PRACTICAL ALTERNATIVES FOR MANAGING CASTRATION PAIN IN PIGLETS

A Thesis Submitted to the

College of Graduate and Postdoctoral Studies

In Partial Fulfillment of the Requirements

For the Degree of Master's

In the Department of Large Animal Clinical Sciences

University of Saskatchewan

Saskatoon

By

Erin Davis

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Head of the Department of Large Animal Clinical Sciences
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OR

Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatoon, Saskatchewan, S7N 5C9, Canada

ABSTRACT

Surgical castration of piglets is a painful procedure used to reduce aggression, sexual behaviours, and boar taint, and is often completed on young piglets. Recent requirements in Canada state that an analgesic must be administered to control the pain of the procedure. The thesis objectives were to evaluate the effects of age and timing of analgesic administration on piglets' response to castration. Three-day-old PIC Landrace x Large White male piglets were enrolled in study 1 or 2. For study 1, piglets were split into two groups: handling chute and average daily gain (ADG, n=115) or serum cortisol and pen behaviour (n=96), and assigned within litter to six treatments: 1) castration with ketoprofen at 3 days old (YK, n=35), 2) castration with ketoprofen at 10 days old (OK, n=36), 3) castration at 3 days old (YC, n=35), 4) castration at 10 days old (OC, n=35), 5) sham castration at 3 days old (YS, n=35), and 6) sham castration at 10 days old (n=35). From the ages of 9 to 13 days old, older piglets had a higher ADG than younger piglets (0.27±0.01 kg/day vs. 0.25±0.01 kg/day respectively, LSM±SEM; $P<0.05$). Serum cortisol concentration 24 hours after treatment was higher in young piglets than old piglets (77.8±9.2 nmol/L vs. 36.1±8.9 nmol/L respectively, $P<0.05$). Older piglets were observed tail wagging at a higher frequency 24 and 25 hours after castration than younger piglets ($P<0.05$). For study 2, piglets were split into two groups: handling chute (n=76) or serum cortisol and pen behaviour (n=103) and assigned within litter to five treatments: 1) castration with ketoprofen one hour prior (HK, n=37), 2) sham castration with saline one hour prior (HS, n=34), 3) castration with ketoprofen immediately prior (IK, n=37), 4) sham castration with saline immediately prior (IS, n=35), and 5) castration with saline immediately prior (CA, n=36). HK had lower cortisol concentrations than CA, IK, and IS 45 minutes after treatment (90.2±26.4 nmol/L vs. 206.7±26.4 nmol/L, 147.5±25.6 nmol/L, 158.0±26.4 nmol/L respectively, $P<0.05$). These studies provide evidence that providing ketoprofen one hour before castration reduces cortisol concentrations and is beneficial to piglet welfare. More research is needed to compare piglet pain responses when castrated at different ages.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisors, Dr. Yolande Seddon and Dr. Jennifer Brown. Your patience and commitment into helping ensure I completed on time was unprecedented. I will forever be grateful for how hard you both worked to complete edits on all my chapters as quickly as you could and helping me to better understand the science of ethology. You both stuck with me to the bitter end, and I can't thank you enough.

Additionally, I would like to thank Dr. Tanya Duke, my committee member, for her quick response to complete edits on my chapters and her expert guidance in the subjects of post-surgical pain relief, anaesthesia, and pharmacokinetics. Thank you to Dr. John Harding for his counsel on the experimental design of the project and his continuous encouragement throughout the years. A sincere thank you to Dr. Rayappan Cyril Roy for his guidance in behavioural statistical analysis. And to Dr. Suzanne Millman, thank you for serving as my external reviewer.

Thank you, as well, to Saskatchewan ADF for funding this project. Many thanks go to all the staff at Prairie Swine Centre for providing the facilities, animals, and teaching me the skills I needed to complete my experiments. To Adam Marlowe, thank you for encouraging and supporting me throughout the writing process – your positivity aided me tremendously. Laslty, thank you to both Émilie Viczko and Sarah Barnsley for your continuous and unwavering support and friendship – your understanding towards any struggle I faced was more helpful than I can put into words.

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LIST OF ABBREVIATIONS

NFACC	National Farm Animal Care Council
NSAID	Non-steroidal anti-inflammatory drug
CNS	Central nervous system
ADG	Average daily gain
GnRH	Gonadotropin-releasing hormone
FSH	Follicle-stimulating hormone
LH	Luteinizing hormone
SAM	Sympathetic adrenal medullary
HPA	Hypothalamic-pituitary-adrenal
SNS	Sympathetic nervous system
ACTH	Adrenocorticotropic hormone
CRH	Corticotropin releasing hormone
CBG	Corticosteroid-binding globulin
NT	Navigation time
mg	Milligram
kg	Kilogram
h	Hour
min	Minute
mL	Millilitre
$T_{1/2}$	Plasma elimination half-life
$T_{1/2abs}$	Plasma absorption half-life
Cl/F	Plasma clearance rate
IM	Intramuscular
CO_2	Carbon dioxide
CVMA	Canadian Veterinary Medical Association
m	Meter
YK	Surgical castration with ketoprofen at 3 days of age
OK	Surgical castration with ketoprofen at 10 days of age
YC	Surgical castration with no pain control at 3 days of age
OC	Surgical castration with no pain control at 10 days of age

YS	Sham castration at 3 days of age
OS	Sham castration at 10 days of age
K	Surgical castration with ketoprofen
SD	Standard deviation
cm	Centimeter
RPM	Revolutions per minute
µg	Microgram
dl	Decilitre
nmol	Nanomol
L	Litre
CV	Coefficient of variation
s	Seconds
SEM	Standard error of means
C	Surgical castration with no pain control
S	Sham castration
O	Treated at 10 days of age
Y	Treated at 3 days of age
Tr	Treatment
MESOR	Rhythm adjusted mean
HK	Surgical castration with ketoprofen administered 60 min prior to castration
HS	Sham handling with saline administered 60 min prior to handling
IK	Surgical castration with ketoprofen administered immediately prior to castration
IS	Sham handling with saline administered immediately prior to handling
CA	Surgical castration with saline administered immediately prior to castration

1 INTRODUCTION

Surgical castration of male pigs is a common procedure in North America that is used to reduce aggressive and sexual behaviours, as well as the incidence of boar taint (Vanheukelom et al., 2012). The practice, however, is associated with welfare concerns largely centered around pain and stress both during and after the procedure. In 2014, the National Farm Animal Care Council (NFACC) released the Code of Practice for the care and handling of pigs, which is a document outlining requirements and recommendations for pork producers within Canada. The 2014 Code requires all producers to provide analgesics to help control post-procedural pain, and piglets older than 10 days of age must also be given an anaesthetic (NFACC, 2014). However, the Code fails to provide recommendations on what age is optimal to castrate a piglet, or the optimal timing of administration of analgesics.

To determine optimal practices for pain control, reliable measurements of piglet pain must be established. There are currently two types of measures commonly used to determine if a piglet is in pain: physiological and behavioural responses. Physiological responses include determining increases of stress-related hormones such as cortisol (Prunier et al., 2005; Carroll et al., 2006), or increases in inflammatory substances such as serum-amyloid A (Sutherland et al., 2012). Other physiological measures related to stress and pain include an increased heart rate, temperature, respiratory rate, and a decreased body weight gain, etc. (Hansson et al., 2011; Sneddon et al., 2014; Lonardi et al., 2015). To better understand the reliability, repeatability, and accuracy of these measures, the measurements are often described based on how objective they are. To be objective, a measure must be unbiased, therefore not influenced by personal opinions or feelings. A more subjective measure, conversely, is influenced by personal opinions or feelings. Physiological measures are a more objective way to measure pain as compared with behavioural measures, however other stressors such as the environment may compound the results (Bristow and Holmes, 2007). Behavioural measures are another way of determining if an animal is feeling pain. These methods are often more subjective than physiological measures and can contain unwanted bias, however some can be considered objective, such as the navigation time in a

handling chute (Bilsborrow et al., 2016). Behavioural measures of pain include frequency or duration of pen behaviours (Hay et al., 2003), navigation time through a handling chute (Bilsborrow et al., 2016; Davis et al., 2017), and vocalization (Leidig et al., 2009; Kluivers-Poodt et al., 2012).

Although more accurate ways of measuring pain still need to be developed, the current methods used in combination can help researchers learn how to better reduce the pain and stress of castrated piglets. There are many specific topics regarding piglet castration such as age of castration as well as timing of administration of an analgesic that are lacking in informationon methods to help reduce pain and stress. Currently in Canada, castration is typically performed on piglets younger than 10 days of age. Reasons behind this are due to the new requirements of the Code (NFACC, 2014), but also the speed and minimal effort needed to perform the procedure on a younger, smaller animal. Some research, however, has suggested that piglets greater than 1 to 3 days of age are better able to deal with the pain of castration, and therefore have better weight gain following the procedure (McGlone et al., 1993; Kielly et al., 1999). With the limited amount of research in this area, more information is needed to determine the optimal age to perform castration. Regarding the optimal timing of administration of an analgesic, the majority of producers that provide piglets with analgesics do so at the time of castration to reduce labour and handling stress on piglets. However, from a pharmacokinetic standpoint it would be best to administer analgesics like non-steroidal anti-inflammatory drugs (NSAIDs) some time prior to castration for maximum efficacy. By not allowing NSAIDS time to absorb and take effect prior to castration, piglets do not have pain control for a certain period of time after the procedure (Fosse et al., 2008; Fosse et al., 2010a). Exploration of whether a delay in pain control is more detrimental than handling a piglet twice is needed.

Although there are now alternatives to surgical castration available to producers, it is still commonly performed on North American farms. Studies on optimal methods of pain alleviation are needed to help producers determine the most economic, efficient, and welfare friendly option for their animals.

1.1 Thesis Objectives

Changes in the NFACC Code of Practice for the care and handling of pigs (2014) in Canada have encouraged the need for establishing optimal practices to help control post-procedural castration pain in piglets. Chapter 2 of this thesis outlines basic principles of welfare and how to determine whether or not an animal, or specifically a pig, is experiencing pain, and why this is important. This section references important literature in the field that may aid in determining optimal practices for surgical castration and demonstrates the need for experiments that yield practical information regarding the age at which piglets should be castrated and the optimal timing of approved analgesics. Chapter 2 also reviews literature on alternative methods to castration such as rearing entire males and immunocastration. The objective of this review was to determine if there is sufficient evidence to provide producers with recommended best practices and uses of analgesics to help control the post-procedural pain of castration, and to determine where knowledge of the topic is lacking. To fill in these gaps, two studies were performed and are outlined in Chapters 3 and 4.

The objective of the study in Chapter 3 was to evaluate the effect of piglet age on the response to castration. This chapter describes differences in weight gain and pain responses between piglets castrated at 3 days of age and those castrated at 10 days of age. Additionally, the objective of this study was to explore the efficacy of ketoprofen administered intramuscularly 30 minutes prior to castration on the pain responses of young and old piglets. Three different treatments were applied to piglets in each age group: 1) ketoprofen given 30 minutes prior to castration and castrated, 2) castration with no pain control, and 3) handling as if to castrate without castrating.

Analgesics are recommended to be administered to animals prior to a painful experience to allow for absorption and distribution throughout the tissue. However, stress increases with increased handling, making it difficult to determine whether handling a piglet twice to administer an analgesic prior to castration does more harm than good. The objective of the study in Chapter 4 was to compare the pain and stress responses of piglets given ketoprofen either one hour or immediately prior to castration. An additional objective was to determine if handling a piglet twice (to administer an analgesic prior to the procedure and then perform castration) results in an

increased stress response, or if this practice is best performed with a single handling bout by providing an analgesic at the time of castration.

2 LITERATURE REVIEW

2.1 Animal Welfare

The subject of animal welfare is an important and complex field with scientific, ethical, economic, cultural, social, religious and political dimensions (OIE, 2021). In the past, greater focus was directed at the animal's physical state, which is measured by things such as level of production and health (Hewson, 2003). Over the years, the definition of welfare has grown to encompass the animal's emotional state, as well as allowing animals to express and perform normal behaviours (Rushen et al., 2011, OIE, 2021). This change in understanding of what animal welfare means has led to an increase in research and information on ways to improve many aspects of animal welfare in the swine industry. Providing pigs with enrichment has shown positive benefits, where piglets provided with licking blocks or logs as a form of enrichment were found to show less aggression towards conspecifics (Ralph et al., 2018; Giuliotti et al., 2019) and showed improved cognitive function (Ralph et al., 2018). Increasing space in gestation stalls has reduced severity of lesion scores in sows (Salak-Johnson et al., 2015). Surgical castration in young male piglets has also been identified as a major welfare concern due to the pain it causes these animals (Prunier et al., 2005; Marchant-Forde et al., 2009; Sneddon et al., 2014), and the fact that there are alternatives already available to producers such as immunocastration (Amatayakul-Chantler et al., 2012; Brewster and Nevel, 2013).

In Canada, the welfare standards for animal production systems are defined in written guidelines called Codes of Practice. The Code document is made up of requirements and recommendations developed by a Code Development Committee and a Scientific Committee. The two committees are made up of a selection of producers, transporters, veterinarians, processors, retailers, welfare representatives, researchers, and any other stakeholder that specializes in the specific species the Code is targeting. Once a draft of the Code has been completed it is released to the public for 60 days to gather any comments the public may have, which are taken into account for revisions. The Code for each species is reviewed every five

years and updated at least every 10 years. The goal of each Code of Practice is for the committee to review the latest research on priority welfare issues in an industry and to identify solutions based on the review to target any animal care concerns, meet market requirements, and write requirements and recommendations that can be implemented by producers. The document is not only meant as a guideline for producers, but it is also meant to act as a transparent document that consumers can access to better understand how farm animals are cared for in Canada (NFACC, 2019).

In the swine industry, the most recent Code was released in 2014. Regarding surgical castration, the Code states as a requirement that castration can be performed at any age, however it must be completed using an analgesic to control post-procedural pain (NFACC, 2014). Additionally, the Code requires an anaesthetic to be provided to piglets if castrated over 10 days of age, thus encouraging castration to be performed at a younger age. Prior to this update in the Code, most producers performed castration without any pain control to increase efficiency by reducing procedure time and costs in the barn, and because they did not have access to swine approved analgesics. Due to a large amount of research in the area, it is now understood that castration is a highly painful procedure for all ages of piglets and the industry has an opportunity to improve piglet welfare by providing them with appropriate pain relief. Information on best practices for relieving piglet pain during and after castration is still being developed through research, however it can be a challenging task due to the nature of measuring pain, a physiological response that affects each individual differently.

2.2 Pain

Studies on pain in neonatal animals increased in the 1980s. As with many topics in the scientific world, opinions vary between consumers, producers, and scientists on what is known and what is important. In the past it was thought that animals do not have the capacity to feel pain – especially those considered to be young or neonates (Taylor et al., 2001). However, it is now known that animals feel pain as they exhibit nerve fibers and pathways for sensing and

transmitting pain and behaviourally, they have developed a strong motivation and memory to avoid situations that have caused painful sensations in the past (Sneddon et al., 2014).

Pain is defined as the subjective experience of an unpleasant sense or emotion associated with, or resembling that associated with, actual or potential tissue damage (Raja et al., 2020). The pathway that leads to the sensation of pain begins when a noxious stimulus causes the release of ions (hydrogen and potassium) and cytokines such as histamine, serotonin, bradykinin, kallidin, and prostaglandins (Meyr and Steinberg, 2008) that activate primary afferent neurons in peripheral axons. Pain impulses are then transmitted by Aδ- and C-nerve fibers (from the primary afferent neurons) to the dorsal horn of the spinal cord. Aδ-fibers are thinly myelinated, elicit a sharp, intense sensation, and conduct at a higher velocity than C-fibers, therefore are believed to cause the first sensation of localized pain. C-fibers are unmyelinated, elicit a prolonged burning sensation, and are said to cause the throbbing, poorly localized pain following the intense initial sensation of pain (Lee and Neumeister, 2020). These fibers can both be found in the skin and other superficial organs, while C-fibers alone are found in deeper structures such as muscles. These pain impulses are then modified in the dorsal horn and reach the second-order neurons which will travel up the central nervous system (CNS) via the lateral and medial spinothalamic tract. These tracts are responsible for projecting to the thalamus to tell the brain of the duration, location, and intensity of the pain (lateral spinothalamic tract) and project the negative emotional perception of pain (medial spinothalamic tract). Lastly, third-order neurons in the thalamus project this information to the cerebral cortex (Meyr and Steinberg, 2008; Lee and Neumeister, 2020). The result of these pathways is the painful sensation initially triggered by a noxious stimulus.

Pain can be classified into nociceptive, neuropathic, and inflammatory pain. Nociceptive pain is felt when nociceptors (a sensory neuron) are stimulated and transmit this information along Aδ- and C-nerve fibers to indicate actual or potential tissue damage. Neuropathic pain occurs after a nerve injury or impairment and is triggered from a stimulus deemed to be non-painful in normal conditions or can develop spontaneously from an injured site. Inflammatory pain is triggered by inflammatory mediators released from the inflammatory response by tissues in the body to remove necrotic cells and begin tissue repairs. Inflammation will lead to hyperalgesia, or an increased sensitivity to painful stimuli, allodynia and sympathetic maintained

pain, both of which are often associated with neuropathic pain. Pain can be separated into acute and chronic pain, where acute pain occurs over a short period of time and is generally more intense, and chronic pain which is defined as lasting more than 3 months and may not be as intense of a sensation (Yam et al., 2018; Lee and Neumeister, 2020).

The sensation of pain can be split into sensory and affective aspects. The sensory processes will often lead to the detection and identification of a stimulus while the affective aspect is the aversive feeling of the painful stimuli that drives one to terminate the stimuli (Sherwood et al., 2013). In humans, pain tolerance differs widely amongst individuals and can be modified by many traits such as personality, attitude, previous experience, and circumstances.

2.3 Surgical Castration

Surgical castration is a procedure that is performed in almost all domesticated mammals to either minimize the growth of the testes and associated hormones or to remove the testes from male animals. In swine, surgical castration involves making one or two incisions in the scrotal tissue, forcing the testes from the scrotal sac and subsequently tearing or cutting the spermatic cord to remove the testes (Marchant-Forde et al., 2009). This procedure has been documented to be painful during and after in animals such as dogs (Aengwanich et al., 2019), cats (Väisänen et al., 2007), cattle (Fisher et al., 2001; Coetzee, 2013), and pigs (Taylor and Weary, 2000; Hay et al., 2003; Marchant-Forde et al., 2009).

Castration is commonly performed to eliminate the chance of siring, as well as to reduce certain behaviours such as aggression (Vanheukelom et al., 2012). Specifically, in swine, castration serves another major purpose – the removal of boar taint.

