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# Assessment and Mitigation of Pain During and After Castration in Beef Calves

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# Assessment and Mitigation of Pain During and After Castration in Beef Calves

by

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## Abstract

Castration is a painful common husbandry procedure done in order to reduce aggressive behaviour, avoid unwanted mating and improve meat quality. Physiological and behavioural changes indicative of pain/discomfort have been reported after castration; however castration is commonly performed without the use of pain control. There is a lack of science based guidelines on pain mitigation strategies for castration in beef calves. The objective of this thesis was to assess and mitigate pain associated with castration in young and weaned beef calves. The first part of the study focused on assessing acute pain. Indicators of acute pain were evaluated to assess the effect of band and knife castration in 1 week, 2 month and 4 month old calves, the effect of a single dose of subcutaneous meloxicam administered immediately before band and knife castration in 1 week old calves, and the effect of a single dose of subcutaneous meloxicam administered immediately before knife castration or the combination of knife castration and branding in 2 month old calves. The second part of the study consisted of assessing pain in weaned beef calves after knife castration up to 28 days after castration. Indicators of pain were evaluated to assess the effect of a single subcutaneous injection of meloxicam administered 6, 3 and 0 hours prior to knife castration and the administration of lidocaine or meloxicam alone or in combination prior to knife castration in weaned calves. Behavioural and physiological changes were observed after castration at all ages, however, a greater number of physiological and behavioural parameters showed differences in knife castrated compared to band castrated calves, after multiple painful procedures compared to single painful procedures and in older calves compared to young calves. A reduction in behavioural and physiological responses was observed in calves that received pain mitigation at different ages and after different castration methods. Effective and practical pain mitigation strategies identified at different ages and after different

castration methods could be used to improve calf welfare post castration. These include subcutaneous administration of meloxicam to reduce pain and inflammation associated with castration and the use of lidocaine to block procedural pain.

# Preface

The following manuscripts have been published or submitted for publication. Daniela Meléndez Suárez was involved in the study design, data collection and analysis, result interpretation and writing the manuscripts with the guidance of the supervisors and co-authors. All authors contributed important intellectual content and provided critical reviews of the manuscripts. Written permission for reproduction of the article in its entirety for this thesis has been obtained from the publishers and all co-authors.

Published articles:

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# Dedication

**To my family**
*for your unconditional love and support*

‘He counts the stars and calls them all by name’
Psalm 147:4

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# List of Symbols, Abbreviations and Nomenclature

| Symbol | Definition |
|---|---|
| °C | Degree Celsius |
| m | meter |
| rpm | revolutions/minute |
| V | volt |
| s | second |
| min | minute |
| h | hour |
| d | day |
| wk | week |
| mo | month |
| ANOVA | analysis of variance |
| CI | confidence interval |
| CV | coefficient of variation |
| *n* | sample size |
| SEM | standard error of the mean |
| ADG | average daily gain |
| ELISA | enzyme-linked immunosorbent assay |
| i.m. | intramuscular |
| i.v. | intravenous |
| s.c. | subcutaneous |

# Chapter One: General Introduction

## 1.1 Animal Welfare

Today, animal welfare is a cultural, ethical, economic, social, religious, scientific and political issue. Public attention was initially drawn to the welfare of animals by the description of poultry and livestock intensive farming practices in the USA and the UK described by Ruth Harrison in her book *Animal Machines: The New Factory Farming Industry* written in 1964 (Harrison, 1964). British public reaction to the book led the British Ministry of Agriculture to appoint an expert committee which consisted of experts in veterinary science, animal husbandry and agriculture to investigate the conditions of intensively farmed animals. The committee visited different livestock establishments in the United Kingdom, Denmark and The Netherlands for comparison purposes (Eadie, 2012). The investigation led to the 'Report of the Technical Committee to enquire into the welfare of animals kept under intensive livestock husbandry systems' commonly known as the 'Brambell report' (Brambell, 1965). The report stated housing the animals indoors helped to optimize food conversion by 'restraint on exercise and the protection from extremes of climate' however, animals should have the freedom to 'stand up, lie down, turn around, groom themselves and stretch their limbs'. Shortly after, the Farm Animal Welfare Advisory Committee was created in Britain with the purpose of monitoring livestock production practices. In 1979, the name of the committee was changed to Farm Animal Welfare Council (FAWC) and in the same year the Five Freedoms were outlined:

1. **Freedom from Hunger and Thirst**: by ready access to fresh water and a diet to maintain full health and vigor.

2. **Freedom from Discomfort**: by providing an appropriate environment including shelter and a comfortable resting area.
3. **Freedom from Pain, Injury or Disease**: by prevention or rapid diagnosis and treatment.
4. **Freedom to Express Normal Behavior**: by providing sufficient space, proper facilities and company of the animal's own kind.
5. **Freedom from Fear and Distress**: by ensuring conditions and treatment which avoid mental suffering.

The Five Freedoms have been used as an important guideline for veterinarians, research institutions, as well as national and international organizations that have a mandate to ensure animal health and welfare. The Five Freedoms are outcome based aspirations, and have become a useful tool to assess animal welfare due to their simplicity (Webster, 2016). Mellor (2016) criticized that the Five Freedoms are mainly focused on avoidance of bad management practices and that they do not include the concept of positive welfare. In addition, the author states that *freedom from* can be misunderstood or misinterpreted to mean the complete elimination of negative states, and although it is important to minimize conditions which can lead to negative states, the presence of thirst, hunger, pain and fear are vital affective states that elicit important behavioural responses necessary for animal survival. Mellor and Reid (1994) developed 'The Five Domains Model' in New Zealand, inspired by the Five Freedoms which consists of 4 domains: nutrition, environment, health, and behaviour and their impact on the fifth domain: the mental state. The first four domains are evaluated in a 5 non-numerical scale in order to assess the extent of welfare compromise of animals in research, testing and teaching. In addition, Webster, who initially proposed the Five Freedoms when he was part of the UK Farm Animal Advisory Committee (the predecessor of FAWC), recognizes that the Five Freedoms only

evaluate animal welfare in a particular moment but do not take into consideration the effect of stress that can lead to long-term problems such as learned helplessness in sows and metabolic exhaustion in dairy cows, in addition 'freedom to express normal behaviour' should be changed for 'freedom of choice' to avoid behaviours that can be harmful for the animal performing the behaviour or towards other animals (Webster, 2016).

