00^ac</td>
<td>0.02±0.00^b</td>
<td>0.06±0.01^a</td>
<td>0.01±0.00^b</td>
</tr>
<tr>
<td>Walking</td>
<td>2.99</td>
<td>0.0042</td>
<td>0.06±0.01^a</td>
<td>0.10±0.01^ab</td>
<td>0.09±0.02^b</td>
<td>0.09±0.02^ab</td>
<td>0.08±0.01^ab</td>
<td>0.10±0.02^ab</td>
<td>0.07±0.01^ab</td>
<td>0.07±0.01^ab</td>
</tr>
<tr>
<td>Sitting</td>
<td>0.97</td>
<td>0.4532</td>
<td>0.07±0.02</td>
<td>0.06±0.01</td>
<td>0.05±0.02</td>
<td>0.06±0.01</td>
<td>0.05±0.01</td>
<td>0.05±0.01</td>
<td>0.05±0.01</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>Spasms</td>
<td>0.54</td>
<td>0.8040</td>
<td>0.01±0.00</td>
<td>0.00±0.00</td>
<td>0.01±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Kneeling</td>
<td>2.32</td>
<td>0.0261</td>
<td>0.02±0.00^ab</td>
<td>0.01±0.00^a</td>
<td>0.02±0.00^ab</td>
<td>0.00±0.00^b</td>
<td>0.01±0.00^ab</td>
<td>0.01±0.00^b</td>
<td>0.00±0.00^ab</td>
<td>0.00±0.00^ab</td>
</tr>
<tr>
<td>Playing</td>
<td>1.52</td>
<td>0.1669</td>
<td>0.03±0.03</td>
<td>0.02±0.01</td>
<td>0.05±0.03</td>
<td>0.02±0.01</td>
<td>0.00±0.00</td>
<td>0.02±0.01</td>
<td>0.02±0.01</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Scratching</td>
<td>0.94</td>
<td>0.4813</td>
<td>0.00±0.01</td>
<td>0.01±0.00</td>
<td>0.00±0.00</td>
<td>0.01±0.00</td>
<td>0.01±0.00</td>
<td>0.01±0.00</td>
<td>0.01±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Isolated</td>
<td>1.08</td>
<td>0.3794</td>
<td>0.04±0.03</td>
<td>0.13±0.06</td>
<td>0.03±0.02</td>
<td>0.12±0.05</td>
<td>0.19±0.07</td>
<td>0.09±0.05</td>
<td>0.09±0.04</td>
<td>0.18±0.07</td>
</tr>
<tr>
<td>Desynchronized</td>
<td>1.69</td>
<td>0.1146</td>
<td>0.13±0.12</td>
<td>0.09±0.05</td>
<td>0.05±0.07</td>
<td>0.12±0.06</td>
<td>0.17±0.07</td>
<td>0.07±0.04</td>
<td>0.09±0.04</td>
<td>0.09±0.05</td>
</tr>
<tr>
<td>Stiffness</td>
<td>0.98</td>
<td>0.4449</td>
<td>0.01±0.00</td>
<td>0.00±0.00</td>
<td>0.02±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Chewing</td>
<td>1.20</td>
<td>0.3100</td>
<td>0.02±0.00</td>
<td>0.02±0.00</td>
<td>0.02±0.00</td>
<td>0.02±0.00</td>
<td>0.02±0.00</td>
<td>0.02±0.00</td>
<td>0.00±0.00</td>
<td>0.02±0.00</td>
</tr>
<tr>
<td>Trembling</td>
<td>0.07</td>
<td>0.9991</td>
<td>0.02±0.08</td>
<td>0.04±0.35</td>
<td>0.04±0.19</td>
<td>0.08±0.62</td>
<td>0.00±0.02</td>
<td>0.02±0.05</td>
<td>0.02±0.05</td>
<td>0.00±0.02</td>
</tr>
<tr>
<td>Running</td>
<td>0.02</td>
<td>1.0000</td>
<td>0.00±0.06</td>
<td>0.00±0.04</td>
<td>0.00±0.03</td>
<td>0.01±0.07</td>
<td>0.00±0.03</td>
<td>0.01±0.08</td>
<td>0.01±0.07</td>
<td>0.00±0.08</td>
</tr>
<tr>
<td>Agonistic</td>
<td>1.51</td>
<td>0.1883</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Active</td>
<td>0.47</td>
<td>0.8557</td>
<td>0.41±0.06</td>
<td>0.32±0.04</td>
<td>0.36±0.06</td>
<td>0.34±0.04</td>
<td>0.34±0.04</td>
<td>0.32±0.04</td>
<td>0.32±0.03</td>
<td>0.31±0.04</td>
</tr>
<tr>
<td>Pain</td>
<td>7.97</td>
<td>&lt;.0001</td>
<td>0.07±0.01^a</td>
<td>0.02±0.00^b</td>
<td>0.08±0.02^a</td>
<td>0.02±0.00^b</td>
<td>0.05±0.00^a</td>
<td>0.02±0.00^b</td>
<td>0.07±0.01^a</td>
<td>0.02±0.00^b</td>
</tr>
</tbody>
</table>

^4Means with different superscripts in the same row differ significantly (P < 0.05)  
^1Significant effects are indicated in bold  
^2Total number of observations for each treatment group  
^3Active behaviors include: nosing, suckling, walking, chewing, playing, and running  
^4Pain behaviors include: stiffness, trembling, spasms, tail wagging and scratching
Table 2.5. Behavioral analysis of piglets (n = 120) pre-treatment and post-treatment across all litters, replicates, and time points. Values presented are the proportional means ± SE

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Pre-Castration</th>
<th></th>
<th>Post-Castration</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment P-value</td>
<td>Pre-treatment (115)^2</td>
<td>Treatment P-value</td>
<td>Time P-value</td>
<td>Time*Treatment P-value</td>
<td>NSAID-castrated (402)</td>
<td>NSAID-uncastrated (405)</td>
</tr>
<tr>
<td>Awake inactive</td>
<td>0.8992</td>
<td>0.55±0.05</td>
<td>0.6807</td>
<td>&lt; 0.0001</td>
<td>0.0581</td>
<td>0.50±0.03</td>
<td>0.52±0.02</td>
</tr>
<tr>
<td>Lying</td>
<td>0.7540</td>
<td>0.66±0.05</td>
<td>0.6996</td>
<td>&lt; 0.0001</td>
<td>0.1350</td>
<td>0.65±0.04</td>
<td>0.68±0.04</td>
</tr>
<tr>
<td>Nosing</td>
<td>0.2154</td>
<td>0.05±0.01</td>
<td>0.2732</td>
<td>&lt; 0.0001</td>
<td>0.0039</td>
<td>0.03±0.00</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Sleeping</td>
<td>0.1341</td>
<td>0.39±0.04</td>
<td>0.3097</td>
<td>&lt; 0.0001</td>
<td>0.0077</td>
<td>0.53±0.03</td>
<td>0.47±0.03</td>
</tr>
<tr>
<td>Standing</td>
<td>0.1922</td>
<td>0.30±0.05</td>
<td>0.6248</td>
<td>&lt; 0.0001</td>
<td>0.1666</td>
<td>0.32±0.03</td>
<td>0.34±0.03</td>
</tr>
<tr>
<td>Suckling</td>
<td>0.3407</td>
<td>0.16±0.03</td>
<td>0.3568</td>
<td>&lt; 0.0001</td>
<td>0.2430</td>
<td>0.18±0.02</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>Tail wagging</td>
<td>0.0900</td>
<td>0.02±0.00</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.0006</td>
<td>0.05±0.00a</td>
<td>0.02±0.00b</td>
</tr>
<tr>
<td>Walking</td>
<td>0.0934</td>
<td>0.09±0.01</td>
<td>0.0347^4</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.08±0.00</td>
<td>0.08±0.00</td>
</tr>
<tr>
<td>Desynchronized</td>
<td>0.6852</td>
<td>0.11±0.05</td>
<td>0.0404^4</td>
<td>0.0447</td>
<td>0.3329</td>
<td>0.14±0.03</td>
<td>0.09±0.02</td>
</tr>
<tr>
<td>Active^7</td>
<td>0.6597</td>
<td>0.34±0.04</td>
<td>0.6936</td>
<td>&lt; 0.0001</td>
<td>0.1528</td>
<td>0.35±0.03</td>
<td>0.37±0.03</td>
</tr>
<tr>
<td>Pain^8</td>
<td>0.4968</td>
<td>0.03±0.00</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.0001</td>
<td>0.06±0.00a</td>
<td>0.02±0.00b</td>
</tr>
</tbody>
</table>

^a^b Means with different superscripts in the same row differ significantly (P < 0.05)

^1^Only significant behavior variables are presented

^2^Total number of observations for each treatment group

^3^Significant effects are indicated in bold

^4^Not significant after Tukey-Kramer adjustment

^5^NSAID-castrated includes: 0.4 mg/kg mel cast, 1.0 mg/kg mel cast, and 6.0 mg/kg ket cast piglets

^6^NSAID-uncastrated includes: 0.4 mg/kg mel uncast, 1.0 mg/kg mel uncast, and 6.0 mg/kg ket uncast piglets

^7^Active behaviors include: nosing, suckling, walking, chewing, playing, and running

^8^Pain behaviors include: stiffness, trembling, spasms, tail wagging and rump scratching
Table 2.6. Piglet Grimace Scale (PGS) scores of piglets ($n = 120$) within each treatment group, across all litters, replicates, and time points. Values presented are the mean score ± SE.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean PGS score (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4mg/kg MEL cast</td>
<td>3.11±0.17ab (215)</td>
</tr>
<tr>
<td>0.4mg/kg MEL uncast</td>
<td>2.45±0.14ac (131)</td>
</tr>
<tr>
<td>1.0mg/kg MEL cast</td>
<td>3.28±0.17b (145)</td>
</tr>
<tr>
<td>1.0mg/kg MEL uncast</td>
<td>2.47±0.15ac (135)</td>
</tr>
<tr>
<td>6.0mg/kg KETO cast</td>
<td>2.80±0.13bc (144)</td>
</tr>
<tr>
<td>6.0mg/kg KETO uncast</td>
<td>2.67±0.15ab (116)</td>
</tr>
<tr>
<td>Saline</td>
<td>2.64±0.11ab (155)</td>
</tr>
<tr>
<td>Sham</td>
<td>2.41±0.13a (115)</td>
</tr>
</tbody>
</table>

*Means with different superscripts in the same row differ significantly ($P < 0.05$)

1Total number of facial images scored for each treatment group
CHAPTER 3

Efficacy of buprenorphine for management of surgical castration pain in piglets

This chapter has been submitted for publication as: Viscardi, A.V., and Turner, P.V. 2018. Efficacy of buprenorphine for management of surgical castration pain in piglets. BMC Vet Res, in review
Abstract

Surgical castration is a painful procedure, performed routinely on commercial pig farms to prevent boar taint and reduce aggression. The objectives of this study were to assess the efficacy of 0.04 mg/kg buprenorphine (BUP) in reducing pain in castrated piglets, using behavioral indicators and vocalization analysis. This study also sought to further validate the Piglet Grimace Scale (PGS) as a pain assessment tool. A pilot study first assessed the safety of BUP or 0.2 mg/kg butorphanol administration to piglets (n = 4 per treatment). When no adverse side effects were noted with BUP administration, ten litters of 5-day old piglets (n = 60 total, 15 per treatment group) were used, and randomly assigned to one of four possible treatments: BUP (castrated or uncastrated), saline, or sham. Treatments were administered as an intramuscular injection 20 min prior to surgical castration. Piglets were video recorded 1 h pre-procedure, post-castration for 8 h and for another hour, 24 h post-procedure. Behaviors were scored continuously for the first 15 mins of each hour and 511 still-images of piglet faces were scored using the PGS. Vocalizations were recorded from each piglet at three points in the study: at initial handling, injection, and castration. Butorphanol caused some piglets to become groggy and vomit and was not further evaluated. BUP-castrated piglets demonstrated significantly fewer pain behaviors and less facial grimacing compared to saline-treated pigs (P < 0.0001 and P = 0.0073, respectively). There was no difference between the pain behaviors displayed by BUP-castrated piglets compared to BUP-uncastrated and sham piglets (P = 0.9986 and P = 0.7484). There was also no difference in PGS score between BUP-castrated and BUP-uncastrated piglets (P = 0.9376). Piglets in the BUP-castrated group produced vocalizations of similar frequency, amplitude, power, and energy to saline-treated piglets. Buprenorphine was highly effective in alleviating castration-associated pain behaviors and facial grimacing in piglets, without causing any obvious side effects. Its administration did not reduce piglet vocalizations at the time of castration. The PGS corresponded well to piglet pain behaviors and has utility as a pain assessment tool.

Keywords: animal welfare, analgesia, buprenorphine, pain assessment, piglet, castration, behavior, Piglet Grimace Scale
Background

Surgical castration is a procedure performed routinely on piglets in North America to prevent boar taint and minimize agonistic behaviors [1]. It causes acute pain in piglets, as evidenced by behavioral and physiologic alterations after castration, including rump scratching, body spasms, high-frequency vocalizations, and increased blood cortisol levels [2-4]. However, piglets are generally not provided analgesia or anesthesia for pain relief. Canada and countries in the EU have recognized this as a significant piglet welfare concern and have guidelines that now require analgesia administration [5, 6]. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as meloxicam and ketoprofen, are currently recommended for use in piglets to manage pain, yet previous research found the label dose (0.4 mg/kg) of meloxicam, a high dose (1.0 mg/kg) of meloxicam, or 6.0 mg/kg ketoprofen to be ineffective at alleviating surgical castration pain in piglets [4, 7]. The analgesic capacity of an NSAID is limited by the degree of tissue trauma caused by the surgical castration procedure, as a significant mechanism underlying NSAID-induced pain mitigation is suppression of pro-inflammatory prostaglandin synthesis [8]. Opioids, such as buprenorphine and butorphanol, are more potent analgesic drugs, binding to μ, δ, and κ opioid receptors in the brain, spinal cord, and peripherally to suppress central pain signal transmission [9]. Butorphanol has been used in combination with various drugs, such as xylazine-ketamine, medetomidine, azaperone-detomidine-ketamine, and midazolam-ketamine, in pigs to prolong sedation [10-13]. Butorphanol alone was found to be ineffective at reducing pain behaviors of piglets castrated at 8 weeks-old [14]. Buprenorphine has demonstrated efficacy in reducing pain and lameness in pigs [15, 16]. The ability of butorphanol and buprenorphine to alleviate pain in 5-day-old piglets following castration has not been assessed.

The Piglet Grimace Scale (PGS) is a novel pain assessment tool that examines specific facial feature alterations in piglets in response to an acutely painful event [17]. Similar species-specific scales have been developed for mice, rats, rabbits, horses, sheep, and lambs [18-23]. These scales are of interest for their non-invasive nature and ability to rapidly detect pain [24]. For appropriate validation of these scales, they must correspond well to known indicators of pain, such as behavior.
The objectives of this study were first to determine the safety of buprenorphine and butorphanol administration to piglets, and then to assess their efficacy in reducing pain in castrated piglets, using behavioral indicators and vocalization analysis. We hypothesized that piglets receiving an opioid pre-castration would show a significant reduction in vocalizations and pain behaviors. This study also sought to further validate the PGS by comparing it against castration-related pain behaviors. The findings of this work will be important for appropriate analgesic recommendations to alleviate piglet pain post-castration, leading to improved animal welfare, a topic of increasing societal concern [25].

**Methods**

**Part I- Opioid Pilot Study**

All animal use and procedures were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #3350). The institution is registered under the Animals for Research Act of Ontario and holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care.

**Animals and Treatments**

A total of 8 Yorkshire-Landrace x Duroc male piglets (5-days-old, average BW = 2.19 ± 0.07 kg) from 2 different litters were used in this pilot study. Sows and piglets were housed in farrowing pens at the University of Guelph Arkell Swine Research Station (Arkell, ON, Canada). The floor space for each pen was 1.8 m x 2.4 m (6 ft x 8 ft) and the farrowing crate was 0.8 m x 2.3 m (2.5 ft x 7.5 ft). The farrowing rooms were maintained at ambient temperature (23°C ± 0.5°C) with lights on/off at 7:00 am/9:00 pm, and additional natural light was provided by windows in each room. Sows were fed ab libitum beginning 4 days after farrowing. The creep areas for piglets were heated to approximately 30-35°C by means of a heat lamp.

Four piglets from each litter were used and randomly assigned one of two treatments: 0.04 mg/kg buprenorphine (Vetergesic 0.3 mg/mL; Champion Alstoe Animal Health Inc., Whitby, ON, Canada; extra-label use) or 0.2 mg/kg butorphanol (Torbugesic 10 mg/mL; Zoetis
Inc., Kalamazoo, MI; extra-label use) Both drugs were administered intramuscularly (IM).
Treatment groups were identified by a symbol (‘C’ or ‘D’) marked on the piglet’s back with a
permanent marker prior to injection, to ensure that those involved in post-castration observations
were blinded to treatment. A number was also marked on the back leg of each piglet for
individual identification purposes.

Processing Procedure

Piglets were weighed approximately 24 h prior to the study start for drug dose
calculations, and then marked with a symbol and number. On the day of castration, male piglets
were removed from their pen, placed in a transport cart, and treatments were administered.
Approximately 20 mins later, piglets were surgically castrated by making one vertical incision
over each testicle using a scalpel and tearing the spermatic cords. The piglets were then returned
to their home pen. Castrations occurred between 8:00 am and 8:30 am and were all done by the
same individual (AVV).

Behavior Recording

Live observations were conducted for the first 1 h post-injection; if piglets responded
negatively to the administered drug, they were quickly removed from their pen and assisted. One
experienced observer blinded to treatment was placed outside each litter of piglets in this study
and was instructed to note any unusual piglet behavior (e.g. grogginess, vomiting, distress, or
lying isolated from littermates for an extended period). Video cameras (JVC GZ-E200 full HD
Everio Camcorder, Yokohama, Japan) were also placed on tripods outside of the farrowing pens
and piglets were video recorded during and after the live observations for 7 h. An individual not
involved in the live observations assessed the video footage for behavioral signs of distress
related to opioid administration.
Part II- Buprenorphine Definitive Study

Animals and Treatments

A total of 60 Yorkshire-Landrace x Duroc male piglets (5-days-old, 1.07 to 3.34 kg BW) from 10 different litters were used in this study. Sows and piglets were housed in farrowing pens at the University of Guelph Arkell Swine Research Station.

Within each litter, piglets were randomly assigned to one of the following treatments: 0.04 mg/kg buprenorphine- castrated, 0.04 mg/kg buprenorphine- uncastrated, saline (castrated control), or sham (uncastrated control). Buprenorphine (BUP) was administered IM at 0.04 mg/kg (range: 0.2-0.5 mL/piglet). Saline was given IM at 0.2 mL/piglet. The sham treatment group was handled only and did not receive an injection. Treatment groups were identified by a symbol (‘V’, ‘X’, circle or diamond) marked on each piglet’s forehead and back with a black permanent marker prior to castration. This was to ensure that the individual involved in post-castration observations and behavior scoring was blinded to animal treatment. A number was also marked on the back leg of each piglet for individual identification purposes. Fifteen piglets were assigned to each treatment group. Group size was based on a sample size estimate, using $\alpha = 0.05$, population $\sigma = 0.1$ (determined from a pilot study) and 5% precision [17, 49].

Processing Procedure

Piglets were processed as described for the pilot study. Castrations occurred between 8:00 am and 10:00 am. The sham treatment group were the only non-castrated piglets that underwent a simulated castration. All handling and technical procedures were conducted by female researchers to eliminate the potential confound of increased stress and an altered pain response in piglets exposed to male researchers, as reported in mice [26].

Behavior Recording and Scoring

Video cameras were placed on tripods outside of the farrowing pens and piglets were video recorded pre-procedure for 1 h. Immediately post-castration, piglets were video recorded continuously for 8 h, and 24 h post-procedure, piglets were recorded for 1 h (10 h of video data were collected in total for each pen of pigs). Videos were randomized using a random number
generator (random.org) and the behavior of each piglet was scored continuously by one experienced observer for the first 15 mins of every hour of video collected using the Observer XT program (Version 12.0: Noldus Information Technology, Wageningen, The Netherlands) according to a detailed ethogram adapted from Hay et al. [2] (Table 2.1). The observer was blinded as to time point, litter, and piglet treatment; however, castrated piglets could be clearly distinguished from those that had not been castrated. A total of 9000 min (150 h) of behavior recordings were scored and analyzed.

Piglet behaviors were analyzed individually and then grouped into active, inactive and pain categories, to assess the piglet’s activity level throughout the observation period and the total amount of pain behaviors displayed [7]. Active behaviors included walking, running, playing, nosing, chewing, and suckling. Inactive behaviors included sleeping and awake inactive. Postures were used for this behavioral analysis; piglets that were standing or sitting were scored as active and lying piglets were scored as inactive. Sitting was scored in the active category because most piglets exhibited this posture when rump scratching or suckling and these were considered active behaviors. Pain behaviors included trembling, stiffness, spasms, tail wagging, and rump scratching [2].

**Piglet Grimace Scale and Scoring**

Still images of piglet faces were taken from the first 30 min of every hour of video data by an individual blinded as to time point and animal treatment using the Everio MediaBrowser 4 program (Pixela Corporation, Osaka, Japan). Whenever a piglet face was in view, the video was paused, and the still image was captured (excluding times when piglets were lying with their head down or sleeping). Taking at least one facial image of each piglet per time point in this study was attempted. A total of 511 images were captured (Table 3.1). The images were uploaded to Photoshop (Adobe Systems Incorporated, San Jose, CA) prior to scoring to blur the symbol marked on each piglet’s forehead. This was to ensure that those scoring the faces were blinded as to treatment. Faces were then randomized into files using a random number generator (random.org).
Four individuals with extensive animal experience were trained to use the Piglet Grimace Scale (Fig. 2.1) in a 30 min interactive training session prior to scoring study faces. If an image could not be scored reliably, those scoring were instructed to exclude it (3 images were removed in total because of poor image quality). The PGS score for each image was calculated by summing the scores given to each of the facial action units (ear position, cheek tightening/nose bulge and orbital tightening). If more than one image was pulled from the same piglet within one time point, the PGS scores were averaged across images prior to analysis, to prevent issues with pseudo-replication. The final PGS score of each piglet per time point was calculated as a mean of the scores from the four individuals.

**Vocalizations**

Vocalizations of each piglet were collected at three points during the study, at initial handling when they were marked with a symbol (marking), when they received their treatment injection (injection) and when they were surgically castrated (incision and castration). A video camera on a tripod was placed as close to the focal piglet’s face as possible and recorded each procedure. Vocalizations from the recorded videos were analyzed using Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca, NY) by two individuals who were blinded as to procedure and piglet treatment. From the spectrograms, maximum frequency (Hz), maximum amplitude (µ), maximum power (dB) and energy (dB) of each call was determined [27, 28].