2.3.1 Boar Taint

Boar taint is a substance consisting mainly of 5α-androst-16-en-3-one (androstenone) and 3-methlindole (skatole) that develops in the fatty tissue of many sexually mature boars (Bonneau, 1982). Androstenone is a steroid synthesized in the testis that increases in production as a boar

sexually matures (Robic et al., 2008) and is highly heritable (Parois et al., 2015). Skatole is an indole which is produced in the colon by bacteria when the amino-acid tryptophan is broken down (Robic et al., 2008) and is only moderately heritable (Parois et al., 2015). Production of skatole can be affected by environment, diet, and genetics (Bonneau, 1982). Androstenone and skatole concentrations are positively correlated in boars (Borrisser-Pairó et al., 2016). These two compounds act synergistically, where the urine-like smell from androstenone is strengthened with the high production and deposition of the faecal-like smell of skatole. This means that boars who produce high amounts of skatole have higher boar taint levels, and those with lower amounts of skatole have reduced boar taint (Bonneau, 1982). In those pig carcasses with high levels of skatole, the combination of skatole and androstenone causes a foul smell and taste in the pork (Robic et al., 2008). In Canada, pork found to contain boar taint is labeled unfit for human consumption and is condemned (CFIA, 2017). To eliminate this problem, boars can either be slaughtered at an earlier age before they reach sexual maturity, or they can be castrated prior to sexual maturity (Bonneau, 1982), which allows them to grow to their full market weight without the production of boar taint compounds. Castration can be completed either surgically or chemically using immunocastration.

The most common method of eliminating boar taint in the past has been surgical castration, however the pain and reduced welfare that the procedure causes piglets has encouraged the development and use of alternative methods. These methods include chemical castration, or immunocastration, and rearing entire males for slaughter.

2.3.2 Alternatives to Surgical Castration

Rearing entire males for slaughter is the most economically and least invasive alternative to surgical castration. Compared with gilts, boars have a higher growth rate during the finishing phase due to the presence of natural steroids in entire males (Vanheukelom et al., 2012). Rearing boars does have some negative implications, however. Agonistic behaviour such as mounting, knocking, and fighting is more common in entire males than gilts or barrows, and biting behaviour in boars was found to increase after alterations to their housing groups occurred (Bünger et al., 2015). Entire males are also observed to have more skin lesions than barrows and

gilts, however these skin lesions were rarely severe (Bünger et al., 2015). Aggression and skin lesions can also be linked to group composition, where entire males raised in sibling groups are less aggressive than those raised in mixed groups (Fredriksen et al., 2008). This shows that management plays a large role in the success of raising entire males. As boar taint severity is partially environmentally influenced (Bonneau, 1982), using different fibrous materials as feed, using feed additives and formulating feed to control the fiber and protein in a boar's diet can reduce the severity of boar taint (Heyrman et al., 2018). For example, feeding a 5% chicory root diet for the two weeks leading up to slaughter reduced olfactory boar taint and skatole concentration (Heyrman et al., 2018). Another way to reduce boar taint is through genetics, as it is highly heritable (Bonneau, 1982; Haberland et al., 2014), however genetic selection must be evaluated with caution, as the genetic markers may be linked to other important characteristics such as average daily gain (ADG), intramuscular fat percentage, and fertility traits (Haberland et al., 2014).

Another alternative to surgical castration is immunocastration. This method is vaccinating against gonadotropin-releasing hormone (GnRH) which is the precursor for both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Both gonadal steroid secretion and gamete development rely on these two hormones, therefore when immunized against GnRH, the lack of production of these hormones results in castration of the animal (Brunius et al., 2011). Immunization against GnRH requires two injections of the vaccine in order to build up antibodies, and these antibodies are present for at least 22 weeks after the second injection (Zamaratskaia et al., 2008; Brunius et al., 2011). This essentially means that boars will need four months to regain full reproductive function after two doses, and the second dose of vaccination can be given up to six weeks before slaughter (Zamaratskaia et al., 2008). Immunocastrated pigs have also shown less aggressive and manipulating behaviours, as well as reduced sexual behaviour (Zamaratskaia et al., 2008). Currently in Canada, the Code of Practice for the care and handling of pigs recommends considering non-surgical methods of castration once they become available (NFACC, 2014). Presently, Health Canada has registered veterinary prescription products (Improvest^{TM}) that can be used for immunocastration and are available for producer use (CFIA, 2017; Health Canada, 2019).

Both rearing entire males and use of immunocastration are available for producers to begin to implement, however making these adjustments are not so simple. In Europe, the European Declaration on alternatives to surgical castration of pigs was created in 2010 and a goal set to phase out surgical castration by 2018 (European Commission, 2010). An online survey was distributed across Europe to determine what progress has been seen at the farm level regarding the move away from surgical castration. The survey determined that 61% of pigs were still being surgically castrated, 36% were being raised as entire males, and only 3% were using immunocastration in Europe (De Briyne et al., 2016). Although implementation of these methods seems realistic, this survey serves as evidence that alternatives to surgical castration are not widely accepted, and this is largely due to lack of consumer acceptance in the case of immunocastration. Until these alternative methods are widely used, there is still much research required to evaluate the best methods of alleviating surgical castration pain, and to do that, researchers need tools on identifying the presence of pain in piglets.

2.3.3 Measuring Castration Pain

In animal pain and welfare research several methods are used to evaluate pain. The two most common ways pain is measured are through physiological measures and animal behaviour. Physiological methods include growth changes as well as acute and chronic markers of stress. The rate, duration, and frequency of various behaviours are also evaluated. Both approaches provide useful information; however, both have flaws, and many of these methods measure animal stress rather than pain specifically. While both methods are incomplete regarding measuring pain, a combination can provide better assessment of pain. Alternatively, the development of a novel measure that is specific to pain that could be implemented in scientific research is needed.

2.3.3.1 Physiological Measures

When evaluating physiological stress there are two main pathways, those of the sympathetic adrenal medullary (SAM) axis and the hypothalamic-pituitary-adrenal (HPA) axis.

The SAM axis is a neuroendocrine stress response system where a stressor increases sympathetic nervous system (SNS) tone and releases catecholamine hormones from the medulla of the adrenal gland. A stressful stimulus activates the CNS and releases hormones from the hypothalamus, pituitary gland, and adrenal cortex (Sherwood et al., 2013). When the SAM axis is activated, responses can be measured using several methods such as measuring increased concentrations of circulating catecholamines (adrenaline and noradrenaline), increases in heart rate, blood pressure, body temperature, respiratory rate, and evaluating body weight change (Hansson et al., 2011; Sneddon et al., 2014; Lonardi et al., 2015). Activation of the HPA axis results in increases in adrenocorticotropic hormone (ACTH), cortisone, and cortisol, which can be measured in blood (Coetzee et al., 2008; Lonardi et al., 2015), saliva (González et al., 2010; Leslie et al., 2010), and hair (Creutzinger et al., 2017; Meléndez et al., 2017).

The most common physiological measurement used in pain and stress research is circulating serum or plasma cortisol concentration. Stress can be linked to many different emotions and feelings such as pain, fear, joy and exertion. Initially, a stressful stimulus causes activation of the HPA axis by activation of the SNS which sends a signal to the hypothalamus (Sherwood et al., 2013). The hypothalamus releases corticotropin releasing hormone (CRH), which travels to the anterior pituitary. CRH then activates specific corticotrope cells in the anterior pituitary to release ACTH into the systemic circulation to the adrenal cortex. Here, ACTH activates the adrenal gland to increase the activity of cholesterol desmolase. Pregnenolone is then produced from cholesterol in the adrenal cortex, which is converted using the cholesterol desmolase. Pregnenolone can then be converted into cortisol, a glucocorticoid. Once released, cortisol travels through the blood stream in an inactive form bound to either a corticosteroid-binding globulin (CBG) or albumin. The inactive cortisol is then activated by 11-beta-hydroxysteroid dehydrogenase 1. Cortisol can affect many organ systems through attachment to glucocorticoid receptors present in almost all tissues in the body. Cortisol can act on the immune response by causing apoptosis of proinflammatory T cells, suppression of B cell antibodies, and reducing the migration of neutrophil migration. Upon activation of production and release due to stress, cortisol can also increase the concentration of energy substrates (e.g., glucose) to the body and brain at an acute level (Sherwood et al., 2013; Thau et al., 2021).

Handling can act as a stressor to animals and can be observed when an increase in cortisol concentration is found. A scientific study comparing the stress responses of piglets given an ear tag, an ear notch, an intraperitoneal injection, or handled without a painful procedure found no difference in salivary cortisol concentrations after the procedures between the treatment groups, however found that in comparison with baseline salivary cortisol sampled 15 minutes prior, all groups had a significant increase in cortisol concentration 15 minutes after treatment (Leslie et al., 2010). Pain will also elicit a stress response, demonstrated where circulating ACTH and plasma cortisol levels were higher in surgically castrated piglets from 5 to 60 minutes and 15 to 90 minutes after castration respectively, compared with those that were handled but not castrated or not handled (Prunier et al., 2005). Castration is known to cause an increase in cortisol in bovine species 14 days after the procedure (Creutzinger et al., 2017) and swine between 20 and 45 minutes after the procedure (Lonardi et al., 2015; Davis et al., 2017). Additionally, when comparing the cortisol response of female and male piglets after teeth clipping, ear tagging or notching, administration of an iron injection, castration in males, and tail docking, the male piglets had a higher total plasma cortisol response after all the procedures, likely due to the only difference between processing of these two sexes – castration (Marchant-Forde et al., 2014).

A reduction in weight gain has also been linked to stress and pain. When wild house sparrows were captured and housed in individual cages, their circulating corticosterone concentration reached peak levels seven days after capture and they experienced weight loss over the first four weeks of captivity (Fischer et al., 2018). Friesian-Jersey cross calves disbudded without pain control at 3 to 6 weeks of age showed a decreased daily weight gain up to 15 days after the procedure compared with calves administered a sedative (xylazine), a local anaesthesia (lignocaine hydrochloride) and/or an analgesic (meloxicam or ketoprofen; Bates et al., 2016). Holstein steers dehorned at 6 months of age without pain control had lower ADG up to zero and seven days after treatment than those provided with pain control (Glynn et al., 2013). Nine-week-old Yorkshire-cross barrows had a lower ADG over two weeks when groups were moved to a pen with higher stocking density and new pigs as compared with pigs left in a standard pen (Li et al., 2017). Three to six days after surgical castration, 3-day-old piglets were found to have a lower weight gain than piglets not castrated (Kielly et al., 1999).

Physiological measures are not the only way to measure pain, however. Measuring pain via cortisol, ACTH, or body weight gives some insight into the effects stress and pain have on the body, however depending on different environmental factors or even the level of stress the procedure in question may cause, any of these physiological measures may be influenced. A combination of physiological and behavioural measures, therefore, can provide a better overall interpretation of what the animal is experiencing.

2.3.3.2 Behaviour

Behaviour is the occurrence of an action or reaction to a situation, a pattern developed from an external or internal stimulus, a group action, or the manipulation of an object (Lazzeri 2014). Behaviour can provide insight into the emotional state of an animal to a stimulus by analyzing their unique responses. There are many different types of behaviour that can be used when measuring pain, especially with piglets. Vocalization, pen-behaviour, and even a specially designed "handling chute" can be used to determine pain in piglets during and after castration.

Pigs are a social species that often vocalize to communicate their emotional state to conspecifics. Using this vocalization behaviour, researchers can determine a lot about their wellbeing and emotional state. Piglet vocalization frequency, pitch, and pattern often changes depending on the situation. Higher frequency calls are associated with pain in piglets, where piglets castrated at 3 to 10 days of age had a higher pitch to their calls during castration than piglets not castrated (Taylor and Weary, 2000; Kluivers-Poodt et al., 2012). Piglets castrated at 3 to 5 days of age showed a higher summed total of durations of higher frequency calls at the time of the procedure than piglets not castrated (Leidig et al., 2009; Kluivers-Poodt et al., 2012). It must be noted, however, that while vocalizations are valuable for determining if a piglet is experiencing immediate pain, vocalizations have not been found useful at determining pain lasting more than a few hours or days.

Piglet behaviours are often split into two different categories when examining pain; pain-related behaviours and non-specific behaviours (Hay et al., 2003). This idea was founded by Hay et al. (2003), who created an ethogram specific to piglet pain. Some examples of non-specific

behaviours may include walking, playing with other piglets, suckling or appearing awake, but inactive. Suckling and udder massaging have been observed in lower frequency for 2.5 hours after castration at 5 to 9 days of age as compared with piglets not castrated (McGlone et al., 1993; Hay et al., 2003) even though other research has shown that castrated piglets spend more time with their sow in the three hours after castration (Taylor et al., 2001; Llamas Moya et al., 2008). This combination of alteration in behaviour could be an indication that piglets are resting to recover from the painful procedure and may be seeking their sow for comfort. Locomotory activities can also be altered, where piglets castrated at 5 days of age were observed engaging in less locomotory behaviour in the three-hour period after castration as compared with piglets not castrated (Llamas Moya et al., 2008). These behaviours support the theory that piglets are recovering from the painful procedure by resting. Piglets castrated at 5 days of age are also found to isolate themselves from their littermates more frequently up to three hours after the procedure than piglets not castrated (Hay et al., 2003). Isolation could be a behavioural protective adaptation, as it is an unusual behaviour seen in social animals such as pigs, of avoiding conspecifics that could generate pain through manipulation of the painful area (Hay et al., 2003). Piglets were also observed spending more time sitting or standing inactive in the first two hours following castration as compared with piglets not castrated (Taylor et al., 2001), while other studies have shown no changes in these postures following castration (Hay et al., 2003). This could be an indication that posture changes are not a reliable measure of pain in piglets.

Pain-related behaviours in piglets are behaviours that have shown to occur in the highest frequency in the presence of pain such as rump scratching, tail wagging, or trembling. Piglets are more often observed prostrated and trembling immediately after castration (Hay et al., 2003), whereas at two to four days after treatment, castrated piglets display increased frequency of behaviours such as rump scratching and tail wagging (Hay et al., 2003; Llamas Moya et al., 2008; Viscardi and Turner, 2018). Some studies have grouped multiple behaviours together to show that pain-related behaviours as a whole are increased after piglet castration (Llamas Moya et al., 2008; Marchant-Forde et al., 2009; Kluivers-Poodt et al., 2013; Viscardi and Turner, 2018). Tail wagging and rump scratching behaviours are associated with castration pain due to the location of the incision and the idea that an incision at the tissue level causes irritation in a localized area.

Behaviour measures are useful for not only measuring if a certain procedure causes immediate pain, but also if these painful procedures alter behaviour long-term (Hay et al., 2003). The efficacy of this kind of measurement on its own, however, is not always accurate, as some studies have found opposing results (Llamas Moya et al., 2008; Billsborrow et al., 2016). This dilemma has created the need for an alternative behavioural measure – one that is more easily repeatable and contains less observer bias.

A new objective method to evaluate pain referred to as the "handling chute" was developed in 2016 (Bilsborrow et al.). The handling chute's efficacy to evaluate pain accurately remains to be determined, however both Bilsborrow et al. (2016) and Davis et al. (2017) found similar results that indicates that the method is repeatable. The handling chute is a small tunnel containing two hurdles that piglets must navigate to arrive back in their farrowing pen (Billsborrow et al., 2016; Davis et al., 2017). The ability of the piglet to cross the two hurdles in the chute were assessed with the assumption that piglets in pain would hesitate to complete the jumps and therefore take a longer amount of time to complete the chute. Piglets are first trained in the chute by allowing them to navigate it over the course of four runs. This is to ensure that the piglets are familiar with how the chute works and understand that completing the chute allows them to return back to the farrowing pen. Initially in training, piglets navigate the chute with no hurdles present to familiarize themselves with the object. Once they have completed two runs, hurdles of a shorter height were then introduced for one training run, and finally the last training run was completed with the hurdle height that would be present in the experiment. After these four runs, piglets were considered trained. For the experimental period, piglets are then placed in the handling chute at certain time points preceding castration and their navigation time (NT) is recorded (Bilsborrow et al., 2016). This method has been successful at distinguishing piglets in pain from those not, with castrated piglets having a longer NT than those handled and not castrated at 0 and 15 minutes after castration (Billsborrow et al., 2016; Davis et al., 2017). As with many behavioural measures, it must be noted that observer biases can arise when using this method as the observer can visualize which piglets are castrated and which are not.

Behavioural measures, on their own, may not be reliable due to individual differences between piglets and researcher bias. To enhance behavioural measures, they can be paired with physiological measures to create a stronger argument and help eliminate bias. With a

combination of physiological and behavioural measures of pain, researchers can begin using them to evaluate ways to mitigate pain at the time of and after a procedure such as castration.

2.3.4 Age and Pain

In Canada, the Code of Practice for the care and handling of pigs states that piglets greater than 10-days-old who are undergoing castration must be provided with both an anaesthetic and an analgesic, whereas younger piglets are only required to receive an analgesic (NFACC, 2014).

Piglets castrated at ages 3, 10, and 17 were all recorded vocalizing at higher rates regardless of the age they were castrated at (Taylor et al., 2001). These same piglets also did not show any changes in lying, sitting or standing inactive, udder massaging, or active behaviours up to 24 hours after castration depending on their age (Taylor et al., 2001). This is in accordance with Carroll et al. (2006) that found piglets castrated at 3, 6, 9, and 12 days of age showed no difference in behaviours for two hours after castration. Castrating piglets at different ages (3, 6, 9, and 12 days of age) have also shown no effect of growth performance in the two days following the procedure when comparing the different ages (Carroll et al., 2006). These findings suggest that piglet age does not influence the amount of pain experienced at the time of, or after, castration. Other studies have found that weight gain is affected by the age at which piglets are castrated. Piglet weight gain was greater in piglets castrated at 14 days of age than 1 day of age from castration to weaning by (McGlone et al., 1993). A similar study by Kielly et al. (1999) found that piglets castrated at a younger age (3 to 5 days of age) gained less weight as compared with control littermates, whereas piglets castrated at an older age (10 to 14 days of age) had no difference in weight gain compared to control littermates. While piglet age at castration may not alter pain behaviour as described from the stated evidence, piglets castrated at older ages may be better able to deal with the stressful procedure than young piglets as seen with a greater weight gain after the procedure. In contrast to this evidence, piglets aged 9- and 12-days-old had overall greater serum cortisol concentrations than 3-day-old piglets after castration (Carroll et al., 2006).

These results indicate that it is still unknown which age castration is best performed at due to conflicting results between scientific studies. More research is needed to come to a

definitive conclusion as to whether younger or older piglets experience castration pain to different degrees. From a practical standpoint, castrating piglets at a younger age is easier for producers, in which case a better understanding of the pain response and effective alleviation of pain is needed.

2.4 Pain Control

In companion animals such as dogs and cats, castration is completed by a veterinarian using general anaesthesia and the animals are given appropriate pain medication for post-procedural pain. In North America, in farm animals such as cattle and pigs, the procedure is commonly completed on farm by barn staff while the animal is conscious, and many of these animals will not receive post-procedural pain medication. In Canada, however, due to the Code requirements (NFACC, 2014), the use of pain control and analgesics is increasing. There are two main categories of drugs used to treat pain: analgesics and anaesthetics (general and local). Analgesics will block the pain response and reduce inflammation, while local anaesthesia will stop all sensations in a localized area. General anaesthesia causes an animal to lose consciousness and will still allow nociceptive transmission.