There are different opinions on what the definition of animal welfare should take into account, Broom suggested that 'the welfare of an animal is its state as regards its attempt to cope with its environment' (Broom, 1986) and suggested that the welfare of an animal can vary along a continuum from very poor to very good (Broom, 1991). Duncan emphasized the importance of feelings when considering animal welfare, not only the absence of negative feelings but also the presence of positive feelings such as pleasure (Duncan, 2005). McGlone disagreed with the importance of feelings and suggested that poor welfare is only present when the animals physiology is compromised which can lead to reproductive and survival challenges (McGlone, 1993). While, Kiley-Worthington suggests that welfare can be affected by the ability of the animal to display natural behaviours (Kiley-Worthington, 1989). A broader definition was suggested by Fraser et al. (1997) which integrates the feelings-based, physical-based and the behaviour-based definitions. Two main circles and three areas within the circles aim to cover the major ethical concerns of animal welfare. Circle A consists of the 'adaptations possessed by the animal', circle B consists of the 'challenges faced by the animal in its current circumstances'. The areas in which the circles do not overlap consist of 1) 'adaptations that no longer serve an important function', 2) 'challenges for which the animal lacks corresponding adaptations' and the area in the middle where the circles overlap, 3) 'challenges for which the animal has

corresponding adaptations'. The quality of life of the animals is affected by the challenges animals are exposed to and their ability to adapt to these challenges.

The World Organization of Animal Health (OIE) is an international organization which develops science-based animal welfare standards that play an important role in international trade. The OIE's definition of animal welfare encompasses all of those previously mentioned definitions: 'Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and appropriate veterinary treatment, shelter, management and nutrition, humane handling and humane slaughter or killing. Animal welfare refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment.'

## 1.2 Animal welfare legislation

Canada has three animal welfare laws to protect animals. The first two are regulated by the Canadian Food Inspection Agency (CFIA), the Health of Animals Act (Health of Animals Act, 2015) which ensures the adequate treatment of animals on farm and during transportation and the Meat Inspection Act (Meat Inspection Act, 2015) which protects animals when they are off-loaded at the slaughter facility. Finally, the Criminal Code (Criminal Code, 2017) which punishes deliberate cruelty to animals, and is enacted by different professionals depending on the province including the provincial Society for the Prevention of Cruelty to Animals (SPCA), appointed veterinarians, Royal Canadian Mounted Police (RCMP) and/or police officers.

Canada has codes of practice specific to farmed animals (veal, sheep, poultry, pigs, mink, goats, fox, rabbit, deer, horses, bison, dairy and beef cattle), which identify recommended best management practices (NFACC, 2013) and are used as regulations in some provinces. The Beef Codes of Practice recommend painful procedures (dehorning, castration, spaying) be done using pain control under veterinary advisement. In the case of castration, calves 6 months or older must be provided pain mitigation (as of January 2018), for dehorning, pain control must be used once the horn bud is attached (as of January 2016) and for spaying, veterinary consultation for pain mitigation is required.

## 1.3 Pain

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage by the International Association for the Study of Pain (Merskey, 1986). Nociception is the process by which the body recognizes a noxious stimulus and the pathways by which the message travels to reach the brain, while pain is defined as the perception of a noxious stimulus (Muir III and Woolf, 2001).

Noxious stimuli are detected by specialised free nerve endings called nociceptors, which convert mechanical, thermal or chemical stimuli of sufficient intensity into action potentials (Tranquilli et al., 2013). Nociceptors can be divided into: Aδ fibers, which are highly myelinated fibers that carry sharp localized pain information at a high speed (2.5 - 30 m/s), and C fibers, which are non-myelinated fibers which carry dull localized pain information at a slower speed (< 2.5 m/s) (Torebjork, 1985; Muir III and Woolf, 2001). Afferent nociceptors carry information to the dorsal horn where they synapse with neurons that carry nociceptive information from the spinal cord towards the brain (Besson and Chaouch, 1987). Two main tracts described in mammals are the spinocervicothalamic tract, which carries superficial pain to the somatosensory

cortex (Kennard, 1954; Ha and Liu, 1966), and the spinoreticular tract which carries visceral sensations and deep pain to higher centres (Milne et al., 1981; Besson and Chaouch, 1987). Pain can be modulated by the body in the spinal cord or in the brain through the release of endorphins, serotonin and norepinephrine which are neurotransmitters that inhibit the transmission of nociceptive information in pain pathways (Tranquilli et al., 2013).

Nociceptive pain is characterized by a high threshold and has a protective role that activates withdrawal responses. Inflammatory pain, on the contrary, is a low threshold pain that protects the body during the healing process and it occurs due to the presence of inflammatory substances as a result of tissue damage (Woolf, 2010). Inflammatory pain is capable of reducing the pain threshold by activating silent nociceptors that are only activated when there are inflammatory mediators (Schaible and Schmidt, 1988; Handwerker et al., 1991). This can lead to an increased responsiveness to noxious pain (hyperalgesia) or responsiveness to non-noxious stimuli (allodynia) (Tranquilli et al., 2013). Pain can be further categorized into acute and chronic pain. Acute pain has a protective role as it enables healing and tissue repair, while chronic pain has no protective value and can induce biochemical and phenotypical changes in the nervous system (Muir III and Woolf, 2001).

It is a challenge for veterinarians and scientists to identify pain in animals as pain is a subjective experience. There are certain methods, such as pain scales, which have been used in human medicine and have been adapted for their use in veterinary medicine. Pain scales include the visual analogue scale (VAS), numerical rating scales (NRS), simple descriptive scales (SDS) and dynamic interactive visual analogue scale (DIVAS). Observers place a mark along a continuum (VAS), select a number (NRS) or a description (SDS) of their perception of the amount of pain the patient is experiencing. Grimace scales are also used in research as a

valuable tool to assess pain in mice and rats, as facial expressions change when animals are in pain. Research is being done in developing similar methods in cows, sheep, and horses (Gleerup et al., 2015a; Gleerup et al., 2015b; McLennan et al., 2016). Validated pain scales are scarce but have been reported in dogs, cats, and cattle (Reid et al., 2007; Brondani et al., 2013; de Oliveira et al., 2014), however, these are species and scenario specific. There is no specific measurement of pain, however there are physiological and behavioural parameters associated with pain that can be used as indicators to objectively assess pain.

## 1.4 Measuring pain associated with castration

The subjective state of the animal after a painful stimulus is unknown, therefore assessment of pain in animals should be based on changes that can be observed and measured (Sneddon, 2009). Excellent reviews on pain assessment after castration in cattle have been previously described by Stafford and Mellor (2005), Bretschneider (2005) and Coetzee (2011).