**Data and Statistical Analysis**

The total duration of behaviors was converted into a proportion of time that piglets spent demonstrating each behavior prior to analysis to account for periods of time when piglets were out of view and unable to be scored. Normality was evaluated using the univariate procedure in SAS (Statistical Analysis System 9.4, SAS Institute Inc., NC). Data was analyzed with a GLIMMIX procedure with a beta distribution, including time, treatment, litter, and the time x treatment interaction. Litter was included as a random effect and time was a repeated measure with piglet as the experimental unit. Post hoc tests were conducted on significant factors using the Tukey-Kramer adjustment. Statistical significance was set at \( P < 0.05 \).
The PGS scores were analyzed using a mixed model procedure, including litter, time, treatment, and time x treatment interaction. Litter was included as a random effect, time was a repeated measure, and piglet was the experimental unit. A post-hoc Tukey’s test was conducted for significant outcomes.

Vocalization data were analyzed using a mixed procedure, including litter, treatment, and procedure in the model. Litter was included as a random effect and piglet was the experimental unit. Significant outcomes were further analyzed using a post-hoc Tukey’s test. Behavior, PGS and vocalization data were used to assess the effectiveness of buprenorphine treatment in reducing surgical castration pain.

To determine if piglet weight was balanced across treatment groups, post-hoc analysis was performed using a GLM procedure, including litter and treatment in the model.

Results

Part I- Opioid Pilot Study

Behavioral Observations

Approximately 30 min post-injection, piglets administered butorphanol became groggy, unable to stand or walk, and two of the four animals vomited. They remained in the farrowing pen, and the observers ensured there was enough distance between them and the sow to eliminate their risk of being crushed. They fully recovered approximately 1.5 h post-injection. There were no side effects noted with buprenorphine administration.

Part II- Buprenorphine Definitive Trial

Behavioral Observations

Four individual behaviors (lying: $P < 0.0001$, standing: $P < 0.0001$, tail wagging: $P < 0.0001$, and walking: $P < 0.0001$) and both grouped behaviors (active: $P < 0.0001$, and pain: $P < 0.0001$), were affected by treatment across all time points. Piglets in the BUP-castrated and BUP-unciastrated treatment groups spent significantly less time lying and more time standing,
walking, and engaged in active behaviors than piglets in the saline and sham treatment groups \((P < 0.05)\) (Fig. 3.1). Saline-treated piglets wagged their tails and demonstrated significantly more pain behaviors than piglets in all other treatment groups \((P < 0.05)\) (Fig. 3.2).

Eight individual behaviors and both grouped behaviors (active and pain) were significantly affected by time across the observation period: awake inactive \((P < 0.0001)\), lying \((P = 0.0016)\), sleeping \((P < 0.0001)\), standing \((P = 0.0004)\), suckling \((P = 0.0288)\), tail wagging \((P < 0.0001)\), walking \((P < 0.0001)\), chewing \((P = 0.0324)\), active \((P = 0.0030)\), and pain \((P < 0.0001)\). Regardless of treatment, at 0 h post-castration, piglets were significantly more active, spending more time standing and walking, and less time lying and sleeping compared to piglets at 4 h, 5 h, and 7 h post-procedure \((P < 0.05)\). No rump scratching or trembling behavior was observed from any piglet pre-procedure and there were no significant behavioral differences between any of the treatment groups pre-castration \((P > 0.05)\) (Table 3.2). Suckling and chewing behaviors were not significant after the Tukey-Kramer adjustment.

A significant time x treatment effect was found for lying \((P = 0.0300)\), standing \((P = 0.0228)\), tail wagging \((P = 0.0488)\), active \((P = 0.0259)\), and pain \((P = 0.0002)\). At 4 h post-castration, sham piglets were significantly less active, spending more time lying and less time standing than BUP-castrated piglets at 0 h, 2 h, and 3h, and BUP-uncastrated piglets at 0 h and 1 h \((P < 0.05)\) (Fig. 3.3). At 24 h post-castration, saline-treated piglets demonstrated significantly more tail wagging and pain behaviors than BUP-castrated piglets at 3 h, 5 h, 6 h, and 24 h, BUP-uncastrated piglets at 6 h and 24 h, saline-treated piglets at 0 h, 1 h, 3 h, 5 h-7 h, and sham piglets at all post-castration time points \((P < 0.05)\) (Fig. 3.4).

**Piglet Grimace Scale**

There was a significant treatment effect on PGS score \((P = 0.0003)\) (Fig. 3.5). BUP-castrated and BUP-uncastrated piglets grimaced significantly less than both saline (BUP-castrated: \(P = 0.007\), and BUP-uncastrated: \(P = 0.001\)) and sham (BUP-castrated: \(P = 0.049\), and BUP-uncastrated: \(P = 0.013\)) treatment groups. Across all time points, there was no significant difference in PGS score between BUP-castrated and BUP-uncastrated piglets \((P = 0.944)\), nor
was there a difference found between piglets in the saline and sham treatment groups ($P = 0.974$).

**Vocalization**

The scrotal incision produced piglet vocalizations that were significantly lower in frequency, amplitude, and power compared to the IM injection ($P = 0.0003$, $P = 0.007$, and $P = 0.0001$, respectively). When compared to castration (i.e., pulling and tearing of the spermatic cord to remove testicles), the scrotal incision produced piglet vocalizations significantly lower in frequency and power ($P < 0.0001$ for both). Injecting piglets resulted in vocalizations that were significantly higher in frequency, amplitude and power compared to marking piglets ($P = 0.02$, $P = 0.04$, and $P = 0.02$, respectively) (Fig. 3.6).

Piglets in the BUP-castrated treatment group produced vocalizations significantly higher in frequency, power, and energy compared to piglets in the sham group ($P = 0.04$, $P = 0.02$, and $P = 0.04$, respectively) (Fig. 3.7). Buprenorphine administration did not reduce piglet vocalizations at the time of castration; piglets in the BUP-castrated group produced vocalizations of similar frequency, amplitude, power, and energy to saline-treated piglets ($P = 1.00$ for all).

**Weight Analysis**

Sham piglets, with an average BW of $1.95 \pm 0.14$ kg, weighed significantly less than piglets in all other treatment groups (BUP-castrated: $2.48 \pm 0.13$ kg, $P = 0.0044$; BUP-uncastrated: $2.32 \pm 0.12$ kg, $P = 0.045$; saline: $2.44 \pm 0.14$ kg, $P = 0.009$).

**Discussion**

This study examined buprenorphine efficacy in mitigating post-castration pain in piglets. Buprenorphine significantly reduced piglet pain behaviors such that no differences were observed between BUP-castrated and BUP-uncastrated or sham piglets at any post-castration time point (up to 24 h). Castrated and uncastrated buprenorphine-treated piglets were significantly more active than saline-treated pigs, showing no sedative effect (and possibly a
stimulatory effect) with the 0.04 mg/kg dose. Animals often become less active but more restless when in pain [29]. Restlessness was difficult to assess with the ethogram used, but increased pain behavior and decreased activity was noted in saline-treated piglets in this study. Results from the treatment control group (i.e., piglets given buprenorphine but uncastrated) also verified no negative behavioral side effects are associated with buprenorphine administration. Saline-treated piglets wagged their tails significantly more than all other treatment groups. An increase in tail wagging after a painful event, such as castration or dehorning, has been observed in piglets, lambs, and calves [2, 7, 30-33], suggesting that this may be a useful pain indicator. It should be noted that piglets in this study had not been tail docked and thus increases in tail wagging could not be attributed to tail stump hyperalgesia [34].

Increased piglet activity was observed immediately post-castration (at 0 h). This was attributed to pain or restlessness after castration as well as the handling, IM injection and separation from the sow. At 24 h post-castration, saline-treated piglets demonstrated significantly more tail wagging and pain behaviors than any other treatment group and at most other time points. This increase in pain may be due to progression of the inflammatory process [35]. Buprenorphine-castrated piglets might have been expected to display more pain behaviors at 24 h post-castration, since the maximum duration of action of buprenorphine in swine is 12 h [36], but this was not observed. Future work should assess piglet pain beyond 24 h post-castration, to determine whether a single dose of buprenorphine provides sufficient peri-castration analgesia for piglets.

After observing the significant sedative and emetic effects associated with butorphanol administration to piglets in the pilot study, this drug was not tested further. The sedative effects put piglets at greater risk of hypothermia or being crushed if they are immediately placed back in their pen, making it an inappropriate opioid for use in piglets. Buprenorphine was determined to be safe and there were no evident side effects in the pilot or definitive studies.

NSAIDs, such as meloxicam and ketoprofen, are licensed for use in swine and are the most practical drugs available for producers to administer to piglets for pain relief [37]. In terms
of efficacy, both NSAIDs have had variable success in significantly reducing post-castration pain [4, 38]. A review of the existing literature by a group of swine experts determined the quality of evidence to support NSAID use was low, and they gave a weak recommendation for the use of NSAIDs to mitigate surgical castration pain [39]. It is clear that a more potent drug class, such as opioids, may be required for appropriate pain control in pigs, further evidenced by the results of this study.

Facial action unit analysis is an increasingly popular method to assess pain in animals, because it is non-invasive, quick, and easy to use [40]. To become a validated pain assessment tool, the PGS must be comparable to known indicators of pain. In this study, we compared PGS scores to the pain behaviors displayed by piglets. The observed pain behaviors corresponded perfectly to PGS results for buprenorphine-castrated, buprenorphine-unastrated, and saline-castrated piglets (e.g., an increase in pain behavior corresponded to higher facial grimacing), and buprenorphine significantly reduced facial grimacing in castrated piglets. However, this was not noted for the sham-unciastrated group. Sham piglets were expected to demonstrate low facial grimacing, high activity, and low pain behaviors, as this group did not receive an IM injection and were not castrated. Instead, they demonstrated high facial grimacing, low activity, and low pain behaviors. The post-study weight analysis revealed that sham piglets as a group weighed significantly less on average than piglets in all other treatment groups. Low body weight (LBW) piglets have low survival rates through the first week of life [41], as they are at greater risk of crushing, starvation, and disease than piglets of average body weight [42, 43]. LBW piglets tend to miss more nursing bouts and spend more time alone [44]. It may be that the increase in facial grimacing and decrease in activity level of these piglets was due to weakness and discomfort, not pain. It may also be that LBW piglets have cheeks that are more sunken, giving the illusion of cheek tightening/nose bulge, and causing observers to assign higher PGS scores to facial images from these piglets. This is a confounder in this study and future work should ensure piglet weights are balanced across treatment groups.

Piglets emit distinct vocalizations associated with castration that have been attributed to pain [3, 28]. Buprenorphine did not reduce the frequency, amplitude, power, or energy of these
vocalizations at the time of castration. Therapeutic concentrations of buprenorphine (0.1 ng/mL) are reached rapidly in pigs after IM injection (between 5 and 30 min) [36] and piglets were castrated 20 min after its administration. This suggests that buprenorphine alone does not provide sufficient analgesia to fully mitigate pain associated with surgical castration in conscious piglets. Previous studies have found that surgical castration-related stress vocalizations are reduced by CO₂ anesthesia, a combination of ketamine-climazolam-azaperone anesthesia or intratesticular lidocaine injection [45-47]. However, these agents or combinations provide minimal post-operative analgesia and present greater limitations for on-farm use (e.g., sedated piglets can not be returned to the sow until after drug recovery).

The practicality of buprenorphine use on-farm currently is low. While it was highly effective, easy to administer, and one injection provided pain relief for at least 24 h post-procedure, it is a controlled substance that must be administered by a veterinarian and is not licensed for use in pigs or other food-producing animals [48]. Despite this, identification of buprenorphine as a drug that significantly reduced surgical castration pain may encourage other researchers to focus on how to make this highly effective option for piglet pain management practical for use in swine production.

Conclusions

Buprenorphine, when administered at 0.04 mg/kg IM, significantly reduced pain behaviors and facial grimacing in surgically castrated piglets for up to 24 h post-procedure, without evident adverse effects. It was not able to reduce vocalizations at the time of castration. The PGS corresponds well to the pain behaviors of piglets and has utility as a pain assessment tool. Future work should focus on potential solutions to the current limitations of using buprenorphine on-farm, as it is a highly effective analgesic agent that could improve the welfare of millions of piglets undergoing painful procedures each year.
Declarations

Ethics approval and consent to participate
All animal use and procedures were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #3350). The institution is registered under the Animals for Research Act of Ontario and holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care.

Consent for publication
Not applicable.

Availability of data and material
The datasets used and analysed during the current study are available from the corresponding author on request.

Competing interests
The authors declare that they have no competing interests.

Funding
Funding was provided by Ontario Pork (052367), National Pork (052457) and the Ontario Ministry of Agriculture Food and Rural Affairs (27295).

Authors’ contributions
AVV and PVT conceived of and designed the experiments. AVV performed the experiments and analyzed the data. Both authors prepared and edited the final manuscript.

Acknowledgements
The authors wish to thank Brianne Mercer, Stephanie Cervi, Anna Maystrenko, Tim Thalen, and personnel at the Arkell Swine Research Station for technical assistance.
References


Figure 3.1: Mean proportion of time (± SE) piglets engaged in active behaviors in each treatment group. Different letters indicated significance.
Figure 3.2: Mean proportion of time (± SE) piglets displayed pain behaviors in each treatment group. Different letters indicated significance.
**Figure 3.3:** Mean proportion of time (± SE) piglets engaged in active behaviors within each treatment group across the observation period.
Figure 3.4: Mean proportion of time (± SE) piglets demonstrated pain behaviors within each treatment group across the observation period.
Figure 3.5: Mean Piglet Grimace Scale scores (± SE) in each treatment group. Different letters indicate significance.
Figure 3.6: Vocalization (a) frequency, (b) amplitude, and (c) power (± SE) of all piglets undergoing each procedure. Different letters indicate significance.
Figure 3.7: Vocalization (a) frequency, (b) power, and (c) energy (± SE) of piglets in each treatment group. Different letters indicate significance.
Table 3.1. Total number of piglet faces captured for Piglet Grimace Scale scoring

<table>
<thead>
<tr>
<th>Time point (h)</th>
<th>Treatment</th>
<th>0.04mg/kg BUP cast</th>
<th>0.04mg/kg BUP uncast</th>
<th>Saline</th>
<th>Sham</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td></td>
<td>17</td>
<td>14</td>
<td>17</td>
<td>9</td>
<td>57</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>18</td>
<td>17</td>
<td>15</td>
<td>11</td>
<td>61</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>12</td>
<td>21</td>
<td>3</td>
<td>10</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>18</td>
<td>19</td>
<td>11</td>
<td>6</td>
<td>54</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>23</td>
<td>12</td>
<td>7</td>
<td>8</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>12</td>
<td>15</td>
<td>7</td>
<td>5</td>
<td>39</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>19</td>
<td>19</td>
<td>10</td>
<td>7</td>
<td>55</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>14</td>
<td>15</td>
<td>10</td>
<td>8</td>
<td>47</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>12</td>
<td>13</td>
<td>9</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>18</td>
<td>21</td>
<td>15</td>
<td>12</td>
<td>66</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>163</td>
<td>166</td>
<td>104</td>
<td>78</td>
<td>511</td>
</tr>
</tbody>
</table>
Table 3.2. Behavioral analysis of piglets (n = 60) pre-treatment and post-treatment across all litters and timepoints. Values presented are the proportional means ± SE

<table>
<thead>
<tr>
<th>Behavior (duration)</th>
<th>Pre-Castration</th>
<th>Post-Castration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment P-value</td>
<td>Pre-Treatment</td>
</tr>
<tr>
<td>Awake inactive</td>
<td>0.9704</td>
<td>0.64±0.05</td>
</tr>
<tr>
<td>Lying</td>
<td>-</td>
<td>0.53±0.08</td>
</tr>
<tr>
<td>Nosing udder</td>
<td>0.0551</td>
<td>0.23±0.05</td>
</tr>
<tr>
<td>Sleeping</td>
<td>0.6062</td>
<td>0.28±0.06</td>
</tr>
<tr>
<td>Sleeping</td>
<td>0.7142</td>
<td>0.44±0.08</td>
</tr>
<tr>
<td>Tail wagging</td>
<td>0.4929</td>
<td>0.02±0.00</td>
</tr>
<tr>
<td>Walking</td>
<td>0.2945</td>
<td>0.16±0.04</td>
</tr>
<tr>
<td>Active</td>
<td>0.6394</td>
<td>0.47±0.07</td>
</tr>
<tr>
<td>Pain</td>
<td>0.2859</td>
<td>0.02±0.00</td>
</tr>
</tbody>
</table>

*Means with different superscripts in the same row differ significantly (P < 0.05)

1Only behavior variables that were significant post-treatment are presented
2Active behaviors include: nosing, suckling, walking, chewing, playing, running
3Pain behaviors include: stiffness, trembling, spasms, tail wagging and scratching
CHAPTER 4

Use of meloxicam, buprenorphine, and Maxilene® to assess a multimodal approach for piglet pain management after surgical castration

This chapter has been submitted for publication as: Viscardi, A.V., and Turner, P.V. 2018. Use of meloxicam, buprenorphine, and Maxilene® to assess a multimodal approach for piglet pain management after surgical castration. J Anim Sci, in review
Abstract

Surgical castration of piglets is a routine procedure on commercial pig farms, to prevent boar taint and reduce aggression. This procedure is known to cause pain, yet piglets are often not provided appropriate analgesia for relief. The objective of this study was to assess a multimodal approach to managing post-castration pain in piglets, using 0.4 mg/kg meloxicam (MEL), 0.04 mg/kg buprenorphine (BUP), and Maxilene® (MAX). Efficacy was evaluated using behavioral indicators, vocalization, and facial grimace analysis. Male piglets from 25 different litters (150 animals total, five days-old) were used and piglets within a litter were randomly assigned to one of 10 possible treatments: MEL + BUP + MAX (castrated or uncastrated), MEL + BUP (castrated or uncastrated), BUP + MAX (castrated or uncastrated), MEL + MAX (castrated or uncastrated), saline (castrated control), or sham (uncastrated control). Treatments were administered intramuscularly (MEL, BUP, saline) or topically on the scrotal surface (MAX) 20 min prior to surgical castration. Piglets were video recorded 1 h pre-procedure, immediately post-castration for 8 h and for another hour, 24 h post-procedure. Behavior was scored continuously for the first 15 min of every hour and 1118 still-images of piglet faces were captured from the first 30 min of each hour of video data collected and scored using the Piglet Grimace Scale (PGS). Vocalizations were recorded from each piglet at three points in the study: at initial handling, injection, and castration. Castrated piglets in the MEL + BUP + MAX, MEL + BUP, and BUP + MAX treatment groups displayed significantly fewer pain behaviors than piglets administered saline ($P < 0.0001$). MEL + MAX was insufficient in reducing surgical castration pain behaviors. At 24 h post-procedure, saline-castrated and MEL + MAX-castrated piglets displayed significantly more pain behaviors than all other treatment groups and time points ($P < 0.01$). PGS scoring indicated that MEL + MAX-castrated piglets grimaced significantly more than MEL + BUP (castrated and uncastrated) and BUP + MAX-unastrated piglets ($P < 0.05$ for all). There were no significant differences in emitted vocalizations between the analgesia-treated and saline-castrated piglets ($P > 0.05$). All treatment groups with buprenorphine were effective in alleviating castration-associated pain behaviors, suggesting that opioid administration is highly effective for managing piglet castration pain.

Keywords: analgesia, multimodal, piglet, castration, pain assessment, Piglet Grimace Scale
Introduction

Surgical castration of piglets is performed on commercial pig farms in North America to prevent boar taint and reduce aggressive behavior (Rault et al., 2011). The procedure causes acute pain, based on specific behavior and physiologic alterations, such as rump scratching, increased blood cortisol, and high frequency vocalizations (Hay et al., 2003; Sutherland et al., 2012). This acute pain may persist beyond 24 h post-castration (Hay et al., 2003; Moya et al., 2008). Both Canada and the EU have animal care guidelines that require analgesia administration to alleviate piglet castration pain (EU Commission, 2010; NFACC, 2014). Nonsteroidal anti-inflammatory drugs (NSAIDs) are recommended for use on-farm; however, recent research examining meloxicam and ketoprofen use found them both to be ineffective at alleviating post-procedural pain in piglets (Chapter 2: Viscardi and Turner, 2018a). Combining an NSAID with a more potent analgesic, such as an opioid, is common practice in companion animal medicine for post-operative pain management (Shih et al., 2008; Epstein et al., 2015). The efficacy of such an approach to control pain in piglets following castration has not been assessed; however, it could provide piglets with more appropriate, longer-term pain control, improving their post-operative well-being (Keita et al., 2010; Thiede et al., 2014).

The objective of this study was to assess a multimodal approach to managing surgical castration pain in piglets, using 0.4 mg/kg meloxicam, 0.04 mg/kg buprenorphine, and topical Maxilene®. The efficacy of each drug combination was evaluated using behavioral indicators, vocalization, and facial grimace analysis. We hypothesized that piglets receiving meloxicam, buprenorphine, and Maxilene® would have the greatest reduction in pain behaviors and facial grimacing post-castration and would emit lower frequency vocalizations at the time of the procedure compared to the other treatment combinations used.

Materials and Methods

All animal use and procedures were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #3350). The institution is registered under the Animals
for Research Act of Ontario and holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care.

**Animals and Treatments**

A total of 150 Yorkshire-Landrace x Duroc male piglets (5 days-old, average BW = 2.15 ± 0.04 kg) from 25 different litters were used in this study. Sows and piglets were housed in farrowing pens at the University of Guelph Arkell Swine Research Station. The floor space for each pen was 1.8 m x 2.4 m (6 ft x 8 ft) and the farrowing crate was 0.8 m x 2.3 m (2.5 ft x 7.5 ft). Farrowing rooms were maintained at ambient temperature (23°C ± 0.5°C) with lights on/off at 07:00/21:00, and natural light was provided by windows in each room. Sows were fed ad libitum beginning 4 days after farrowing. The creep areas for piglets were heated to approximately 30-35°C by means of a heating pad or lamp. Cross-fostering of piglets did occur on-farm when necessary; however, for this study, we chose litters of piglets that had remained with their biological sow.