2.4.1 Non-Steroidal Anti-Inflammatory Drugs

Non-steroidal anti-inflammatory drugs are the most common type of drug used for reducing inflammation and controlling pain (Health Canada, 2006) in both animals and humans. There are many NSAIDs approved for use in swine (Canadian Pork Council, 2019), however, only meloxicam is approved for use to relieve post-operative pain associated with soft tissue surgery in pigs (Canadian Pork Council, 2019). NSAIDs approved for use in swine include meloxicam, ketoprofen, paracetamol, and flunixin meglumine. All have been shown to be effective at reducing pain and inflammation in certain species for certain situations. NSAIDs work by blocking the production of prostaglandins through inhibiting the two cyclooxygenase enzymes that biosynthesize them. By blocking the production of prostaglandins, these drugs can

reduce the inflammation, blood flow, and formation of blood clots that are the result of prostaglandins (Gunaydin and Bilge, 2018).

2.4.1.1 Pharmacokinetics

Pharmacokinetics is the study of how drugs are absorbed and move throughout the body, as well as the various pathways of absorption, metabolism and elimination. These indices are different not only in different species, but in different ages of the same species. Unfortunately, there are few research studies available on pharmacokinetics of NSAIDs in neonatal pigs.

Intravenous injection of meloxicam (0.4 mg/kg) administered to 19-day-old pigs (5.5 kg) results in a plasma elimination half-life ($T_{1/2}$) of 2.7 h, a plasma absorption half-life ($T_{1/2abs}$) of 42 min, and a plasma clearance rate (Cl/F) of 60.6 mL/kg/min (Fosse et al., 2008). These values were similar to administration of meloxicam (0.6 mg/kg) injected intramuscularly to smaller pigs (2.7 kg; Fosse et al., 2010a; Cl/F: 60.4 mL/kg, $T_{1/2}$: 2.6 h), however values differed from large sows (217.3 kg) administered 0.5 mg/kg meloxicam intravenously (Pairis-Garcia et al., 2014; Cl/F: 37.8 mL/kg/h, $T_{1/2}$: 6.15 h). It was also found that prostaglandin E_2 concentration was 46% lower in pigs given meloxicam compared to a placebo group, whereas approximately 80% inhibition is required to produce antipyretic, anti-inflammatory, or analgesic effects (Fosse et al., 2008; Fosse et al., 2010a). These studies suggest that meloxicam is eliminated within approximately six hours in 3-week-old piglets when given intravenously.

Ketoprofen is an NSAID that has two enantiomer forms, S-ketoprofen and R-ketoprofen, due to an asymmetric carbon (Fosse et al., 2010b). Although these enantiomers will have identical physio-chemical properties, they can have different pharmacokinetics (Fosse et al., 2010b; Knych et al., 2016). Ketoprofen is sold as a mixture of the two enantiomers, however S-ketoprofen has proven to be the better of the two at inhibiting cyclo-oxygenase, the most common prostaglandin that ketoprofen will target (Fosse et al., 2010b). Ketoprofen is most often prescribed for animals suffering musculoskeletal inflammation (Knych et al., 2016). Intramuscular injection of the racemic ketoprofen (6 mg/kg) into 11.7-day-old pigs (4.5 kg) resulted in different pharmacokinetics for each enantiomer (Fosse et al., 2010b). S-ketoprofen

had the highest concentration compared with R-ketoprofen, with clearance after oral administration (Cl/F) being 39.1 mL/kg/h, $T_{1/2}$ being 3.51 h, and $T_{1/2abs}$ 6.6 min, and R-ketoprofen had a Cl/F of 399.8 mL/kg/h, a $T_{1/2}$ of 30.6 min, and a $T_{1/2abs}$ of 3 min (Fosse et al., 2010b). These results show a short absorption half-life for both enantiomers, however the elimination half-life of R-ketoprofen was much shorter than that of S-ketoprofen. Skin temperatures of kaolin-injected pigs showed that piglets given the racemic ketoprofen (6 mg/kg) had significantly lower skin temperatures than the placebo at 2 and 12 hours after injection (Fosse et al., 2010b). It was also found that piglets treated with ketoprofen had a higher mechanical nociceptive threshold for up to 24 hours than those in the placebo group (Fosse et al., 2010b). Intramuscular injection of racemic ketoprofen (3 mg/kg) administered to 4-week-old Holstein-Friesian calves resulted in unique pharmacokinetic values, with both enantiomers having similar half-lives than previous research in different species, and much lower Cl/F (S-ketoprofen: $T_{1/2}$: 3.02 h, Cl/F: 32.8 mL/kg/h; R-ketoprofen: $T_{1/2}$: 2.45 h, Cl/F: 139 mL/kg/h; Plessers et al., 2014). In 60 kg pigs, the bioavailability of racemic ketoprofen seems to be higher when given intramuscularly than when given orally using the same dose (3 mg/kg), however when the dose of orally administered ketoprofen increases, bioavailability becomes similar (Raekallio et al., 2008). Ketoprofen given intramuscularly had a faster mean absorption time than when administered orally (Raekallio et al., 2008).

With less research completed on pharmacokinetics of ketoprofen than meloxicam in young piglets, it is difficult to know which drug is more effective, and which drug will be most effective at treating castration pain in neonatal piglets.

2.4.1.2 Use of NSAIDs at Castration

The majority of research concerning the control of castration pain in piglets has focused on the use of the NSAID meloxicam. Other NSAIDs such as ketoprofen and flunixin meglumine have also been studied, although, to a much lesser extent.

Piglets given meloxicam one hour prior to castration were observed performing rooting behaviour more frequently than those not given any pain control (Bilsborrow et al., 2016). This

may indicate reduced pain, as rooting is a natural behaviour that piglets will often perform. Piglets given 0.2 ml meloxicam (5 mg/ml) intramuscularly (IM) immediately after castration performed similarly by both showing less pain-related behaviours (huddled up, spasms, rump scratching, stiffness, prostrated, trembling) than piglets castrated without any pain control (Hansson et al., 2011). Kluivers-Poodt et al. (2013) observed piglets showing fewer pain-related behaviours in the first three hours following castration when given a 0.4 mg/kg dose of meloxicam (5 mg/ml) IM 15 minutes prior to the procedure. The use of a standardized behavioural test utilizing a handling chute resulted in longer NTs 15 minutes after the procedure for piglets castrated with no pain control compared to piglets that were administered a 0.2 mg/kg dose of meloxicam (5 mg/ml) one hour prior to castration (Bilsborrow et al., 2016). Immediately after castration in the same study however, piglets given meloxicam one hour prior had NTs that were no different than control castrates (Bilsborrow et al., 2016). This may be an example of how meloxicam is effective at controlling post-procedural pain, but it is not effective at alleviating the pain at the time of castration when administered one hour prior. In another study, piglets castrated without or with meloxicam given 15 minutes prior to the procedure have longer vocalizations compared with piglets handled without being castrated (Kluivers-Poodt et al., 2012). Conversely, piglets given 0.4 mg/kg meloxicam (5 mg/ml) IM 10 minutes before castration spent more time at the teat after the procedure than those handled, suggesting they are better able to recover from a painful procedure and return to normal (Schmidt et al., 2012). Sixty minutes after castrating or handling, serum cortisol concentration of piglets given a 0.06 ml/piglet dose of meloxicam (20 mg/ml) IM 10 minutes before were similar to those sham handled (Gottardo et al., 2016).

Piglets castrated at 7-days-old that were given a 3 mg/kg dose of ketoprofen (100 mg/ml) IM 30 minutes prior to castration had lower plasma cortisol levels at 30, 60, and 90 min after castration than those not given ketoprofen, which would suggest possible pain relief for the first hour or two after the procedure (Cassar et al., 2014). Up to 90 minutes after castration, piglets given a 0.1 ml/piglet dose of ketoprofen (10 mg/ml) IM 10 minutes before castration were observed isolating themselves less and performing pain-related behaviours less frequently than those castrated without ketoprofen (Gottardo et al., 2016). Alternatively, pain-related behaviours did not differ between piglets given 0.2 ml of ketoprofen (80 mg/ml) IM 20 minutes prior to castration and those not given pain medication when castrated (Viscardi and Turner, 2018). In beef cattle, 3 mg/kg ketoprofen administered IM 30 minutes prior to castration resulted in a trend

towards lower salivary cortisol concentration five hours after castration, however, overall showed minimal effects at mitigating indicators of pain (Moya et al., 2014). Plasma fibrinogen, an acute-phase protein that increases during inflammation, has a smaller increase in cattle given 3 mg/kg ketoprofen (10 mg/ml) intravenously 20 minutes prior to castration in combination with 2% lidocaine hydrochloride than the castration control or cattle castrated with just 2% lidocaine hydrochloride (Earley and Crow, 2002). Thus, there are conflicting research results on the effectiveness of ketoprofen for the treatment of post-procedural pain after castration, and more research is needed in this area.

Giving an analgesic to reduce post-procedural pain is crucial for piglets undergoing castration, but there is more to consider than just administering the drugs. For one, dose of the analgesic must be calculated, as all piglets will be of different body weight at the time of castration. If a uniform dose is used, the larger piglets may not be provided with therapeutic analgesic levels. Another factor influencing the effectiveness of analgesic treatment is timing of administration of the drug. In beef cattle, giving meloxicam six and three hours prior to castration resulted in higher meloxicam levels in blood 60 minutes after castration than in cattle given meloxicam at the time of the procedure (Meléndez et al., 2017).

2.4.1.3 Timing of Administration

Pharmacokinetic evidence suggests that analgesics should be administered prior to a painful procedure in order for complete absorption into the body and distribution into tissues, resulting in a more effective use of drug (Nixon et al., 2020). However, analgesics are often given at the time of castration to reduce any economic costs due to the time required for barn staff to complete a procedure on each individual piglet. It is also not well understood whether handling an animal more than once is more stressful and therefore harmful than handling the animal once, because a single handling bout can cause an increase in salivary cortisol (Leslie et al., 2010). Scientific evidence is needed to determine whether handling a piglet twice and having an increased stress response or giving an analgesic at the time of the procedure with poor analgesia is more detrimental to a piglet's welfare.

Regarding piglet processing, which includes procedures like teeth clipping, tail-docking, ear-notching, iron-injections, and castration, it has been found that the processes that take a shorter amount of time and result in minimal tissue damage cause less stress to piglets than those that have longer duration (Marchant-Forde et al., 2009). This may suggest that prolonging the castration procedure by handling piglets more than once to administer an analgesic prior to the procedure may result in an increased stress response. It has also been found that administering meloxicam or ketoprofen 15 to 20 minutes before castration did not reduce the plasma cortisol concentration 20 minutes after castration (Kluivers-Poodt et al., 2012) or behavioural response (Viscardi and Turner, 2018) of piglets compared with those castrated without analgesics.

Other evidence suggests providing pain control prior to castration is more effective at relieving pain than providing it at the time of castration. Providing meloxicam 10 to 30 min before castration results in lower serum cortisol and ACTH levels 30 min after the procedure than those not given NSAIDs (Keita et al., 2010). Similarly, providing meloxicam 15 minutes prior to castration resulted in less pain-related behaviours in the hours following castration (Kluivers-Poodt et al., 2013). In beef cattle, administration of ketoprofen with 2% lidocaine hydrochloride 20 minutes prior to castration resulted in a decreased plasma cortisol response 0 to 2.5 hours after the procedure (Earley and Crowe, 2002). It is also shown that 15 minutes after castration, piglets given meloxicam 1 hour prior to the procedure have a shorter NT in the handling chute, indicating they are feeling less pain than those not given meloxicam (Bilsborrow et al., 2016). This is significant as it shows that as early as 15 minutes after castration, supplying animals with an analgesic prior to a painful procedure can result in reduced pain.

The majority of research regarding the use of NSAIDs to reduce post-procedural pain administer analgesics 10 to 60 minutes prior to castration and make comparisons to piglets not receiving any pain control when castrated. More scientific research is needed on comparing the pain responses of piglets provided with an analgesia at different times prior to a painful procedure.

2.4.2 Anaesthetics

Although NSAIDs may be valuable for controlling post-procedural pain, they are not capable of controlling pain at the time of castration. There are two types of anaesthetics: general and local. A general anaesthetic renders an animal unconscious for the required time which prevents cognitive perception of pain (Armstrong and Mouton, 2018). A local anaesthetic is used to prevent nerve transmission in specific areas of the body to eliminate all sensation within a localized area, but it does not render the animal unconscious (Armstrong and Mouton, 2018). The NFACC (2014) Code of Practice for the care and handling of pigs now requires producers in Canada to provide piglets older than 10-days-old with both a local anaesthetic drug and an analgesic for the castration procedure. Although many producers will likely castrate piglets earlier than 10-days-old, it is still an important topic to evaluate, and the information gained will also be helpful when surgically castrating younger piglets.

General anaesthesia for a procedure such as surgical castration on young piglets is not a commonly used practice on farm due to the increase in labour requirements and procedure time, resulting in a higher cost to producers. Instead, research into general anaesthesia during surgical castration is largely centered around using carbon dioxide (CO_2) to render an animal unconscious. CO_2 is currently being used on farm in some countries to stun an animal prior to slaughter, therefore is available to many producers (Van Beirendonck et al., 2011). Piglets rendered unconscious by 100% carbon dioxide (CO_2) for 30 seconds and castrated, spent more time with their sow as compared with piglets given no pain control or anaesthesia at castration 60 to 150 minutes after castration (Sutherland et al., 2012). In another study, piglets castrated under 100% CO_2 for 25 seconds displayed more interactive type behaviours such as nosing, chewing, licking, and playing, over the eight days following the procedure (Van Beirendonck et al., 2011). These behaviours may indicate a reduction in pain and stress following castration with CO_2-induced unconsciousness, as the pigs performed higher levels of normal behaviours. Despite this, CO_2 must be used carefully as it can cause respiratory acidosis (Patel and Sharma, 2021). Administering a mix of azaperone and ketamine (general anaesthesia) intramuscularly to piglets 10 minutes prior to castration in combination with meloxicam resulted in piglets spending more time active and away from their sow up to 3 hours after the procedure compared with 3 hours prior to the procedure (Schmidt et al., 2012). Although these piglets were observed to be more

active following the procedure, this result indicate that these piglets spent significantly less time suckling after castration. This change in behaviour could indicate that piglets anaesthetized using azaperone and ketamine have a more difficult time re-integrating into the litter up to 3 hours after castration (Schmidt et al., 2012).

Administration of the local anaesthetic, lidocaine, into the testis of piglets 3 to 30 minutes prior to surgical castration produced lower frequency calls and less resistance movements when compared to those not receiving lidocaine (Hansson et al., 2011). Although local anaesthesia does show some benefits in piglet castration, there are some exceptions, mainly regarding post-procedure behaviour. Piglets given meloxicam 15 minutes before castration and those not handled at all showed less pain-related behaviours than piglets castrated with lidocaine administered into each testi 15 minutes before (Kluivers-Poodt et al., 2013). Even during the procedure, stress call duration increased for piglets regardless of whether another local anaesthetic drug, procaine, was administered into each testi or not (Leidig et al., 2009).

Using a local anaesthetic may be feasible, however it can be time-consuming to administer, and similar to analgesics it takes time to take effect. It can be difficult to properly administer, as not only does the location of the scrotal incision need to be anaesthetized, but also the spermatic cord in order to eliminate the pain from tearing or cutting. Using a general anaesthetic is not only more time-consuming but may be more harmful on the piglets as they come back into consciousness due to problems reintegrating into the litter. As no technique has yet been discovered to both reduce pain at the time of and after surgical castration, the optimal procedure may not be surgical castration.

2.5 Conclusion

Optimal practices regarding the use of analgesics to control post-procedural castration pain in piglets have not yet been found. Piglets may be better able to cope with the pain when castrated at an older age, however research on the topic is minimal, and more needs to be done before recommendations can be made. Similarly, more research is needed for determining the optimal analgesic to use in combination with castration, and how to best administer the analgesic.

Most studies use analgesics as prescribed by administering them prior to the procedure to allow for absorption, however this requires additional handling and labour, and thus clear benefits of the procedure need to be identified before producers can be expected to adopt this practice. Similarly, both local and general anaesthesia use require more handling and labour, and without clear evidence of how it benefits the piglets at the time of castration, the use of them may not be feasible at the farm level. To make suggestions on optimal timing of administration of these drugs, more needs to be done to provide producers with the knowledge of why we administer analgesics before a painful procedure.

3 AN INVESTIGATION INTO THE PAIN RESPONSES OF PIGLETS WHEN CASTRATED AT 3 OR 10 DAYS OF AGE WITH AND WITHOUT PAIN CONTROL

This chapter explores an experiment performed to investigate the physiological and behavioural pain responses of piglets surgically castrated at either 3 or 10 days of age with and without ketoprofen administered.

Chapter 3 is prepared for submission for publication. The journal it is published in will have copyrights to this chapter.

Davis, E., J. Brown, T. Duke, and Y. Seddon. An investigation into the pain responses of piglets when castrated at 3 or 10 days of age with and without pain control

Drafting of the manuscript was completed by Erin Davis and Drs. Yolande Seddon, Jennifer Brown, Tanya Duke, and Suzanne Millman gave suggestions for any necessary revisions. Experimental design was completed by Erin Davis with suggestions from Drs. Yolande Seddon and Jennifer Brown. Animal handling and data collection was completed by Erin Davis. Cortisol analysis was completed at Prairie Diagnostic Services. Statistical analysis was completed by Erin Davis with suggestions given from Drs. Yolande Seddon and Jennifer Brown.

3.1 Introduction

Surgical castration is a common procedure often performed on young piglets as it was assumed that neonatal animals have a reduced capability of perceiving pain (Taylor et al., 2001). However, research has shown evidence of physiological and behavioural changes that indicate pain in the neonatal piglet (Taylor et al., 2001; Marchant-Forde et al., 2009; Bovey et al., 2014; Lonardi et al., 2015). The NFACC in Canada has recently revised the required and recommended practices in regard to the practice of castration in the swine industry. It is now required of producers to use an analgesic for piglets at all ages when they are surgically castrated and an anesthetic to piglets that are over the age of 10 when they are castrated (NFACC, 2014). The Canadian Veterinary Medical Association (CVMA) has also recently updated their position statement to encourage the use of analgesics and anesthetics for any age of piglets at the time of castration (CVMA, 2016). With the new NFACC requirement in mind, most producers have had to update their standard operating procedures to account for the change. These guidelines, however, do not give producers guidance on what age is most appropriate to perform castration.