The cortisol response after castration has been suggested as an indicator of nociception (Stafford and Mellor, 2005) due to the reduction observed in cortisol concentrations in calves receiving lignocaine (Fisher et al., 1996). Reduction in the cortisol response has also been reported after administration of a local anesthetic, an analgesic or the combination of a local anesthetic and an analgesic (Earley and Crowe, 2002; Ballou et al., 2013; Webster et al., 2013). Surgical castration has been shown to elicit a greater cortisol response compared to rubber ring castration (Fell et al., 1986; Robertson et al., 1994; Molony et al., 1995), Burdizzo castration (Fisher et al., 1996) and compared to uncastrated calves (Earley and Crowe, 2002; Ballou et al., 2013; Webster et al., 2013). In addition, timing and concentration of peak plasma cortisol concentrations vary after surgical, band and Burdizzo castration (Stafford et al., 2002). Bretschneider (2005) recommends castrating calves as young as possible based on increased

weight loss in older cattle and a trend for cortisol concentrations to be lower in young calves compared to older calves following castration. However, others have reported no differences in the cortisol response between different castration methods in young and weaned calves (Robertson et al., 1994; Molony et al., 1995; Fisher et al., 1996).

Production parameters are highly valued by livestock producers (Coetzee, 2011) but effects of castration on weight gain may not mirror the pain experienced by calves during and after castration (Stafford and Mellor, 2005). Production parameters have been reported to decrease as the age of castration increases, independent of the method of castration (Bretschneider, 2005). Reduction in ADG has been reported during the first week after surgical castration, while lower ADG has been reported 2 to 4 weeks after band castration in 214 to 275 kg calves (Moya et al., 2014). Slower growth rates have been reported in banded calves than surgically castrated calves, and both surgically castrated and band castrated calves had slower growth rates compared to intact bulls in 9 and 14 month old calves (Fisher et al., 1996). No differences were reported on live weight or average daily gain after Burdizzo or surgical castration in 78 day old calves (King et al., 1991) or in 2-3 month old calves (Stafford et al., 2002; Webster et al., 2013).

Acute phase proteins have been assessed after castration as indicators of infection, inflammation or trauma (Petersen et al., 2004). Haptoglobin levels have been reported to increase after 2 to 3 days castration (Earley and Crowe, 2002; Ting et al., 2003) and the haptoglobin response has been attenuated when administering a non-steroidal anti-inflammatory drug (NSAID) (Brown et al., 2015; Roberts et al., 2015). Substance P concentrations were greater in surgically castrated than uncastrated calves (Coetzee et al., 2008), however no differences were reported in band castrated calves compared to control calves (Repenning et al., 2013). Plasma

catecholamines have also been reported to increase after castration, however there are few studies assessing catecholamines due to their short half-life (Stewart et al., 2010).

Other physiological parameters include infrared thermography of the eye, which has been reported to increase after surgical castration in 4 mo old dairy calves, possibly due to autonomic nervous system-related vasodilation and the release of vasodilating substances in response to pain (Stewart et al., 2010). Heart rate has been previously reported to increase after surgical castration as a response of the sympathetic nervous system to pain caused by the incision of the scrotum (Stewart et al., 2010). However, heart rate was reported to decrease after surgical castration in 47 to 80 day old dairy calves but the authors cautioned that the results may have been confounded by a previous painful experience (Schwartzkopf-Genswein et al., 2005). Heart rate variability is used to assess the balance between the sympathetic and the parasympathetic activity (Von Borell et al., 2007) and in a study assessing surgical castration with or without local anaesthesia it was suggested that visceral pain might be associated with an increase in parasympathetic activity (Stewart et al., 2010). Electrodermal activity measures the electrical resistance between two electrodes applied to the skin and has been associated with pain (Dowling, 1982). However, it was reported to be an unreliable indicator of pain for dehorning and castration in 2 to 4 mo old dairy calves (Baldridge et al., 2011). Electroencephalography measures the sensory component of pain, excluding the behavioural and emotional component (Lehmann et al., 2017). Changes in the electroencephalogram of surgically castrated dairy calves (low-frequency before castration to a high frequency after castration) have been reported (Coetzee et al., 2011).

Behavioural changes at the time of castration have been previously assessed in different castration studies. Movement in the chute assessed using a strain gauge system and the visual

analog scale (VAS) have been reported to be greater in surgically castrated beef calves than those that were banded (Moya et al., 2014). Behaviour such as kicking and struggling during castration has been reported to be greater in surgically castrated dairy and beef calves, followed by band castrated calves and least by uncastrated calves (Fell et al., 1986). Accelerometers, used to measure standing and lying duration, have shown greater standing duration after castration (White et al., 2008), while pain related behaviour such as tail flicks, restlessness, head turning, stretching and easing quarters generally increase after castration in dairy calves (Robertson et al., 1994; Molony et al., 1995). Reduction in stride length after castration in calves has been described as a behavioural modification to discomfort associated with castration (Currah et al., 2009; González et al., 2010). The von Frey anesthesiometer is a device used to automatically record pain thresholds by recording the amount of pressure (grams) applied to the animal before a reaction occurs. Pain thresholds have been reported to be lower in the wound and the surrounding skin after surgical castration in 3 to 4 month old beef calves receiving Tri-solfen® compared to untreated castrated calves (Lomax and Windsor, 2013).

## 1.5 Castration

Castration is a painful husbandry procedure which consists of damaging or removing the testicular parenchyma to stop the production of testosterone, a male hormone mainly produced in the testicles and responsible for undesired behaviours as well as unwanted meat characteristics in production animals. Castration decreases the number of on-farm injuries (Price et al., 2003) as it reduces mounting and aggressive behaviours, avoids reproduction of genetically inferior males and facilitates herd management (Stafford and Mellor, 2005). Meat quality is also affected by testosterone as intact bulls are more likely to produce dark meat, decreased marbling and tenderness, consequently affecting the overall meat quality grade (Jacobs et al., 1977).

## 1.6 Castration methods

The most common castration techniques used in beef cattle are band, surgical and Burdizzo castration. Band castration consists of placing a band above the testicles which interrupts the blood supply, causing cell damage due to a lack of oxygen and nutrients, consequently causing cell death. Burdizzo castration interrupts the blood flow to the testicles by crushing the blood vessels using a clamp over the skin of the scrotal neck, it is referred to as a closed technique as no incision or cuts are made. Surgical castration on the other hand consists of making an incision on the scrotum in order to externalize and remove the testicles, which can be done with the help of an emasculator that seals the blood vessels and severs the spermatic cord, or by tearing the spermatic cord by pulling on the testicles (Weaver et al., 2008).