Ten treatments were used and each treatment group was identified by a unique letter or symbol (‘H’, ‘T’, ‘V’, ‘X’, ∞, asterisk, circle, triangle, square or squiggle) written on the piglet’s forehead and back with a black marker prior to castration. This was to ensure individuals involved in behavior and facial grimace scoring remained blind to treatment. For individual identification, a number was written on the back leg of each piglet. Fifteen piglets were assigned to each treatment group. Group size was based on a sample size estimate, using α = 0.05, population σ = 0.1 (determined from a pilot study) and 5% precision (Suresh and Chandrashekara, 2012; Viscardi et al., 2017). Within each litter, piglets were randomly assigned to one of the following treatments: 0.4 mg/kg meloxicam + 0.04 mg/kg buprenorphine + Maxilene®-castrated, 0.4 mg/kg meloxicam + 0.04 mg/kg buprenorphine + Maxilene®-uncastrated, 0.4 mg/kg meloxicam + 0.04 mg/kg buprenorphine-castrated, 0.4 mg/kg meloxicam + 0.04 mg/kg buprenorphine-unastraated, 0.4 mg/kg buprenorphine + Maxilene®-castrated, 0.4 mg/kg buprenorphine + Maxilene®-uncastrated, 0.4 mg/kg meloxicam + Maxilene®-castrated, 0.4 mg/kg meloxicam + Maxilene®-uncastrated, saline (castrated control), or sham (uncastrated control). Meloxicam (MEL) (Metacam 20 mg/mL; Boehringer Ingelheim Ltd., Burlington, ON,
Canada), buprenorphine (BUP) (Vetergesic 0.3 mg/mL; Champion Alstoe Animal Health Inc., Whitby, ON, Canada; extra-label use), and saline were injected intramuscularly (IM). Maxilene® (MAX) (Maxilene® 4% lidocaine; RGR Pharma Ltd., Windsor, ON, Canada; extra-label use) was applied topically to the scrotal surface.

**Processing Procedures**

Piglets were weighed 24 h prior to the start of the study, for drug dose calculations. They were then marked with the symbol that corresponded to their treatment group (treatments were not piglet weight-balanced; mean piglet weight in each treatment group is presented in Table 4.1). On the day of castration, piglets were removed from their litter, placed in a transport cart, and administered their assigned treatments 20 min pre-procedure. Piglets were then surgically castrated using two vertical incisions and tearing of the spermatic cord before being returned to their home pen. Castrations occurred between 08:00 and 10:00 and were conducted by one individual (AVV). All handling and technical procedures were done by female researchers, to eliminate the potential risk of piglets altering their pain response due to stress of exposure to male researchers, as has been demonstrated in mice (Sorge et al., 2014). Piglets in the sham treatment group were the only non-castrated pigs that underwent a simulated castration.

**Behavior Recording and Scoring**

Video cameras (JVC GZ-E200 full HD Everio Camcorder, Yokohama, Japan) were placed on tripods outside of each farrowing pen. Piglets were video recorded pre-procedure for 1 h, immediately post-castration for 8 h, and for another hour at 24 h post-procedure (i.e., 10 h of video data was collected in total from each litter of pigs). The videos were randomized across litters and time points using a random number generator (random.org) prior to being scored. Piglet behavior was scored continuously by two trained observers for the first 15 min of every hour of video data collected using Observer XT (Version 12.0: Noldus Information Technology, Wageningen, The Netherlands) and a detailed ethogram adapted from Hay et al. (2003) (Table 2.1). Observers were blind as to treatment, time point, and litter; however, they were able to see which piglets had been castrated. Interobserver scoring reliability was assessed at 3 times during the behavior scoring period (once monthly), by having them score the same piglet in a video and
calculating the intraclass correlation coefficient (ICC). All reliability tests produced an ICC above 0.9, indicating excellent correlation between scorers. A total of 22,500 min (375 h) of behavior recordings were scored and analyzed for this study.

Piglet behaviors were analyzed individually and then grouped into active, inactive, and pain categories, to assess the activity level of piglets and the total proportion of pain behaviors displayed. Active behaviors and postures included playing, running, walking, suckling, nosing, chewing, sitting, and standing. Inactive behaviors and postures included lying, sleeping, and awake inactive. Sitting was placed in the active category, as most piglets assumed this posture when suckling or scratching the rump (both considered active behaviors). Pain behaviors included stiffness, spasms, trembling, tail wagging, and rump scratching (Hay et al., 2003; Chapter 2: Viscardi and Turner, 2018a; Chapter 3: Viscardi and Turner, 2018b).

**Piglet Grimace Scale Recording and Scoring**

Still images of piglet faces were captured from the first 30 min of every hour of video data collected, by an individual blinded as to piglet treatment, litter, and time point using the Everio MediaBrowser 4 program (Pixela Corporation, Osaka, Japan). Whenever a piglet face was in view and clear, the video was paused, and the image was collected (excluding times when piglets were lying with their head down or sleeping). An attempt was made to take one facial image of each piglet per time point during the study. A total of 1118 images were captured (Table 4.1). The symbol marked on each piglet’s forehead was blurred prior to scoring using Photoshop (Adobe Systems Incorporated, San Jose, CA), to ensure volunteer scorers were blinded to treatment. Faces were randomized by litter, treatment, and time point for scoring using a random number generator (random.org).

Four individuals were trained to use the Piglet Grimace Scale (PGS) (Viscardi et al., 2017) in an interactive 30 min session before scoring study images. The PGS score was calculated for each image by summing the scores assigned to the 3 facial action units (ear position, cheek tightening/nose bulge, and orbital tightening). If more than one image was taken from the same piglet at the same time point, PGS scores were averaged prior to analysis to
produce one score per piglet per time point, preventing the potential for pseudo-replication. The final PGS score of each piglet per time point was calculated as a mean of the scores from the four individuals.

**Vocalizations**

Piglet vocalizations were measured at three points in the study: at initial handling when they were marked with a symbol (marking), when they received an intramuscular injection (injection), and when they were surgically castrated (incision and castration). A video camera was placed on a tripod and positioned as close to the focal piglet’s face as possible to record each procedure. The resulting video files were converted to audio files and vocalizations were analysed using the sound analysis software Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca, NY) by two individuals blinded as to piglet treatment and procedure. From the spectrograms, maximum frequency (Hz), maximum amplitude (µ), maximum power (dB) and energy (dB) of each call was determined (Taylor and Weary, 2000; Marx et al., 2003).

**Data and Statistical Analysis**

The total duration of behaviors was converted into proportions of time prior to analysis (to account for periods when piglets were out of view and unable to be scored). Normality was evaluated using the univariate procedure in SAS (Statistical Analysis System 9.4, SAS Institute Inc., NC, USA). Data were analyzed with a GLIMMIX procedure with a beta distribution, including time, treatment, litter, and the time x treatment interaction. Litter was included as a random effect and time was a repeated measure with piglet as the experimental unit. Post hoc tests were conducted on significant factors using the Tukey-Kramer adjustment, to control the false-positive rate (i.e., incidence of Type I error) for multiple comparisons (Ranganathan et al., 2016). Statistical significance was set at $P < 0.05$.

The grimace scale scores were analyzed using a mixed model procedure, including litter, time, treatment, and time x treatment interaction. Litter was included as a random effect, time was a repeated measure, and piglet was the experimental unit. A post-hoc Tukey’s test was conducted for significant outcomes.
The treatment variable was first set as each treatment combination included in the study. When no significant treatment and treatment*time interaction was found on any behavior variable between BUP + MEL + MAX-castrated, MEL + BUP-castrated, and BUP + MAX-castrated, they were pooled into a “BUP-castrated” group for further analysis. Similarly, no significant treatment and treatment*time interaction was found between MEL + BUP + MAX-unastrated, MEL + BUP-unastrated, and BUP + MAX-unastrated, and they were pooled into a “BUP-unastrated” group. These groups were compared to MEL + MAX-castrated and saline-castrated for treatment and treatment*time effects.

Vocalization data were analyzed using a mixed procedure, including litter, treatment, and procedure in the model. Litter was included as a random effect and piglet was the experimental unit. Significant outcomes were further analyzed using a post-hoc Tukey’s test. Behavior, PGS, and vocalization data were used to assess each treatment’s effectiveness in reducing surgical castration pain.

Results
Behavioral Observations
Comparison between analgesia-treated and control piglets

There were eight individual behaviors and two grouped behaviors (active and pain) significantly affected by treatment across the observation period: awake inactive \((P < 0.0001)\), lying \((P < 0.0001)\), nosing \((P < 0.0001)\), nosing udder \((P < 0.0001)\), sleeping \((P = 0.0015)\), standing \((P < 0.0001)\), tail wagging \((P = 0.0002)\), walking \((P < 0.0001)\), active \((P < 0.0001)\), and pain \((P < 0.0001)\) (Table 4.2). Saline-castrated piglets wagged their tails significantly more than all other treatment groups, except MEL + MAX-castrated piglets \((P < 0.05)\). MEL + MAX-castrated piglets also wagged their tails significantly more than all treatment groups, except MEL + BUP + MAX-unastrated, BUP + MAX-unastrated, and saline-castrated piglets \((P < 0.05)\). Saline-castrated piglets displayed significantly more pain behaviors than MEL + BUP + MAX-castrated, MEL + BUP-castrated, and BUP + MAX-castrated piglets \((P < 0.0001)\) (Figure 4.1). There was no significant difference in pain behavior between MEL + MAX-castrated and saline-
castrated piglets ($P = 0.1269$). Saline-castrated piglets spent significantly more time lying and less time walking, standing, and engaged in fewer active behaviors than piglets in the MEL + BUP + MAX (castrated and uncastrated), MEL + BUP (castrated and uncastrated), and BUP + MAX (castrated and uncastrated) groups ($P < 0.05$). MEL + MAX-castrated piglets spent significantly more time lying and less time standing than MEL + BUP + MAX-castrated, MEL + BUP-castrated, and BUP + MAX-castrated piglets ($P < 0.05$). Saline-castrated and sham piglets spent significantly less time awake inactive than piglets in the MEL + BUP + MAX-castrated, MEL + BUP-castrated, and BUP + MAX-castrated treatment groups ($P < 0.01$). MEL + MAX-castrated, saline-castrated, and sham piglets spent significantly less time nosing than MEL + BUP + MAX-castrated, MEL + BUP-castrated, and BUP + MAX-castrated piglets ($P < 0.01$). Sham piglets spent significantly more time nosing the udder than MEL + BUP + MAX-castrated, MEL + BUP-castrated, and BUP + MAX-castrated piglets ($P < 0.05$). Saline-castrated and sham piglets spent significantly more time sleeping than MEL + BUP + MAX-castrated piglets ($P < 0.05$). There were no significant behavioral differences between any of the treatment groups pre-castration ($P > 0.05$).

**Comparison between buprenorphine-treated, non-buprenorphine-treated and control piglets**

After analyzing the effect of each treatment combination with buprenorphine on behavior and identifying no significant treatment-related effects, data was collapsed into two groups: BUP-castrated and BUP-uncastrated piglets to facilitate analysis of time*treatment interactions. The comparison focus was between BUP-castrated, BUP-uncastrated, MEL + MAX-castrated, and saline-castrated piglets. There were significant time*treatment differences found for awake inactive ($P < 0.0001$), lying ($P < 0.0001$), nosing ($P < 0.0001$), nosing udder ($P = 0.0133$), sleeping ($P = 0.0061$), standing ($P < 0.0001$), tail wagging ($P = 0.0197$), walking ($P < 0.0001$), active ($P < 0.0001$), and pain ($P < 0.0001$) (Table 4.3). At 0 h post-castration, BUP-castrated piglets spent significantly less time lying and more time awake inactive, standing, and engaged in active behaviors than BUP-uncastrated piglets at 4 h to 7 h, MEL + MAX-castrated piglets at 0 h, 3 h, 4 h, 6 h, and 7 h, and saline-castrated piglets from 0 h to 5 h ($P < 0.05$). BUP-castrated piglets also spent significantly more time nosing at 0 h than MEL + MAX-castrated and saline-castrated piglets at the same time point and walked significantly more than MEL + MAX-
castrated piglets at 3 h ($P < 0.05$). Activity level of BUP-castrated piglets did not decrease 1 h post-castration, with piglets spending significantly less time lying and more time awake inactive, standing, and engaged in active behaviors than BUP-unastrated piglets at 6 h, MEL + MAX-castrated piglets at 3 h, 5 h, and 6 h, and saline-castrated piglets at 1 h, 3 h and 5 h ($P < 0.05$). BUP-castrated piglets also spent significantly less time sleeping at 1 h than BUP-unastrated piglets at 4 h post-procedure ($P < 0.05$). At 5 h post-castration, BUP-castrated piglets spent significantly less time nosing the udder than MEL + MAX-castrated piglets at 1 h and 4 h ($P < 0.05$). At 24 h post-castration, MEL + MAX-castrated piglets demonstrated significantly more pain behaviors than BUP-castrated piglets at 6 h, 7 h, and 24 h, BUP-unastrated piglets at 24 h, MEL + MAX-castrated piglets from 0 h to 6 h, and saline-castrated piglets at 0 h, 1 h, and 5 h. At 24 h post-castration, saline-castrated piglets also displayed significantly more pain behaviors than BUP-castrated piglets at 0 h, 3 h, 6 h, 7 h, and 24 h, BUP-unastrated piglets at 6 h and 24 h, MEL + MAX-castrated piglets from 0 h to 7 h and saline-castrated piglets from 0 h to 2 h and 5 h ($P < 0.0001$) (Figure 4.2). Across all time*treatment interactions, there were no significant differences in any behavioral variable at the same time point between MEL + MAX-castrated and saline-castrated piglets ($P > 0.05$).

**Piglet Grimace Scale**

**Comparison between analgesia-treated and control piglets**

There was a significant treatment effect on PGS score ($P = 0.0013$) (Figure 4.3). Piglets in the MEL + MAX-castrated group grimaced significantly more than MEL + BUP-castrated, MEL + BUP-unastrated, and BUP + MAX-unastrated piglets ($P = 0.0394$, $P = 0.0011$, and $P = 0.0184$, respectively). BUP + MAX-castrated piglets also grimaced significantly more than MEL + BUP-unastrated piglets ($P = 0.0150$).

**Comparison between buprenorphine-treated, non-buprenorphine-treated and control piglets**

Collapsing data into BUP-castrated and BUP-unastrated groups resulted in a significant effect of treatment on PGS score ($P = 0.0029$), with MEL + MAX-castrated piglets grimacing significantly more than BUP-unastrated piglets ($P = 0.0010$).
Vocalization

Castration (pulling and tearing the spermatic cord) resulted in piglet vocalizations significantly higher in frequency, amplitude, energy, and power compared to all other procedures measured \( (P < 0.01) \). Marking piglets resulted in vocalizations that were significantly lower in frequency, amplitude, energy, and power compared to the intramuscular injection and scrotal incision \( (P < 0.001) \).

Piglets in the sham group emitted vocalizations significantly lower in frequency than MEL + BUP + MAX-uncastrated, MEL + BUP (castrated and uncastrated), and MEL + MAX (castrated and uncastrated) piglets \( (P < 0.05) \) (Figure 4.4). Sham piglets also produced vocalizations significantly lower in power than all treatment groups, except BUP + MAX-uncastrated \( (P < 0.05) \). None of the analgesic combinations reduced piglet vocalizations at the time of castration; all castrated piglets produced vocalizations similar in frequency, amplitude, energy, and power.

Discussion

This study examined a multimodal approach for mitigating surgical castration pain in piglets. Buprenorphine treatment (i.e., MEL + BUP + MAX, MEL + BUP, BUP + MAX) resulted in significantly reduced pain behaviors in piglets up to 24 h post-castration. Buprenorphine has proven efficacy in alleviating pain in piglets and growing swine without significant adverse effects (Hermansen et al., 1986; Rodriguez et al., 2001; Meijer et al., 2015; Chapter 3: Viscardi and Turner, 2018b). Results from the treatment control groups (i.e., piglets administered drugs and not castrated) also confirmed there were no behavioral side effects associated with providing a single dose of buprenorphine, meloxicam, and Maxilene® to piglets; however, the addition of meloxicam and Maxilene® did not appear to provide any significant benefit to the pigs. Saline-castrated piglets were significantly less active than most other treatment groups. Animals often show a decrease in general activity level when in pain (Berger and Eeg, 2008). No reduction in activity of MEL + BUP + MAX-, MEL + BUP-, and BUP + MAX-castrated piglets was observed, further supporting the analgesic efficacy of these drug
combinations. Buprenorphine may also have a stimulatory effect on piglet activity. Saline-castrated piglets also wagged their tails significantly more than all other treatment groups. An increase in tail wagging has been observed in piglets, lambs, and calves after castration or dehorning (Robertson et al., 1994; Graf and Senn, 1999; Hay et al., 2003; Rault and Lay, 2014; Jongman et al., 2016; Chapter 2: Viscardi and Turner, 2018a; Chapter 3: Viscardi and Turner, 2018b). This may be a useful and specific indicator of piglet pain for future studies or assessments.

At 24 h post-castration, saline and MEL + MAX-castrated piglets demonstrated significantly more pain behaviors than all other treatment groups. Progression of the postsurgical inflammatory process may have caused this increase in pain (Kumar et al., 2015). Previous research has indicated that meloxicam alone was insufficient in providing piglets post-castration pain relief (Kluivers-Poodt et al., 2012; Chapter 2: Viscardi and Turner, 2018a). The addition of topical Maxilene® to meloxicam did not reduce the pain behaviors displayed. A more invasive application of lidocaine (the active ingredient in Maxilene®) via intratesticular injection with meloxicam IM has been shown to effectively reduce castration pain in piglets (Hansson et al., 2011); however, this route of administration may be painful and significantly increases the castration time for each piglet, limiting on-farm practicality (Leidig et al., 2009). MEL + BUP + MAX-, MEL + BUP-, and BUP + MAX-castrated piglets were expected to display more pain behaviors 24 h post-castration, as the maximum duration of action of buprenorphine in swine is 12 h (Thiede et al., 2014); however, this was not observed, suggesting a single dose of buprenorphine may provide sufficient peri-operative analgesia for piglets. Future work should assess piglet pain beyond 24 h post-castration to determine whether pain recurs outside of this period of assessment.

Facial grimace analysis is used to assess pain in animals, such as mice and rabbits, and non-verbal humans (Langford et al., 2010; Herr et al., 2011; Keating et al., 2012). The Piglet Grimace Scale developed by Viscardi et al. (2017) evaluates changes in ear position, cheek tightening/nose bulge, and orbital tightening to assess piglet facial expressions of pain. These facial expressions correspond well to observed pain behaviors (Chapter 2: Viscardi and Turner,
2018a; Chapter 3: Viscardi and Turner, 2018b). In this study, there was not a strong correlation between displayed pain behaviors and facial grimacing in piglets. PGS scoring did not detect any significant difference in facial grimacing between saline-castrated piglets and piglets in any other treatment group. Therefore, relying only on piglet facial expressions to detect pain could risk false negative results for the presence of pain. When pain assessments are conducted by trained but inexperienced observers, behavioral analysis appears to be a more sensitive tool. Modifications to the PGS training session may be needed to improve individual scoring success.

Piglets undergoing surgical castration emit distinct vocalizations associated with procedural pain (Marx et al., 2003; Leidig et al., 2009). None of the drug combinations studied reduced the frequency, amplitude, power or energy of these vocalizations at the time of castration. Tearing of the spermatic cord is thought to be the most painful aspect of the castration procedure, eliciting the strongest vocal response from piglets (Leidig et al., 2009). This suggests that piglets may need to be anesthetized to eliminate vocalizations and fully mitigate pain associated with castration (Sutherland et al., 2012). Piglets administered Maxilene® were expected to vocalize less at incision, but this was not observed. Maxilene® is recommended for use with a 30 min application time (Eichenfield et al., 2002) but was administered only 20 min prior to surgical castration in this study. Thus, it is possible that the full topical anesthesia potential of Maxilene® was not reached. As expected, the castration procedure caused the greatest vocal response from piglets compared to all other procedures measured. The IM injection also elicited a strong vocal response, suggesting it caused acute pain. Two of the four drug combinations evaluated in this study required two injections (meloxicam and buprenorphine). As buprenorphine provided sufficient pain relief when administered with Maxilene® alone, the acute pain caused by the second injection of meloxicam was deemed to be unnecessary. This also eliminates the associated cost of a second drug. Future work assessing a multimodal approach to piglet castration should focus on relieving the immediate pain of castration, as sufficient post-operative pain relief can be provided by buprenorphine alone.

In veterinary clinical practice, it is common for dogs and cats undergoing ovariohysterectomy or castration to receive multimodal analgesia to alleviate peri-operative pain
(Hewson et al., 2006). This approach is difficult to replicate in a farm setting, primarily because of the cost, time, effort, and equipment required for each surgery (Rault et al., 2011). This study found minimal benefit to providing piglets with meloxicam and Maxilene® pre-castration. While buprenorphine was determined to be most effective at alleviating surgical castration pain in piglets, it is the least practical drug to use on-farm and a limitation of this study. Currently, buprenorphine is a controlled substance that can only be administered by a veterinarian and is not currently licensed for use in pigs or other food-producing animals (FDA, 2014). However, buprenorphine is a highly effective option for piglet pain management and measures to make it practical for use on-farm should be explored further.