There is evidence that the age at which piglets are castrated impacts their response to castration, with piglets castrated at 14 days of age showing greater weight gain to weaning than those castrated at 1 day of age (McGlone et al., 1993). Similarly, piglets castrated at 3 days of age were found to have slower weight gain between days 3 and 4 of age compared to piglets not castrated, while piglets castrated at 10 days of age had no difference in weight gain between days 10 and 11 of age compared to piglets not castrated (Kielly et al., 1999). This scientific evidence suggests that older piglets are better able to withstand the stress and pain of castration. However, this effect has not been consistently found, with studies that suggest no differences in weight gain between piglets castrated at 3, 10, or 17 days of age as compared with piglet's sham handled at this age (Lessard et al., 2002). Short term effects have also been disputed. For example, Carroll et al. (2006) found no difference in weight gain between 24 and 48 hours after castration between piglets castrated at 3, 6, 9, or 12 days of age. Other evidence presents physiological data that suggest older piglets (ages 9 and 12 at castration) find the procedure more stressful, with serum cortisol concentrations of older piglets being greater at 48 hours after castration or handling than those that are 3 days old (Carroll et al., 2006).

Behavioural responses such as suckling, huddling, trembling, awake but inactive, tail wagging and rump scratching have also shown evidence of being linked to the stress and pain of castration when changes in frequency are observed (Hay et al., 2003; Llamas Moya et al., 2008; Schmidt et al., 2012). These behaviours are most commonly studied in piglets aged 3 to 5 days of age (Hay et al., 2003, Llamas Moya et al., 2008), however, and may not be a proper representation of how older piglets' express pain behaviourally. Few scientific studies have investigated behaviour differences of older piglets. One study found that piglets castrated at 3 days of age spent more time standing in the two hours after castration than piglets castrated at 6, 10, and 12 days of age (Carroll et al., 2006). Another study comparing piglets castrated at 3, 10, or 17 days of age, age did not find any changes to behavioural responses to pain up to 24 hours after castration (Taylor et al., 2001). More scientific research is needed to compare the pain responses of different ages of piglets' multiple days after surgical castration, particularly in regards to pain behaviour, as research in this area is limited. The differences or similarities of behavioural and physiological responses of piglets castrated at different ages is still unclear and needs to be considered further.

There remain questions over the age at which castration can be performed with reduced consequences for piglet welfare. The objective of this study was to determine at which age castration is best performed to protect piglet welfare and help reduce economic loss associated with reduced growth rates post castration. A second objective was to determine whether there are differences in the way younger and older piglets respond to pain, whether it be behaviourally or physiologically. Lastly, this study will compare the effect of an analgesic given 30 minutes prior to castration between piglets castrated at 3 days of age or 10 days of age. The hypothesis of this study was that older piglets would perform a higher frequency of behaviours and give a more pronounced behavioural response to post-procedural pain. As well, it was hypothesized that administering ketoprofen prior to a painful procedure would reduce the pain responses of piglets at both a young and old age after surgical castration.

3.2 Materials and Methods

This research was approved by the University of Saskatchewan's Animal Research Ethics Board (AUP# 20160022) and followed the Canadian Council on Animal Care guidelines regarding the ethical use of animals.

3.2.1 Animals and Facility

The study was carried out at the Prairie Swine Centre's research facility, a 300-sow farrow-to-finish unit in Saskatchewan, Canada. A total of 211 PIC Landrace x Large White male piglets were used from 28 litters. Sows were housed within five farrowing rooms containing 16 farrowing crates. Farrowing pens measured 2.44 m x 1.83 m with a 1.98 m x 0.86 m crate on tri-bar metal slatted floors. Each pen contained a hooded creep area with one heat lamp and a rubber mat. Sows were fed a commercial lactation diet ad libitum. Piglet needle teeth were clipped within hours of birth, and iron injections were administered at 3 to 4 days of age. No further processing (tail docking or ear notching) was carried out on the piglets until after completion of the trial in order to minimize other sources of pain to where any pain responses observed would be a result of castration alone. Additionally, tail docking was not performed in order for a more complete and accurate observation of natural piglet tail positions and how this may indicate pain. Cross-fostering was allowed from 1 to 2 days of age to ensure that all piglets had access to a viable teat.

3.2.2 Study Design

Litters were split into two sections: handling chute and average daily gain (14 litters, n = 115), or blood collection and pen behaviour observations (14 litters, n = 96). Litters were selected if they had greater than or equal to six male piglets. Male piglets of 2 to 3 days of age and greater than or equal to 1 kg of weight were selected and randomly assigned using a random number generator (Microsoft ® Excel 2016, Microsoft Corporation) to one of six treatments in a 3 x 2 design. The six treatments were as follows: 1) Castration with ketoprofen at 3 days of age (YK,

ranging from 3 to 4 days of age, n = 35), 2) Castration with ketoprofen at 10 days of age (OK, ranging from 10 to 11 days of age, n = 36), 3) Castration with no pain control at 3 days of age (YC, ranging from 3 to 4 days of age, n = 35), 4) Castration with no pain control at 10 days of age (OC, ranging from 10 to 11 days of age, n = 35), 5) Sham castration at 3 days of age (YS, ranging from 3 to 4 days of age, n = 35), and 6) Sham castration at 10 days of age (OS, ranging from 10 to 11 days of age, n = 35). All treatments were represented within each litter. Anafen ® (100 mg/kg ketoprofen, Merial Canada Inc., Baie-D'Urfé, QC, Canada; extra label use) was the ketoprofen used in this trial and was diluted with distilled water using a ratio of 1:10 and administered intramuscularly to all K piglets 30 minutes prior to castration. Timing was selected on the basis of a previously conducted pilot study where ketoprofen was administered 15 minutes prior to castration. Lower NTs and serum cortisol concentrations as compared to castration controls were observed (unpublished data), therefore to obtain similar or a more conclusive result, timing of ketoprofen administration prior to castration was increased. Ketoprofen concentration was administered per recommended dose usage for swine at 3 mg/kg and administered dependent on piglet body weight (volume injected = 0.47 ± 0.09 ml, mean ± SD). Piglets were marked with a Sharpie® Magnum marker (Newell Brands, Atlanta, GA, USA) upon entrance into the trial and remarked every two to three days as the markings faded.

Control treatment piglets (OC, YC, OS, YS) in the blood collection and pen behaviour observation section of the trial received a dose of intramuscular saline 30 minutes prior to castration, given at 0.3 ml/kg.

3.2.3 Handling Chute and Average Daily Gain

A sub-sample of 115 piglets from 14 litters was selected to complete a specialized behavioural test referred to as a handling chute. The handling chute was a rectangular white box made of plywood, 18 x 177 cm (Figure 3.1A). Two hurdles could be placed in the chute with the ability to adjust the hurdle height. The chute was placed at the back of the farrowing crate after removal of the back gate, with an opening at the end of the chute to allow piglets to exit back into the pen (Figure 3.1B). Piglets were placed in the handling chute one by one and timed using a stopwatch to record NT. This standardized behavioural test has previously been used to identify

piglets experiencing pain from castration, where castrates had longer NT than uncastrated piglets for up to 30 minutes after the procedure (Bilsborrow et al., 2016; Davis et al., 2017).

Piglets were trained to navigate the chute one day prior to treatment, with the 3-day-old piglets trained at 2 days of age and the 10-day-old piglets trained at 9 days of age. Training consisted of four chute runs at 0, 15, 30, and 45 minutes. For 3-day-old piglets, the first chute run had no hurdles, while the second, third and fourth had increasing hurdle heights of 5.1, 7.6, and 10.2 cm respectively. For 10-day-old piglets, the first chute run had no hurdles, while the second, third and fourth had increasing hurdle heights of 5.1, 10.2, and 12.7 cm respectively. For training, in the 30 seconds before each run, piglets were placed in a plastic tote box at the starting point of the chute. One at a time, piglets were removed, placed in the chute, and their NT was recorded. If piglets lay down or turned around in the handling chute, they were picked up and placed standing in the same spot to encourage them to finish the run. At this time, piglets were not pushed through the chute. After two minutes, piglets that had not completed the chute were encouraged to complete the chute by lightly pushing and tapping them. After the last run on training day, piglets were considered trained.

On treatment day, piglets ran a baseline chute run 30 minutes prior to treatment and NT was recorded. Immediately after completion of this chute run, YK and OK piglets were administered a dose of diluted ketoprofen and returned back to the pen. At the time of treatment, all piglets were castrated and released back into the farrowing pen with the exception of YS and OS piglets, who were handled for 30 seconds (simulating the castration procedure) before being released into the pen. Chute runs were repeated at 15, 40, 60, and 120 minutes after treatment, and NT was recorded. All trial piglets were caught 30 seconds prior to chute runs and placed in the same plastic tote box. Once collected, piglets remained in the tote box until their chute run was completed. For 3-day-old piglets, each hurdle reached 10.2 cm in height while for 10-day-old piglets, each hurdle reached 12.7 cm in height throughout the trial. Treatments were carried out on each individual piglet at a randomized specific time point, then the same order was maintained for each chute run. If piglets had not completed the handling chute after two minutes, they were encouraged to complete the course by lightly pushing and tapping them. These piglets were recorded to have a NT of 120 seconds and remained in the trial data set.

As six piglets were removed from this section of the experiment at various times throughout the experiment, as described below, 109 of the piglets were followed in the weighing section of the trial. The piglets were weighed one day prior to both 3-day-old and 10-day-old treatment days (2 to 3 and 9 to 10 days old), and 3 days after the treatments. In addition, all piglets were weighed at 20 to 21 days of age, prior to weaning. To summarize, piglets were weighed at five time points: 2 to 3, 6 to 7, 9 to 10, 13 to 14, and 20 to 21 days of age. From this point on, these ages will be referred to by the younger of the two ages. ADG was calculated using the equation:

$$ADG = \frac{w_1 - w_2}{a_1 - a_2} \quad \text{.....(3.1)}$$

where a_1 represents piglet age and w_1 represents the corresponding piglet. Similarly, a_2 represents a second piglet age and w_2 represents the corresponding piglet weight. Piglets were removed from the experiment after collection of weight at 20 days of age.

Piglets (n= 6) were removed from handling chute and/or ADG measures due to several reasons. Of piglets assigned to the handling chute, one was removed prior to treatment due to starvation (OC) and one from meningitis (YC). Two were removed before collection of weights at 20 to 21 days of age due to rupture (scrotal hernia) (OK and OS) and one from sow crushing (OS). One piglet was treated for an inflamed front limb, and was later laid on (YS), therefore was removed from the trial before collection of final weight.

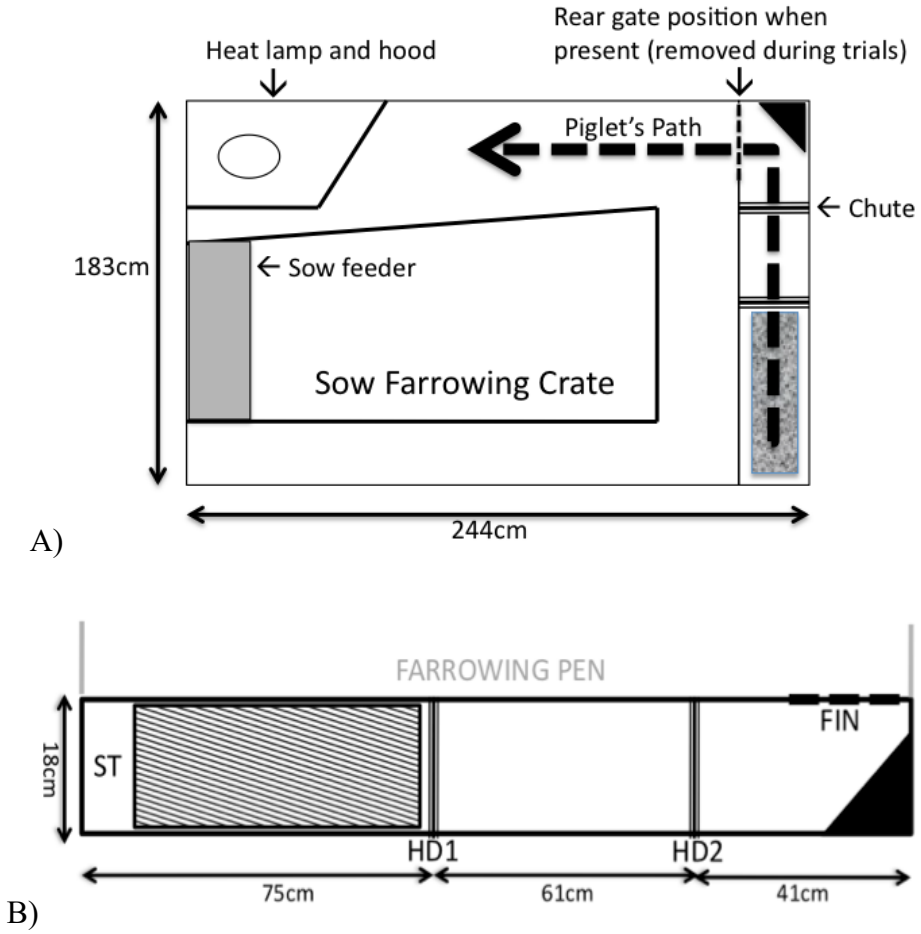

Figure 3.1 A diagram showing the handling chute. A) A diagram showing the positioning of the handling chute in relation to the farrowing crate. B) Piglet handling chute containing two hurdles placed 61 cm apart. ST represents the starting point at which the piglets were released. FIN represents the exit point where piglets exited the chute after completion of a run to re-enter the farrowing crate (Billsborrow et al., 2016).

3.2.4 Serum Cortisol

A sub-sample of piglets (n = 96) in 14 litters not included in handling chute or ADG measures were subjected to two post-treatment blood collections: one taking place 45 minutes post-treatment, the other at 24 hours post-treatment. A sub-set of these piglets (n = 53) was subjected to an additional blood collection (baseline measure) one day prior to treatment.

To collect blood, the entire litter was restricted to the area underneath the heat lamp using a plywood board in an attempt to reduce stress. Once corralled, study piglets were picked up one at a time and held upside down underneath the arm of one collector. The head of the piglet was restrained and a 22-gauge collection needle (Monoject™, Covidien™, Dublin, Ireland) was inserted into the orbital sinus of the piglet. The same eye was not used over consecutive days. Blood was then collected into a 10 ml BD Vacutainer® Serum Blood Collection Tube (Beckton, Dickinson and Company, Franklin Lakes, NJ, USA) until approximately 5 ml of blood was taken. Each collection was timed from the point of handling until the needle was removed, and each collection was completed within two minutes. After collection, piglets were returned to the farrowing pen. Once collection was complete per litter, blood was placed in a refrigerator at approximately 4°C for 30 minutes to allow for clotting, then removed and centrifuged for 15 minutes at 3000 RPM. Serum was extracted and placed into serum storage vials, then frozen at -20°C. Serum was analyzed for cortisol via a solid-phase chemiluminescent enzyme immunoassay (Immulite 1000 Cortisol, Siemens Healthcare Ltd., Oakville, ON) with a detection limit of 0.2 µg/dl (5.5 nmol/L). This immunoassay has been validated in swine (Escribano et al., 2012). Analysis was completed at Prairie Diagnostic Services Inc. in Saskatoon, SK, Canada, and the intra-assay mean coefficient of variation (CV) was 5.7%.

Two (YK) piglets were removed prior to the third blood collection due to death from rupture (scrotal hernia).

3.2.5 Pen Behaviour

Piglets that underwent blood collection were also subjected to behavioural observations (n = 96, litters = 14). A total of six live observations and video recordings occurred immediately after treatment and at 15, 30, 120 minutes, 24, and 25 hours after treatment. A camcorder was mounted on a tripod and was placed at the back of the farrowing crate (Figure 3.2). The behaviours selected (Table 3.1) were those commonly used to successfully measure piglet pain in the literature (Hay et al., 2003; Kluivers-Poodt et al., 2013). Total frequency of trembling behaviour was collected through live observation (due to the challenge of properly observing and

recording this behaviour over a video recording) over 10-minute intervals at each sampling period, and the total frequency of tail wagging, rump scratching, playing, and suckling behaviours were collected through continuous video observations over the same 10 minutes at each sampling period. Behaviours prostrated, sleeping, and awake inactive and piglet location (udder, heat lamp, or other) were recorded by scan sampling every one minute over the 10-minute recorded video (11 observations every 10 minutes).

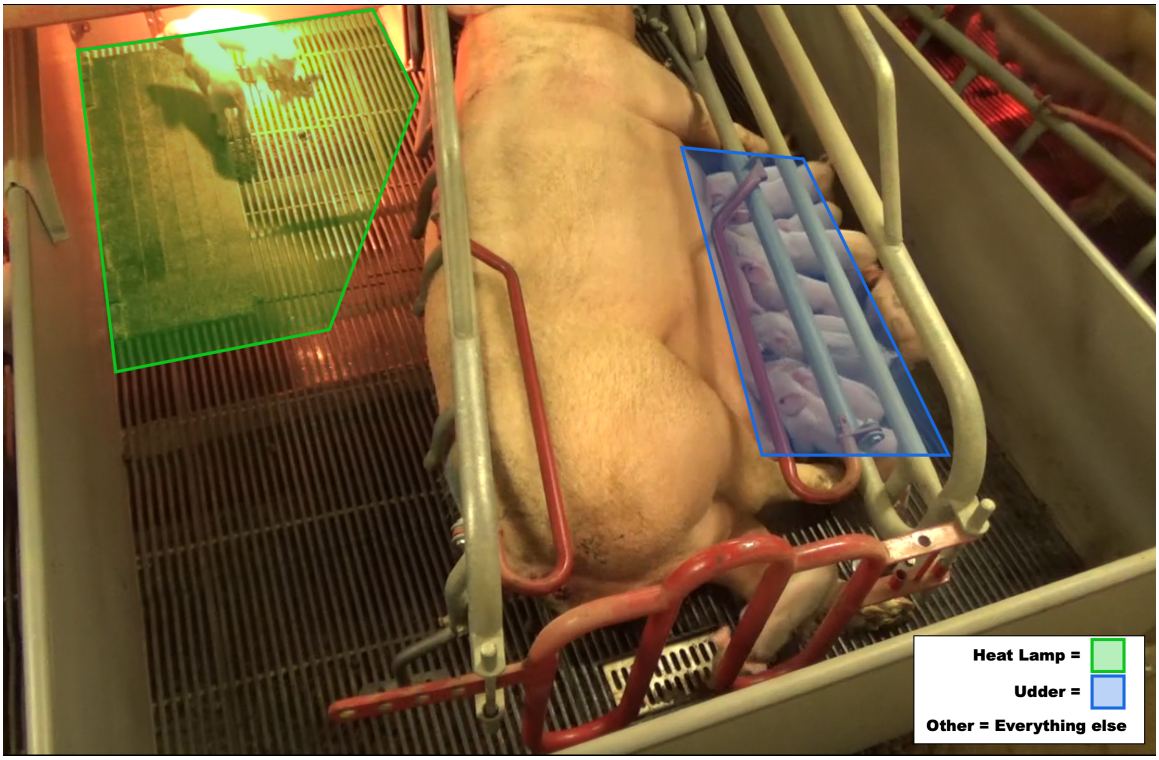

Figure 3.2 The standard field of view for video collected for behavioural observations. Highlighted areas: Green = shows the area in which piglets were counted as being located at the heat lamp; Blue = shows the area in which piglets were counted as being located at the udder. Any other location in the farrowing crate was considered "other".