Studies assessing different castration methods in young calves include a comparison of Burdizzo, rubber ring and surgical castration in dairy calves at 6, 21 and 42 days of age (Robertson et al., 1994) as well as a comparison between Burdizzo, rubber ring and a combination of both methods in 1-week old calves (Molony et al., 1995). Robertson et al. (1994) concluded all castration methods were painful across ages, however 6 day old calves spent less time in abnormal postures compared to 21 and 42 day old calves; while Molony et al. (1995) suggested rubber ring castration is associated with chronic pain compared to Burdizzo and surgical castration, but recognized the need for additional validated physiological and behavioural indicators of pain.

## 1.7 Pain mitigation during and after castration

In western Canada 95 % of beef calves are castrated before 3 months of age and only 10% of the producers use pain mitigation (Moggy et al., 2017). Although there are different types of analgesic and anesthetic drugs available for use in large animals, the use and

combination of drugs varies greatly between countries due to product availability, veterinary advice and federal regulations. Commonly used NSAID's in food animals in North America include Flunixin meglumine, Ketoprofen, and Meloxicam (Metacam®) (Schwartzkopf et al., 2012). The half-life of these products includes 22 ± 3 h for intravenous or subcutaneous meloxicam (Metacam®), 0.42 h for intravenous or intramuscular ketoprofen, and 3-8 h for intravenous flunixin meglumine (Stock and Coetzee, 2015). Routes of administration include subcutaneous, intravenous and oral for meloxicam (Metacam®), intramuscular and intravenous for ketoprofen and intravenous for flunixin meglumine.

Meloxicam is an attractive analgesic option in production animals due to the ease of administration and long lasting effect (Stock and Coetzee, 2015). Contrary to the United States, Meloxicam is approved for its use in cattle in Canada, however only oral meloxicam is labelled for mitigating pain associated to castration. Oral meloxicam did not mitigate pain indicators associated with band castration compared to uncastrated weaned calves (Repenning et al., 2013) while oral meloxicam was effective at mitigating the acute inflammatory response associated with surgical castration in yearling bulls (Roberts et al., 2015) and decreased the incidence of respiratory disease in feedlot cattle when administered prior to castration (Coetzee et al., 2012). Oral meloxicam has also been tested in neonatal calves but a lack of physiological changes after castration, with or without oral meloxicam administration, suggests the possibility that stress caused by castration is reduced in neonates compared to older calves (Brown et al., 2015).

Effective pain control consists of a combination of an anesthetic to block the conduction of pain signaling during a painful procedure and an analgesic to mitigate the pain associated with inflammation as a result of tissue damage. Lidocaine is a local anesthetic commonly used at the time of castration while commonly used analgesics include flunixin meglumine and ketoprofen.

Previous papers have studied the effect of the combination of an analgesic and a local anesthetic, as an effective way of reducing pain at the time of castration (Stafford et al., 2002; Webster et al., 2013; Ballou et al., 2013; Sutherland et al., 2013).

Lidocaine eliminated the cortisol response of 2-4 month old Holestein calves after band and ring castration while the combination of lidocaine and ketoprofen eliminated the cortisol response after ring, band, surgery cut and surgery pull, as well as clamp castration (Stafford et al., 2002). Mean cortisol concentrations were lower in 2-3 month old Holestein sham calves and calves that received lidocaine (LA) and flunixin meglumine (FM) compared to calves that did not receive medication (Webster et al., 2013) and no differences were seen in 3 month old Holstein calves in cortisol levels between sham and castration + LA + FM (Sutherland et al., 2013). Leukocyte response in 3 month old Holstein calves also decreased after castration when lidocaine and flunixin meglumine where used in combination, suggesting that analgesia and anesthesia can prevent future disease by preventing reduced numbers of white blood cells (Ballou et al., 2013).

The combination of analgesia and local anesthesia has been reported to decrease the leukocyte response caused by castration (Ballou et al., 2013) and the administration of analgesia on its own (oral meloxicam) on weaned calves prior to castration decreased the incidence of respiratory disease at the feedlot (Coetzee et al., 2012).

## 1.8 Effect of multiple painful procedures

Production animals usually undergo several painful husbandry procedures without the use of analgesia and/or anesthesia to mitigate pain. During processing, calves can be castrated, dehorned, branded, vaccinated and ear tagged on the same day. Branding is a common

husbandry procedure used to permanently identify animals as ear tags can be lost. Branding can be done either by freeze or hot-iron branding, however hot-iron branding is more commonly used due to its lower cost and as it requires less handling time than freeze branding (Schwartzkopf-Genswein et al., 1997).

Only two previous studies focused on alleviating pain caused by multiple stressors reveal that the combination of an anesthetic and an analgesic can suppress the leucocyte response following castration, dehorning and the combination of castration + dehorning (Ballou et al., 2013). Similarly, the combination of an anesthetic and an analgesic suppressed the cortisol response after castration, dehorning and castration + dehorning (Sutherland et al., 2013).

## 1.9 Thesis aim and overview

This thesis will address two significant gaps of knowledge in the beef-welfare-pain management literature. The first is the assessment of acute pain caused by knife and band castration at three different industry relevant ages and the efficacy of meloxicam at reducing pain responses related to castration and multiple stressors in young calves. The second is the assessment of meloxicam administration timing and the efficacy of the combination of meloxicam and lidocaine at mitigating pain related responses associated with surgical castration in weaned calves.

This project has two main components, one focused in young calves (Chapter 2 to 4) and one focused in weaned calves (Chapter 5 to 6). In Chapter 2, assessment of acute pain associated with band and knife castration in beef calves at 1 week, 2 months and 4 month old calves. In Chapter 3, assessment and mitigation of acute pain with a subcutaneous injection of meloxicam prior to band and knife castration in 1 week old calves. In Chapter 4, assessment and mitigation

of acute pain with a subcutaneous injection of meloxicam prior to knife, and the combination of knife and branding in 2 month old calves. In Chapter 5, assessment of subcutaneous meloxicam administered 6, 3 and 0 hours prior to surgical castration in 7-8 month old calves. In Chapter 6 assessment of the effect of lidocaine and meloxicam, alone or in combination in mitigating pain associated to knife castration in 7-8 month old calves. Finally, Chapter 7 which contains the main findings, a general discussion and concluding remarks.

This project will provide information on pain-related responses that can be used as reference indicators when assessing pain and testing new pain mitigation protocols.