In conclusion, buprenorphine was highly effective at reducing surgical castration pain behaviors in piglets. A multimodal approach with meloxicam and Maxilene® did not provide significant pain-relieving benefits to the piglet to justify the added cost and time required for their administration. None of the analgesia-treatment groups reduced vocalizations that occurred at the time of castration. The PGS may be used to compliment pain scoring in piglets but should be used in combination with other pain-specific behavioral assessments.
Literature Cited


Figure 4.1: Mean proportion of time (± SE) piglets demonstrated pain-related behaviors (trembling, stiffness, spasms, tail wagging and rump scratching) in each treatment group. MEL = 0.4 mg/kg meloxicam, BUP = 0.04 mg/kg buprenorphine, and MAX = Maxilene®. Control groups included saline-castrated and sham-uncastrated piglets (n = 15 piglets/treatment group). Observers (n = 2) were unaware of piglet treatment, litter, and time point when scoring. Different letters (a, b, c) indicate significant differences between treatments ($P < 0.05$).
Figure 4.2: Mean proportion of time (± SE) piglets demonstrated pain-related behaviors (trembling, stiffness, spasms, tail wagging and rump scratching) within each treatment group and time point. MEL = 0.4 mg/kg meloxicam, BUP = 0.04 mg/kg buprenorphine, and MAX = Maxilene®. BUP cast represent all the piglets administered buprenorphine in their treatment regimes (MEL + BUP + MAX, MEL + BUP, and BUP + MAX) and castrated (n = 45). BUP uncast represent all the piglets administered buprenorphine in their treatment regimes and uncastrated (n = 45). Observers (n = 2) were unaware of piglet treatment, litter, and time point when scoring. Different letters (a, b) indicate significant differences between treatment groups within a time point (P < 0.05).
**Figure 4.3:** Mean Piglet Grimace Scale (PGS) scores (± SE) in each treatment group. Higher PGS scores indicate increased pain expression. MEL = 0.4 mg/kg meloxicam, BUP = 0.04 mg/kg buprenorphine, and MAX = Maxilene®. The control groups were saline-castrated and sham-uncastrated (n = 15 piglets/treatment group). Volunteers scoring faces (n = 4) were unaware of piglet treatment, litter, and time point. Different letters (a, b, c) indicate significant differences between treatments ($P < 0.05$).
Figure 4.4: Mean vocalization (a) frequency (Hz) and (b) power (dB) ± SE of piglets across all procedures in each treatment group. MEL = 0.4 mg/kg meloxicam, BUP = 0.04 mg/kg buprenorphine, and MAX = Maxilene®. The control groups were saline-castrated and sham-uncastrated (n = 15 piglets/treatment group). Individuals (n = 2) scoring data were unaware of piglet treatment, litter, and procedure when analyzing vocalizations. Different letters (a, b) indicate significant differences between treatments (P < 0.05).
Table 4.1. Total number of piglet faces captured for Piglet Grimace Scale scoring

<table>
<thead>
<tr>
<th>Time point (h)</th>
<th>1 (2.04±0.0 kg)$^1$</th>
<th>2 (2.05±0.0 kg)</th>
<th>3 (2.24±0.1 kg)</th>
<th>4 (2.18±0.2 kg)</th>
<th>5 (2.18±0.1 kg)</th>
<th>6 (2.23±0.2 kg)</th>
<th>7 (2.16±0.1 kg)</th>
<th>8 (2.10±0.2 kg)</th>
<th>9 (2.27±0.1 kg)</th>
<th>10 (2.13±0.1 kg)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>9</td>
<td>7</td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>11</td>
<td>82</td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>11</td>
<td>18</td>
<td>7</td>
<td>22</td>
<td>13</td>
<td>16</td>
<td>8</td>
<td>18</td>
<td>15</td>
<td>143</td>
</tr>
<tr>
<td>1</td>
<td>19</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>22</td>
<td>14</td>
<td>14</td>
<td>4</td>
<td>15</td>
<td>14</td>
<td>142</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>14</td>
<td>20</td>
<td>10</td>
<td>16</td>
<td>11</td>
<td>9</td>
<td>5</td>
<td>10</td>
<td>4</td>
<td>118</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>11</td>
<td>17</td>
<td>9</td>
<td>14</td>
<td>14</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>8</td>
<td>101</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>12</td>
<td>14</td>
<td>6</td>
<td>14</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>12</td>
<td>12</td>
<td>103</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>7</td>
<td>18</td>
<td>5</td>
<td>17</td>
<td>15</td>
<td>11</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>107</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>7</td>
<td>15</td>
<td>4</td>
<td>18</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>9</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>7</td>
<td>14</td>
<td>9</td>
<td>13</td>
<td>9</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>91</td>
</tr>
<tr>
<td>24</td>
<td>18</td>
<td>10</td>
<td>19</td>
<td>9</td>
<td>19</td>
<td>12</td>
<td>13</td>
<td>11</td>
<td>13</td>
<td>12</td>
<td>136</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>96</td>
<td>161</td>
<td>74</td>
<td>164</td>
<td>113</td>
<td>104</td>
<td>55</td>
<td>112</td>
<td>91</td>
<td>1118</td>
</tr>
</tbody>
</table>

$^1$Mean weight of piglets ± SE (n=15) in each treatment group

1 = meloxicam + buprenorphine + Maxilene®. castrated; 2 = meloxicam + buprenorphine + Maxilene®, unastrated; 3 = buprenorphine + meloxicam, castrated; 4 = buprenorphine + meloxicam, unastrated; 5 = buprenorphine + Maxilene®, castrated; 6 = buprenorphine + Maxilene®, unastrated; 7 = meloxicam + Maxilene®, castrated; 8 = meloxicam + Maxilene®, unastrated; 9 = saline, castrated; 10 = sham, unastrated
### Table 4.2. Behavioral analysis of piglets (n = 150) across all litters and time points. Values presented represent the proportional mean ± SE

<table>
<thead>
<tr>
<th>Behavior</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake inactive</td>
<td>0.62±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.62±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.65±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.61±0.03&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.57±0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.52±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.57±0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.48±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.50±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lying</td>
<td>0.45±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43±0.06&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.40±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48±0.04&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.45±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.62±0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.59±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.66±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.62±0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Nosing</td>
<td>0.09±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08±0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.11±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.10±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08±0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.04±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.04±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Nosing udder</td>
<td>0.22±0.04&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.22±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.23±0.04&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.24±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.20±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.30±0.04&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.34±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.27±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.33±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sleeping</td>
<td>0.45±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.44±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.47±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.49±0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.51±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.44±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.55±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.49±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.60±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.58±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Standing</td>
<td>0.52±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.55±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.57±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49±0.04&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.54±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48±0.04&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.37±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.32±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.37±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Tail wagging</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.01±0.00&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.02±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.04±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02±0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.06±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.03±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0002</td>
</tr>
<tr>
<td>Walking</td>
<td>0.09±0.01&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.11±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09±0.01&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.12±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10±0.01&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.11±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05±0.01&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.04±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Active&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.55±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56±0.05&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.60±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52±0.04&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.55±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.38±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.34±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Pain&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.01±0.00&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.02±0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.02±0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.02±0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.01±0.00&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.05±0.00&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.02±0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.07±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.03±0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

1 = meloxicam + buprenorphine + Maxilene®<sup>®</sup>, castrated; 2 = meloxicam + buprenorphine + Maxilene®, uncastrated; 3 = buprenorphine + meloxicam, castrated; 4 = buprenorphine + meloxicam, uncastrated; 5 = buprenorphine + Maxilene®<sup>®</sup>, castrated; 6 = buprenorphine + Maxilene®, uncastrated; 7 = meloxicam + Maxilene®, castrated; 8 = meloxicam + Maxilene®, uncastrated; 9 = saline, castrated; 10 = sham, uncastrated

<sup>a</sup>-<sup>c</sup>Values within a row with different superscripts differ significantly (P < 0.05)

<sup>1</sup>Only significant behavior variables are presented

<sup>2</sup>Proportion of time piglets engaged in each behavior

<sup>3</sup>Active behaviors include: nosing, suckling, walking, chewing, playing, running

<sup>4</sup>Pain behaviors include: stiffness, trembling, spasms, tail wagging and rump scratching
**Table 4.3.** Behavioral analysis of piglets (n = 150) pre-treatment and post-treatment across all litters and time points. Values represent the proportional mean ± SE

<table>
<thead>
<tr>
<th>Behavior¹</th>
<th>Pre-Castration</th>
<th>Post-Castration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment P-value</td>
<td>Pre-Treatment</td>
</tr>
<tr>
<td>Awake inactive</td>
<td>0.2475</td>
<td>0.58±0.09</td>
</tr>
<tr>
<td>Lying</td>
<td>0.8864</td>
<td>0.52±0.05</td>
</tr>
<tr>
<td>Nosing</td>
<td>0.0833</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>Nosing udder</td>
<td>0.1324</td>
<td>0.25±0.04</td>
</tr>
<tr>
<td>Sleeping</td>
<td>0.3436</td>
<td>0.48±0.10</td>
</tr>
<tr>
<td>Standing</td>
<td>0.8515</td>
<td>0.46±0.05</td>
</tr>
<tr>
<td>Tail wagging</td>
<td>0.2967</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Walking</td>
<td>0.1126</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>Active³</td>
<td>0.8349</td>
<td>0.48±0.05</td>
</tr>
<tr>
<td>Pain⁴</td>
<td>0.2414</td>
<td>0.04±0.00</td>
</tr>
</tbody>
</table>

<sup>a</sup>b Values within a row with different superscripts differ significantly (*P < 0.05*)

¹ Only significant behavior variables are presented

² Proportion of time piglets engaged in each behavior

³ Active behaviors include: nosing, suckling, walking, chewing, playing, running

⁴ Pain behaviors include: stiffness, trembling, spasms, tail wagging and rump scratching
CHAPTER 5

Opioid analgesia for pain management of piglets after tail docking
Abstract

Piglets on commercial pig farms are often tail docked to reduce tail biting among pigs. While this procedure is known to be painful, piglets are often not provided analgesia or anesthesia for pain relief. The objectives of this study were to assess a multimodal approach to managing tail docking pain in piglets, using 0.4 mg/kg meloxicam (MEL), 0.04 mg/kg buprenorphine (BUP), and/or Maxilene® (MAX). The effectiveness of each drug and drug combination was evaluated using behavioral indicators, vocalization, and facial grimace analysis. This study also assessed whether male and female piglets responded differently to pain or pain treatments. Four day-old piglets from 14 litters (n= 165 total) were used and piglets within a litter were randomly assigned to one of six possible treatments: MEL, BUP, MEL + BUP, MEL + BUP + MAX, no treatment (tail docked control), or sham (non-tail docked control). Treatments were administered intramuscularly (MEL, BUP) or topically on the tail (MAX) 20 min prior to tail docking. Piglets were video recorded for 1 h pre-procedure, immediately after tail docking for 8 h and for another 1 h at 24 h post-procedure. Behavior was scored continuously for the first 15 min of each hour and 674 still-images of piglet faces were scored using the Piglet Grimace Scale (PGS). Vocalizations were recorded from each piglet at three points in the study: at initial handling, injection, and tail docking. Piglets administered MEL + BUP and BUP demonstrated significantly fewer pain behaviors than piglets in the MEL and no treatment group (p < 0.05). MEL + BUP + MAX and BUP piglets also grimaced significantly less than piglets in the no treatment group (p < 0.05). There were no significant differences in emitted vocalizations between the analgesia-treated piglets and the no treatment group and both injection and tail docking elicited piglet vocalizations of similar frequency, power, and energy (p > 0.05). There were no significant differences in behavior, facial grimacing or emitted vocalizations between male and female piglets. BUP provides sufficient analgesia for at least 24 h post-procedure and may be all that is required to alleviate tail docking pain.

Keywords: analgesia, opioid, buprenorphine, piglet, tail docking, pain assessment, Piglet Grimace Scale
Introduction

Piglets are tail docked on commercial farms in North America to minimize tail biting among pigs (Sutherland et al., 2008). This procedure is known to cause pain, based on behavior changes and physiologic measures, including an increase in tail wagging, tail jamming (tucking the tail stump between the hind legs), increased blood cortisol levels, and high-frequency vocalizations (Noonan et al., 1994; Sutherland et al., 2008; Torrey et al., 2009). Analgesia or anesthesia is not given routinely to alleviate pain; however, Canada requires analgesia administration to reduce tail docking pain in piglets, regardless of age at docking (NFACC, 2014). There is limited research regarding effective pain mitigation strategies for piglets post-procedure (Sutherland, 2015). Nonsteroidal anti-inflammatory drugs (NSAIDs), such as meloxicam, administered alone have been unsuccessful in reducing post-surgical pain behaviors caused by tail docking (Herskin et al., 2016). Injecting a local anesthetic into the base of the tail or applying a topical anesthetic to the tail-docked wound were also insufficient in alleviating piglet pain post-procedure (Sutherland et al., 2011). Buprenorphine is effective at reducing surgical castration pain in piglets without causing any obvious side effects (Chapter 3: Viscardi and Turner, 2018b; Chapter 4: Viscardi and Turner, 2018c). The analgesic capacity of buprenorphine to mitigate tail docking pain alone, or in combination with an NSAID, has not been assessed. Multimodal analgesia is common to alleviate post-operative pain in veterinary clinical practice (Hewson et al., 2006).

Sex-related differences in pain and analgesia sensitivity have been reported in mice, rats and humans (Mogil et al., 2000; Craft, 2003; Fillingim et al., 2009). Females have largely been found to have greater sensitivity to procedural and postoperative pain (Fillingim et al., 2009). Differences in pain and analgesia sensitivity between male and female piglets after tail docking have not been examined. Understanding potential sex-related differences is important for proper administration of pain treatments and maintenance of good animal welfare on-farm.

The objectives of this study were to assess the efficacy of 0.04 mg/kg buprenorphine, used alone or in combination with 0.4 mg/kg meloxicam and topical Maxilene® to manage tail docking pain in piglets. The effectiveness of each drug and drug combination was evaluated
using behavioral indicators, vocalization, and facial grimace analysis (using the Piglet Grimace Scale). This study also assessed whether male and female piglets responded differently to pain and analgesic therapies. Based on the results from a previous study (Chapter 4: Viscardi and Turner, 2018c), we hypothesized that piglets receiving buprenorphine alone, or in combination with other drugs, would have the greatest reduction in pain behaviors and facial grimacing after tail docking and would emit lower frequency vocalizations at the time of tail docking compared to other non-opioid treatments. We also hypothesized that female piglets would demonstrate more pain behaviors and facial grimacing when in pain compared to male piglets.

**Materials and Methods**

All animal use and procedures were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #3350). The institution is registered under the Animals for Research Act of Ontario and holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care.

**Animals and Treatments**

Yorkshire-Landrace x Duroc male and female piglets (n=165, 4 days-old, average BW = 1.87 ± 0.03 kg) from 14 different litters over three trials were used. Sows and piglets were housed in farrowing pens at the University of Guelph Arkell Swine Research Station (Arkell, ON, Canada). The floor space for each pen was 1.8 m x 2.4 m (6 ft x 8 ft) and the farrowing crate was 0.8 m x 2.3 m (2.5 ft x 7.5 ft). Farrowing rooms were maintained at ambient temperature (23°C ± 0.5°C) with lights on/off at 7:00 am/9:00 pm, and additional natural light was provided by windows in each room. Sows were fed ad libitum beginning 4 days after farrowing. The creep areas for piglets were heated to approximately 30-35°C by means of a heat pad or lamp. Cross-fostering of piglets did occur on-farm when necessary; however, only litters of piglets remaining with their biological sow were selected for this study.
Within each litter, piglets were randomly assigned to one of six possible treatments: 0.4 mg/kg meloxicam, 0.04 mg/kg buprenorphine, 0.4 mg/kg meloxicam + 0.04 mg/kg buprenorphine, 0.4 mg/kg meloxicam + 0.04 mg/kg buprenorphine + Maxilene®, no treatment (tail docked control), and sham (non-tail docked control) (n= 15 male piglets and n= 15 female piglets per treatment group, except the sham group, which contained n= 8 male piglets and n= 7 female piglets). Group size was based on a sample size estimate, using α = 0.05, population σ = 0.1 (determined from a pilot study) and 5% precision (Suresh and Chandrashekara, 2012; Viscardi et al., 2017). Meloxicam (MEL) (Metacam 20 mg/mL; Boehringer Ingelheim Ltd., Burlington, ON, Canada) was administered as an intramuscular (IM) injection at the label dose of 0.4 mg/kg. Buprenorphine (BUP) (Vetregesic 0.3 mg/mL; Champion Alstoe Animal Health Inc., Whitby, ON, Canada; extra-label use) was also administered IM at 0.04 mg/kg. 1.0 g Maxilene® (MAX) (Maxilene 4% lidocaine; RGR Pharma Ltd., Windsor, ON, Canada; extra-label use) was applied topically to the entire tail using a swab. The no treatment group was tail docked without an analgesic or topical anesthetic. The sham treatment group underwent a simulated tail docking without topical application or injection. The treatment groups were identified by a unique symbol (‘H’, ‘V’, ‘X’, square, triangle or circle) marked on the piglet’s forehead and back with a black permanent marker for males and a red permanent marker for females prior to tail docking. This was to ensure that individuals scoring post-procedure behaviors and facial grimacing were blinded as to animal treatment. Numbers were also written on the back leg of piglets for individual animal identification.

**Processing Procedures**

Twenty-four hours prior to the trial, piglets were weighed and marked with the symbol that corresponded to their treatment group (treatments were not piglet weight-balanced; mean piglet weight in each treatment group is presented in Table 5.1). On the day of tail docking, all piglets were removed from their pen and placed in a transport cart. Treatments were administered and 20 min later, piglets were tail docked using side-pliers before being returned to their home pen. All procedures occurred between 8:00 am and 10:00 am and were done by one individual (AVV). Handling and technical procedures were conducted by female researchers to eliminate the potential confound of increased stress and an altered pain response in piglets.
exposed to male researchers, as has been reported in mice (Sorge et al., 2014). Piglets in this study were not ear notched, teeth clipped, given an iron injection or castrated previous to this research project.

**Behavioral Recording and Scoring**

High definition video cameras (JVC GZ-E200 full HD Everio Camcorder, Yokohama, Japan) were placed on tripods outside of each farrowing pen. Piglets were video recorded pre-procedure for 1 h, immediately post-tail docking for 8 h, and then again for 1 h at 24 h post-procedure (i.e., a total of 10 h of video data was collected for each litter of pigs). Videos were randomized across litters and time points using a random number generator (random.org) and the behavior of each piglet was then scored continuously for the first 15 min of each hour of data collected by one of six trained observers using Observer XT (Version 12.0: Noldus Information Technology, Wageningen, The Netherlands) and a detailed ethogram adapted from Hay et al. (2003) (Table 2.1). Observers were blinded as to time point, pen, and piglet treatment; however, they could observe which piglets had been tail docked as well as differentiate piglet sex. Four observers scored 1.5 pens, one observer scored 1 pen, and one observer scored 7 pens. Interobserver reliability was assessed by having all participants score the same piglet in a video and calculating the intraclass correlation coefficient (ICC). All interobserver reliability tests produced an ICC above 0.9, indicating excellent correlation between scorers. A total of 24,750 min (412.5 h) of behavior recordings were scored and analyzed.

Piglet behaviors were assessed individually and then grouped into active, inactive and pain categories, to analyze the activity level of piglets across the observation period and the total amount of pain behaviors displayed (Chapter 2: Viscardi and Turner, 2018a). Active behaviors and postures included running, walking, playing, nosing, suckling, chewing, sitting, and standing. Inactive behaviors and postures included sleeping, awake inactive, and lying. Sitting was placed in the active category, as most piglets assumed this posture when suckling or scratching the rump (both considered active behaviors). Pain behaviors included stiffness, spasms, trembling, tail wagging, and rump scratching (Hay et al., 2003).
Piglet Grimace Scale Scoring

Still-images of piglet faces were captured from the first 30 min of every hour of video data collected by an individual blinded as to piglet litter, treatment, and time point. Videos were uploaded to the Everio MediaBrowser 4 program (Pixela Corporation, Osaka, Japan) and whenever a piglet face was in view, the video was paused, and the still image was collected (excluding times when piglets were lying with their head down or sleeping). An attempt was made to take one facial image of each piglet per time point. A total of 674 images were captured (Table 5.1). Prior to scoring, facial images were uploaded to Photoshop (Adobe Systems Incorporated, San Jose, CA) and the symbol marked on each piglet’s forehead was blurred to ensure volunteer scorers were unbiased. Faces were then randomized into files using a random number generator (random.org).

Three individuals were trained to use the Piglet Grimace Scale (PGS) (Viscardi et al., 2017) prior to scoring in an interactive 30 min session. The PGS score was calculated for each image by summing the scores given to each of the facial action units (ear position, cheek tightening/nose bulge, and orbital tightening). The final PGS score of each piglet per time point was calculated as a mean of the scores from the three individual scorers. If more than one image had been pulled from the same piglet at the same time point, the PGS scores were averaged across images prior to analysis to produce one score per piglet per time point and avoid pseudo-replication.

Vocalizations

Vocalizations of each piglet were measured at three points during the study: at initial handling when they were marked with a symbol (marking), when they received their treatment injection (injection) and when they were tail docked (tail docking). A video camera on a tripod was placed as close to the focal piglet’s face as possible to record each procedure-induced vocalization. Audio clips from the recorded videos were analyzed using Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca, NY, USA) by three individuals blinded as to procedure and piglet treatment. From the spectrograms, maximum frequency (Hz), maximum amplitude (µ),
maximum power (dB) and energy (dB) of each call was determined (Taylor and Weary, 2000; Marx et al., 2003).

**Data and Statistical Analysis**

The total duration of behaviors was converted into proportion of time piglets engaged in each behavior prior to analysis (to account for periods of time when the piglet was out of view and could not be scored). Normality was evaluated using the univariate procedure in SAS (Statistical Analysis System 9.4, SAS Institute Inc., NC, USA). Data were analyzed using a GLIMMIX procedure with a beta distribution, including treatment, time, litter, sex, treatment x time, time x sex, and treatment x sex interaction. Litter was included as a random effect and time was a repeated measure with piglet as the experimental unit. Post hoc tests were conducted using the Tukey-Kramer adjustment, to control the false-positive rate (i.e., incidence of Type I error) for multiple comparisons (Ranganathan et al., 2016). Statistical significance was set at $p < 0.05$.

The grimace scale scores were analyzed using a mixed procedure, including litter, time, treatment, sex, and treatment x sex interaction. Litter was included as a random effect and time was a repeated measure with piglet as the experimental unit. A post hoc Tukey’s test was conducted for significant outcomes.

Vocalization data were analyzed using a mixed procedure, including litter, treatment, and procedure in the model. Litter was included as a random effect and piglet was the experimental unit. Significant outcomes were further analyzed using a post-hoc Tukey’s test. Behavior, PGS, and vocalization data were used to assess each treatment’s effectiveness in reducing tail docking pain and to determine if male and female piglets responded differently to pain and pain treatments.

**Results**

**Behavioral Observations**

Prior to tail docking, male piglets spent significantly more time sitting than female piglets and female piglets engaged in more agonistic behaviors ($p= 0.0031$ and $p= 0.0438$, respectively).
After tail docking, there was no significant difference in behavior between male and female piglets. Because of this, treatment groups were combined across sexes.

There were no significant behavioral differences between any of the treatment groups pre-tail docking (p > 0.05) (Table 5.2). Four individual behaviors and two grouped behaviors had significant treatment effects after tail docking: lying (p < 0.0001), standing (p < 0.0001), tail wagging (p < 0.0001), walking (p < 0.0001), active (p < 0.0001), and pain (p = 0.0002) (Table 5.3). MEL and no treatment piglets displayed significantly more pain behaviors than piglets in the MEL + BUP, BUP, and sham treatment groups (p < 0.05) (Figure 5.1). Female piglets administered BUP demonstrated significantly fewer pain behaviors across the observation period than both male and female piglets in the MEL and tail docked control groups (p < 0.05). There were no other treatment*sex differences found. There was also no effect of time on the amount of pain behaviors displayed (Figure 5.2). Piglets in the MEL, tail docked control, and sham groups spent significantly more time lying and less time standing, walking, and engaged in fewer active behaviors than piglets in all other treatment groups (p < 0.05) (Figure 5.3). There were no sex differences in activity level (p > 0.05). MEL piglets wagged their tails significantly more than MEL + BUP, BUP, and sham piglets (p < 0.05).

Regardless of treatment, all piglets spent significantly less time lying and more time standing, walking and engaged in active behaviors at 0 h compared to all other time points (p < 0.05). All piglets also spent significantly less time lying and sleeping and more time standing at 1 h vs. 2 h and 4 h to 24 h (p < 0.05).