Table 3.1 Piglet behaviour descriptions (modified from Hay et al., 2003)

Behaviour	Description
Pain-related behaviour	
Trembling	Shivering as with cold. May be standing, sitting, or lying.
Tail wagging	Rapid tail movements from side to side or up and down.
Rump scratching	Scratching the rump by rubbing against a surface.
Prostrated	Sitting or standing motionless with the head down, lower than the shoulder level.
Non-specific behaviours	
Playing	Head shaking, springing, (sudden jumping or leaping), running with vertical and horizontal bouncy movements. Can involve partners
Sleeping	Lying down, eyes closed.
Awake Inactive	No special activity, but awake (eyes open or half closed). Lying, sitting, or standing.
Suckling	Teat in mouth, actively suckling.
Location (see Figure 2)	
Udder	Suckling, nosing, massaging the udder, or searching for a teat.
Heat lamp	Standing, sitting, lying, or sleeping underneath the heat lamp.
Other	Located anywhere other than the udder or heat lamp.

3.2.6 Statistical Analysis

All statistical analysis was completed using SAS ® 9.4 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina, USA). A Proc univariate test was used on all data sets prior to analysis to determine if the data were normally distributed. Residuals of all data sets using the Proc Mixed model were checked for normality after analysis.

Handling chute data was found to be non-normal, therefore was log-transformed prior to analysis. An ANOVA model with repeated measures (Proc Mixed) was used to determine the difference in total mean NT between treatments over time, with a maximum NT of 120 min. Treatment, time, age, and their significant interactions were included in the model as fixed effects, with piglet weight and pre-treatment navigation time as covariates. Piglet nested within litter was included as a random effect.

Average daily gain from piglets 2 days of age and 20 days of age were calculated for each treatment. Additionally, ADG was calculated between the ages of 2 and 6 days old and between 9 and 13 days of age. A Proc Mixed model was used to explore differences in ADG between treatments across each of the calculated time points. Treatment, age, and their interaction were used as fixed effects with piglet birth weight as a covariate. Piglet nested within litter was included as a random effect.

Serum cortisol data was determined to be non-normal and was subsequently log-transformed. Mixed models (Proc Mixed) were used to determine the difference in cortisol levels between treatment groups at each sampling time point (24 hours pre-treatment, 45 minutes post-treatment, and 24 hours post-treatment). Treatment, age, and the interaction between the two were used as fixed effects. Piglet weight and the duration of the blood draw were used as covariates. Pre-treatment cortisol levels were used as covariates in both post-treatment cortisol models. Piglet nested within litter was used as a random effect in all.

Pen behaviour continuous observations and scan sampling was analyzed using a Proc Glimmix model as count data with a Poisson distribution to determine any differences in frequency of each behaviour or pen location. Treatment, age, and time were used as fixed effects

in all models. Interactions were used on an individual basis dependent on significance seen in individual behaviours. Piglet nested within litter was used as a random effect.

All log-transformed data is presented as back-transformed data in the results section. Statistical significance was set to a $P \leq 0.05$. Statistical tendencies were set to $P \leq 0.10$ and were considered where necessary.

3.3 Results

3.3.1 Handling Chute

Handling chute data showed no differences in the three-way interaction of age, treatment and time ($P > 0.05$), however there was a significant interaction between age and treatment ($P < 0.05$, Figure 3.3). There was no individual effect of treatment, age or time ($P > 0.05$).

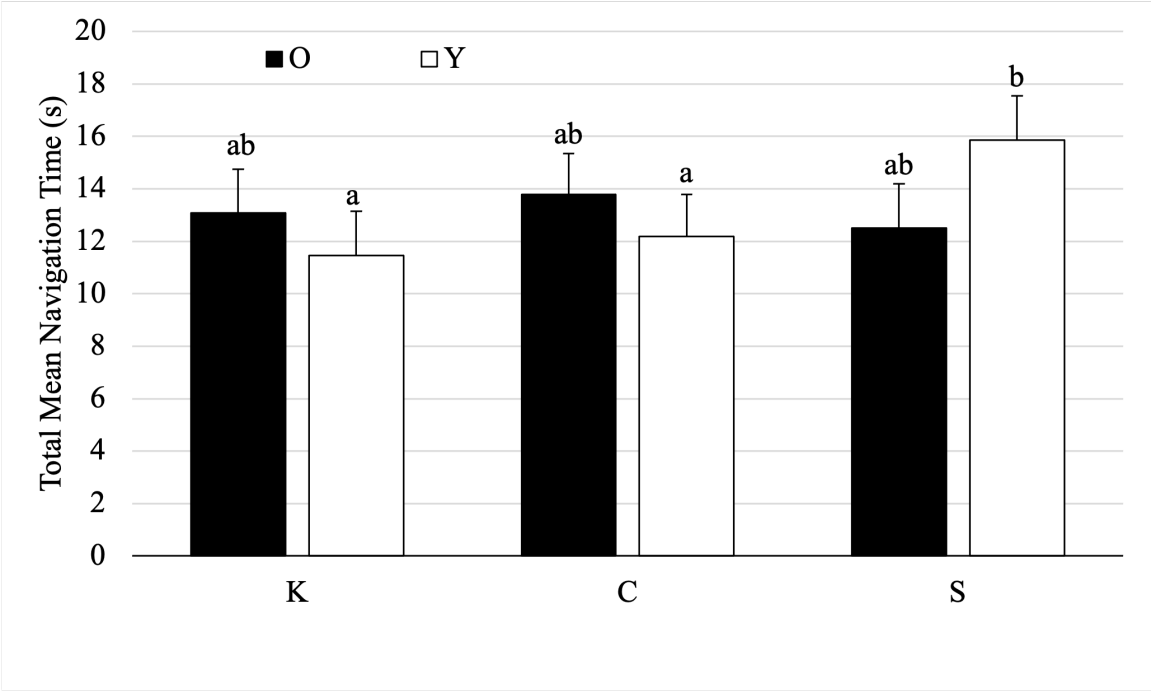

Figure 3.3 Total average chute navigation times (s) for three treatments, with piglets castrated at 3 or 10 days of age (least squared means + SEM). Treatments: K = piglets given ketoprofen 30 minutes prior to treatment; C = piglets castrated; S = piglets sham handled; O = treated at 10 days of age; Y = treated at 3 days of age. Differences in lettering signify statistical significance between treatments within age groups ($P < 0.05$).

3.3.2 Average Daily Gain

The calculated total ADG between the ages of 2 and 20 days old showed no significant difference between treatments or interaction between treatment and age ($P > 0.05$, Table 3.2). Similarly, the ADG of piglets from 2 to 6 days of age showed no difference between treatments

nor an interaction with age ($P > 0.05$). From the ages of 9 to 13 days old, there was no interaction between age and treatment ($P > 0.05$), but age was statistically significant. Piglets treated at 10 days of age had significantly higher ADG (0.27 ± 0.01 kg per day) between the ages of 9 and 13 days old compared to piglets treated at 3 days of age (0.25 ± 0.01 kg per day; $P < 0.05$).

Table 3.2 The effects of ketoprofen and age of surgical castration on ADG of piglets between three different time points[1]

	Treatment[2]									
	O			Y				*P*-value		
ADG, age, kg[3]	K	C	S	K	C	S	SEM	Tr[4]	Age	Tr×Age
ADG, d 2 to 6	0.21	0.18	0.17	0.18	0.20	0.18	0.01	0.226	0.803	0.366
ADG, d 9 to 13	0.27	0.27	0.28	0.25	0.27	0.23	0.01	0.768	0.048	0.253
ADG, d 2 to 20	0.26	0.24	0.26	0.24	0.24	0.24	0.01	0.960	0.158	0.594

[1]Data expressed as least squared means.

[2]O = piglets treated at age 10 days; Y = piglets treated at age 3 days; K = piglets given ketoprofen 30 minutes prior to treatment; C = piglets castrated with no pain-control; S = piglets sham castrated.

[3]kg = kilograms per day

[4]Tr = treatment

3.3.3 Serum Cortisol

Analysis of serum cortisol concentrations 24 hours prior to treatment found no effect of treatment (K: 102.82 ± 20.70 nmol/L; S: 78.61 ± 21.53 nmol/L; C: 64.98 ± 20.42 nmol/L; $P > 0.05$), age (Y: 82.83 ± 27.20 nmol/L; O: 81.44 ± 26.57 nmol/L; $P > 0.05$), or their interaction (YK: 132.05 ± 34.80 nmol/L; OS: 88.90 ± 34.59 nmol/L; OC: 81.85 ± 32.95 nmol/L; OK: 73.59 ± 34.45 nmol/L; YS: 68.32 ± 34.49 nmol/L; YC: 48.11 ± 34.98 nmol/L; $P > 0.05$). At 45 minutes

after treatment there was no effect of the interaction between treatment and age (OC: 203.11 ± 41.77 nmol/L; YC: 182.17 ± 43.84 nmol/L; OK: 153.23 ± 42.86 nmol/L; OS: 125.85 ± 43.65 nmol/L; YK: 110.22 ± 45.30 nmol/L; YS: 45.58 ± 43.88 nmol/L; $P > 0.05$), nor age alone (O: 160.73 ± 31.37 nmol/L; Y: 112.66 ± 32.30 nmol/L, $P > 0.05$), however there was a significant treatment effect, with C piglets having higher cortisol levels than S piglets (Figure 3.4; $P < 0.05$). At 24 hours post treatment there was no effect of the interaction between treatment and age (YK: 80.48 ± 14.24 nmol/L; YS: 78.78 ± 11.86 nmol/L; YC: 74.03 ± 12.36 nmol/L; OC: 49.08 ± 11.47 nmol/L; OS: 37.86 ± 13.13 nmol/L; OK: 21.27 ± 12.14 nmol/L; $P > 0.05$), nor treatment alone (C: 61.56 ± 6.97 nmol/L; S: 58.32 ± 7.69 nmol/L; K: 50.87 ± 7.75 nmol/L; $P > 0.05$). There was an age effect, however, with Y piglets having higher cortisol levels than O piglets (Figure 3.5; $P < 0.05$).

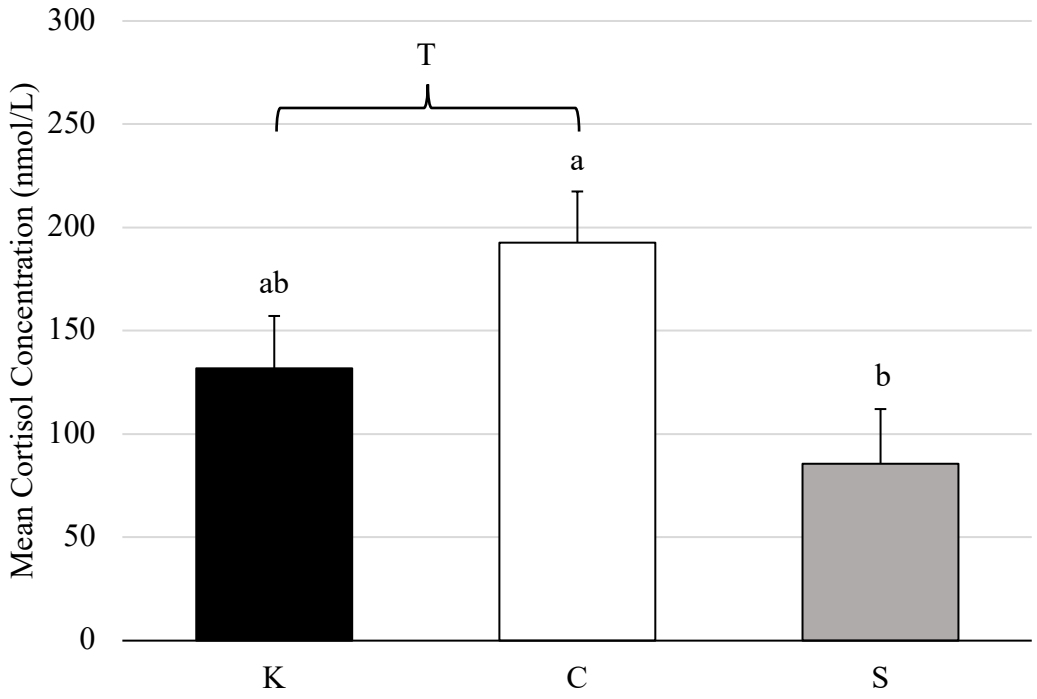

Figure 3.4 Serum cortisol concentrations (nmol/L) for three treatments sampled 45 minutes after treatment (least squared means + SEM). Treatments: K = piglets given ketoprofen 30 minutes prior to treatment; C = piglets castrated; S = piglets sham handled. Differences in lettering signify statistical significance ($P < 0.05$). A 'T' signifies statistical tendency ($P < 0.10$).

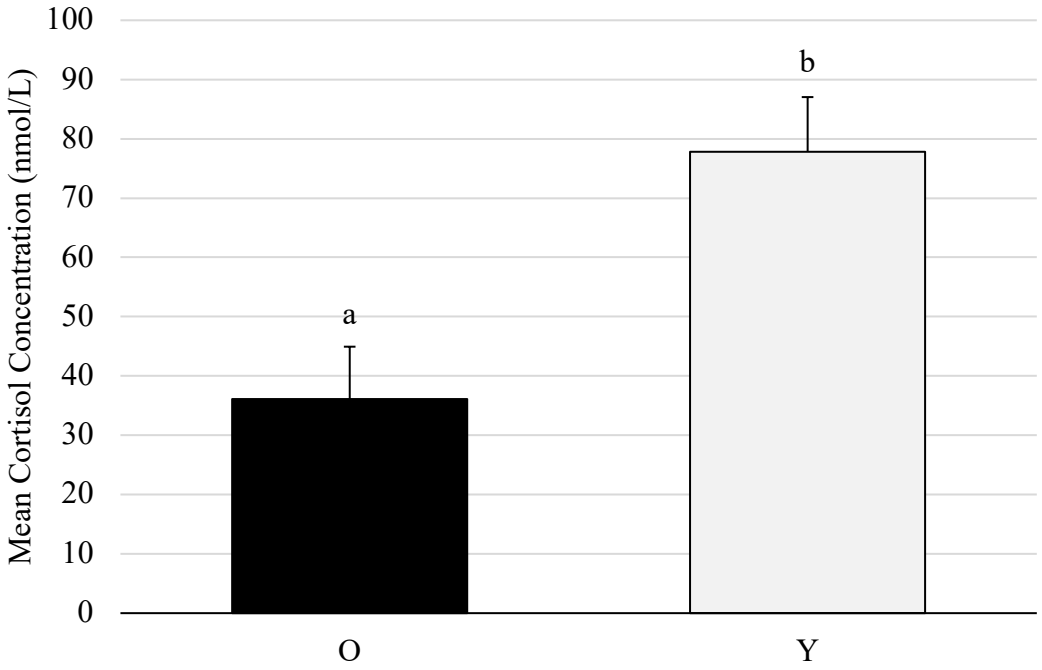

Figure 3.5 Serum cortisol concentrations (nmol/L) for two treatments sampled 24 hours after treatment (least squared means + SEM). Treatments: O = piglets treated at 10 days of age; Y = piglets treated at 3 days of age. Differences in lettering signify statistical significance ($P < 0.05$).

3.3.4 Pen Behaviour

There was a significant interaction between age, treatment, and time for pen behaviours tail wagging, sleeping, suckling, and 'other' behaviours, however not for the behaviour awake inactive (Table 3.3). There was a significant interaction between age and time for prostrated behaviour, where older piglets were observed prostrated at a higher frequency than younger piglets 25 hours after treatment (O = 0.27 ± 0.08 per pig/10 min; Y = 0.02 ± 0.02 per pig/10 min; $P < 0.05$). There was also a significant interaction between age and time for sleeping ($P < 0.001$) and other behaviours ($P < 0.001$). The interaction between age and time was not found to be significant for suckling ($P = 0.11$), however was trending for tail wagging ($P = 0.05$). There was a significant interaction between treatment and time for behaviours tail wagging ($P < 0.001$) and other behaviours ($P < 0.001$), whereas for sleeping, the interaction was trending ($P = 0.07$). Age had an effect on tail wagging, rump scratching, playing, sleeping, awake inactive, suckling, and other behaviours (Table 3.4). Treatment had an effect on tail wagging (Table 3.4). Time had an

effect on trembling, tail wagging, rump scratching, prostrated, playing, sleeping, awake inactive, and other behaviours ($P < 0.05$). All piglets were observed trembling at a higher frequency 120 minutes after treatment than all other time points (120 min = 0.26 ± 0.06 per pig/10 min; 15 min = 0.09 ± 0.04 per pig/10 min; 24 hr = 0.08 ± 0.02 per pig/10 min; 25 hr = 0.07 ± 0.02 per pig/10 min; 0 min = 0.06 ± 0.02 per pig/10 min; 30 min = 0.04 ± 0.01 per pig/10 min; $P < 0.05$). Rump scratching was observed at a higher frequency by all piglets 24 hours after treatment than all other time points (24 hr = 0.21 ± 0.05 per pig/10 min; 25 hr = 0.13 ± 0.03 per pig/10 min; 15 min = 0.06 ± 0.02 per pig/10 min; 30 = 0.04 ± 0.02 per pig/10 min; 0 min = 0.03 ± 0.01 per pig/10 min; 120 min = 0.01 ± 0.01 per pig/10 min; $P < 0.05$). Similarly, all piglets were observed playing more frequently 24 hours after treatment than all other time points (24 hr = 0.10 ± 0.03 per pig/10 min; 25 hr = 0.04 ± 0.02 per pig/10 min; 0 min = 0.04 ± 0.02 per pig/10 min; 30 min = 0.02 ± 0.01 per pig/10 min; 15 min = 0.01 ± 0.01 per pig/10 min; 120 min = 0.004 ± 0.004 per pig/10 min; $P < 0.05$). All piglets were observed awake inactive at a higher frequency immediately after treatment than all other time points, while the lowest frequency of this behaviour was observed 120 minutes after treatment as compared with all other time points (0 min = 2.30 ± 0.20 per pig/10 min; 30 min = 1.69 ± 0.16 per pig/10 min; 15 min = 1.50 ± 0.15 per pig/10 min; 25 hr = 1.49 ± 0.15 per pig/10 min; 24 hr = 1.47 ± 0.15 per pig/10 min; 120 min = 1.04 ± 0.12 per pig/10 min; $P < 0.05$).