# Chapter Two: Effect of band and knife castration of beef calves on welfare indicators of pain at three relevant industry ages: I Acute Pain

## 2.1 Abstract

Three experiments evaluated the effect of band and knife castration on acute pain for the first 7 d after the procedure on 1 wk, 2 mo and 4 mo calves. All calves were blocked by age and weight and randomly assigned to one of three treatments: sham castration (control, **CT**), band castration (**BA**), and knife castration (**KN**). Experiment 1, evaluated 1 wk old Angus bull calves ($n$ = 34; 43.0 ± 6.61 kg BW), Experiment 2, 2 mo old Angus bull calves ($n$ = 34; 91.5 ± 11.93 kg BW) and Experiment 3, 4 mo old Angus bull calves ($n$ = 35; 157.6 ± 22.52 kg BW). For all experiments, physiological and behavioral parameters were collected before (d −1 and immediately before castration (T0)), and after (60, 120 min and on d 7) castration to assess acute pain. Physiological measures included complete blood cell count (CBC), cortisol, substance P and scrotal temperature (SCT). Behavioral measures consisted of a visual analog scale (VAS), stride length, as well as time and frequency budgets for walking, standing, lying, tail flicking, foot stamping and head turning. Performance parameters included initial and final BW and average daily gain (ADG). In Exp. 1, tail flicking was greater ($P$ = 0.02) in KN calves compared to BA calves 2 to 4 h after castration, although no differences were seen between BA and KN compared to CT calves. In Exp. 2, a treatment × time interaction ($P$ = 0.02) was observed for cortisol, where KN calves had greater cortisol concentrations 120 min after castration compared to BA and CT calves, KN calves also laid down and ate less ($P$ < 0.01; $P$ = 0.02), and stood and walked more ($P$ < 0.01; $P$ = 0.05) compared to BA and CT calves 2 to 4 h after castration. In Exp. 3, a treatment × time interaction ($P$ < 0.01) was observed for cortisol concentrations where

all treatments were different from one another at 60 and 120 min, with BA calves having the greatest concentrations, KN calves were intermediate and CT had the lowest concentrations. Overall, KN and BA castrated calves presented physiological and behavioral changes associated with acute pain, however 2 mo old BA calves presented the fewest behavioral changes and no physiological changes associated with acute pain compared to CT calves.

## 2.2 Introduction

Castration is a routine management procedure used in beef production intended to stop the production of testosterone, a hormone responsible for undesirable behaviors and unwanted meat characteristics (Jacobs et al., 1977; Stafford and Mellor, 2005). The most common castration techniques include band, surgical and Burdizzo castration (Weaver et al., 2008).

The *OIE Terrestrial Animal Health Code* (Chapter 7.9) and Canadian Beef Codes of Practice (NFACC, 2013) recommend castration be done in calves as young as practically possible; however age of castration varies across production systems. One wk, 2 mo and 4 mo of age were selected as ages of interest as cow-calf operations commonly castrate calves during the first wk after birth, at 2 mo of age during processing, or at 4 mo of age when unfit bulls intended for breeding are identified. Although there are several studies comparing different castration methods (Robertson et al., 1994; Fisher et al., 2001; Pieler et al., 2013), there are few studies comparing different castration techniques in beef calves at young ages (< 6 mo of age).

Studies assessing different castration methods in young calves include a comparison of Burdizzo, rubber ring and surgical castration in calves of 6, 21 and 42 d of age (Robertson et al., 1994) and a comparison between Burdizzo, rubber ring and a combination of both methods in 1-wk old calves (Molony et al., 1995). Although detailed behavioral evaluations were done in both studies, plasma cortisol was the only physiological parameter measured. Moreover the animals

used in both studies were bottle fed dairy bull calves separated from their dams instead of beef calves that remain with their dams until weaning.

The aim of this study was to identify which method of castration causes less acute pain and distress during the first 7 d after castration in beef calves at 1 wk, 2 mo and 4 mo of age. We hypothesize that younger calves will present less indicators of acute pain after band castration.

## 2.3 Materials and Methods

This protocol was approved by the Animal Care Committees of the Lethbridge Research Centre (ACC # 1410) and the University of Calgary (AC14-0159), all animals were cared for in accordance with the (CCAC, 2009). This paper is part of a larger study evaluating acute pain (described in the present paper), and chronic pain (Marti et al., 2017) in order to determine the optimal method of castration at three different ages.

### 2.3.1 Animal housing and management

One hundred and eight Angus and Angus crossbred bull calves were used in three separate experiments based on age (described in detail following this section) at the Agriculture and Agri-Food Canada Lethbridge Research Centre (LRC; Lethbridge, Alberta, Canada). All cow-calf pairs were transported 30 km to the LRC from a neighboring ranch between April 3$^{rd}$ and April 8$^{th}$, 2014 when calves were 1 to 5 d of age. Calves were vaccinated at 3 mo of age with a single s.c. dose of a 7-way clostridial vaccine (Ultrabac®/Somubac®, Zoetis Canada Inc., Kirkland, Canada). Animals were divided into 3 groups of 36 animals to facilitate castrating the calves at different ages; 1wk, 2 mo and, 4 mo of age. Cow-calf pairs that were not in the study, were housed in holding pens (36 m × 22 m) or in the pasture immediately adjacent to the LRC feedlot facility that measured 76.9 ha and consisted of a perennial pasture mix of brome grass, orchard and creeping red fescue and annual cereal crops including oats, spring and fall triticale.

Cow-calf pairs on the study were housed in experimental pens (40 m × 27 m), each with a calf shelter (2.4 m × 3.6 m × 1.4 m) and straw bedding to protect the calves from inclement weather.

Calf diet consisted of milk from suckling and free choice alfalfa grass hay that was also fed to the cows. Free choice salt blocks and loose minerals containing a coccidiostat (Diluted Rumensin Drug Premix 1100 (Medicated), HI-PRO FEEDS, Okotoks, Alberta, Canada) were provided for the prevention of diarrhea caused by coccidiosis. Fresh water was available at all times through a centrally located water system. When housed on pasture, calf diet also consisted of suckling and free choice access to grass, salt blocks and loose minerals containing a coccidiostat (Diluted Rumensin Drug Premix 1100 (Medicated), HI-PRO FEEDS, Okotoks, Alberta, Canada) was provided. Water was available ad libitum in troughs (80 cm long × 40 cm wide × 50 cm high) that were monitored and cleaned regularly.