**Piglet Grimace Scale**

There were no significant time or time*treatment interactions found for PGS score (p = 0.2993 and p = 0.5651, respectively). There was a significant treatment effect (p = 0.0018) (Figure 5.4). Male and female piglets in the no treatment-docked group grimaced significantly more than MEL + BUP + MAX, BUP only and sham piglets (p = 0.0024, p = 0.0153 and p = 0.0153, respectively). There was a trend for MEL + BUP piglets of both sexes to grimace less
than no treatment-docked piglets (p= 0.0836). Similarly, there was a trend for male piglets to grimace more than female piglets, irrespective of treatment (p= 0.0639).

Vocalization

There were significant procedure*treatment effects on the frequency, power, and energy of piglet vocalizations (p < .0001, p= 0.0009 and p < .0001, respectively) (Figure 5.5). All tail docked piglets, regardless of treatment group produced significantly higher vocalizations than the sham treatment group during tail docking (p < 0.05). There were no treatment differences in vocalizations during marking and injection (p > 0.05). Tail docking and injection produced vocalizations of significantly higher frequency, energy, and power than marking (p <.0001). Injection and tail docking produced similar vocalizations. There were no sex differences in emitted vocalizations (p > 0.05).

Discussion

This study examined several approaches to mitigate tail docking pain in male and female piglets. Buprenorphine, when administered alone as a single IM injection, was the only treatment to significantly reduce both facial grimacing and piglet pain behaviors. Buprenorphine has previously been shown to alleviate pain in piglets and growing swine without causing any adverse effects (Hermansen et al., 1986; Rodriguez et al., 2001; Meijer et al., 2015; Chapter 3: Viscardi and Turner, 2018b). All piglets that were tail docked and administered buprenorphine (i.e., MEL + BUP + MAX, MEL + BUP, and BUP alone) were significantly more active than the MEL, no treatment-docked, and sham-docked groups, further supporting its efficacy, since animals will often show a decrease in activity level when in pain (Berger and Eeg, 2008) and reinforcing the lack of sedative side effects (and demonstrating a possible stimulatory effect) at this dose. Unfortunately, buprenorphine is a controlled drug that must be administered by a veterinarian and is not currently licensed for use in food animals, limiting its practicality for use on-farm (FDA, 2014)
Increased piglet activity was observed immediately after tail docking (at 0 h). This was likely due to stress from piglet handling, injection, short-term separation from the sow or pain from the procedure. Our work examining piglet pain behavior after surgical castration found a significant increase in pain behaviors displayed by saline-treated piglets at 24 h post-procedure (Chapter 3: Viscardi and Turner, 2018b). In the current tail docking study, there was no significant time effect on pain behavior, and by comparison, male piglets tail docked without analgesia displayed significantly fewer pain behaviors 24 h post-procedure than castrated piglets without analgesia at the same time point (0.04 ± 0.01 vs. 0.26 ± 0.04). This suggests that surgical castration is a more painful procedure for piglets to undergo than tail docking. Tail wagging and tail jamming, key tail docking-related pain behaviors, were difficult to assess after docking and this may have also contributed to the reduction in observed pain behaviors (Noonan et al., 1994).

Facial grimace analysis is a recent technique that has been developed to assess pain in animals. Species-specific scales have been developed for mice, rats, rabbits, horses, sheep, lambs, and piglets (Langford et al., 2010; Sotocinal et al., 2011; Keating et al., 2012; Costa et al., 2014; Guesgen et al., 2016; McLennan et al., 2016; Viscardi et al., 2017). Grimace scales involve identifying and quantifying facial features (or facial action units) that change in response to pain; they are non-invasive and permit rapid pain detection (Miller and Leach, 2015). PGS results corresponded well to overall pain behavior results in this study. This is consistent with previous piglet pain studies where the PGS was used (Chapter 2: Viscardi and Turner, 2018a; Chapter 3: Viscardi and Turner, 2018b). However, the PGS results have yet to correspond perfectly with observed pain behaviors of piglets. This is important for PGS validation and use for pen side treatment decision-making. The ability of swine producers, technical caregivers or veterinarians working with swine to accurately use the PGS to score piglet facial expressions has not been assessed. This should be evaluated in a future study to assess on-farm applicability of the PGS.

Piglets emit distinct vocalizations associated with tail docking that have been attributed to pain (Marchant-Forde et al., 2009; Torrey et al., 2009). None of the treatments in this study reduced the frequency, amplitude, power or energy of these vocalizations at the time of tail
docking. Piglets that had Maxilene® applied to the tail were expected to vocalize less, but this was not observed. Perhaps waiting the recommended 30 min (instead of 20 min in this study) or wrapping the tail after application of the topical might have enhanced the local numbing effect of Maxilene® (Eichenfield et al., 2002). However, a topical agent is unlikely to provide sufficient analgesia for tail removal. IM injection of any drug elicited a similar vocal response as the tail docking procedure, suggesting it also causes acute pain, although likely of very short duration. Small, sharp needles (25 G) were used for injections in this study and discarded after one use, to eliminate unnecessary pain caused by blunt-needle injection. As two of the four drug combinations evaluated required two injections (meloxicam and buprenorphine), and the addition of meloxicam and Maxilene® did not provide piglets any significant benefit, a single IM injection of buprenorphine is recommended to alleviate tail docking pain. The longer handling time required for multiple drug administration was also likely to have contributed to increased piglet stress (Marchant-Forde et al., 2009).

Sex differences in pain and sensitivity to analgesia have been reported in rodents and humans (Greenspan et al., 2007; Fillingim et al., 2009; Sorge and Totsch, 2017). It is generally accepted that females have a higher sensitivity to pain than males (Goffaux et al., 2011). Previous work found sex was not a factor affecting nociceptive thresholds in piglets (Janczak et al., 2012). No differences were seen in the proportion of pain behaviors displayed, PGS scores or vocalizations between male and female piglets in the current study. Sex-related differences in pain sensitivity have not been described previously in pigs and is an area of future work.

Tail docking, or removal of the distal portion of the tail, is often performed using side-pliers, a scalpel blade, scissors, or electrical cautery iron (Sutherland et al., 2008; Sutherland et al., 2015). Tail docking does not eliminate tail biting on-farm but does reduce its prevalence and is the most practical solution to this problem with current North American swine management systems (Hunter et al., 2001; Sutherland et al., 2009). This study confirmed that tail docking causes acute pain in piglets. It is unknown whether tail docked pigs experience chronic pain (Nannoni et al., 2014) and is an area of future work.
Conclusion

Buprenorphine, when administered as a single IM injection 20 min prior to tail docking, did not cause a decrease in piglet activity or any unwanted side effects, and is recommended for alleviating tail docking pain. PGS results corresponded well to piglet pain behaviors and may have utility as a pain assessment tool. None of the treatments evaluated reduced pain-related vocalizations at the time of tail docking. Male and female piglets in this study responded to painful procedures and analgesic drugs similarly. Future work should focus on making buprenorphine a practical drug to administer on-farm, as it has proven efficacy in alleviating both tail docking and castration pain in piglets.
References


Figure 5.1: The mean proportion of time (± SE) piglets demonstrated pain-related behaviors (spasms, scratching the rump, tail wagging, trembling, stiffness) after tail docking (n= 15 piglets/sex/treatment group, except sham: n= 8 male and n= 7 female piglets). Different letters (a, b) indicate significant differences between treatment groups (p < 0.05).
Figure 5.2: The mean proportion of time (± SE) piglets demonstrated pain-related behaviors (spasms, scratching the rump, tail wagging, trembling, stiffness) after tail docking across time (n= 15 piglets/sex/treatment group, except sham: n= 8 male and n= 7 female piglets). There were no significant differences between treatment groups at the same time point (p > 0.05).
Figure 5.3: The mean proportion of time (± SE) piglets engaged in active behaviors (playing, walking, suckling, nosing, chewing and running) after tail docking (n= 15 piglets/sex/treatment group, except sham: n= 8 male and n= 7 female piglets). Different letters (a, b) indicate significant differences between treatment groups (p < 0.05).
Figure 5.4: Mean Piglet Grimace Scale (PGS) scores (± SE) within each treatment group after tail docking (n= 15 piglets/sex/treatment group, except sham: n= 8 male and n= 7 female piglets). Observers (n= 3) were unaware of piglet treatment, litter, and time point when scoring. Different letters (a, b) indicate significant differences between treatment groups (p < 0.05).
Figure 5.5: The (a) frequency (Hz), (b) power (dB), and (c) energy (dB) of vocalizations emitted by piglets during marking, injection and tail docking ± SE. \(n=30\) piglets/treatment group, except sham: \(n=15\) piglets). Different letters (a, b) show significance between treatment groups \((p < 0.05)\). Asterisks indicate significant differences between procedures \((p <.0001)\).
Table 5.1. Total number of piglet faces captured for Piglet Grimace Scale scoring. Black numbers are from male piglets and red numbers are from female piglets.

<table>
<thead>
<tr>
<th>Time point (h)</th>
<th>Treatment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEL (1.99±0.0 kg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BUP (1.87±0.0 kg)</td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>3 9 5 6 6 3 7 1 3 4 1 2</td>
<td>50</td>
</tr>
<tr>
<td>0</td>
<td>10 10 14 8 10 5</td>
<td>12 9 12 11 4 2</td>
</tr>
<tr>
<td>1</td>
<td>2 5 12 11 11 3 10 7 3 4</td>
<td>2 7</td>
</tr>
<tr>
<td>2</td>
<td>3 5 11 6 9 8 7 12 5 1</td>
<td>2 1</td>
</tr>
<tr>
<td>3</td>
<td>6 6 9 7 7 5 10 8 7 5 2 1</td>
<td>73</td>
</tr>
<tr>
<td>4</td>
<td>5 4 9 4 10 4 5 10 2 8</td>
<td>1 1</td>
</tr>
<tr>
<td>5</td>
<td>8 4 12 4 6 3 8 5 5 2 1</td>
<td>63</td>
</tr>
<tr>
<td>6</td>
<td>4 2 7 3 4 7 10 6 . 6</td>
<td>2 1</td>
</tr>
<tr>
<td>7</td>
<td>1 1 11 3 4 3 4 7 2 2</td>
<td>3 1</td>
</tr>
<tr>
<td>24</td>
<td>8 4 13 9 8 10 12 9 4 5</td>
<td>1 1</td>
</tr>
<tr>
<td>Total</td>
<td>50 50 103 61 75 51</td>
<td>85 74 43 51 20 11</td>
</tr>
</tbody>
</table>

1Mean weight of piglets ± SE (n=15) in each treatment group
Table 5.2. Behavioral analysis of piglets (n= 165) pre-treatment and post-treatment across all litters and time points. Values presented are the proportional means ± SE.

<table>
<thead>
<tr>
<th>Behavior¹</th>
<th>Proportion of time in each behavior</th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
<th>Sex</th>
<th>Time*Treatment</th>
<th>Sex*Time</th>
<th>Sex*Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake inactive</td>
<td>0.8612 (0.46±0.05)</td>
<td>0.2625 (&lt;.0001)</td>
<td>0.4065</td>
<td>0.2298</td>
<td>0.8384</td>
<td>0.8938</td>
<td></td>
</tr>
<tr>
<td>Lying</td>
<td>0.9294 (0.69±0.07)</td>
<td>&lt;.0001</td>
<td>0.5485</td>
<td>0.2050</td>
<td>0.1331</td>
<td>0.3647</td>
<td></td>
</tr>
<tr>
<td>Nosing udder</td>
<td>0.3624 (0.18±0.05)</td>
<td>0.9400 (0.0315)</td>
<td>0.6081</td>
<td>0.6259</td>
<td>0.9664</td>
<td>0.4857</td>
<td></td>
</tr>
<tr>
<td>Sleeping</td>
<td>0.5793 (0.50±0.05)</td>
<td>0.0662 (0.0004)</td>
<td>0.2116</td>
<td>0.5548</td>
<td>0.5676</td>
<td>0.6074</td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td>0.5319 (0.25±0.05)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.4255</td>
<td>0.3460</td>
<td>0.2503</td>
<td></td>
</tr>
<tr>
<td>Tail wagging</td>
<td>0.0928 (0.01±0.00)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.2536</td>
<td>0.8309</td>
<td>0.0364</td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td>0.2142 (0.07±0.02)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.5127</td>
<td>0.0626</td>
<td>0.0988</td>
<td></td>
</tr>
<tr>
<td>Sitting</td>
<td>0.1828 (0.04±0.00)</td>
<td>0.1008</td>
<td>0.0607</td>
<td>0.3603</td>
<td>0.6446</td>
<td>0.0384</td>
<td></td>
</tr>
<tr>
<td>Spasms</td>
<td>0.9306 (0.00±0.00)</td>
<td>0.9339</td>
<td>0.3460</td>
<td>0.2684</td>
<td>0.2076</td>
<td>0.3769</td>
<td></td>
</tr>
<tr>
<td>Playing</td>
<td>0.2518 (0.02±0.00)</td>
<td>0.8993</td>
<td>0.0441</td>
<td>0.6888</td>
<td>0.6958</td>
<td>0.8662</td>
<td></td>
</tr>
<tr>
<td>Active²</td>
<td>0.3767 (0.29±0.06)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.5573</td>
<td>0.2949</td>
<td>0.1697</td>
<td></td>
</tr>
<tr>
<td>Pain³</td>
<td>0.0832 (0.01±0.00)</td>
<td>0.0050</td>
<td>0.9547</td>
<td>0.2956</td>
<td>0.0239</td>
<td>0.0693</td>
<td></td>
</tr>
</tbody>
</table>

¹Only behavior variables that were significant post-treatment are presented
²Significant effects (p < 0.05) are indicated in bold
³Proportion of time piglets engaged in each behavior
⁴Active behaviors include: nosing, suckling, walking, chewing, playing, running
⁵Pain behaviors include: stiffness, trembling, spasms, tail wagging and scratching
### Table 5.3. Behavioral analysis of piglets (n= 165) across all litters and time points. Values presented are the proportional means ± SE.

<table>
<thead>
<tr>
<th>Behavior¹</th>
<th>F value</th>
<th>Pr¹ &gt; F</th>
<th>Post-Tail Docking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MEL</td>
</tr>
<tr>
<td>Lying</td>
<td>12.68</td>
<td>&lt;.0001</td>
<td>0.64±0.03b</td>
</tr>
<tr>
<td>Standing</td>
<td>14.36</td>
<td>&lt;.0001</td>
<td>0.33±0.02b</td>
</tr>
<tr>
<td>Tail wagging</td>
<td>5.93</td>
<td>&lt;.0001</td>
<td>0.02±0.00b</td>
</tr>
<tr>
<td>Walking</td>
<td>20.45</td>
<td>&lt;.0001</td>
<td>0.08±0.01b</td>
</tr>
<tr>
<td>Active³</td>
<td>12.43</td>
<td>&lt;.0001</td>
<td>0.36±0.03b</td>
</tr>
<tr>
<td>Pain⁴</td>
<td>4.88</td>
<td>0.0002</td>
<td>0.02±0.00b</td>
</tr>
</tbody>
</table>

¹²Means with different superscripts in the same row differ significantly (p < 0.05)
¹Only behavior variables that were significant post-treatment are presented
²Proportion of time piglets engaged in each behavior
³Active behaviors include: nosing, suckling, walking, chewing, playing, running
⁴Pain behaviors include: stiffness, trembling, spasms, tail wagging and scratching
CHAPTER 6

General Discussion

Surgical castration and tail docking of piglets on commercial pig farms is routine practice in North America to prevent boar taint and to minimize aggression and tail biting among littermates (Sutherland et al., 2015). While these procedures are known to cause pain, piglets are generally not provided analgesia or anesthesia, which is an important pig welfare concern. Guidelines in Canada now require analgesia administration prior to surgical castration and tail docking to reduce post-procedural pain (NFACC, 2014); however, the literature is lacking research results that strongly support drug efficacy to mitigate post-procedural pain in piglets, making recommendations to producers to comply with these new regulations difficult. This thesis examines the use of nonsteroidal anti-inflammatory drugs (meloxicam, ketoprofen), opioids (butorphanol, buprenorphine), and topical anesthesia (Maxilene®), used alone and in combination, for their ability to reduce surgical castration and tail docking pain in piglets using behavioral measures, facial grimacing and vocalization. Across four large-scale research studies, 330 piglets were castrated and 165 piglets were tail docked. In total, 1237.5 h of behavioral data were scored and analyzed using a comprehensive ethogram, and 3459 facial images were scored using the Piglet Grimace Scale (PGS). With regards to all in-vivo work in this project, to minimize potential confounding variables, all handling and technical procedures (injection, tail docking, castration) were conducted by the PhD student, who had been extensively trained by a veterinarian to optimize piglet care and minimize procedural stress. We used small 25 G (16 mm x 0.5 mm) needles for injections and they were discarded after one use. We also used new scalpel blades for castrations, cleaned them thoroughly with an alcohol wipe after each use, and discarded the blade after one litter of pigs had been castrated, to ensure they remained sharp. We are confident the results presented in this thesis reflect the true efficacy of the drugs examined to reduce surgical castration and tail docking pain, will strengthen the literature regarding piglet
pain control and will allow for the most current and complete recommendation on piglet pain management.

6.1. Evaluation of nonsteroidal anti-inflammatory drugs to reduce surgical castration pain

This work found a single intramuscular (IM) administration of meloxicam (at the label dose of 0.4 mg/kg or a high dose of 1.0 mg/kg) or 6.0 mg/kg ketoprofen were ineffective at alleviating behavioral indicators of castration pain in piglets, including tail wagging, spasms, trembling, scratching the rump, and stiffness. They were also ineffective at reducing facial grimacing, as assessed using the Piglet Grimace Scale (Viscardi et al., 2017). These findings are consistent (Kluivers-Poodt et al., 2012) and inconsistent with previous work that found meloxicam was effective at reducing behavioral indicators of surgical castration pain in piglets (Keita et al., 2010; Kluivers-Poodt et al., 2013); however, these studies had significant design and methodology limitations that weaken the validity of their findings. Keita et al. (2010) only observed piglet behavior “for a few minutes” at each time point in their study (at 0.5, 1, 2, 4, or 24 h after castration) and used a very simple ethogram, scoring piglets on the presence or absence of four behaviors (prostration, tremors, tail movements, and isolation). They did not account for trembling, scratching the rump, or stiffness, which are important castration-related pain behaviors (Hay et al., 2003). With such a short observation period and limited ethogram, the conclusion that meloxicam was efficacious at relieving post-surgical pain should be challenged. Kluivers-Poodt et al. (2013) used a more detailed ethogram but employed a scan sampling method of scoring at two periods in the day, once in the morning and once in the afternoon, which may have resulted in a large amount of pain behaviors being missed. Both studies used direct observation in the farrowing rooms to score behavior, with no indication as to whether sows and piglets were habituated to their presence. (Keita et al., 2010; Kluivers-Poodt et al., 2013). An observer effect has been shown to alter animal behavior (Iredale et al., 2010). Overall, the methodology of behavioral pain assessment was weak in these studies and does not strongly support the conclusion that meloxicam effectively reduced surgical castration pain behaviors. To address these limitations, we employed a much more comprehensive behavior scoring system (continuous observation of each piglet for 15 min per hour at pre- and 0, 1, 2, 3, 4, 5, 6, 7, and 24 h post-castration), used a detailed ethogram of over 20 behaviors and postures, and video
cameras were placed on tripods outside of each farrowing pen to reduce the potential observer effect on animal behavior. We ensured every individual involved in behavior and facial scoring were blinded as to treatment and thoroughly randomized videos and images across time point and litter. We ensured our methodology and study design were sound so that the results obtained would accurately address the analgesic capacity (or lack thereof) of both meloxicam and ketoprofen.

This work found that castrated piglets (administered an NSAID or saline) had a significant increase in pain behavior 24 h post-castration compared to every other time point observed. This may have been due to progression of the inflammatory processes, causing an increase in pain (Kumar et al., 2015). Persistence of castration pain can extend beyond 24 h and may be present up to 4 days post-procedure (Hay et al., 2003). Therefore, appropriate analgesia should not simply target the immediate post-procedural pain of castration. Castrated piglets (administered an NSAID or saline) also wagged their tails significantly more than uncastrated piglets in this study. An increase in tail wagging behavior after a painful event, such as castration and dehorning, has been observed in piglets, lambs, and calves (Robertson et al., 1994; Graf and Senn, 1999; Hay et al., 2003; Rault and Lay, 2014; Jongman et al., 2016), which suggests that it may be a useful, non-invasive pain indicator.

Meloxicam and ketoprofen are commonly used analgesics in food animals and both are licensed for use in pigs (CPC, 2016). A group of swine experts reviewed the existing literature and determined the quality of evidence to support NSAID use was low, and they gave a weak recommendation for the use of NSAIDs to mitigate piglet surgical castration pain (O’Connor et al., 2014). Based on the results of our studies, we would agree with this recommendation. The Canadian Veterinary Drug Directorate approved a label claim for piglets for Metacam® (meloxicam) of 0.4 mg/kg to relieve surgical castration pain in September, 2016 (Garbutt, 2016). This drug is currently being recommended by the Canadian Pork Council to comply with the recent Codes of Practice and NSAIDs are the most widely used drugs in Europe (NFACC, 2014; Briyne et al., 2016; CPC, 2016). There are ethical concerns that must now be considered
regarding drug administration to alleviate piglet pain that, as outlined in Chapter 2, were as effective as an IM injection of saline.

6.2. Evaluation of opioids to reduce surgical castration pain

Our work found 0.04 mg/kg buprenorphine was highly effective at alleviating surgical castration pain in piglets. It reduced piglet pain behaviors and facial grimacing to the level of uncastrated piglets. The maximum duration of action of buprenorphine in piglets is 12 h (Thiede et al., 2014); however, our results suggest a single 0.04 mg/kg IM dose was able to maintain analgesia in piglets beyond 24 h post-administration. Perhaps in providing piglets with appropriate pre-emptive analgesia, central sensitization (or spinal cord “wind-up”) did not occur, reducing the increased pain expression observed in Chapter 2 (Hanania and Argoff, 2009). Therapeutic concentrations of buprenorphine (0.1 ng/mL) are also reached rapidly in pigs, taking only 5-30 min after IM administration (Thiede et al., 2014). Castrated piglets administered buprenorphine were significantly more active across the entire observation period than castrated piglets administered saline. There have been contradictory results regarding the effect (if any) buprenorphine has on activity level in rats (Roughan and Flecknell, 2002; Hansen et al., 2002). Animals often become less active when in pain (Berger and Eeg, 2006), and the increased activity observed in piglets administered buprenorphine could therefore be related to their reduced pain state. These factors all support buprenorphine being an efficient, and highly effective analgesic to administer prior to piglet surgical castration and one that we could recommend; however, currently it is a controlled substance that must be administered by a veterinarian and it cannot be used on commercially-raised piglets in North America or the EU, making it impractical for use on-farm (EFSA, 2004; FDA, 2014; CVMA, 2016).