There was a significant interaction between age, treatment, and time for pen location at the udder and other location, however not for the heat lamp (Table 3.3). There was a significant interaction between age and time for other location ($P < 0.05$). There was a significant interaction between treatment and time for both udder and heat lamp location ($P < 0.05$). Piglets given ketoprofen and castrated were observed underneath the heat lamp at a lower frequency immediately after castration than at all other time points (0 min = 3.29 ± 0.44 per pig/10 min; 30 min = 4.27 ± 0.54 per pig/10 min; 15 min = 4.45 ± 0.55 per pig/10 min; 120 min = 5.16 ± 0.62 per pig/10 min; 25 hr = 5.42 ± 0.66 per pig/10 min; 24 hr = 5.53 ± 0.67 per pig/10 min; $P < 0.05$). Piglets given ketoprofen and castrated were also observed underneath the heat lamp at a higher frequency 24 hours after treatment than 15 and 30 minutes after ($P < 0.05$). Piglets castrated with no pain control and those sham handled were observed underneath the heat lamp at a higher frequency immediately after treatment than piglets given ketoprofen and castrated (0 min: C = 4.96 ± 0.61 per pig/10 min; S = 5.04 ± 0.61 per pig/10 min; K = 3.29 ± 0.44 per pig/10 min; $P <$

0.05). Piglets castrated with no pain control were recorded under the heat lamp at a higher frequency 30 minutes after castration than piglets given ketoprofen and castrated (30 min: C = 6.44 ± 0.75 per pig/10 min; K = 4.27 ± 0.54 per pig/10 min; $P < 0.05$). Time had an effect on piglet location at the heat lamp, udder, and other location ($P < 0.05$). Age had an effect on piglet location at the heat lamp and other location ($P < 0.05$), but not the udder ($P > 0.05$; Table 3.4).

Table 3.3 LSMeans of frequency (per piglet/10 minutes) of pen behaviours in six treatment groups measured at six sampling time points.

	Time							*P*-value
Treatment[1], freq[2,3]	0 min	15 min	30 min	120 min	24 hr	25 hr	SEM[4]	Age×Tr×Time[5]
Pain-related behaviour								
Tail wagging[2]								
OK	1.63d	2.12c	1.18bd	1.58cd	7.49c	6.36c	0.61	
OC	0.37bc	1.15bc	1.73d	2.43d	8.19c	5.27c	0.60	
OS	0.80cd	0.76b	1.08bd	0.60bc	1.51b	1.59b	0.61	0.001
YK	0.44bc	0.47ab	0.21a	0.21ab	1.34b	0.65ab	0.67	
YC	0.03a	0.82bc	0.60ab	0.38ab	0.38a	0.94ab	0.72	
YS	0.16ab	0.11a	0.16a	0.11a	0.11a	0.34a	0.70	
Non-specific behaviour								
Sleeping[3]								
OK	1.66a	3.56a	5.64	4.57a	1.72ab	2.73a	0.61	
OC	2.78ab	4.09ab	4.88	4.60a	1.13a	3.35ab	0.60	
OS	2.14a	3.48a	5.19	5.25ab	2.46b	4.01abc	0.61	0.003
YK	2.55ab	5.04abc	4.71	5.48ab	5.88cd	6.30d	0.67	
YC	4.91c	6.03bc	6.74	7.39b	6.56d	4.81bcd	0.72	
YS	3.86bc	6.99c	4.94	5.55ab	4.10c	5.58cd	0.68	

Awake inactive[3]								
	OK	3.42	2.84	2.20	1.91	3.07	1.33	0.61
	OC	3.51	3.16	2.63	1.76	2.63	2.63	0.60
	OS	4.22	1.97	1.97	1.41	1.91	2.48	0.61
	YK	1.54	0.53	1.38	0.64	1.10	0.87	0.67
	YC	1.62	1.23	1.12	0.84	1.17	1.94	0.72
	YS	1.17	0.99	1.30	0.49	0.49	0.75	0.70
Suckling[2]								
	OK	1.09ab	1.15	0.60ab	0.66	0.78ab	1.51b	0.61
	OC	1.09ab	0.54	0.79ab	0.54	1.57b	0.97ab	0.60
	OS	1.91b	0.84	0.79ab	0.67	1.07ab	1.07ab	0.61
	YK	0.99a	0.83	1.16b	0.59	0.50a	0.47a	0.67
	YC	0.47a	0.65	0.35a	0.71	0.47a	1.41b	0.72
	YS	0.71a	0.52	0.84ab	0.52	0.52a	0.50a	0.70
Other[3]								
	OK	4.40c	3.87b	2.35ab	2.64	4.05cd	4.05b	0.61
	OC	2.63ab	2.57ab	2.40ab	2.52	5.03d	3.39b	0.60
	OS	3.58bc	3.74b	2.31ab	3.14	5.17d	2.86b	0.61
	YK	4.53c	3.68b	3.41b	2.21	1.42a	1.52a	0.67
	YC	1.60a	2.06a	1.94a	1.66	2.06ab	3.48b	0.72
	YS	3.38bc	1.51a	3.56b	2.59	3.01bc	1.46a	0.70

0.154	
0.036	
0.001	

Location									
Udder[3]									
	OK	2.83ab	2.98	2.78ab	2.38	2.23	3.37bc	0.61	
	OC	2.47a	2.63	2.42ab	3.14	1.90	3.45bc	0.60	
	OS	2.81ab	2.66	2.61ab	3.45	3.11	2.17ab	0.61	
	YK	4.89b	3.65	3.50b	3.68	1.86	1.29a	0.67	0.001
	YC	2.07a	2.72	1.47a	3.59	2.66	4.27c	0.72	
	YS	2.96ab	2.13	3.12b	3.23	3.34	1.99ab	0.73	
Heat Lamp[3]									
	OK	2.43	3.59	2.93	4.53	3.65	3.48	0.61	
	OC	3.50	4.51	4.83	4.14	3.82	4.19	0.60	
	OS	3.94	4.14	4.81	4.40	3.63	4.40	0.61	
	YK	4.45	5.51	6.23	5.88	8.38	8.43	0.67	0.111
	YC	7.03	7.33	8.59	5.71	7.51	5.54	0.72	
	YS	6.46	7.69	6.15	6.28	6.28	7.62	0.70	
Other Location[3]									
	OK	3.89c	2.65b	3.48b	2.39c	3.38b	2.34b	0.76	
	OC	3.06c	1.97b	1.87b	1.82c	3.30b	1.48b	0.59	
	OS	2.57c	2.53b	1.94b	1.51bc	2.57b	2.77b	0.59	
	YK	0.29a	0.55a	0.18a	0.28a	0.04a	0.32c	0.12	0.004
	YC	0.94b	0.18a	0.27a	0.67ab	0.09a	0.44c	0.17	
	YS	0.42ab	0.06a	0.48a	0.24a	0.12a	0.13c	0.13	

abcd = Values within a behaviour and column not sharing a lowercased letter differ significantly at the $P < 0.05$ level.

[1]OK = piglets given ketoprofen 30 minutes prior to castration at 10 days of age; OC = piglets given saline 30 minutes prior to castration at 10 days of age; OS = piglets given saline 30 minutes prior to sham handling at 10 days of age; YK = piglets given ketoprofen 30 minutes prior to castration at 3 days of age; YC = piglets given saline 30 minutes prior to castration at 3 days of age; YS = piglets given saline 30 minutes prior to sham handling at 3 days of age.

[2]freq = total mean frequency of behaviour per pig/10 minutes (continuous observation).

[3]freq = mean frequency of behaviour per pig/10 minutes (scan sampling at 11 time points per 10-minute period).

[4]SEM = pooled standard error of means.

[5]Statistical interaction between age, treatment, and time.

Table 3.4 LSMeans for frequency (per piglet/10 minutes) of pen behaviours in three treatment groups and two age groups.[1]

	Treatment[5]				Age[7]			*P*-value		
Pen Behaviours[2], freq[3,4]	K	C	S	SEM[6]	O	Y	SEM[6]	Tr[8]	Age	Time
Pain-related behaviour										
Trembling[3]	0.05	0.12	0.09	0.04	0.09	0.08	0.03	0.381	0.752	<0.001
Tail wagging[3]	1.08b	0.86b	0.39a	0.18	1.74b	0.29a	0.18	0.007	<0.001	<0.001
Rump scratching[3]	0.07	0.07	0.03	0.02	0.10b	0.03a	0.02	0.061	0.001	<0.001
Prostrated[4]	0.15	0.22	0.12	0.04	0.19	0.13	0.03	0.108	0.222	<0.001
Non-specific behaviours										
Playing[3]	0.02	0.02	0.03	0.01	0.06b	0.01a	0.01	0.585	0.001	<0.001
Sleeping[4]	3.79	4.34	4.24	0.37	3.22a	5.26b	0.31	0.529	<0.001	<0.001
Awake inactive[4]	1.49	1.84	1.33	0.17	2.39b	0.99a	0.14	0.098	<0.001	<0.001
Suckling[3]	0.81	0.72	0.77	0.09	0.92b	0.63a	0.07	0.783	0.007	0.086
Other[4]	2.97	2.48	2.86	0.26	3.26b	2.33a	0.22	0.370	0.003	0.006
Location										
Udder[4]	2.80	2.63	2.76	0.36	2.71	2.75	0.29	0.939	0.930	0.007
Heat Lamp[4]	4.62	5.34	5.31	0.51	3.89a	6.63b	0.43	0.506	<0.001	0.018
Other Location[4]	0.81	0.84	0.65	0.15	2.44b	0.24a	0.18	0.629	<0.001	0.001

ab = Values within a behaviour and row not sharing a lowercased letter differ significantly at the $P < 0.05$ level.

[1]Data expressed as least squared means

[2]See Table 1 for descriptions

[3]freq = total mean frequency of behaviour per pig/10 minutes (continuous observation).

[4]freq= mean frequency of behaviour per pig/10 minutes (scan sampling at 11 time points per 10-minute period).

[5]K = piglets given ketoprofen 30 minutes prior to castration; C = piglets castrated with no pain control; S = piglets sham castrated

[6]SEM = pooled standard error of means

[7]O = piglets treated at 10 days of age; Y = piglets treated at 3 days of age

[8]Tr = treatment

3.4 Discussion and Conclusions

This study was performed because the optimal age at which castration should be performed in piglets is not well understood. The assumption that younger piglets feel less pain is not widely accepted due to advances in understanding the physiology of neonatal piglets. The result is that many countries, including Canada, are requiring that producers use an NSAID to treat post-procedural pain following castration (NFACC, 2014). However, it is still uncertain if castrating piglets at a younger age rather than an older age is more beneficial for the growth and overall welfare of the animal. Some physiological markers like ADG and serum cortisol concentration have been used in previous studies to compare piglets castrated at different ages (McGlone et al., 1993; Kielly et al., 1999; Carroll et al., 2006). Studies have shown that over a four-day period, piglet weight gain after castration at 5 days of age had not been affected (Hay et al., 2003). In the present study there were no differences in ADG between piglets castrated at 3 and 10 days of age over the period from 2 to 20 days of age. However, between the ages of 9 and 13 days, piglets treated at 10 days of age showed significantly higher ADG than those treated at 3 days of age, regardless of treatment. Kielly et al. (1999) found that piglets castrated at 3 days of age gained less weight between castration day and the following day than weight matched control littermates, whereas there was no difference in weight gain at the same time points between the piglets castrated at 10 days of age. It was hypothesized that piglets surgically castrated at a younger age would have a lower ADG as compared with piglets castrated at an older age up until weaning. These results, however, may indicate that although piglet age at castration may have a short-term effect on weight gain, there is unlikely a long-term effect.

Increased serum cortisol levels can be an indicator of physiological stress, which can also indicate pain. The hypothesis was that castrated piglets will have a higher serum cortisol response than sham handled piglets 45 minutes after the procedure, and that piglets given ketoprofen and castrated would have a response similar to sham handled piglets 45 minutes after castration. It was also hypothesized that a similar response would be seen 24 hours after surgical castration or sham handling. Piglets castrated without pain control in this study showed higher serum cortisol concentrations at 45 minutes after castration compared to those not castrated, while those given ketoprofen and castrated tended to have lower cortisol levels. Previous studies show that surgical

castration of piglets causes a significant rise in serum cortisol levels from 20 to 45 minutes afterwards, with peak levels occurring between 30 and 60 minutes (Prunier et al., 2005; Carroll et al., 2006; Marchant-Forde et al., 2014). The evidence of the present study supports these conclusions by indicating that the pain of castration causes a higher stress response than handling alone. It also shows that ketoprofen may have had a beneficial effect when given 30 minutes prior to castration as piglets in this group tended to have lower cortisol levels than those not given pain control. These results are similar to Davis et al. (2017), who found cortisol concentrations higher in castrated piglets 30 and 45 minutes after the procedure compared with those sham handled. While castration resulted in changes in serum cortisol 45 minutes after castration in the present study, age did not affect the concentration of cortisol at this time. Regardless of whether treatment was performed on day 3 or day 10 of age, piglets showed similar cortisol responses. Conversely, treatment did not have an effect on cortisol levels 24 hours after treatment in the present study while age did. Piglets handled at 10 days of age had a lower cortisol concentration than piglets handled at 3 days of age, regardless of treatment. These results may indicate that older piglets are better able to handle stressful events than younger piglets and cortisol concentrations may return to their resting levels quicker after castration. Alternatively, these results could be reflecting evidence that resting cortisol levels decrease as pigs age, as supported by measures in 12-, 16-, 20-, and 24-week-old growing pigs where the rhythm adjusted mean (MESOR) decreased between the ages 12 to 16 weeks and 16 to 20 weeks (Ruis et al., 1997). This is unlikely, however, as comparing neonatal piglet plasma cortisol concentration from the day of birth to 40 days after birth has shown no change in cortisol concentrations (Heo, 2001). Similarly, the baseline cortisol measured 24 hours prior to treatment in the present study showed no age effect, supporting there being no decrease in resting cortisol of piglets between the ages of 2 and 10 days.

Age has shown to have a small effect on pen behaviour, where piglets 3 days of age were observed standing more frequently after handling than piglets 6, 9, and 12 days of age (Carroll et al., 2006). It was hypothesized that both young and old piglets would show a higher frequency of pain-related behaviours like tail wagging and rump scratching after surgical castration as compared to those sham handled, with a more pronounced response seen in older piglets. Piglets provided with ketoprofen were hypothesized to show a response similar to piglets that were sham handled. In the present study, treatment, regardless of age or time, had an effect such that all

castrated piglets with or without pain control tail wagged more frequently than the sham handled group. This could indicate that regardless of whether pain control in the form of ketoprofen was given, piglets may still experience pain or discomfort near the incision, causing them to wag their tail. When analyzing the interaction between age, treatment, and time, older piglets given ketoprofen prior to castration had a higher frequency of tail wagging than all other groups apart from the older sham handled piglets immediately after treatment. Similarly, young piglets given ketoprofen wagged their tails more immediately after castration than those in the control castrate group. The increased wagging behaviour could be showing an irritation to ketoprofen, however there is little evidence in literature to support this. Older piglets given ketoprofen did not tail wag significantly more than their sham handled counterpart immediately after treatment, which is replicated in the young groups as well. This evidence is more likely to support the idea that tail wagging immediately after a stressful or painful procedure is more indicative of increased activity compared to the control castrate group. It is also worth noting that time had an effect on tail wagging, where tail wagging behaviour was lowest immediately after treatment compared with all other time points. Tail wagging was greatest at 24 and 25 hours after treatment and was observed more often in older castration control piglets than those sham handled at the same age. These results suggest that some pain-related behaviours may not start occurring until at least one day after treatment. This conclusion is in accordance with Hay et al. (2003), who found increased tail wagging by castrates at one day after treatment but not before. Age also influenced tail wagging frequency in this study, where older piglets performed more of this behaviour than young piglets.

In the present study, piglets given ketoprofen and castrated were located underneath the heat lamp more at all time points other than immediately after castration. Conversely, piglets castrated without pain control and those sham handled were observed under the heat lamp sooner after treatment than those in the ketoprofen group. This may indicate that piglets are seeking comfort following the stress of handling by staying warm underneath the heat lamp. Piglets given ketoprofen may have chosen to seek comfort at the sow rather than the heat lamp immediately after castration as expressed by this evidence. These results could indicate that piglets given ketoprofen are more likely to seek comfort from their sow rather than the heat lamp. Alternatively, this preference for the sow could have resulted by chance as stressed piglets could be looking for any form of warmth. An age, treatment, and time interaction was observed for

piglets near the udder and in 'other' locations. Young piglets that were castrated with no pain control showed an increased frequency of being at the udder 25 hours after treatment compared to young sham handled piglets and those given ketoprofen. The results of the present study do not agree with other studies (Taylor et al., 2001; Hay et al., 2003; Llamas Moya et al., 2008), where no difference was found in piglets' location after being handled or castrated. These previous studies used scan sampling, as did the present study, however they recorded pigs' locations more frequently and over a longer time period. The current study used scan sampling every minute for a 10-minute period beginning at 0, 15, 30, and 120 minutes, and 24 and 25 hours after treatment, so it is difficult to make direct comparisons between these studies.

The handling chute is a recently developed method used to empirically and objectively determine pain in piglets after castration (Bilsborrow et al., 2016; Davis et al., 2017). In these previous studies, pigs experiencing pain following castration spent more time navigating the handling chute compared to sham-handled controls (Bilsborrow et al., 2016; Davis et al., 2017). However, in the current trial an age by treatment interaction was found, where young sham handled piglets had a much longer NT than piglets castrated with or without ketoprofen. Young piglets that were sham handled may have taken a longer time to complete the chute as they were experiencing less stress and felt no urgency to return to their farrowing crate and sow. For young, castrated piglets, it can be hypothesized that they may have a faster NT if they feel stressed in order to return to their farrowing crate to seek comfort from their sow or the heat lamp. It is important to note that there were no differences in chute time among older piglets, regardless of treatment. It is uncertain why the conclusions of this study regarding the handling chute are so different than those of previous studies as the procedures for collection of NTs were similar between all three studies. This could be an indication that the handling chute is not a reliable method for detecting pain-behaviour in piglets.

In conclusion, the present study showed that castration is painful and causes an increase in serum cortisol levels up to 45 minutes afterwards. The procedure can also affect piglet behaviour up to 24 hours afterwards, causing an increase in painful behaviours such as tail wagging and rump scratching. Some age effects were seen when comparing cortisol concentrations taken 24 hours after treatment. Serum cortisol levels were higher in younger piglets than older piglets at that time point, but baseline levels were similar for both ages,

suggesting that stress levels will not drop as quickly back to normal in young piglets' castration. Handling may also briefly affect piglet ADG at a young age, which could also be an indication that young piglets are more affected by pain and stress than older piglets. In the present study no consistent differences between pain responses were observed across young and old piglets at castration, therefore an optimal age to perform castration at can not be identified. A recommendation can only be made based on level of difficulty to perform this procedure by barn staff, where younger and therefore smaller piglets are more easily castrated by barn staff than older piglets. Lastly, ketoprofen usage for control of pain was successful to a small degree, where piglets may have seen some relief shown in serum cortisol levels. More needs to be done to determine if ketoprofen can be used in neonatal piglets for relieving post-procedural pain.