Calves of different ages were restrained differently during castration due to their variation in size as a safety precaution. One wk old calves were placed on their right side on a platform (100 cm × 100 cm) made of straw bales and manually restrained by one person holding the head while restraining the animal by bending the front right leg, and two persons restrained the back legs. Two mo old calves were caught, sampled and restrained using a tip table (Calf Roper, Ram-Bull Ltd, Barons, Alberta, Canada). Calves were tipped on their right side and their hind left leg was tied with a rope to the tipping table. Four mo old calves were caught, sampled and restrained in a hydraulic squeeze chute (Cattlelac Cattle, Reg Cox Feedmixers Ltd, Lethbridge, Alberta, Canada), haltered and castrated while standing in the chute. Calves were sampled for a period of 3 to 5 min while standing and manually restrained in the holding pen (1 wk and 2 mo) or standing in the chute (4 mo). One wk and 2 mo old calves were band castrated using an elastrator (Elastrator Pliers and Rings, Kane Veterinary Supplies Ltd., Edmonton, Alberta, Canada) which

stretches the band to facilitate the placement above the testicles, while a Callicrate bander (Callicrate® Bander, No-Bull Enterprise, St Francis, Kansas, USA) was used to achieve optimal tightening of the band in 4 mo old calves due to greater testicle size. Surgical castration was performed by making a latero-lateral incision on the scrotum with a scalpel in 1 wk calves, or using a Newberry castration knife (Syrvet Inc., Waukee, IA) in 2 mo and 4 mo old calves. Testicles were then externalised and the spermatic cord was crushed and cut with an emasculator. Sham control calves were handled in a similar manner as castrated calves and the testicles of the control calves were manipulated for a similar amount of time as the band and knife castrated calves to differentiate stress caused by handling. The same veterinarian performed castrations for all age groups to ensure consistency throughout the experiments. One day before castration calves and cows were fitted with matching number penning tags (between 1 and 12) applied to their backs using tag cement (Livestock Identification tag cement, W.J. Ruscoe Company, Akron, Ohio) to facilitate individual identification for behavioral observations.

### 2.3.2 Experiments design and treatments

*Experiment 1: Effect of band and knife castration in 1 wk old beef calves*

Thirty-six 1 wk old Angus bull calves (43.0 ± 6.61 kg of BW; 5.1 ± 1.16 d of age) were used in a 7-d experiment conducted between April 9$^{th}$ and 17$^{th}$, 2014. Immediately following arrival to the research feedlot, 12 cow-calf pairs were adapted to their experimental pens for 2 d before castration. The adaptation time for this group was shorter compared to Exp. 2 and 3 because calves had to be castrated within the first wk of age. Two calves became sick for reasons unrelated to the experiment on d -1 and d 7 of the trial and were removed from the study. Calves were blocked by weight and randomly assigned to one of three different treatments mixed within

the pen: sham castration (control calves, **CT**; *n* = 11), band (**BA**; *n* = 11) or knife (**KN;** *n* = 12) castration.

*Experiment 2: Effect of band and knife castration in 2 mo old beef calves*

Thirty-six 2 mo old Angus bull calves (91.5 ± 11.93 kg of BW; 62.5 ± 1.0 d of age) were used in a 7-d experiment conducted between May 25$^{th}$ and June 3$^{rd}$, 2014. Cow-calf pairs were adapted to experimental pens for a period of 5 d before castration. Three calves (between 8 and 42 d of age) died for reasons unrelated to the experiment before the start of the trial. Calves were blocked by weight and randomly assigned to one of three different treatments: sham castration (control calves, **CT**; *n* = 10), band (**BA**; *n* = 12) or knife (**KN**; *n* = 11) castration.

*Experiment 3: Effect of band and knife castration in 4 mo old beef calves*

Thirty-six 4 mo old Angus bull calves (157.6 ± 22.50 kg BW; 131.3 ± 1.34 days of age) were used in a 7-d experiment conducted between August 4$^{th}$ and 12$^{th}$, 2014. Cow-calf pairs were adapted to the experimental pens for a period of 5 d before castration. One calf (19 d of age) died for reasons unrelated to the experiment before the start of the trial. Calves were blocked by weight and randomly assigned to one of three different treatments: sham control (**CT**; n=12); band (**BA;** n=11); or knife castration (**KN**; n=12).

### 2.3.3 Measurements of acute pain and sample collection

*Cortisol*

Saliva samples were collected on d -1, immediately before castration (T0), and at 60 and 120 min and 7 d after castration. Saliva collection was done by swabbing the oral cavity with a cotton swab applicator that was stored in a plastic tube, and immediately frozen at –20°C for

further cortisol analysis using an enzyme immunoassay kit (Salimetrics LLC, State College, PA). Inter-assay and intra-assay variability values were 9.1 % and 7.9 % respectively.

*Complete blood count (CBC) and substance P*

Blood was obtained via jugular venipuncture from all calves on d -1, immediately before castration (T0), 60 and 120 min after castration, and on d 7. Blood samples were collected into two 6-mL vacuum tubes containing EDTA (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ). One EDTA tube was analyzed to determine total blood cell count using HemaTrueHematology Analyzer (Heska, Lobeland, Co) and the neutrophil-to-lymphocyte (N: L) ratio was calculated. Complete blood cell count (CBC) included red blood cells (RBC), white blood cells (WBC) and platelets. Benzamidine was added to the second blood sample (to reduce substance P degradation), centrifuged at 1.5 *g* for 15 min at 0 ºC and the serum was decanted and frozen at -80 ºC. Substance P was analyzed at Iowa State University, College of Veterinary Medicine (Ames, IA) as previously described by Van Engen et al. (2014) with some modifications. The range of detection for substance P was between 10-320 pg/mL, with an average $R^2$ =0.98. The coefficient of variation for intra-assay variability was 10.7% and the inter-assay variability was calculated at 21.0%. Limit of detection was 5 pg/mL and limit of quantitation was 10 pg/mL.

*Scrotal area temperature (SCT)*

Thermographic images of the scrotum were obtained immediately before castration (T0), 60 and 120 min, and on d 7 after castration, using a FLIR I60 infrared camera (FLIR Systems Ltd Burlington, Ontario, Canada). Images were taken from behind the animal at a distance of 1m from the scrotal area in all animals. Additional temperature recordings of the area of the scrotum

were possible in KN castrated calves after castration as an incision was made versus cutting the lower part of the scrotum. Scrotal temperature data was obtained using FLIR Tools v.5.1 (FLIR Systems Ltd Burlington, Ontario, Canada) to delineate the area of the scrotum and record the temperature. An emissivity coefficient of 0.98 was used to analyze the images for maximum temperatures.

*Rectal temperature (Temp)*

Rectal temperature was measured on d -1, T0 and on d 7 using a digital thermometer (GLA M750 Livestock Thermometer, San Luis Obispo, CA).