Piglets emit distinct vocalizations associated with surgical castration that have been attributed to pain (Marx et al., 2003; Leidig et al., 2009). In this study, buprenorphine did not reduce the frequency, amplitude, power, or energy of piglet pain vocalizations at the time of castration. It is likely that piglets require full anesthesia prior to surgical castration to eliminate this vocal response and associated surgical pain (Axiak et al., 2007; Sutherland et al., 2012), and
this presents greater limitations for on-farm use (e.g. sedated piglets could not be returned to the sow until full drug recovery occurs and specialized equipment would be needed).

Opioids, such as buprenorphine and butorphanol, are more potent analgesic drugs than NSAIDs that suppress central pain signal transmission by binding to μ, δ, and κ opioid receptors in the brain, spinal cord and peripherally (Chahl, 1996). The positive outcomes observed in this study were not unexpected. Buprenorphine has demonstrated efficacy in reducing pain and lameness in pigs under experimental conditions (Hermansen et al., 1986; Meijer et al., 2015). Butorphanol has previously been used in combination with anesthetic drugs to prolong pig sedation in biomedical research studies (Sakaguchi et al., 1992; Heinonen et al., 2009). This study found piglets administered 0.2 mg/kg butorphanol IM became groggy and vomited. These side effects put piglets at greater risk of hypothermia or being crushed by the sow, and therefore, we do not recommend butorphanol use in piglets on-farm. There were no adverse effects observed with 0.04 mg/kg buprenorphine IM, further supporting our recommendation for its use should opioid administration on-farm become a practical option.

6.3. A multimodal approach to reduce surgical castration pain

In veterinary clinical practice, it is standard for dogs and cats undergoing castration or ovariohysterectomy to receive general anesthesia as well as multimodal analgesia to address and alleviate peri-operative pain (Hewson et al., 2006). To simulate this multimodal analgesia approach on-farm, we administered combinations of 0.4 mg/kg meloxicam IM, 0.04 mg/kg buprenorphine IM, and/or topical Maxilene® to the scrotal surface. Piglets administered any treatment containing buprenorphine had a significant reduction in pain behavior beyond 24 h after castration and were significantly more active across the study period. The addition of meloxicam or Maxilene® did not provide piglets with any significant benefit. Meloxicam + Maxilene® alone were insufficient in reducing pain. These results are consistent with the outcomes observed in Chapters 2 and 3.

Piglets administered Maxilene® were expected to vocalize less at incision, yet this was not observed. A more invasive anesthesia application, such as an intratesticular injection of
lidocaine (the active ingredient in Maxilene®), has been shown to effectively reduce the vocal response and castration pain in piglets (Hansson et al., 2011). However, this route of administration is painful, requires technical precision to ensure lidocaine properly diffuses into the spermatic cords, and would greatly increase the castration time for each piglet, limiting its on-farm practicality (Leidig et al., 2009). Tearing the spermatic cords elicited the strongest vocal response from piglets, regardless of the treatment administered. Unless pain directly caused by severing the spermatic cord is targeted (or piglets are anesthetized), it is unlikely that procedural castration pain will be completely mitigated with any currently available analgesic (Leidig et al., 2009). The Canadian Code of Practice for the Care and Handling of Pigs was carefully worded, stating piglets must be administered an analgesic drug to control post-procedural pain of surgical castration and tail docking (NFACC, 2014), and we therefore focused our studies on post-procedural pain management and selected analgesics that may not prevent pain of the actual procedure, but will alleviate longer-term pain. Optimal piglet welfare should eventually focus on complete peri-operative pain management, both during and after surgical castration and tail docking.

The IM injection of an opioid or NSAID elicited a strong vocal response from piglets, reinforcing that this procedure causes acute pain, although likely of very short duration. Regardless of whether certain injections are necessary for analgesia administration (or other treatments, such as iron injection), it is important for veterinarians and producers to be mindful of all procedures that are done to piglets. Two of the four drug combinations in our multimodal approach required two IM injections. Since buprenorphine was able to provide significant pain control when administered alone, the acute pain caused by the secondary injection of meloxicam was unnecessary. This further supports the notion that buprenorphine is all that is required for piglet post-operative pain relief.

6.4. A multimodal approach to reduce tail docking pain

Similar results to Chapter 4 were observed when a multimodal approach to alleviate tail docking pain was used. Buprenorphine, administered alone, significantly reduced facial grimacing and piglet pain behaviors. All piglets administered buprenorphine (alone or in
combination with meloxicam or Maxilene®) were also significantly more active than piglets in other treatment groups, suggesting that they were less painful (Berger and Eeg, 2006). The addition of meloxicam or Maxilene® did not benefit piglets. The IM injection elicited a similar vocal response from piglets as the tail docking procedure, further discouraging use of a second injection of meloxicam when one injection of buprenorphine was effective.

Piglets administered Maxilene® on their tail did not vocalize less at tail docking than piglets who did not receive a topical anesthetic prior to docking. It is unlikely a topical agent would have provided piglets sufficient analgesia at the time of tail removal, as they affect the surface tissue only, leaving deep tissue in the area of application poorly anesthetized (Dhawan and Dhawan, 2011). Injecting a local anesthetic into the base of the tail pre-procedure or applying a topical anesthetic to the tail-docked wound were previously found to be insufficient in alleviating procedural pain as well (Sutherland et al., 2011). Unless pain directly caused by docking the tail is targeted (or piglets are anesthetized), it is unlikely that tail docking pain will be fully mitigated.

Tail docked piglets did not display a significant increase in pain behaviors at 24 h post-procedure as was noted in untreated, castrated piglets. Examining proportion of pain behaviors displayed by piglets after tail docking and comparing them to the results from the castration studies, we found that piglets exhibited significantly more pain behaviors after surgical castration than tail docking. This suggests that surgical castration is a more painful procedure for piglets to undergo.

Sex differences in pain and sensitivity to analgesia have been reported in humans and rodents (Greenspan et al., 2007; Fillingim et al., 2009; Sorge and Totsch, 2017). In this study, no differences were seen in the proportion of pain behaviors displayed, facial grimacing or vocalizations between male and female piglets. Further, both sexes had a similar reaction to the analgesic drugs used. Gonadal hormones (e.g., estradiol, testosterone) and estrogen in females are functionally important for the differences observed in pain sensitivity and analgesia (Craft et
al., 2004; Sarajari and Oblinger, 2010). As the animals in this study were sexually immature, this is likely why we did not observe any sex differences in pain expression.

6.5. Validation of the Piglet Grimace Scale

The Piglet Grimace Scale (PGS) was developed in a pilot study, prior to undertaking this thesis work (Viscardi et al., 2017; Appendix A). The PGS uses quantifiable changes to piglet facial features (ear position, cheek tightening/nose bulge, orbital tightening) to assess pain. In our study, we retrospectively took still-images of piglet faces from the video data collected to be scored using the PGS, making it an ideal tool to use without concerns of behavior disruption. Other studies have used grimace scales for live, pen-side detection of pain (Miller and Leach, 2015; Leung et al., 2016). Similar scales have been developed for mice, rats, rabbits, horses, sheep, and lambs ((Langford et al., 2010; Sotocinal et al., 2011; Keating et al., 2012; Costa et al., 2014; Guesgen et al., 2016; McLennan et al., 2016). To validate the PGS as a pain assessment tool, we had to demonstrate that the facial grimace scores were comparable to the amount of pain behaviors displayed by piglets.

Overall, the PGS results corresponded well to the proportion of pain behaviors displayed by piglets across all studies; an increase in facial grimacing related to an increase in pain behavior. This is what we anticipated to observe if the PGS was able to detect piglet pain and is a huge step towards validation of the PGS as a pain assessment tool. For now, we recommend it be used as an adjunct for pain assessment with ongoing validation, as it does have utility for non-invasive pain detection but is not yet as sensitive a tool as behavioral analyses. Real-time scoring using the PGS was not assessed but would further expand its application and make it clinically relevant, allowing for more rapid intervention to occur, tighter endpoints for research animals and a general increase in piglet welfare.

There were several factors we found affected the PGS results. The PGS was used by different groups of volunteers in each study. All volunteers went through an interactive 30 min training session to teach them how to properly use the scale. We found that volunteers with more animal, and specifically, pig, experience (DVM students, for example) produced PGS results that
were more consistent with piglet pain behavior expression (Chapters 3 and 5). When volunteers had little animal experience, the PGS scores did not correspond well to pain behavior (Chapter 4). We also found that volunteers early in their undergraduate degree (first or second year students) were less consistent in scoring than DVM or graduate animal science students, suggesting that maturity may also play a role (or perhaps this was a confounding variable to animal experience). Our aim is to have all individuals, regardless of animal experience or age, use the PGS to accurately score piglet pain faces. Piglet weight also had a significant effect on PGS score (Chapter 3). Low body weight piglets that underwent no painful procedure or treatment had significantly higher levels of facial grimacing. Piglets in this weight range have low survival rates during the first week of life, miss more nursing bouts and are at greater risk of starvation (Marchant et al., 2000; Pedersen et al., 2011; Bovey et al., 2014). It is likely they had high levels of facial grimacing due to weakness and discomfort, not pain. Not balancing piglet weight across treatment groups was an inadvertent limitation of this study.

6.6. Pain assessment tools

There were three pain assessment tools used in this thesis project: behavior, facial grimace analysis, and vocalization. Of these tools, the PGS appears most prone to false negative results (not detecting pain when it is present), especially when inexperienced scorers are used (Table 6.1). Without the use of sound analysis software, vocalization appears most prone to false positive results (detecting pain when it is absent). Had we used vocalization analysis as a pain assessment tool post-procedure, we would have also produced a high rate of false negative results, as piglets rarely vocalized after surgical castration and tail docking. Behavior analysis in this research project was the best stand-alone measure to detect piglet pain and is least likely to generate false positive or negative results.

6.7. Future directions

The persistence of surgical castration pain (beyond 24 h post-procedure) should be examined in piglets. As well, determining how long a single dose of 0.04 mg/kg buprenorphine alleviates surgical castration and tail docking pain would better inform recommendations. In our studies, we evaluated surgical castration and tail docking as procedures independent from one
another; however, piglets often undergo them together, along with teeth clipping, ear tagging/notching and an iron injection. Future work could assess analgesic efficacy after piglets are exposed to all painful procedures at the same time to see if drug recommendations change. Finally, the utility of using tail wagging behavior as a non-invasive indicator of pain could be explored.

The biggest limitation to the application of these research results is the current inability to use opioids, particularly buprenorphine, on-farm. Future work should explore ways (perhaps through novel formulation to limit potential for abuse) to make buprenorphine a practical drug to administer to piglets in a production setting. As well, there is a need to establish drug or treatment combinations that effectively manage peri-operative castration and tail docking pain to optimize piglet welfare. Finally, before abandoning NSAID use, there may be potential to improve drug potency based on the route of administration (e.g., oral, transdermal, transmammory via the sow) and these should be explored further.

Future directions for the Piglet Grimace Scale involve assessing whether it can be effectively used for real-time detection of piglet pain and if it can accurately detect pain in sows and grower pigs. For assessment of clinical and on-farm applicability of the PGS, the ability of producers, technical caregivers and veterinarians working with swine to accurately measure facial grimacing should be evaluated.

6.8. Conclusions

A single dose of buprenorphine (0.04 mg/kg IM) was highly effective at reducing behavioral signs of surgical castration and tail docking pain in piglets for at least 24 h post-procedure without any adverse side effects. Meloxicam (0.4 mg/kg or 1.0 mg/kg IM) and ketoprofen (6.0 mg/kg IM) were ineffective at alleviating piglet pain. The addition of 0.4 mg/kg meloxicam and Maxilene® administration with buprenorphine did not provide significant pain-relieving benefits post-castration or tail docking. None of the analgesia treatments or combinations were able to reduce piglet vocalizations at the time of surgical castration or tail docking. Male and female piglets respond to tail docking pain and analgesic drugs similarly.
LBW piglets produce high grimace scores, independent of pain state. The PGS corresponds well to piglet pain behavior expression when experienced scorers are used and has utility as a pain assessment tool.
References


162


<table>
<thead>
<tr>
<th>Pain Assessment Tool</th>
<th>False Positive</th>
<th>False Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavior analysis</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td></td>
<td>Behaviors, such as trembling and tail wagging, are not specific to pain. The</td>
<td>Prey animals may be stoic and mask their behavioral expression of pain (likely</td>
</tr>
<tr>
<td></td>
<td>increase in these behaviors can be indicative of pain, which is why we</td>
<td>not a significant factor in this research project)</td>
</tr>
<tr>
<td></td>
<td>assessed the proportion of time piglets displayed each behavior and not just</td>
<td></td>
</tr>
<tr>
<td></td>
<td>their presence or absence in this research project.</td>
<td></td>
</tr>
<tr>
<td>Piglet Grimace Scale</td>
<td>Medium risk</td>
<td>High risk</td>
</tr>
<tr>
<td></td>
<td>Low body weight piglets had higher grimace score (Ch. 3)</td>
<td>Inexperienced scorers are prone to assign lower grimace scores (Ch. 4)</td>
</tr>
<tr>
<td>Vocalization analysis</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td></td>
<td>We were able to distinguish vocalization frequency, amplitude, power, and</td>
<td>Only because we did not use vocalization analysis post-procedure (piglets</td>
</tr>
<tr>
<td></td>
<td>energy using sound analysis software. Without this technology, vocalization</td>
<td>rarely vocalized after castration and tail docking; vocalization assessment</td>
</tr>
<tr>
<td></td>
<td>analysis would produce a high rate of false positive results (vocal response</td>
<td>would therefore produce a high rate of false negative results if used post-</td>
</tr>
<tr>
<td></td>
<td>of piglets during handling, injection, tail docking and castration would be</td>
<td>surgically)</td>
</tr>
<tr>
<td></td>
<td>difficult to distinguish)</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX A

Development of a Piglet Grimace Scale to evaluate piglet pain using facial expressions following castration and tail docking: a pilot study

A.V. Viscardi 1*, M. Hunniford2, P. Lawlis3, M. Leach4, P.V. Turner1

1Department of Pathobiology, University of Guelph, Guelph, ON, Canada
2Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada
3Ontario Ministry of Agriculture, Food, and Rural Affairs, Guelph, ON, Canada
4School of Agriculture, Food, and Rural Development, Newcastle University, Newcastle upon Tyne, United Kingdom

*Correspondence: Abbie Viscardi, aviscard@uoguelph.ca

Abstract

Facial expressions are increasingly being used to assess pain in non-human species, including rodents, horses and lambs. The development of these species-specific grimace scales has allowed for more rapid pain detection, which can lead to better animal welfare if intervention promptly occurs. For grimace scales to ever be used as a stand-alone measure of pain, it is important they correlate with established pain assessment tools, such as behavioral analysis. This preliminary study aimed to determine whether piglets exhibit pain grimacing and if these facial expressions correlate with their behavior. It also assessed and compared the behavior of boar piglets given an analgesic and topical anesthetic prior to surgical castration and tail docking to piglets that did not receive anything for pain relief. Five-day-old male Yorkshire piglets (n = 19) from four pens were randomly assigned, within their pen, to one of five possible treatments: meloxicam (0.4 mg/kg, intramuscularly) + EMLA® cream, meloxicam (0.4 mg/kg, intramuscularly) + non-medicated cream, saline (intramuscularly) + EMLA® cream, saline (intramuscularly) + non-medicated cream, or no treatment prior to surgical castration and tail docking. Piglet behaviors were video recorded for 8 h immediately after castration, as well as for 1 h at 24 h pre- and post-castration. Their individual behaviors were scored continuously for the first 15 mins of every hour of video collected. Facial images were also captured across all time points. A Piglet Grimace Scale (PGS) was developed and used by two observers blinded to treatment, time, and procedure to score over 600 piglet faces. All piglets displayed significant behavioral changes up to 7 h post-castration when compared to baseline, and the use of meloxicam and EMLA® cream was not associated with a reduction in painful behaviors. Significantly higher PGS scores were noted at 0, 3, 4, and 5 h post-castration when compared to PGS scores at 7 h and there was no effect of treatment. PGS scores significantly correlated with piglet behavioral activity. The results suggest that the PGS may have utility for pain evaluation in neonatal pigs.

Keywords: analgesia, animal welfare, grimace scale, piglet, pain, refinement
**Introduction**

Facial expression analysis is widely used to assess pain in non-verbal humans (1). It has only recently been validated as a tool to evaluate pain in animals, with the development of species-specific grimace scales. Since the first grimace scale was introduced for mice in 2010, there have been scales developed for rats, rabbits, horses, sheep and lambs (2-7). Grimace scales require identification of specific facial action units (FAUs) that change when animals are in pain, such as ear position, orbital tightening, and nose bulge. At least four FAUs have been described for each scale and all scales have demonstrated high inter-observer reliability among participants, suggesting they are accurate and easy to use. Their potential clinical application and ability to permit rapid pen-side detection of pain have generated interest in scale development for other species and further refinement of those that are pre-existing (8, 9). However, there are ethical issues with subjecting animals to unnecessary painful procedures for the development of a grimace scale.

Surgical castration and tail docking are routinely performed on commercially raised piglets in North America to reduce boar taint (an unpleasant odor and flavor associated with pork from intact males), aggression, and tail biting (10, 11). These procedures are known to cause acute pain persisting beyond 24 h, as indicated by behavioral and physiologic changes in piglets, including increased blood cortisol concentrations, high-frequency vocalizations, and trembling (10, 12-14). Behavioral analysis is often regarded as the gold standard for pain evaluation in animals (15), yet it is extremely laborious and impractical on a large-scale commercial farm. A grimace scale for piglets, if correlated with pain-related behaviors, would have great utility for on-farm assessments for its speed and ease of use. To determine whether these correspond, piglet behaviors and facial expressions before and after a known painful event (such as castration and tail docking) would need to be evaluated.

Castration and tail docking of piglets are often done without the use of an analgesic or anesthetic agent for pain relief. This may be due to drug approval limitations for food animals, or the added time, cost and effort involved with implementing analgesia into routine practice (16). There are analgesics licensed for use in piglets in North America and the European Union,
including meloxicam, a non-steroidal, anti-inflammatory drug, which has pharmacologic effects lasting for at least 4 h (17). It has been shown to be efficacious in mitigating pain for procedures such as dehorning in calves as well as castration and tail docking in lambs (18, 19). Meloxicam has been used in previous piglet castration studies with varying results (14, 17). EMLA® (Eutectic Mixture of Local Anesthetics) cream is a mixture of 2.5% lidocaine and 2.5% prilocaine that works as a topical anesthetic (4). It hasn’t been used on piglets, but it has been shown to reduce the pain of a scrotal injection prior to vasectomy in humans and tattooing in rabbits (4, 20). Legislation in Canada now mandates that analgesia be provided to piglets prior to surgical castration and tail docking (21) and there is an urgency to identify appropriate and effective analgesics and anesthetics to relieve postsurgical piglet pain.

The objectives of this preliminary study were to develop a pain scoring scale for piglets based on their facial expressions and to assess the effectiveness of 0.4 mg/kg of meloxicam and EMLA® cream (individually and in combination) in reducing pain behaviors and facial grimacing of piglets following castration and tail docking. A decrease in pain behaviors and facial grimacing was expected in piglets receiving both the meloxicam injection and EMLA® cream compared with piglets that did not receive any form of analgesic or anesthetic.

**Animals and Methods**

All animal use and procedures were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #3350). The institution is registered under the ‘Animals for Research Act’ of Ontario and holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care.

**Animals and Treatments**

Nineteen 5-day-old Yorkshire piglets from four different litters, weighing between 1.02 and 3.20 kg, were used. Sows and piglets were housed in farrowing pens at the University of Guelph Arkell Swine Research Station (Arkell, ON, Canada). The floor space for each pen was 6 ft x 8 ft (1.8 m x 2.4 m) and the farrowing crate was 2.5 ft x 7.5 ft (0.8 m x 2.3 m). The farrowing
rooms were maintained at ambient temperature (23°C ± 0.5°C) with lights on/off at 07:00/21:00, and natural light was provided by windows in each room. Sows were fed ad lib 4 days after farrowing. The creep areas for piglets were heated to approximately 30-35°C by means of a heat lamp.

Five treatments were used and each treatment group was identified by a pre-determined symbol that was marked on the piglet’s forehead and back prior to castration. This was to keep individuals performing castrations and those involved in post-castration observations and behavioral scoring blinded as to animal treatment. Within each pen, piglets were randomly assigned to a treatment group (Table A.1), and all treatments were represented in each litter (except for those that only had four boars). Meloxicam (Metacam 20mg/mL; Boehringer Ingelheim Ltd, Burlington, ON, Canada) was administered at 0.4mg/kg (liquid volume range of 0.1-0.32 mL) as an intramuscular injection. Saline was given intramuscularly at 0.2 mL. Then, 1.0 g of EMLA® cream (EMLA®; Oak Pharmaceuticals Inc, Lake Forest, IL, USA; extra-label use) or 1.0 g of a sterile, non-medicated ointment (Life Brand Personal Lubricant Jelly; Shoppers Drug Mart Inc, Guelph, ON, Canada) was applied topically to cover the entire scrotal surface. The treatment groups were meloxicam + EMLA® cream, meloxicam + non-medicated cream, saline + EMLA® cream, saline + non-medicated cream, and no treatment (where no injection or cream was applied to the piglets prior to surgical castration and tail docking).

**Processing Procedures**

Twenty-four hours prior to study initiation, piglets were weighed and the meloxicam dose was calculated. On the day of the procedure, boar piglets were separated from their littermates, marked with a symbol using a black marker, and their assigned treatments were applied. Piglets were dosed at slightly more than 0.4 mg/kg meloxicam to account for the expected weight gain over the past 24 h. Approximately 20 min later, piglets were surgically castrated using a horizontal incision and tearing of the spermatic cord (16, 22). All piglet castrations occurred between 7 a.m. and 8 a.m. on the same day.
At the time of castration, the piglets also had their tails docked using blunt trauma cutters and were given iron intramuscularly (Iron Dextran; Dominion Veterinary Laboratories Ltd, Winnipeg, MB, Canada). The piglets were then returned to their pens. One piglet in the meloxicam + non-medicated cream treatment group was euthanized 5 h post-castration because of intestinal herniation through the castration site; all other piglets (n = 18) recovered from surgery without incident.