4 AN INVESTIGATION INTO THE PHYSIOLOGICAL AND BEHAVIOURAL RESPONSE OF PIGLETS ADMINISTERED KETOPROFEN OR SALINE EITHER ONE HOUR OR IMMEDIATELY BEFORE CASTRATION

The current chapter examines an experiment performed to investigate the stress and pain responses of piglets that were given either a ketoprofen or saline intramuscular injection either one hour, or immediately before castration. Physiological and behavioural responses explored were serum cortisol concentrations, pen behaviour, and navigation time in a specially designed handling chute.

Chapter 4 is prepared for submission for publication. The journal it is published in will have copyrights to this chapter.

Davis, E., J. Brown, T. Duke, and Y. Seddon. An investigation into the physiological and behavioural response of piglets administered ketoprofen or saline either one hour or immediately before castration.

Drafting of the manuscript was completed by Erin Davis and Drs. Yolande Seddon, Jennifer Brown, Tanya Duke, and Suzanne Millman gave suggestions for any necessary revisions. Experimental design was completed by Erin Davis with suggestions from Drs. Yolande Seddon and Jennifer Brown. Animal handling and data collection was completed by Erin Davis. Cortisol analysis was completed at Prairie Diagnostic Services. Statistical analysis was completed by Erin Davis with suggestions given from Drs. Yolande Seddon and Jennifer Brown.

4.1 Introduction

In Canada, analgesics are now required when performing castration on male piglets at any age (NFACC, 2014). Scientific evidence from physiological measures show increases in cortisol (Lonardi et al., 2015) between 20 and 45 minutes after castration (Davis et al., 2017). Behavioual evidence also suggests castration is painful with castrated piglets more commonly found standing or sitting hunched and showing signs of being awake but inactive as compared with piglets handled without being castrated (Taylor et al., 2001; Hay et al., 2003; Viscardi et al., 2018).

NSAIDs are the most commonly used analgesic in piglet castration (Viscardi et al., 2018) and have been shown to be effective at reducing piglet pain after the procedure (Keita et al., 2010; Kluivers-Poodt et al., 2012). However, pharmacological evidence indicates that these drugs take time, in some cases more than one hour, for full absorption and distribution into tissue (Nixon et al., 2020). It may therefore be necessary that these analgesics are administered one hour prior to castration, providing time for active compounds to take effect before castration is performed. This would require producers to handle piglets more than once, which not only increases labour requirements, but handling a piglet can play a role in increasing its stress levels (Marchant-Forde et al., 2014; Brajon et al., 2015).

Analgesic use in piglet castration has shown benefits in the scientific literature such as decreasing cortisol levels (Keita et al., 2010) and reducing pain-related behaviours (Kluivers-Poodt et al., 2013) as compared with piglets not receiving any pain control at castration. However, comparisons in research between the timing of analgesic administration and the effects this may have on physiological and behavioural pain responses are lacking. These effects are relevant to producers as it may be necessary to administer analgesics at a certain time prior to castration to optimize their pain-relieving effect not only after the procedure, but at the time of the procedure. However, administering an analgesic prior to castration would require two handling bouts, and therefore has the potential to increase piglet stress due to multiple instances of handling. Scientific evidence has found that procedures that take a longer amount of time to complete increase a piglet's stress level than procedures that take a shorter amount of time (Marchant-Forde et al., 2014). Therefore, the question remains is it more beneficial for piglets to receive a more effective pain relief at the time of castration and increase handling time, or to risk

reduced effectiveness of an analgesic and be handled once. The objective of this study was to determine whether the administration of ketoprofen one hour prior or immediately prior to castration was most effective at pain and stress in piglets. The hypothesis of this study was that piglets provided with ketoprofen one hour prior to surgical castration would have a lesser physiological and behavioural pain response following castration as compared with piglets not provided with pain control or provided with ketoprofen immediately prior to surgical castration. It was also hypothesized that piglets handled twice as compared to once would have a higher stress response following handling.

4.2 Materials and Methods

This research was approved by the University of Saskatchewan's Animal Research Ethics Board (AUP# 20160022).

4.2.1 Animals and Facility

The study was carried out at the Prairie Swine Centre research facility, a 300-sow farrow-to-finish unit. A total of 179 PIC Landrace x Large White male piglets in 31 litters were used, weighing an average of 1.75 ± 0.29 kg (mean ± SD). Sows were housed within five farrowing rooms, each containing 16 farrowing pens that measured 2.44m x 1.83m with a 1.98m x 0.86m sow crate on tri-bar metal slatted floors. All pens had a hooded creep area with one heat lamp and a rubber mat. Sows were fed ad libitum on a commercial lactation diet. Iron injections were delayed until removal from the trial, while clipping needle teeth was performed within hours of birth. No further processing (tail docking or ear notching) was carried out on the piglets until after experimental procedures were completed. Cross-fostering was allowed from 1 to 2 days of age to ensure all piglets were given access to a viable teat.

4.2.2 Study Design

Litters were split into two sections: handling chute (13 litters, n = 76), or blood collection and pen behaviour observations (18 litters, n = 103). Litters were selected if they had greater than or equal to five male piglets. Trial piglets were weighed at 2 to 3 days of age and individually marked with a Sharpie® Magnum marker (Newell Brands, Atlanta, GA, USA). All piglets were required to weigh ≥ 1.00 kg. Piglets were randomly assigned using a random number generator (Microsoft ® Excel 2016, Microsoft Corporation) to one of five treatments: 1) Ketoprofen administered 60 minutes prior to surgical castration (HK, n = 37), 2) Saline administered 60 minutes prior to sham castration (handling as if to castrate, but not) (HS, n = 34), 3) Ketoprofen administered immediately prior to surgical castration (IK, n = 37), 4) Saline administered immediately prior to sham castration (handling as if to castrate, but not) (IS, n = 35), 5) Saline administered immediately prior to surgical castration (CA, n = 36). Each treatment was represented within each litter and was performed at 3 to 4 days of age. Injectable Anafen ® (100 mg/kg ketoprofen, Merial Canada Inc., Baie-D'Urfé, QC, Canada; extra label use) was the ketoprofen used throughout the trial and was diluted with distilled water using a 1:10 ratio. Anafen ® was administered at 3 mg/kg, as per the recommended dose for swine. Anafen ® and saline were administered intramuscularly dependent on piglet weight (volume injected: 0.53 ± 0.09 ml, mean ± SD).

4.2.3 Handling Chute

A sub-sample of 76 piglets was selected from 13 litters to complete a specialized behavioural test called a handling chute. The handling chute was a rectangular white box (18 x 177 cm) made of plywood (Figure 3.1A), and two hurdles could be placed in the chute. The chute was attached to the back of the farrowing crate once the back gate had been removed, with an opening that led back into the pen (Figure 3.1B). Piglets were placed into the handling chute and timed using a stopwatch. This behavioural test has been used to measure piglet pain using NT where castrated piglets without pain control had a slower NT in the handling chute than those sham handled up to 30 minutes after the procedure (Bilsborrow et al., 2016; Davis et al., 2017).

Piglets were trained to navigate the handling chute over two consecutive days prior to trial start. For both days, training consisted of three chute runs at 0, 15, and 30 minutes. On the first day of training, no hurdles were used in the chute. On the second day, the first, second, and third chute runs had increasing hurdle heights of 5.1, 7.6, and 10.2 cm respectively. In the 30 seconds before each run, all piglets per litter were restrained underneath the heat lamp in the farrowing crate using a pig board. One at a time piglets were placed in the chute and their NT was recorded. If a piglet laid down or turned around in the handling chute it was picked up and placed standing in the same location where it performed the unwanted action, however it was not pushed in any way at this time. If a piglet had not completed the run in two minutes, they were encouraged out of the handling chute by gently pushing their rump the rest of the way. After the last run on the second training day, piglets were considered trained.

On the treatment day, a baseline NT was recorded for all piglets 60 minutes prior to treatment. HK and HS piglets were administered ketoprofen or saline respectively, immediately after the initial chute run. At the time of treatment, IK, IS, and CA piglets were administered ketoprofen or saline intramuscularly, then were immediately castrated or sham handled for approximately 30 seconds before their NT was recorded in the first post-treatment chute run. HK and HS piglets were immediately castrated or sham handled (simulating the castration procedure) for 30 seconds before their NT was recorded in the first post-treatment chute run. Chute runs were repeated at 15, 40, and 60 minutes after treatment and NT was recorded for each run. The entire litter of piglets was restrained underneath the farrowing crate heat lamp using a pig board for 30 seconds before each chute run. Each hurdle was 10.2 cm in height. Treatments were carried out on each individual piglet at the specific time point and run order was maintained for each chute run. Once the last chute run at 60 minutes post-treatment was completed, piglets were removed from the trial. If piglets did not complete the handling chute within two minutes, they were removed from the chute and placed back in the farrowing crate. These piglets were recorded to have a NT of 120 seconds and their data remained in the data set.

A single piglet was removed from the handling chute portion of the trial due to death prior to second handling chute training day.

4.2.4 Blood Collection

The remaining 103 piglets from 18 litters were selected for blood collection. At 2 to 3 days of age, a sub-set of these piglets (n = 51, from nine litters) were subjected to blood collection 24 hours prior to treatment that served as a baseline measure. All piglets were weighed at this time. On treatment day, ketoprofen or saline was administered intramuscularly to HK and HS piglets, respectively at 60 minutes prior to treatment. At the time of treatment, IK, IS, and CA piglets were administered ketoprofen or saline intramuscularly, then were immediately castrated or sham handled (to simulate castration) for 30 seconds and released. HK and HS piglets were immediately castrated or sham handled (to simulate castration) for 30 seconds and released. Forty-five minutes after treatment completion, all piglets underwent blood collection. Once this blood collection was completed, piglets were removed from the trial.

For blood collections, the entire litter was first restrained underneath the heat lamp using a plywood board in an attempt to reduce stress. Once corralled, trial piglets were picked up one at a time and held upside down underneath the arm of one collector. The head of the piglet was restrained and a 22-gauge collection needle (Monoject™, Covidien™, Dublin, Ireland) was inserted into the orbital sinus of the piglet. The same eye was not used on consecutive days. Blood was allowed to drain into a 10 ml BD Vacutainer® Serum Blood Collection Tube (Beckton, Dickinson and Company, Franklin Lakes, NJ, USA) until approximately 5 ml of blood was obtained. Each collection was timed from the point of handling until the needle was removed, and each collection completed within two minutes. After collection, piglets were returned to the farrowing pen. Once collection was complete per litter, blood was placed in a refrigerator at approximately 4°C for approximately 30 minutes to allow for clotting, then removed and centrifuged for 15 minutes at 3000 RPM. Serum was then removed and placed into serum storage vials, then frozen at -20°C until analysis. A solid-phase chemiluminescent enzyme immunoassay (Immulite 1000 Cortisol, Siemens Healthcare Ltd., Oakville, ON) with a detection limit of 0.2 µg/dl (5.5 nmol/L) was used to analyze serum samples for cortisol. This immunoassay has been validated in swine (Escribano et al., 2012). Analysis was completed at Prairie Diagnostic Services Inc. in Saskatoon, SK, Canada, and the mean intra-assay CV was 5%.

4.2.5 Pen Behaviour

Pen behaviour observations were also collected from piglets that underwent blood collection (n = 103, 18 litters). A total of five video recordings occurred on treatment day at -60, -30, 0, 20, and 120 minutes in regard to treatment time. At -60 minutes, piglets were injected prior to behavioural observations, and at 0 minutes piglets were treated prior to behavioural observations. A camcorder mounted on a tripod was placed behind the farrowing crate (Figure 3.2). The behaviours selected (Table 4.1) were those observed in literature to significantly indicate piglet pain (Hay et al., 2003; Kluivers-Poodt et al., 2013). Frequency of behaviours was measured using either continuous observations lasting 10 minutes at each time point or scan sampling observations taken every one minute over 10 minutes (11 observations every 10 minutes). A single observer was used for all observations, and this observer was blind to treatments within reason (surgical castration incision sites could often be visualized in recordings). Behaviours recorded by continuous observation were tail wagging, rump scratching, playing and suckling, while scan sampling was used on prostrate, sleeping and awake inactive. Piglet location (udder, heat lamp or other) was also recorded at each scan sampling point in the 10-minute period.

A total of two (HK) piglets were removed from blood collection and pen behaviour measures. Removal of one piglet was due to death from sow crushing on the day of treatment prior to any handling. The second piglet was removed due to cryptorchidism determined at time of castration.

Table 4.1 Piglet behaviour descriptions (modified from Hay et al., 2003)

Behaviour	Description
Pain-related behaviour	
Tail wagging	Rapid tail movements from side to side or up and down.
Rump scratching	Scratching the rump by rubbing against a surface.
Prostrated	Sitting or standing motionless with the head down, lower than the shoulder level.
Non-specific behaviours	
Playing	Head shaking, springing, (sudden jumping or leaping), running with vertical and horizontal bouncy movements. Can involve partners
Sleeping	Lying down, eyes closed.
Awake Inactive	No special activity, but awake (eyes open or half closed). Lying, sitting, or standing.
Suckling	Teat in mouth, actively suckling.
Location (see Figure 2)	
Udder	Suckling, nosing, massaging the udder, or searching for a teat.
Heat lamp	Standing, sitting, lying, or sleeping underneath the heat lamp.
Other	Located anywhere other than the udder or heat lamp.

4.2.6 Statistical Analysis

All statistical analysis was performed using SAS ® 9.4 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina, USA). A Proc univariate test was run on all data sets prior to analysis to determine whether the data was normally distributed. After analysis, all residuals were checked for normality.

Navigation time data was determined to be non-normal and was log-transformed prior to analysis. An ANOVA model with repeated measures (Proc Mixed) was run to determine the

differences in NT between treatments over time. Maximum NT was recorded as 120 min. Treatment, time, and the interaction between the two were used as fixed effects, with time as a repeated measure. Piglet weight and pre-treatment navigation time were used as covariates, and piglet nested within litter was used as a random effect.

Serum cortisol data was log-transformed as it was determined to be non-normal. Mixed models (Proc Mixed) were used to determine the differences in serum cortisol concentrations between treatment groups at each sampling time point (24 hours pre-treatment and 45 minutes post-treatment). Treatment was the only fixed effect used, while piglet weight and blood draw duration were covariates. For serum cortisol data 45 minutes post-treatment, pre-treatment cortisol levels were used as a covariate as well. Lastly, litter was used as a random effect.

A Proc Glimmix model was used with a poisson distribution to run both the continuous pen behaviour data and scan sampling observations to determine the differences in frequency of pen behaviours between treatments at each sampling time point. Piglet location data was also analyzed using a Proc Glimmix model with a poisson distribution to determine the differences in frequency of location between treatments at each sampling time point. Treatment and time were fixed effects. The interaction between treatment and time was not included in any of the behaviour or location models as it was not significant in any of them or did not allow the model to converge when included. Litter was included as a random effect.

4.3 Results

4.3.1 Handling Chute

There was no treatment by time interaction effect ($P > 0.05$, Table 4.2), however there was a difference in the fixed effect time ($P < 0.05$, Figure 4.1). At time 0, total mean NT was significantly lower than 15 and 60 minutes after treatment (0 min = 14.9 ± 1.6 s; 60 min = 17.7 ± 1.6 s; 15 min = 21.3 ± 1.6 s; $P < 0.05$).

Table 4.2 Navigation time (NT) of piglets in five treatment groups at each time point with no significant treatment effect found.[1]

	Treatment[4]					
Time[2], s[3]	CA	HK	HS	IK	IS	SEM[5]
0 minutes	19.2	11.5	16.0	15.5	12.2	3.6
15 minutes	28.5	22.1	19.0	22.3	14.5	3.6
40 minutes	19.5	15.2	19.8	16.3	14.4	3.7
60 minutes	17.3	15.7	18.2	17.6	19.5	3.7

[1]Data expressed as least squared means.

[2]Sampling time points in regards to time after castration.

[3]s = seconds

[4]CA = piglets given saline immediately before castration; HK = piglets given ketoprofen one hour before castration; HS = piglets given saline one hour before handling; IK = piglets given ketoprofen immediately before castration; IS = piglets given saline immediately before handling.

[5]SEM = pooled standard error of means

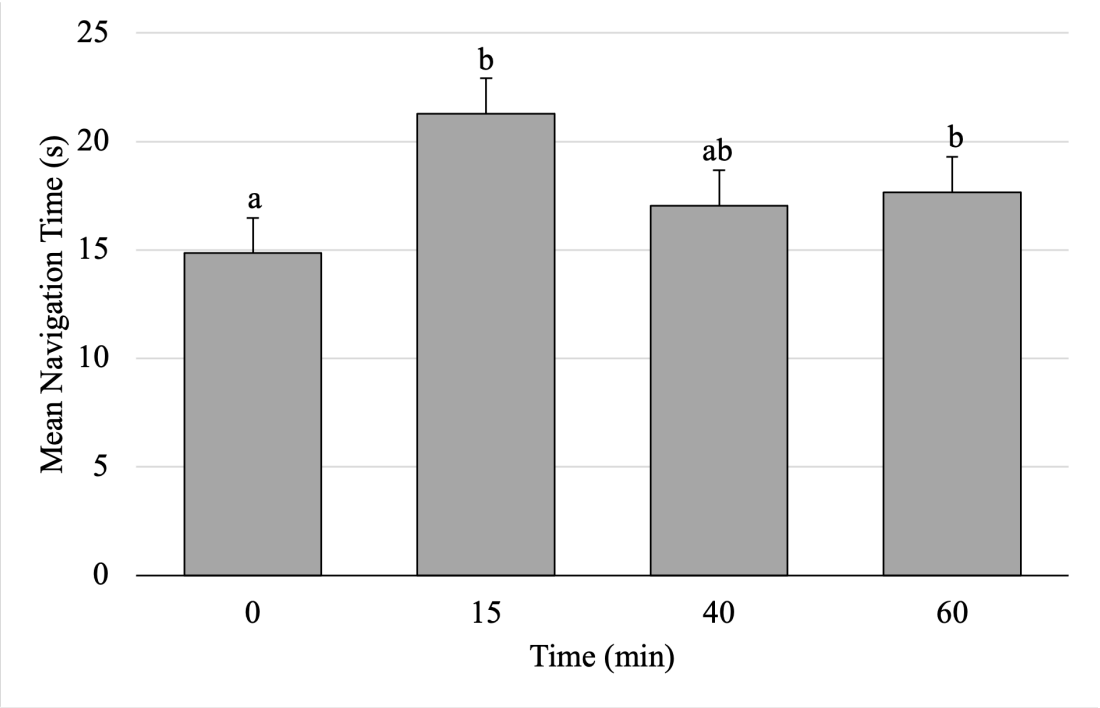

Figure 4.1 Mean navigation time (s) of trial piglets regardless of treatment over four time points (least squared means + SEM). Differences in lettering between time points signify statistical significance ($P < 0.05$).

4.3.2 Serum Cortisol

Baseline serum cortisol concentrations 24 hours prior were no different between treatments ($P > 0.05$). A treatment effect was observed at 45 minutes after treatment as shown in Figure 4.2 ($P < 0.05$). Average serum cortisol levels 45 minuts after treatment were significantly greater for CA piglets than in HK or HS piglets ($P < 0.05$), while IK and IS were not different than CA. Piglets in the HK treatment had the lowest cortisol levels and were significantly lower than CA, IK, and IS treatments ($P < 0.05$).