*Performance*

Animals were weighed on d -1 (initial BW) as well as at the end of the experiment on d 7 (final BW) using a portable scale and ADG (kg/d) was calculated by subtracting the d 7 weights from d -1 weights and dividing the result by the number of days calves were on trial (9 d).

*Visual Analog Scale (VAS)*

Behavioral observations during the time of castration were made by two experienced observers. The observers were located 2 m from the animal and used a VAS sheet to score behavioral responses indicative of pain and discomfort at the time of castration including leg movement (kick or leg lifting), urination, defecation and vocalization. The observers indicated their overall impression of the amount of pain the animal was experiencing by placing a mark along a rating scale (10-cm horizontal line), with the far left indicating no pain response and the far right representing an extreme pain response. The distance from the start of the line to the mark was measured to the nearest 0.5 cm and was used as an indicator of pain response to

castration (Ludington and Dexter, 1998; Moya et al., 2014). Observers could not be blind to treatments due to the nature of the experiment.

*Behavioral observations*

All the calves in the three experimental pens were continuously video recorded using two Avigilon cameras (2.0MP HD IR Bullet Camera, Avigilon®) located on the north and south side of each pen mounted on a 6 m pole. Focal animal sampling and continuous recordings (Martin and Bateson, 2007) were conducted by one experienced observer between 2 and 4 h after castration when calves returned to the experimental pen (after 120 min sampling). In addition, focal sampling and continuous recordings were conducted by a total of 4 experienced observers but limited to 2 observers per experiment for a period of 2 min every 10 min from 0800 to 1400 on d 1, 2, 3 and 5 after castration for a subset of 6 animals per treatment. Duration behaviors included standing, lying (lateral and ventral), walking, eating and not in sight, while tail flick, foot stamping, head turning and lesion licking were classified as frequency behaviors (described in detail in Table 2.1) and were recorded using The Observer® XT (Noldus Information Technology, Wageningen, The Netherlands). Intra-class correlation coefficient values were 0.93 for inter-rater reliability and 0.91 for intra-rater reliability.

*Accelerometer recordings*

On d -1 of each experiment, all calves were fitted with an accelerometer (Hobo pendant G, Onset Computer Corporation, Bourne, MA) to determine total standing and lying duration (min/day) which was converted into a percentage, standing and lying duration (mean standing and lying bout duration (min)) as well as standing and lying bouts (frequency/day (UBC AWP, 2013)). Accelerometers were wrapped with plastic film, to protect the device from moisture, and foam to eliminate rubbing on the hind leg. Vet Wrap was used to strap the accelerometer to the

hind leg. Accelerometers were removed on d 7 post-castration and only information from days where information was collected for 24 h was used (d 0 to d 6).

*Stride length*

Stride length was obtained by video recording the calves walking through an alley (1 m wide and 3 m long) on d -1, immediately after castration, 120 min and on d 7 after castration. GOM Player (GOM Lab, Gretech Corporation, Seoul, South Korea) was used to take 2 pictures of the back legs when both feet were flat on the ground or close to the ground in case animals were running. Stride length was measured using ImageJ (Bethesda, MD) by measuring the distance between the middle of each foot and obtaining the average of both pictures as previously described by (Currah et al., 2009).

### 2.3.4 Statistical Analysis

Data was analysed using the Mixed Models procedure in SAS (SAS 9.4, SAS Inst. Inc., Cary, NC) to assess the effects of castration method and time on all variables. Time and treatment were considered as fixed effects and were tested for interactions, while pen was considered a random effect. Covariance structures included unstructured, compound symmetry and autoregressive order 1. The covariance structure with the lowest Schwarz's Bayesian information criterion was selected as the analysis of preference. Schematic boxplots for outlier detection was used which resulted in the removal of 3 data points across all animals and variables. Non-normally distributed cortisol and substance P data were log transformed, percentage data was arcsine transformed, and the rest of the physiological and behavioral parameters were square-root transformed to achieve normality. Physiological data for T0, 60 and 120 min after castration was analyzed separately from data on d 0 and d 7. Behavioral data from 2 to 4 h after castration was analyzed separately from d 1, 2, 3 and 5 after castration. Results

were presented as LS Mean ± SEM. Data collected on d -1 was used as a covariate for all physiological variables and stride length, however, covariates that were not significant were removed from the model. The experimental unit was the animal, as treatments were mixed within the pen. All data, with the exception of VAS and performance, were analysed in a mixed repeated measures model. Significance was determined when $P \leq 0.05$ and tendencies when $0.05 < P \leq 0.10$. When interactions were significant a post-hoc PDIFF test was used to compare adjusted means. An intra-class correlation coefficient (ICC) with a 95 % CI was used to determine behavior inter-rater and intra-rater reliability using IBM SPSS statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA).

## 2.4 Results and Discussion

### 2.4.1 Experiment 1. Effect of band and knife castration on 1 wk old beef calves

No treatment or interaction effects ($P > 0.10$) were observed for cortisol (Fig. 2.1A) or substance P 60 and 90 min after castration (Table 2.2). No differences ($P > 0.10$) were observed for cortisol, substance P, Temp or CBC on d 0 and d 7, or for performance on d -1 and d 7 (Table 2.3). Lack of differences in cortisol concentrations have been previously reported by Mellor et al. (1991) who did not observe differences between rubber ring and uncastrated 1 wk old calves up to 4 h after castration. The author suggested that a lack of differences could be due to management, as calves were hand reared and isolated from their dams and other calves. In the present study no differences were seen in cortisol, however, KN calves had numerically greater cortisol concentrations, followed by BA calves and CT calves with the lowest concentrations. These findings are in agreement with other studies reporting greater plasma cortisol concentrations after surgical castration compared to Burdizzo and rubber ring castration in 1 wk old calves (Robertson et al., 1994; Molony et al., 1995). Dockweiler et al. (2013) suggested that

young calves may be less distressed by handling and castration compared to older calves, while Stafford and Mellor (2005) suggested that separation from the dam and handling had such an aversive effect that castration did not affect the HPA axis. In the present study, baseline cortisol concentrations for 1 wk old calves were greater (data not shown) than in 2 and 4 mo old calves, supporting the notion that separation from their dams and handling 1 wk old calves is so aversive that a cortisol 'ceiling effect' was reached (Molony and Kent, 1997) before castration.