**Behavioral Recording and Scoring**

Piglets were video recorded 24 h prior to castration for 1 h using a high definition video camera (JVC GZ-E200 full HD Everio Camcorder, Yokohama, Japan) mounted on a tripod. On the day of castration, the cameras were turned on immediately post-procedure and recorded for 8 h continuously. Finally, 24 h post-procedure, the cameras were turned on for 1 h. The behavior of each piglet was scored continuously by a single experienced observer blinded as to piglet treatment and time point for the first 15 min of every hour using the Observer XT program (Version 9.0: Noldus Information Technology, Wageningen, Netherlands) according to an ethogram adapted from a previous study assessing castration pain in piglets (10) (Table 2.1). All video clips were randomized using a random number generator program (www.random.org) prior to being scored. Intra-scorer reliability was tested every 3-4 weeks by having the observer rescore a video they had previously scored. The intraclass correlation coefficient (ICC) was then determined to ensure behaviors were being scored consistently over time and no drift had occurred (all intra-observer reliability tests produced an ICC above 0.9). A total of 2,700 min were observed and scored for this study.

After the piglet behaviors were analyzed separately, they were grouped into “active” and “inactive” categories. Active behaviors included walking, playing, nosing, suckling, and running. Inactive behaviors included sleeping and awake inactive. Postures were used for this behavioral analysis; piglets that were standing or sitting were scored as performing an “active” behavior and piglets that were lying were scored as demonstrating an “inactive” behavior. The sitting posture was placed in the active behavior category because most of the piglets exhibited this posture when suckling or scratching and these were considered active behaviors.
Piglet Grimace Scale (PGS) Recording and Scoring

The PGS was developed using still images of piglet faces taken from the raw video recordings used for behavior scoring. Videos were uploaded to the PlayMemories Home program (Sony Corporation, Tokyo, Japan, 2014) and whenever a piglet face or profile was in view, the video was paused and the still image was saved, omitting times when piglets were lying down to sleep (to prevent facial changes due to tiredness being misinterpreted as pain faces). Images were captured across all time points and treatments, for a total of 627 faces (Table A.2). Prior to scoring, the images were imported into Photoshop (Adobe Systems Incorporated, CA, USA, 2014) and the symbol on the piglet’s forehead was blurred to ensure blind scoring.

To develop the PGS, images of piglet faces (across all treatments) were compared at different post-castration time points to the images pulled from the video recordings 24 h prior to castration (a time point that was assumed to represent a “no pain” state). Based on these comparisons, three significant FAUs were identified: orbital tightening, cheek tightening/nose bulge, and ear position (Figure A.1), which are common to previously developed grimace scales. Cheek tightening/nose bulge and ear position were set to a 3-point scale (0-2), while orbital tightening was set to a 2-point scale (0-1). Therefore, the maximum pain score using the PGS was 5. Two individuals blinded as to piglet treatment and time point used the PGS to score the images. If an image could not be scored reliably, the observers were instructed to exclude it from scoring. The PGS score for each image was calculated by summing the scores allotted to each FAU. These “absolute” grimace scores were analyzed and then grouped into pain categories: scores of 0-1 represented a piglet experiencing “no-to-low pain” and scores of 3-5 represented “moderate-to-high pain”. A PGS score of 2 was excluded from these categories because of interpretation difficulty. The pain categories were transformed into proportions; at each time point, the proportion of piglet faces scored in the “no-to-low pain” category (low) and “moderate-to-high pain” category (high) was calculated. This was to allow correlation analysis between PGS score and active/inactive behavioral activity. A greater proportion of piglets falling in a “moderate-to-high pain” category would also give us information regarding analgesia efficacy, in that the drug (or dose) is insufficiently reducing pain. Appropriate analgesia would keep a majority of piglets in the “no-to-low pain” category.
Data and Statistical Analysis

Behavior Analysis

The frequency and mean duration of the behaviors in Table 2.1 were square root transformed (except for awake inactive, sleeping, lying and standing) to satisfy the assumptions of ANOVA (Statistical Analysis System 9.4, SAS Institute Incorporated, NC, USA, 2014). The normality of these behavior variables was tested using the univariate procedure prior to analysis. Data were analyzed using a mixed model ANOVA with piglet as the experimental unit. The model included pen, treatment, and time and the interaction between treatment and time. Post hoc tukey tests were conducted for significant factors with more than two levels using the least-squared means statement. The values presented here are the untransformed means with SE. Statistical significance was set at p < 0.05.

Durations of time spent active (standing + sitting) and inactive were converted to the proportion of total time observed; activity and inactivity were mutually exclusive. The proportions of activity/inactivity were then assessed to determine if they met the assumptions of ANOVA by testing that the residuals were normally distributed. The residuals of the raw proportions were normal (W = 0.99), so the proportions were not transformed. The proportion of activity and inactivity were analyzed as separate dependent variables using a mixed model analysis of variance; the model included treatment, time, treatment x time, and pen. LS means were calculated for all significant effects and a post hoc tukey adjustment was used. The values presented here are the untransformed means with SE. Statistical significance was set at p < 0.05.

Grimace Scale Analysis

The statistical analyses for the grimace scale scores were conducted using GraphPad Prism (GraphPad Software Incorporated, CA, USA, 2014). A two-way ANOVA was used to evaluate the scores provided for treatment and time and the interaction between them. A post hoc Tukey’s test was conducted for significant factors. SAS was used to determine the ICC of the two scorers. Both the behavioral data and the PGS data were used to determine if there was an effect of treatment in reducing the pain experienced by piglets undergoing castration and tail docking.
Correlation between Behavior and Grimace Scores

Correlation analyses were used to determine if there was an association between behavior and the PGS scores. The proportion of activity/inactivity over time and the proportion of high and low grimace scale scores were sorted by pen, treatment and time. Residuals were analyzed and found to be normal (low: $W = 0.98$; high: $W = 0.99$; active: $W = 0.98$; inactive: $W = 0.99$), and the data was not transformed prior to analysis. The Correlation Procedure (SAS 9.4) and Pearson's correlation coefficient were used to analyze the relationship between the proportion of time spent active or inactive with the proportion of low or high grimace scores. R and p values are presented.

Results

Behavioral Observations after Castration and Tail Docking

There were no litter-associated differences in behavior ($p > 0.05$) and data were combined across litters. Piglets demonstrated significant behavioral changes, when compared to baseline behaviors, up to 7 h post-castration and tail docking (for most behaviors, $p < 0.0001$). Only two behaviors, tail wag ($p = 0.036$) and isolated ($p = 0.002$), were affected by treatment across all time points, but overall, none of the treatments given to the piglets pre-castration and tail docking significantly reduced painful behaviors and postures (Table A.3).

Active and inactive behaviors of piglets were also analyzed. Both behavior categories had a significant time effect (active: $F_{9,130} = 25.75$, $p < 0.0001$; inactive: $F_{9,130} = 26.75$, $p < 0.0001$), with piglets displaying more inactive behaviors than active behaviors up to 6 h post-castration and tail docking (Figure A.2). There was no effect of treatment and no interaction between time and treatment ($p > 0.05$).

Piglet Grimace Scale

Between-litter differences in facial grimacing were not noted and images were combined across litters. There was a significant treatment, time, and time x treatment interaction on PGS score ($p = 0.005$, $p = 0.015$ and $p = 0.003$, respectively). Further analysis found PGS scores at 0,
3, 4 and 5 h to be significantly increased than those at 7 h post-castration ($F_{4,15} > 3.06$, $p < 0.05$; Figure A.2). There was no significant difference in pre-treatment PGS scores and those obtained 7 h after processing ($p = 0.6404$). PGS scores were significantly higher in piglets administered a saline injection and EMLA cream compared to piglets that were given a saline injection and non-medicated cream ($p = 0.0083$). Across all treatments, there was a similar pattern in PGS score, with the highest scores generally noted between 3 to 4 h post-procedure. There was moderate agreement between the scores of the two observers, with an ICC of 0.57.

**Correlation between Behavior and PGS**

Piglet Grimace Scale scores and behavioral activity were strongly correlated (Figure A.2). The proportion of low pain scores had a significant positive correlation with active behaviors ($r = 0.222$; $p = 0.008$) and a significant negative correlation with inactive behaviors ($r = -0.218$; $p = 0.009$). The proportion of high pain scores tended to negatively correlate with active behaviors ($r = -0.159$; $p = 0.058$) and positively correlate with inactive behaviors ($r = 0.158$; $p = 0.061$).

**Discussion**

The results of this study suggest that 0.4 mg/kg of meloxicam and EMLA cream® were unable to reduce piglet pain post-procedure. Meloxicam has been proven to be an effective analgesic for reducing postoperative pain in other food animal species, such as lambs and calves (18, 19), yet it has had limited success in significantly reducing castration pain in piglets (14, 16, 23). The dose of meloxicam used in this study (0.4 mg/kg) is consistent with current recommendations (24); however, this dose was not derived from analgesia studies in piglets. Rather, this dose has been shown to be effective when given one or more times to sows with mastitis-metritis-agalactia or when treating lameness in grower pigs (25). It may be that this study’s sample size was too small to evaluate treatment effects or that the dose of meloxicam provided to the piglets was not sufficient to reduce pain. EMLA® cream also did not prove to be effective at providing topical anesthesia and reducing pain in this study. At the time of castration, piglets had their tails docked, yet EMLA® cream was only placed on the scrotum. It may be that piglets were expressing pain behaviors (such as tail wagging) related to the tail docking.
procedure, for which they were given no local anesthetic (26). The duration of action for EMLA® cream on human male genital skin is 15-30 min (27). Castration occurred 20 min after EMLA® application, so it is possible that its full anesthetic potential was not reached. However, severing of the spermatic cord is known to be the most painful aspect of the castration procedure and EMLA® cream’s anesthetic effects remain localized on the scrotum (28). It would not penetrate into the spermatic cord and is likely an impractical anesthetic to use to significantly reduce castration pain. A testicular injection of lidocaine may be better suited, but is not likely to be used in a commercial system because of the experience and time needed to administer the injection (29, 30).

This study found piglets in the saline and EMLA cream treatment group had the highest PGS scores compared to piglets administered saline and a non-medicated cream. The significance of this is difficult to interpret because of the low animal numbers in each group (3 and 5, respectively). Other studies using EMLA cream to reduce pain in rabbits, mice, rats, dogs, cats, and children have noted only mild irritation, if any, following topical use (4, 31-33). Future studies with larger piglet numbers are needed to evaluate the potential treatment effects on PGS score. Significant correlation was found between piglet behavioral activity and PGS scores; as piglet activity decreased, grimace scores increased and as piglet activity increased, grimace scores decreased. A higher grimace score indicates a greater pain state and a reduction in activity is consistent with pain expression (34). These results suggest that piglet grimaces do indicate pain and the PGS may be useful for future use in piglet pain assessment. Further, there was no difference in PGS score 24 h pre-procedure and 7 h post-procedure, indicating piglet’s facial expressions of pain are reduced to near the baseline level after 7 h (again, consistent with behaviors returning to baseline levels after 7 h). Other studies that have assessed piglets after surgical castration found behaviors indicative of pain persist from 2 h to 4 days (10, 12, 35, 36). This range in results could be because of differing castration techniques, piglet age, or overall study design variance.

Piglet Grimace Scale scores were grouped into pain categories to facilitate a direct comparison with piglet activity level. As this was a small-scale study, we felt it was important to
ensure the scale works broadly before using it in large-scale studies, as data collection is quite
time consuming. Grouping the PGS results also allows for easy interpretation of pain score. For
example, piglets consistently falling in the “moderate-to-high pain” category are a more obvious
cause for concern than piglets repeatedly scoring 3 or 4. Especially, if this is to be used on a
production farm, by individuals with various levels of experience, it may be more clear and
accessible to others to report the proportion of piglets in a pen that fall within each category of
pain, than to report PGS numbers. The preliminary results of the PGS are promising and it will
continue to be used and validated in future research.

The moderate inter-observer agreement found in this study could be a result of only
identifying three FAUs for the PGS. The maximum pain score was significantly reduced from
that reported in the other grimace scales (for example, the Mouse Grimace Scale had a maximum
pain score of 10 versus 5 for the PGS) (2). It also may have been due to some of the images
collected having low resolution, as faces were pulled from video data and not taken with a high-
resolution camera. There was also no formal training session to teach the observers how to use
the PGS, which may have accounted for the moderate inter-observer agreement. As this was a
preliminary study, only two individuals were recruited to score piglet faces; future work will
employ a larger group of scorers from different backgrounds to improve the validity of this scale.

A second grimace scale for piglets has been published recently by Di Giminiani et al. (37).
The complexity of scales differ (10 FAUs were used in its development versus 3 FAUs in our
scale) and orbital tightening was placed on a 3-point scale while we have orbital tightening set to
a 2-point scale. Another study looking at piglet facial expressions used two FAUs only (orbital
tightening and cheek tension) to assess pain (38). The decision to place orbital tightening on a
“present” or “not present” scale and to limit the FAUs included (for example, collapsing cheek
tightening and nose bulge into one FAU) in the current study was to ensure that this scale can be
used easily and rapidly on-farm. Further validation is needed for both scales but having two
available for piglets demonstrate the interest in grimace scale development and their importance
as a tool for pain assessment. A limitation of this study is the small sample size of 19 piglets.
While additional work is needed on a larger scale to further validate the PGS and confirm the
lack of treatment effects, there have been grimace scales developed using less animals. The Lamb Grimace Scale was developed with 16 animals, although the authors do acknowledge that the results should be interpreted with caution due to these low numbers (6). Another study limitation is not having a strong behavioral baseline to compare to post-procedure behaviors. Therefore, it is unknown whether the increase in inactivity noted following castration was solely attributable to the surgical procedure versus partially attributable to normal circadian variation in activity levels of piglets. Future studies should assess baseline piglet behaviors at the same time of day as to minimize this possible confounding variable.

Animal Welfare Implications and Conclusion

This preliminary study was able to demonstrate piglet grimacing in response to pain. It also confirmed that castration and tail docking cause significant pain in piglets lasting up to 7 h, as measured by detailed behavioral analyses and a newly conceived PGS. The current recommended dose of meloxicam (0.4 mg/kg) may not be sufficient at mitigating pain associated with castration and tail docking. The application of a local anesthetic (EMLA®) to the scrotum, with or without meloxicam, appears ineffective in reducing surgical castration pain. The PGS requires further validation but may become a useful tool to identify piglets experiencing acute pain, which will improve their welfare if prompt intervention occurs.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

PVT and PL conceived and designed the experiments. AV, PVT and PL conducted the studies and scoring. Data was analyzed by AV, MH, PVT, and ML. The manuscript was prepared by AV, MH and PVT and edited by all authors.
Funding
Funding was provided by the Campbell Centre for the Study of Animal Welfare (CCSAW), the Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA), and Ontario Pork [52367].

Acknowledgments
The authors wish to thank Tim Thalen and personnel at the Arkell Swine Research Station for technical assistance.
References


Figure A.1: Piglet Grimace Scale with descriptions for each of the 3 facial action units (FAUs) employed: ear position, cheek tightening/nose bulge and orbital tightening. FAUs are scored based on whether it is absent (score of 0), moderately present (score of 1) or obviously present (score of 2), with the exception of orbital tightening, which is scored on a 2-point scale of absent (score of 0) and present (score of 1).
Figure A.2: Comparison of active (walking, playing, suckling, nosing, etc) and inactive (lying, sleeping, isolated, awake inactive, etc) behaviors of piglets at all time points pre- and post-castration (±SE) are represented by the bar graph. Active behaviors decreased significantly following castration, independent of treatment given, and eventually returned to baseline levels after approximately 7 h. Proportion (±SE) of Piglet Grimace Scale (PGS) scores within each pain category across all treatment types are represented by the line graph. Observers (n=2) were unaware of piglet treatment or time point when scoring. PGS scores significantly correlated with piglet behavioral activity.
Table A.1. Number of piglets per pen assigned to each treatment group.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Pen A</th>
<th>Pen B</th>
<th>Pen C</th>
<th>Pen D</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEL + EMLA (meloxicam + EMLA® cream)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>MEL + 0 (meloxicam + non-medicated cream)</td>
<td>1(^a)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SAL + EMLA (saline + EMLA® cream)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>SAL + 0 (saline + non-medicated cream)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>NONE (no treatment)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\)Piglet was euthanized 5 h post-castration.
Table A.2. Total number of facial images captured for Piglet Grimace Scale scoring.

<table>
<thead>
<tr>
<th>Time point (h)</th>
<th>Treatment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEL + EMLA</td>
<td></td>
</tr>
<tr>
<td>-24</td>
<td>*</td>
<td>63</td>
</tr>
<tr>
<td>0</td>
<td>9 14 6 21 7</td>
<td>57</td>
</tr>
<tr>
<td>1</td>
<td>21 18 12 27 11</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>8 12 7 15 2</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>8 13 6 11 5</td>
<td>43</td>
</tr>
<tr>
<td>4</td>
<td>13 13 12 15 7</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>24 12 11 16 9</td>
<td>72</td>
</tr>
<tr>
<td>6</td>
<td>19 6 12 21 8</td>
<td>66</td>
</tr>
<tr>
<td>7</td>
<td>11 4 5 10 5</td>
<td>35</td>
</tr>
<tr>
<td>24</td>
<td>22 20 19 28 9</td>
<td>98</td>
</tr>
<tr>
<td>Total</td>
<td>135 112 90 164 63</td>
<td>627</td>
</tr>
</tbody>
</table>
Table A.3. Behavioral analysis of castrated and tail docked piglets across all treatments and time points.\textsuperscript{d}

<table>
<thead>
<tr>
<th>Behavior</th>
<th>F(_{4,130})</th>
<th>p*</th>
<th>Treatment</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NONE (19)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MEL + 0 (37)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SAL + 0 (50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SAL + EMLA (27)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MEL + EMLA (50)</td>
<td></td>
</tr>
<tr>
<td>Playing</td>
<td>0.62</td>
<td>0.6508</td>
<td>0.90 ± 0.66</td>
<td>2.22 ± 1.25</td>
</tr>
<tr>
<td>Walking</td>
<td>1.34</td>
<td>0.2587</td>
<td>5.78 ± 2.33</td>
<td>9.24 ± 1.84</td>
</tr>
<tr>
<td>Running</td>
<td>0.56</td>
<td>0.6945</td>
<td>0.03 ± 0.02</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Awake inactive</td>
<td>1.36</td>
<td>0.2519</td>
<td>35.75 ± 5.5</td>
<td>45.60 ± 4.3</td>
</tr>
<tr>
<td>Sleeping</td>
<td>1.99</td>
<td>0.0997</td>
<td>57.47 ± 7.00</td>
<td>43.09 ± 5.79</td>
</tr>
<tr>
<td>Suckling</td>
<td>1.05</td>
<td>0.3861</td>
<td>7.83 ± 3.9</td>
<td>14.30 ± 3.35</td>
</tr>
<tr>
<td>Nosing udder</td>
<td>0.95</td>
<td>0.4355</td>
<td>1.64 ± 0.98</td>
<td>3.84 ± 1.16</td>
</tr>
<tr>
<td>Nosing</td>
<td>0.77</td>
<td>0.5474</td>
<td>5.94 ± 3.01</td>
<td>6.74 ± 1.81</td>
</tr>
<tr>
<td>Chewing</td>
<td>1.48</td>
<td>0.211</td>
<td>0.27 ± 0.13</td>
<td>1.23 ± 0.53</td>
</tr>
<tr>
<td>Stiffness</td>
<td>0.75</td>
<td>0.5614</td>
<td>0.93 ± 0.36</td>
<td>0.54 ± 0.13</td>
</tr>
<tr>
<td>Trembling</td>
<td>0.95</td>
<td>0.4373</td>
<td>0.50 ± 0.30</td>
<td>0.40 ± 0.26</td>
</tr>
<tr>
<td>Spasms</td>
<td>0.43</td>
<td>0.7845</td>
<td>1.12 ± 0.25</td>
<td>1.57 ± 0.77</td>
</tr>
<tr>
<td>Scratching</td>
<td>0.14</td>
<td>0.9675</td>
<td>0.38 ± 0.33</td>
<td>0.45 ± 0.27</td>
</tr>
<tr>
<td>Tail wagging</td>
<td>2.65</td>
<td>0.036</td>
<td>\textbf{0.94 ± 0.58}</td>
<td>2.18 ± 0.81</td>
</tr>
<tr>
<td>Lying</td>
<td>0.94</td>
<td>0.4411</td>
<td>75.09 ± 7.19</td>
<td>66.67 ± 5.32</td>
</tr>
<tr>
<td>Sitting</td>
<td>1.17</td>
<td>0.327</td>
<td>4.13 ± 1.61</td>
<td>2.61 ± 0.57</td>
</tr>
<tr>
<td>Standing</td>
<td>1.99</td>
<td>0.0992</td>
<td>19.87 ± 6.70</td>
<td>30.3 ± 4.98</td>
</tr>
<tr>
<td>Kneeling</td>
<td>2.43</td>
<td>0.051</td>
<td>0.92 ± 0.49</td>
<td>0.69 ± 0.42</td>
</tr>
<tr>
<td>Isolated</td>
<td>4.67</td>
<td>0.0015</td>
<td>\textbf{0.0 ± 0.0}\textsuperscript{a}</td>
<td>0.04 ± 0.04\textsuperscript{a}</td>
</tr>
<tr>
<td>Desynchronized</td>
<td>0.45</td>
<td>0.7717</td>
<td>1.94 ± 1.4</td>
<td>2.00 ± 1.68</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Statistical significance after post hoc Tukey test (p < 0.05).

\textsuperscript{b}Sample sizes for each treatment group.

\textsuperscript{c}Interactions were not statistically significant after post hoc Tukey test.

\textsuperscript{d}Twenty-four hour pre-castration for 1 h, 7 h immediately post-castration, and 24 h post-castration for 1 h.

\textsuperscript{*}Significant effects (p < 0.05) are indicated in bold.
APPENDIX B

Pharmacokinetics of 1.0 mg/kg meloxicam in piglets

Introduction

Neonatal pigs often undergo several painful procedures during their first week of life, including surgical castration, tail docking, teeth clipping, and ear notching (Leslie et al., 2010; Sutherland, 2015). The Canadian Code of Practice for the Care and Handling of Pigs as well as animal care guidelines in the EU require analgesia administration prior to processing (EU Commission, 2010; NFACC, 2014). The most practical and widely used analgesic drugs administered to food animals are nonsteroidal anti-inflammatory drugs (NSAIDs), with meloxicam being approved for use in swine. The label dose for pigs is 0.4 mg/kg and it has demonstrated efficacy in reducing pain caused by mastitis-metritis-agalactia syndrome and lameness in sows (Hirsch et al., 2003; Pairis-Garcia et al., 2015). However, there is evidence to suggest this dose of meloxicam is insufficient when administered to piglets to alleviate castration and tail docking pain (Kluivers-Poodt et al., 2012; Herskin et al., 2016; Chapter 2: Viscardi and Turner, 2018). Increasing the dosage of meloxicam administered may provide piglets with more appropriate pain control.