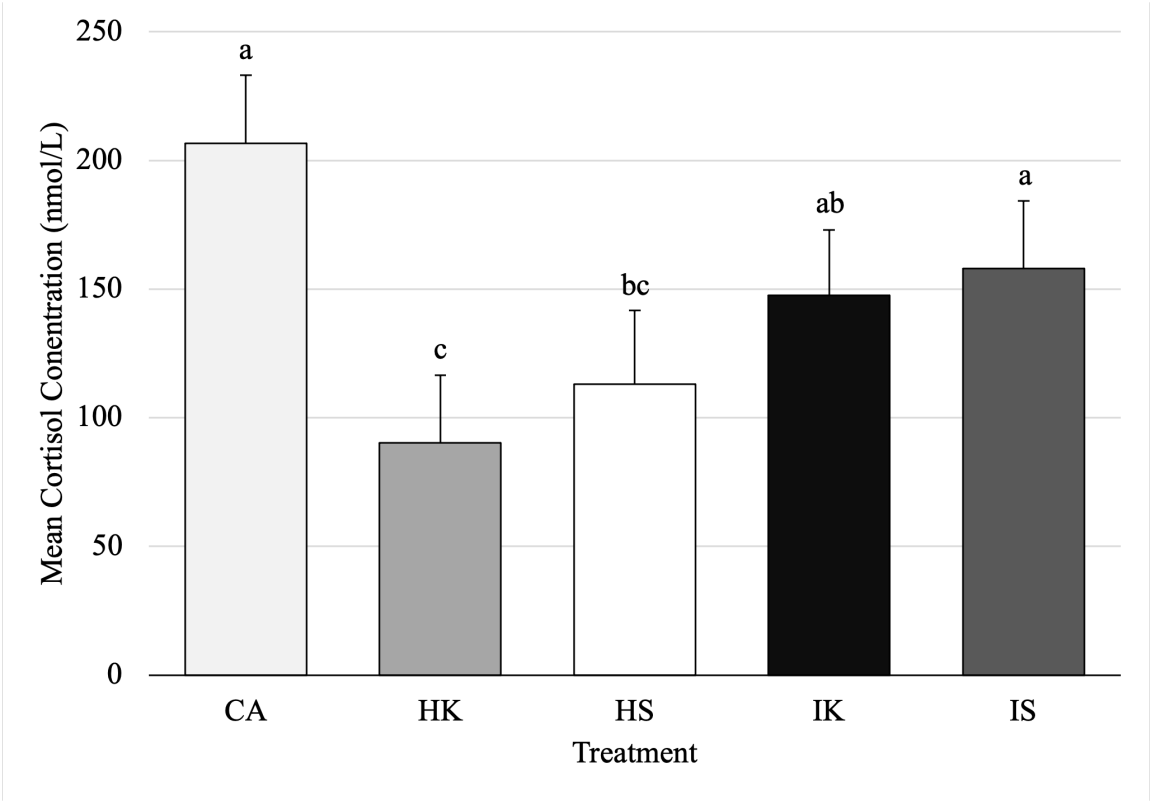

Figure 4.2 Mean serum cortisol concentrations (nmol/L) between five treatments 45 minutes after treatment (least squared means + SEM). Treatments: CA = piglets castrated with saline given immediately before; HK = piglets castrated with ketoprofen given one hour before; HS = piglets sham handled and given saline one hour before; IK: piglets castrated with ketoprofen given immediately before; IS: piglets sham handled and given saline immediately before. Differences in lettering signify statistical significance between treatment groups ($P < 0.05$).

4.3.3 Pen Behaviour and Location

There were no treatment effects or treatment by time interactions for pen behaviours or piglet location ($P > 0.05$). Tailwagging, however, showed a treatment tendency where piglets that were castrated (CA, HK, IK) tended to show more tail wagging than castrates (HS, IS; $P < 0.10$). Time, however, was significant for prostrated, sleeping, awake inactive, and other behaviours ($P < 0.0001$, Table 4.3). Time effects for pen behaviour are summarized below.

Immediately after treatment piglets, regardless of treatment, were recorded standing or sitting prostrated more frequently than 30 minutes before, 20 and 120 minutes after (0 min = 0.50 ± 0.08 per pig/10 min; 20 min = 0.24 ± 0.049 per pig/10 min; -30 min = 0.13 ± 0.035 per pig/10 min; 120 min = 0.096 ± 0.029 per pig/10 min; $P < 0.05$). Similarly, immediately after giving saline or ketoprofen injections (-60 minutes = 0.37 ± 0.065 per pig/10 min) piglets were found prostrated more often than 30 minutes before treatment and 120 minutes after ($P < 0.05$). Sleeping occurred less 60 minutes prior to treatment than 30 minutes before, 0, 20, and 120 minutes after (-60 = 3.45 ± 0.25 per pig/10 min; 0 min = 4.56 ± 0.30 per pig/10 min; 20 min = 5.04 ± 0.33 per pig/10 min; -30 min = 5.21 ± 0.34 per pig/10 min; 120 min = 7.30 ± 0.44 per pig/10 min; $P < 0.0001$). Piglets were also found to sleep the most 120 minutes after treatment than all other time points ($P < 0.0001$). Awake inactive behaviours were seen most frequently 60 minutes before and immediately after treatment than 30 minutes before, 20, and 120 minutes after (0 min = 0.65 ± 0.086 per pig/10 min; -60 min = 0.54 ± 0.077 per pig/10 min; 120 min = 0.35 ± 0.060 per pig/10 min; 20 min = 0.30 ± 0.0056 per pig/10 min; -30 min = 0.22 ± 0.047 per pig/10 min; $P < 0.05$). Behaviours other than those specified occurred most often 60, 30 minutes before, and 20 minutes after treatment than 0 and 120 minutes after (-60 min = 4.04 ± 0.31 per pig/10 min; -30 min = 3.90 ± 0.30 per pig/10 min; 20 min = 3.58 ± 0.28 per pig/10 min; 0 min = 2.91 ± 0.24 per pig/10 min; 120 min = 1.57 ± 0.15 per pig/10 min; $P < 0.05$).

Regarding pen location, there was no interaction effect between treatment and time found, and no treatment effect ($P > 0.05$). Time only showed an effect for the heat lamp location ($P < 0.0001$, Table 4.3), where piglets were most often found underneath the heat lamp 120 minutes after treatment than 60, 30 minutes before, 0, and 20 minutes after (120 min = 7.64 ± 0.51 per pig/10 min; 0 min = 6.28 ± 0.43 per pig/10 min; -60 min = 5.93 ± 0.41 per pig/10 min; 20 min = 5.34 ± 0.38 per pig/10 min; -30 min = 5.23 ± 0.37 per pig/10 min; $P < 0.05$).

Table 4.3 LSMeans of the frequency (per pig/10 minutes) of pen behaviours in five treatment groups after treatment.[1]

	Treatment[5]						*P*-value	
Pen Behaviours[2], freq[3,4]	CA	HK	HS	IK	IS	SEM[6]	Tr[7]	Time[8]
Pain-related behaviour								
Tail wagging[3]	0.16	0.15	0.04	0.14	0.06	0.05	0.086	0.205
Rump scratching[3]	0.03	0.03	0.01	0.02	0.03	0.02	0.875	0.411
Prostrated[4]	0.26	0.27	0.18	0.23	0.19	0.06	0.793	<0.001
Non-specific behaviours								
Playing[3]	0.05	0.11	0.06	0.12	0.05	0.03	0.465	0.253
Sleeping[4]	5.24	5.07	4.99	4.94	4.60	0.58	0.953	<0.001
Awake Inactive[4]	0.45	0.39	0.34	0.34	0.39	0.07	0.815	<0.001
Suckling[3]	0.81	1.07	0.92	0.81	0.94	0.14	0.654	0.265
Other[4]	2.75	3.06	3.12	3.03	3.24	0.45	0.953	<0.001
Location								
Udder[4]	2.00	2.54	2.49	2.20	2.93	0.54	0.784	0.161
Heat Lamp[4]	7.07	6.00	5.77	5.90	5.50	0.81	0.727	<0.001
Other Location[4]	0.65	0.59	0.47	0.57	0.63	0.13	0.888	0.225

[1]Data expressed as least squared means.

[2]See Table 4 for descriptions.

[3]freq = total mean frequency of behaviour per pig/10 minutes (continuous observation).

[4]freq= mean frequency of behaviour per pig/10 minutes (scan sampling at 11 time points per 10-minute period).

[5]CA = piglets given saline immediately before castration; HK = piglets given ketoprofen one hour before castration; HS = piglets given saline one hour before handling; IK = piglets given ketoprofen immediately before castration; IS = piglets given saline immediately before handling.

[6]SEM = pooled standard error of means

[7]Tr = treatment

[8]Time = behaviours were recorded at -60, -30, 0, 20, and 120 minutes relative to treatment

4.4 Discussion and Conclusions

A shorter NT through a handling chute was found in piglets given sucrose immediately after castration as a form of analgesia compared with castrates not given any form of pain management (Davis et al., 2017). The hypothesis of this study was that piglets surgically castrated with no pain control or those provided with ketoprofen immediately prior to surgical castration would have a longer NT in the handling chute 0 and 15 minutes after castration as compared to those receiving ketoprofen one hour prior or sham handled. In the present study, no treatment effects were found, however, regardless of treatment, piglets had shorter NT in the handling chute immediately after treatment compared with 15 and 60 minutes after treatment. From the evidence given in this study, handling stress may have been a factor when longer NT was observed, and the reason why treatment was not shown to cause an effect on NT. Piglets had shorter NT immediately after treatment regardless of treatment, whereas previous studies have shown longer NT immediately after castration when no pain control was provided (Bilsborrow et al., 2016; Davis et al., 2017; Reynolds et al., 2020). The current results could indicate that piglets feel a strong urge to return to the farrowing pen quickly after a traumatic or stressful event like castration or handling, resulting in shorter NT. After the first chute run, the increase in NT may indicate that any shock from the procedure had worn off, and piglets did not need to complete the chute quickly. However, with no treatment effect or similarities with previous research (Bilsborrow et al., 2016; Davis et al., 2017; Reynolds et al., 2020), this indicates that the handling chute had limited value in the present study and may indicate that changes to the methodology are needed.

In the present study, serum cortisol concentrations 45 minutes after treatment were higher in those piglets castrated without pain control than those given ketoprofen or sham handled and given saline immediately before treatment. These results agree with the hypothesis of this study with the exception of sham handled piglets given saline immediately prior to handling having a higher serum cortisol response. It was also expected that sham handled piglets that were handled twice to administer saline one hour prior to handling would have an increased serum cortisol reponse as compared with piglets sham handled and given saline at the same time. In the present study, however, piglets that were handled twice and given either ketoprofen or sham handled and

given saline one hour before had lower serum cortisol concentrations. These findings agree with previous studies where piglets castrated without pain control demonstrated increased cortisol concentrations between 20 and 45 minutes after treatment (Marchant-Forde et al., 2014; Lonardi et al., 2015). Piglets given pain control up to 30 minutes prior to castration had reduced serum cortisol responses 30 minutes after treatment (Langhoff et al., 2009; Keita et al., 2010). These findings indicate that providing piglets with analgesics prior to a painful procedure allows these analgesics proper time for sufficient absorption into tissue and will result in more effective pain control at the time of castration and immediately afterwards.

Certain pen behaviours are seen more or less frequently after a stressful procedure like castration. It was hypothesized that piglets castrated with no pain control and piglets provided with ketoprofen immediately prior to surgical castration would have a higher frequency of pain-related behaviours as compared to piglets provided with ketoprofen one hour before or those sham handled. In the present study, no treatment effects were found, however, there was effect of time. Piglets were found to be prostrated and awake inactive most frequently immediately after treatment, regardless of what treatment group they were in. These results are in accordance with the previous research on piglet behaviour following castration. Studies have shown an increase in standing, walking, and awake inactive behaviours immediately after a stressful procedure (Taylor et al., 2001; Hay et al., 2003; Viscardi et al., 2018). Pain specific behaviours such as tail wagging and rump scratching was most often and consistently seen 24 hours after treatment (Hay et al., 2003; Kluivers-Poodt et al., 2013; Viscardi et al., 2018). Piglets were also prostrated and awake inactive 30 minutes after handling and administered a saline or ketoprofen injection. This is a good indication that the pain of the injection and/or the stress from handling is also enough to cause certain behaviours.

In the present study, the only significant finding regarding pen location was an increase in time spent underneath the heat lamp 120 minutes after treatment, which is consistent with an increase in sleeping behaviour in this study at that time point. Hay et al. (2003), found no differences in time spent in different locations between piglets castrated, sham handled, and left undisturbed in the farrowing pen over five days. As there was no treatment effect for these two measures, it is unlikely that there is a connection with pain. Location of piglets in the farrowing

pen after castration show no real significance or value when used to try to determine pain responses of piglets after castration.

In conclusion, despite the extra time handling, providing piglets with ketoprofen one hour prior to castration was more beneficial for piglet welfare than providing ketoprofen at the time of the procedure. Serum cortisol has been found to be higher in piglets after castration when given an analgesic at the time of the procedure than if an analgesic was administered 30 minutes to one hour prior to castration. Although other measurements such as frequency of pen behaviours and navigation time in a handling chute did not support this finding with treatment effects, the pen behaviour results indicate that handling stress is enough to change piglet behaviour. This is observed 30 minutes after handling to administered injections of saline or ketoprofen with an increase in awake inactive and prostrate behaviours. This implies that although increased handling bouts may not affect a piglet's physiological stress response, they can visibly show behavioural signs of stress up to 30 minutes after handling. These findings would suggest reducing handling bouts is ideal, when necessary, but providing piglets with analgesics prior to a painful procedure to allow them to take effect is the first priority.

5 GENERAL DISCUSSION AND CONCLUSIONS

The objectives of this thesis were to investigate the pain responses of piglets to castration when castrated at 3 and 10 day sof age or when given an analgesic one hour prior to castration than immediately prior to castration. The purpose of the research was to evaluate the effect of piglet age on the response to castration and compare the responses of piglets given an analgesic one hour prior or immediately prior to castration, with the overall goal being to help determine optimal practices to minimize pain and stress in these animals after the procedure.

Surgical castration in the swine industry is a routine procedure that is known to cause pain and stress when measured by both physiological and behavioural measures (Prunier et al., 2005; Hansson et al., 2011; Sneddon et al., 2014; Lonardi et al., 2015). Results generated in this thesis regarding cortisol levels following castration demonstrate the benefit of analgesic use. In Chapter 3, results show that piglets castrated with no pain control had higher serum cortisol levels 45 minutes after castration than those not castrated. It was also found that piglets given ketoprofen as an analgesic 30 minutes prior to castration had lower serum cortisol concentration 45 minutes after castration compared with those castrated with no pain control, although statistically this result was not significant. In Chapter 4, piglets given ketoprofen one hour prior to treatment had lower serum cortisol levels 45 minutes after treatment than those given ketoprofen immediately before castration and those castrated without pain control. Based on this, the timing of administration of ketoprofen prior to surgical castration, in this case one hour prior, makes a difference to the overall effectiveness of the analgesic at the time of and immediately after the procedure. This is in agreement with Cassar et al. (2014) where piglets given ketoprofen 30 minutes prior to castration resulted in lower cortisol levels 30, 60, and 90 minutes following castration compared with piglets castrated without pain control. A pharmacological study of meloxicam, flunixin and ketoprofen concluded that maximum tissue concentrations occur two to four hours after administration, suggesting that for optimal effect, ketoprofen should be administered at least two hours prior to piglet castration (Nixon et al., 2020).

Chapter 3 results show an increase in frequency in tail wagging behaviour for piglets castrated whether they were given an analgesic or not compared with sham handled piglets, which is in accordance with other studies (Hay et al., 2003; Viscardi et al., 2018). The results of

the current work suggest that a piglet is more uncomfortable on the day after the procedure occurred. These results also support the idea that piglets feel the effects of castration many days after the procedure, not just the day after the procedure was performed. It is still not known for exactly how long after the castration procedure pain is present, however a study which followed piglets for five days after the procedure showed behavioural differences between uncastrated animals and castrates were still significant four days after castration (Hay et al., 2003). The combination of behavioural results of Chapter 3 and the study by Hay et al. (2003) provide evidence that pain is still felt multiple days after the procedure, therefore one injection of pain control does not sufficiently provide piglets with pain control after the procedure, and more research needs to be completed in order to determine how long pain control must be provided for.

Previous studies have found that piglet weight gain was reduced up to three days after castration when the procedure took place at 3 days of age (Kielly et al., 1999). The same study found no differences in weight gain after treatment between castrates and non-castrates when piglets were castrated at 10 days of age, and at weaning age, piglets castrated at 3 days of age showed no difference in weight compared with the late castrates (Kielly et al., 1999). McGlone et al. (1993) found that piglets castrated at 14 days of age had a higher ADG from birth until weaning than piglets castrated at 1 day of age. Another study looking at the effects of age concluded that there was no change in weight gain between piglets castrated at 3, 6, 9, and 12 days of age after the first 48 hours after their castration (Carroll et al., 2006). In Chapter 3, no differences were found in ADG between piglets castrated at 3 days or 10 days of age from 2 days of age until weaning. It can then be concluded that castrating at either 3 or 10 days of age has no long-term effects on ADG. Chapter 3 results may indicate, however, that there is a short-term effect on ADG depending on age at processing, as it was found that piglets castrated at 10 days of age had a higher ADG between the ages of 9 and 13 days old than those castrated at 3 days of age, regardless of pain control treatment. Serum cortisol results in this chapter also indicated that at 24 hours after treatment piglets treated at 2 days of age had higher serum cortisol concentrations than piglets treated at 10 days of age, regardless of their assigned treatment group. Although these results do not indicate that younger piglets feel the effect of castration pain more than older piglets 24 hours after the procedure, it does indicate that piglets handled at a younger age are more susceptible to the stress of handling. This information is important when

considering at what age it is most appropriate to castrate at to reduce both pain and stress for the animal, and in turn improving the welfare throughout its life.

In conclusion, research is still needed to help fill in many of the knowledge gaps with regards to optimal procedures to reduce stress and improve welfare when castrating pigs. Results from Chapter 3 may be an indication that piglets handled and castrated at a younger age may be more susceptible to the effects of stress and pain, and that older piglets are better able to manage stressful situations. However, without treatment effects that show evidence that piglets castrated at a young age have a more pronounced pain response than piglets castrated at an older age, a conclusion on which age is best to castrate at cannot be determined and instead, more research is needed. Chapter 4 results indicate that it is important to provide piglets with ketoprofen at least one hour prior to a painful procedure such as castration, and although there is evidence that behaviour can be altered due to handling stress, it is much more beneficial for piglet welfare to handle them twice and allow absorption of the analgesic prior to castration than to handle once and provide an analgesic immediately before castration.

6 REFERENCES

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