Overdosage of an NSAID may cause side effects such as renal toxicity, hepatotoxicity, and injury to the gastrointestinal tract (Davies and Wallace, 1997; Lindsley and Warady, 1990; Boelsterli, 2002). Meloxicam toxicity can occur after administration of a single high dose, with the resulting clinical signs manifesting in as little as 1 h in dogs and cats (Osweiler et al., 2011). An incremental dose escalation approach has been shown to enhance safety and reduce the risk of toxicity in drug trials (Cook et al., 2015). 0.4 mg/kg and 0.6 mg/kg meloxicam have previously been administered to piglets and the pharmacokinetic parameters described; both doses were unable to significantly reduce inflammation (Fosse et al., 2008; Fosse et al., 2011).
The objectives of this study were to characterize the pharmacokinetics of 1.0 mg/kg meloxicam when administered intramuscularly to 8-day old male and female piglets, and to evaluate its potential for causing toxicity via gross examination and histopathology. We anticipate the incremental dose increase of meloxicam will not cause any acute or chronic toxicity to piglets.

Materials and Methods

All animal use and procedures were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #3350). The institution is registered under the Animals for Research Act of Ontario and holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care.

Animals and Procedures

Yorkshire-Landrace x Duroc male and female piglets (n=12, 8 days old, mean BW = 3.44 ± 0.09 kg) from 2 litters were used in this study. Sows and piglets were housed in farrowing pens at the University of Guelph Arkell Swine Research Station (Arkell, ON, Canada). The floor space for each pen was 1.8 m x 2.4 m (6 ft x 8 ft) and the farrowing crate was 0.8 m x 2.3 m (2.5 ft x 7.5 ft). Farrowing rooms were maintained at ambient temperature (23ºC ± 0.5ºC) with lights on/off at 7:00 am/9:00 pm, and additional natural light was provided by windows in each room. Sows were fed ab libitum 4 days after farrowing. Creep areas for piglets were heated to approximately 30-35ºC by means of a heat lamp. All piglets had been ear notched and tail docked at 1 day of age.

Four days prior to study start, piglets were marked on the back using a black permanent marker for individual identification (F1-7 for females and M1-5 for males) and were gently handled for 60 seconds, three times per day for four consecutive days (at 8:30am, 11:30am and 2:30pm) to habituate them to handling needed for blood sample collection. On the day of catheter placement, piglets were placed in a transport cart bedded with wood shavings and an attached heat lamp and fasted for 1 h before surgery. Piglets were anesthetized using 5% isoflurane (Forane; Pfizer Inc., New York, NY, USA) in oxygen. Central venous line catheters (4
Fr. x 8 cm double lumen catheter set; MILA International Inc., Florence, KY, USA) were placed in the external jugular vein using a percutaneous triangulation technique (Fudge et al., 2002; Flournoy and Mani, 2009) (Figure B.1). The catheter was secured to each piglet using three sutures. A 3-way stopcock was attached to the end of each catheter line and covered with elastic bandage. Catheter lines were flushed with 2.0 mL of heparinized saline (10 USP units heparin/1 mL saline) (Heparin 1000 USP units/mL; Pfizer Inc., New York, NY, USA). Piglets were recovered from anesthesia and returned to their home pen. Placement of seven catheters were unsuccessful; the remaining five piglets (n=3 male, n=2 female) were used in the pharmacokinetic portion of this study.

Pharmacokinetic Sampling

The day following catheter placement, all 12 piglets received an intramuscular injection of 1.0 mg/kg meloxicam (Metacam 20 mg/mL; Boehringer Ingelheim Ltd., Burlington, ON, Canada). Blood (1.0 mL) was collected into heparinized tubes (Vacutainer 2 mL; BD, Mississauga, ON, Canada) from the catheterized piglets at 0, 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 h post-injection. At each collection, catheters were flushed with 0.5-1.0 mL of saline and the initial 0.2 mL drawn was discarded to account for heparinized blood in the catheter line. Catheters were filled with 0.2 mL heparinized saline after sample collection. At 36 h and 48 h, blood could only be collected from two piglets, as the catheter lines blocked in the remaining three.

Samples were immediately placed on ice after collection and then centrifuged for 10 min at 2000 x g (Fosse et al., 2008). Plasma was transferred into clean micro polypropylene tubes, frozen at -80°C and shipped to Iowa State University for meloxicam concentration analysis using liquid chromatography-mass spectrometry (LC-MS) (Hormazábal et al., 2006).

Gross and Microscopic Pathology Evaluations

Immediately following blood sample collection at 48 h post-injection, all piglets were sedated with an intramuscular injection of 2.2 mg/kg azaperone (Stresnil 40 mg/mL; Elanco, Guelph, ON, Canada) and euthanized by intracardiac injection of pentobarbital (Euthasol; Virbac AH, Inc., Fort Worth, TX, USA). Post-mortem examinations were conducted on each piglet and
samples from the stomach, liver, and kidneys were collected and placed in containers with 10% formalin. Samples were routinely processed, sectioned, and stained with hematoxylin and eosin for microscopic evaluation. A pathologist blinded as to tissue source reviewed the slides.

**Data Analysis**

The maximal plasma concentration and time to maximal plasma concentration were determined by direct observation of data. Pharmacokinetic parameters (elimination constant; elimination half-life; apparent volume of distribution; area under the plasma concentration-time curve, AUC; and apparent oral clearance) were determined using noncompartmental analyses. The elimination constant was calculated by logarithmic linear regression of the plasma concentration-time curve. The elimination half-life was calculated as 0.693 divided by the elimination constant. The AUC to the final measurable sample was determined using the trapezoidal rule (Gunaratna, 2011) and extrapolated to infinity with the final plasma concentration being divided by the elimination constant, calculated from the apparently linear portion of the log plasma concentration-time curve. The extrapolated area was <5% of the total. Apparent oral clearance was calculated by dividing the dose by AUC, and apparent volume of distribution was calculated by dividing the dose by the product of the elimination constant and AUC.

**Results**

No adverse clinical signs were noted in piglets (those with catheters or those without) for the duration of the trial. After a single intramuscular dose of 1.0 mg/kg meloxicam, maximum plasma concentrations were achieved rapidly at 0.5 ± 0.00 h (Table B.1). Peak plasma meloxicam concentration was 3.12 ± 0.13 µg/mL and area under the plasma concentration-time curve was 17.13 ± 1.61 mg/L·h. The apparent volume of distribution was 0.18 ± 0.01 L/kg and the apparent oral clearance was 0.02 ± 0.00 L/h·kg. The elimination half-life of meloxicam was 3.94 ± 0.41 h. There was minimal interindividual variability in plasma meloxicam concentration. Plasma drug levels decreased to near-undetectable levels by 48 h. A plasma concentration-time curve for 1.0 mg/kg meloxicam in 8 day-old male and female piglets is presented in Figure B.2.
Gross and histologic evaluation of the liver, stomach and kidneys found no signs of toxicity in any piglet (Figure B.3).

Discussion

The pharmacokinetic (PK) parameters of 1.0 mg/kg meloxicam in 8-day old piglets were similar to those described in previous PK studies, using 0.4 mg/kg meloxicam (intravenous, 16-23-day old piglets) and 0.6 mg/kg meloxicam (intramuscular, 14-day old piglets) (Fosse et al., 2008; Fosse et al., 2011). As anticipated, the peak plasma concentration of meloxicam was greater when piglets were administered 1.0 mg/kg IM compared to 0.6 mg/kg IM (3.12 vs. 2.0 ug/mL), as was the area under the plasma concentration-time curve (17.13 vs 9.9 mg/L*h) (Fosse et al., 2011). Thus, piglets administered 1.0 mg/kg meloxicam were exposed to higher levels of drug for a longer period, which may have clinical relevance. Plasma meloxicam levels above 0.2 µg/mL is required for analgesic effects in other species, such as horses, which suggests that a single intramuscular dose of 1.0 mg/kg meloxicam provides some level of analgesia to piglets for approximately 12 h (Kreuder et al., 2012). This was difficult to assess and compare in the plasma concentration-time curves provided from the studies using 0.4 mg/kg and 0.6 mg/kg meloxicam (Fosse et al., 2008; Fosse et al., 2011).

Increasing meloxicam by a semi-log increment to 2.5 times the label dose resulted in no signs of tissue toxicity in piglets. This result was expected, as the oral LD\text{50} of meloxicam following administration to mini-pigs is approximately 1600 mg/kg (4000 times the label dose) (EMA, 2006). However, it is important to increase doses incrementally and evaluate drug safety after each administration. NSAIDs administered at levels 5 times the established therapeutic dose can result in clinical signs of toxicity in dogs, and severe symptoms of drug toxicity (e.g. renal failure, GI perforation) can develop clinically 48-72 h post-administration (Osweiler et al., 2011).

The results of this study indicate 1.0 mg/kg meloxicam, administered IM, may provide piglets with 12 h sustained analgesia without causing tissue toxicity. This may have clinical relevance over the current label dose for pigs of 0.4 mg/kg.
References


Figure B.1: Percutaneous catheterization of an anesthetized piglet (isoflurane/O₂) using the triangulation technique, as described by Flournoy and Mani (2009). The major landmarks of the triangle drawn on the piglet are the caudal ramus of the right mandible, the cranial manubrium, and the cranial point of the right shoulder.
Figure B.2: Plasma concentration-time curve of 1.0 mg/kg meloxicam. Values presented are mean ± SE [n= 5 piglets, except at 36 h and 48 h (n= 2 piglets)].
Figure B.3: Tissue sections 48 h following 1.0 mg/kg meloxicam administration. No lesions were noted in any tissue examined (samples from n= 12 piglets); a) liver, b) stomach, c) kidney (medulla), d) kidney (cortex).
Table B.1. Pharmacokinetic parameters (mean ± SE) of 1.0 mg/kg meloxicam, administered intramuscularly, to 8-day old piglets (n= 5)

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak plasma concentration (ug/mL)</td>
<td>3.12 ± 0.13</td>
</tr>
<tr>
<td>Time to peak plasma concentration (h)</td>
<td>0.5 ± 0.00</td>
</tr>
<tr>
<td>Area under the concentration-time curve (mg/L*h)</td>
<td>17.13 ± 1.61</td>
</tr>
<tr>
<td>Elimination half-life (h)</td>
<td>3.94 ± 0.41</td>
</tr>
<tr>
<td>Apparent volume of distribution (L/kg)</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Apparent oral clearance (L/h*kg)</td>
<td>0.02 ± 0.00</td>
</tr>
</tbody>
</table>
APPENDIX C

Utility of infrared cranial temperature measurements for assessing pain in piglets

Introduction

Pain identification in animals is crucial for timely alleviation, prevention, and overall maintenance of good animal welfare on-farm (Prunier et al., 2013). Measures of general physiologic function and behavior, such as plasma cortisol concentrations, vocalizations and activity-time budgets, are commonly used for animal pain assessments; however, they lack specificity, can be invasive to collect, and can be time consuming to conduct (Anil et al., 2005; Leidig et al., 2009). Repeated handling or manipulation of an animal to collect data for pain evaluation can affect their physiologic response and disrupt their behavior, resulting in confounded and inaccurate results (Prunier et al., 2005). Noninvasive tools, such as grimace scales for facial analysis, are increasingly being developed but require extensive validation (Langford et al., 2010; Sotocinal et al., 2011; Keating et al., 2012; Costa et al., 2014; Guesgen et al., 2016; McLennan et al., 2016; Viscardi et al., 2017). Infrared thermography (IRT) cameras are novel, easy to operate, noninvasive, and have previously been used to detect the physiologic response to stress and pain in animals (Stewart et al., 2005; Edgar et al., 2013; Bates et al., 2014; Lonardi et al., 2015). This pilot study sought to examine IRT utility on piglets administered a nonsteroidal anti-inflammatory drug (NSAID) prior to surgical castration.

Piglets raised commercially in North America and the EU undergo painful procedures, including surgical castration and tail docking. This is to prevent boar taint (an unpleasant taste and odor associated with the meat from intact boars), reduce aggression, and decrease tail biting among littermates (Zonderland et al., 2010; Sutherland, 2015). While these procedures are known to cause pain, piglets are not generally provided analgesia or anesthesia for pain relief. Canada and the EU have recognized this as a serious pig welfare concern and now have guidelines and requirements, respectively, indicating that piglets must be administered analgesia.
prior to surgical castration and tail docking (EU Commission, 2010; NFACC, 2014). NSAIDs, such as meloxicam and ketoprofen, are currently recommended for use in piglets to manage pain, and both are licensed for use in pigs in Canada and the EU. Despite this recommendation, it has been difficult to assess analgesic efficacy in piglets.

The sympathetic nervous system (SNS) is activated when an animal is stressed or in pain, causing increased blood pressure, heart and respiration rate, and peripheral vasoconstriction (Macefield, 2010; Herborn et al., 2015). Vasoconstriction reduces blood flow to the skin, leading to a decrease in cutaneous temperature and an increase in core temperature (Oka et al., 2001). The amount of vasoconstriction is related to the intensity of the stressor or painful event; increased stressor intensity causes a measurable increase in vasoconstriction and a decrease in skin temperature (Herborn et al., 2015). Analgesics reduce pain and, if effective, should decrease the physiologic pain response, including vasoconstriction. Therefore, effective analgesia should maintain skin temperature after a painful event (Bates et al., 2014).

The objective of this pilot study was to assess the cranial temperature of boar (after surgical castration) and gilt (undergoing no painful procedure) piglets administered 0.4 mg/kg meloxicam or 6.0 mg/kg ketoprofen intramuscularly (IM). We hypothesized that boar piglets would have a decreased cranial temperature compared with gilts in response to pain if the NSAIDs were ineffective at providing analgesia. If one or both NSAID was effective, we expected to see no difference in cranial temperatures between boar and gilt piglets. We also investigated the practicality of collecting IRT images from piglets on-farm.

**Materials and Methods**

All animal use and procedures were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #3350). The institution is registered under the Animals for Research Act of Ontario and holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care.
A total of 20 Yorkshire cross piglets (5 days-old, 1.27 to 2.80 kg BW) from 2 different litters were used in this study. Sows and piglets were housed in farrowing pens at the University of Guelph Arkell Swine Research Station. The floor space for each pen was 1.8 m x 2.4 m (6 ft x 8 ft) and the farrowing crate was 0.8 m x 2.3 m (2.5 ft x 7.5 ft). The farrowing rooms were maintained at ambient temperature (23 °C ± 0.5 °C) with lights on/off at 07:00/21:00, and natural light was provided by windows in each room. Sows were fed ad libitum 4 days after farrowing. The creep areas for piglets were heated to approximately 30-35 °C by means of a heating pad or lamp.

Within each litter, boar and gilt piglets were randomly assigned to one of the following treatments: 0.4 mg/kg meloxicam, 6.0 mg/kg ketoprofen or no treatment (Table C.1). All injections were administered IM by one individual. Meloxicam (MEL) (Metacam 20mg/mL; Boehringer Ingelheim Ltd., Burlington, ON, Canada) was diluted to 4 mg/mL to provide an average volume of approximately 0.2 mL to all piglets. Ketoprofen (KET) (Anafen 100mg/mL; Merial Canada Inc., Baie-D’Urfé, QC, Canada) was diluted to 80 mg/mL to administer approximately 0.2 mL to all piglets (range: 0.1-0.2 mL/piglet). The no treatment group (NONE) did not receive an injection.

Twenty-four hours prior to the trial, piglets were weighed to calculate appropriate NSAID dose and marked with a number between 1 and 20 using a black permanent marker. All piglets received a different number for identification. On the day of the trial, piglets were removed from their pen and placed in a holding cart. Pre-injection thermography images were collected from each piglet using an infrared thermography camera (FLIR E340, Systems, Wilsonville, OR). Piglets were placed individually in a separate holding cart for approximately 30 sec, the crosshatch of the IRT camera was aimed at the piglet’s cranium and the temperature was recorded (Figure C.1). All piglets then received an IM injection, except those in the no treatment group. Fifteen minutes post-injection, the boar piglets were surgically castrated using a scalpel to make two vertical incisions on the scrotum. The testicles were removed by tearing the spermatic cord (Rault et al., 2011). Piglets were then returned to their pen. IRT images were
taken of each piglet’s cranium at 11 time points throughout the trial: pre, 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 24 h post-injection.

The temperature data was analyzed using a mixed procedure, including time, treatment, and time x treatment interaction in SAS (Statistical Analysis System 9.4, SAS Institute Inc., NC, USA). Time was a repeated measure in the model with piglet as the experimental unit. Post hoc tests were conducted using the Tukey-Kramer adjustment. Statistical significance was set at p < 0.05.

Results

There were significant time and treatment effects on piglet cranial temperature (p < .0001 for both). Within each sex, ketoprofen administration caused a significant decrease in cranial temperature [boars (MEL: p = 0.0231, NONE: p = 0.0184); gilts (MEL: p = 0.0010, NONE: p = 0.0146)] (Figure C.2). There was no significant difference in temperature between the group of piglets given meloxicam and the no treatment group (boars: p = 0.9868; gilts: p = 1.0000). Boar piglets administered meloxicam or ketoprofen had significantly lower cranial temperatures than gilt piglets administered the same treatment (MEL: p = 0.0007, KET: p = 0.0167). There was no significant difference between boar and gilt piglets in the no treatment group (p = 0.1745). This suggests that castrated boar piglets were experiencing more vasoconstriction as a result of SNS activation after a painful event, which causes decreased blood flow to the skin, thereby reducing cranial temperature (Macefield, 2010). It also suggests that meloxicam may be more effective at relieving pain than ketoprofen.

Piglets were not weight-balanced across treatment groups, and there were significant weight differences found between treatments (p = 0.0003) (Figure C.3). Both boar and gilt piglets in the MEL treatment group and NONE gilts weighed significantly more than both boar and gilt piglets in the KET treatment group and NONE boars (p < 0.05). This is a study confound, as cranial temperature may be attributed to factors other than stress and pain.
All piglets had significantly higher cranial temperatures at 0 h post-injection than at pre, 1, 2, 3, 4, 5, 6, and 7 h ($p < 0.05$) (Figure C.4). This suggests that the restraint required to administer the IM injection at 0 h caused stress-induced hyperthermia, which resulted in a temporary increase in piglet cranial temperature (Watanabe, 2015).

**Discussion**

Pain detection is important for optimal animal care and welfare. Methods commonly used to assess pain, such as drawing blood for plasma cortisol level analysis or palpation of an area to observe an animal’s behavioral response, may cause physiologic or behavioral alternations that confound the outcomes observed (Prunier et al., 2005). As such, the use of noninvasive tools to measure pain are preferred. IRT has been used previously in pig studies, to assess how birth weight affects piglet surface temperature, to examine sow and piglet thermal comfort in different housing systems, and to detect inflammation of the lungs and leg joints in adult pigs (Caldara et al., 2014; Menzel et al., 2014; Soerensen and Pedersen, 2015; Machado et al., 2016). Two studies that used IRT to examine piglet temperature changes after castration found piglets had increased eye temperature and decreased cranial skin temperature (Bates et al., 2014; Lonardi et al., 2015). Piglets treated with meloxicam (mean meloxicam concentration of 0.6 mg/mL at castration; delivered transmammary from the sow) had significantly higher temperatures after surgical castration than piglets not receiving meloxicam, and there was a positive association found between blood plasma meloxicam levels and cranial skin temperature (Bates et al., 2014). This suggests a physiologic benefit to meloxicam administration prior to a painful procedure. We found consistent results in this study; however previous research in our lab found no behavioral differences between piglets administered meloxicam and those given a saline injection prior to surgical castration (Chapter 2: Viscardi and Turner, 2018). Further work is needed before recommendations regarding NSAID use should be given.

An inadvertent confounding factor in this study was the significant weight difference found between treatment groups. While SNS activation can reduce cutaneous temperature, low body weight piglets are at greater risk of hypothermia (Macefield, 2010; Caldara et al., 2014).
This may explain the lower cranial temperature observed in KET gilts. Future studies should ensure piglet weights are balanced across treatment groups to eliminate this potential confound.

Combining the IRT results from all piglets found an increase in cranial temperature at 0 h post-injection than at most other time points in this study. Piglets had to be restrained at this time point to administer the IM injection, presumably causing more stress to the animal. Stress-induced hyperthermia has been observed in restrained mice (Watanabe, 2015). Piglets were not habituated to the handling or physical restraint needed, which may have also caused stress and led to the temporary increase in cranial temperature observed.

To take accurate measurements of piglet cranial temperatures, animals had to be handled multiple times at each time point. This is because piglets often lie in a pile with littermates or under a heat lamp and not removing them could lead to erroneous surface temperature measurements. This repeated handling may alter normal piglet behavior (Prunier et al., 2005), making IRT a difficult tool to validate using behavioral assessments and reducing some of the noninvasive benefits to measure pain. IRT cameras are also very expensive, which may limit their use on-farm. Future work should refine temperature collection techniques to minimize stress or develop another protocol to eliminate the need to handle piglets. It should also focus on physiologic measures, such as blood cortisol, to further validate IRT as a reliable pain assessment tool.

A limitation of this study was the sample size. Because of this, the results should be interpreted with caution. Our aim was to first assess this novel tool in a pilot project before implementing it within a large-scale study. Further research with increased group sizes is needed to confirm the findings of this study.

**Conclusions**

Castrated boars had significantly lower cranial temperatures than gilts administered the same treatment. Meloxicam administration was more effective than ketoprofen at reducing
vasoconstriction; however, additional work is needed to confirm these findings. Infrared thermography may be a useful tool to noninvasively measure physiologic changes related to pain in piglets, although repeated handling needed to take cranial images and the equipment cost limits the applicability on-farm.
References


Figure C.1: Example of an image captured using the IRT camera. The crosshatch was placed on the cranium of the piglet and the temperature was measured in °C.
Figure C.2: Mean cranial temperature (°C ± SE) of piglets in each treatment group (n= 4 meloxicam/sex, n= 4 ketoprofen/sex, n= 2 no treatment/sex). Different letters (a, b, c, d) indicate significant differences between treatments (p < 0.05).
Figure C.3: Mean weight (kg ± SE) of piglets in each treatment group (n= 4 meloxicam/sex, n= 4 ketoprofen/sex, n= 2 no treatment/sex). Different letters (a, b) indicate significant differences between treatments (p < 0.05).
Figure C.4: Mean cranial temperature (°C ± SE) of boar and gilt piglets combined (n= 20) at each time point. Different letters (a, b, c) indicate significant differences between time points (p < 0.05).
Table C.1. Number of piglets per sex assigned to each treatment group

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Boar</th>
<th>Gilt</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 mg/kg MEL</td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td>6.0 mg/kg KET</td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td>NONE</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Piglets were surgically castrated