Scrotal temperature was greater ($P = 0.03$) in CT compared to KN and BA calves 60 and 90 min after castration (Table 2.2). A treatment × time interaction ($P < 0.01$) for SCT indicated that BA calves had lower values compared to CT and KN calves on d 7 (Table 2.3). Lower SCT detected in BA and KN calves may be due to the interruption of blood flow caused by the placement of the band, in BA calves, and the removal of the testicles in KN calves. A rise in temperature indicative of inflammation was not observed in BA or KN calves during the hours following castration. It is possible that the absence of temperature rise in KN calves was due to limited tissue damage because of smaller testicular size. Bretschneider (2005) also suggested that the amount of discomfort experienced by calves after castration could be associated with testicular size whereby older calves with larger, more developed testicles experienced greater discomfort than those with smaller and less developed testicles. In contrast, ST on d 7 in KN calves did not differ from CT calves. This may be indicative of scrotal inflammation as KN calves would have had lower SCT compared to CT calves due to the removal of the testicles.

VAS scores were greater ($P < 0.01$) in KN and BA calves compared to CT calves. No treatment differences ($P > 0.10$) were observed for urination, defecation, leg movement and vocalization at the time of castration (Table 2.4). The KN calves also had greater ($P = 0.02$) tail flick frequencies 2 to 4 h after castration compared to CT calves, however, no differences were

seen between both of those groups and BA calves. No differences ($P > 0.10$) were seen in behavioral observations for lying, standing, walking, eating, foot stamping and head turning 2 to 4 h after castration (Table 2.5). Greater values in VAS scores and tail flicks in KN castrated calves may be explained by the activation of nociceptors at the time of the scrotal incision and the crushing and cutting of the spermatic cords which would have generated immediate discomfort, followed by the activation of chemical nociceptors once inflammatory substances are released as a response to tissue damage (Muir III and Woolf, 2001). Activation of mechanical nociceptors is also expected in band castration due to the force exerted by the band on the neck of the scrotum, while activation of chemical nociceptors would occur at the time of tissue hypoxia and anoxia (Gebhart and Ness, 1991). However, activation of mechanical nociceptors could take longer, especially in young calves, where pressure could be less intense due to the reduced size of the scrotal neck in comparison to older calves.

A treatment × time interaction ($P < 0.01$) was observed for stride length where KN calves had shorter strides compared to BA calves immediately after castration. However, no differences were seen between both groups and CT calves (Table 2.6). Stride length has been previously used as an indicator of pain associated with castration in 3 mo old calves, as castrated calves have been shown to decrease stride length which was assumed to be a behavioral modification to reduce discomfort during walking (Currah et al., 2009). These findings are in agreement with cortisol and VAS, and suggest that KN calves experienced more discomfort immediately after castration.

Accelerometer recordings presented a treatment × time interaction ($P = 0.02$) for lying duration, where BA calves lay down for longer periods of time on d 2 compared to KN and CT calves, and on d 3 compared to CT calves. A treatment × time interaction ($P < 0.01$; $P = 0.03$)

was also observed for standing and lying bouts. BA calves had a lower number of lying and standing bouts on d 2 and 3 compared to CT calves, but no differences were seen between CT and BA calves to KN calves. No effect of castration method ($P > 0.10$) was seen in behavioral observations for lying, standing, walking, eating, tail flicking, foot stamping, head turning and lesion licking on d 1, 2, 3 and 5 after castration or in accelerometer recordings for standing duration, and standing and lying percentage from d 0 to d 6 (Table 2.5).

Behavioral changes in BA calves on d 2 and 3 after castration may have been due to an attempt to avoid stretching the affected tissue, as transitions between standing and lying involves extension of the hind legs when the front legs are bent. This particular position could generate pain and/or discomfort in BA calves and therefore, they may avoid standing resulting in lying down for longer periods of time. Although KN calves did not differ from CT calves, they had numerically greater lying durations and lower standing and lying bouts compared to control calves on d 2 and d 3 after castration, similar to the behavioral changes seen in BA calves. Changes in lying and standing behavior in 1 wk old calves have been reported in previous studies and include reduced sternal lying after surgical castration (Brown et al., 2015) and increased abnormal standing (Robertson et al., 1994). Abnormal standing was defined as postures rarely adopted by control calves, which included standing with a hunched back and trembling or standing with legs stretched back. Although no statistical differences were detected in the number of tail flicks, BA calves had numerically twice as many tail flicks as CT calves. One possible explanation for the lack of differences between BA and CT calves may be the high individual animal variation which could mask treatment effects. This is reflected by the SE which was 1.6 to 11 times greater for tail flicking compared to the rest of the behaviors.

Overall, KN castrated calves exhibited a greater number of pain-related indicators the day of castration (stride length and tail flicking), while BA calves exhibited pain related behaviours on d 2 and 3 (lying duration and standing and lying bouts) after castration. Therefore, knife castration in 1 wk old calves causes discomfort for a shorter period of time than band castration.

### 2.4.2 Experiment 2. Effect of band and knife castration on 2 mo old beef calves

A treatment × time interaction ($P = 0.01$; Fig. 2.1B) was observed for cortisol. The KN calves had greater concentrations of cortisol compared to CT calves 120 min after castration, but KN and CT calves did not differ from BA calves (Table 2.2). Peak cortisol concentrations in KN castrated calves were detected 120 min after castration, contrary to previous studies which have reported maximum cortisol concentrations 60 to 90 min after castration in 2 to 4 mo old calves (Stafford et al., 2002) and 1 h after castration in 4 to 11 wk old calves (Fell et al., 1986). In the present study, peak cortisol concentrations could have occurred before 60 or 120 min sampling, which may explain differences in peak cortisol times reported in previous studies.

No treatment differences ($P > 0.10$) were observed for substance P 60 and 90 min after castration (Table 2.2). There was a treatment × time interaction tendency ($P = 0.09$) in which CT tended to have greater N:L ratio than BA calves 7 d after castration, but no differences were seen between both groups and KN calves. No differences ($P > 0.10$) were observed in cortisol, substance P, Temp, WBC, RBC, platelets or performance on d 0 or d 7 after castration (Table 2.3). Possible explanations for lack of differences include: a low sample size, the possibility that changes in physiology might have occurred between d 1 and 6 when calves were not sampled, the measurements assessed were not sensitive enough to detect pain, or that by d 7 castrated calves were not experiencing pain associated to castration.

The VAS scores differed ($P < 0.01$) between each of the treatments with KN calves having the greatest score, followed by BA calves and CT calves. The KN calves had greater ($P \leq 0.05$) leg movements and vocalizations at the time of castration than BA and CT calves. However, no differences ($P > 0.01$) were seen in urination or defecation at the time of castration (Table 2.4). Consistent with the findings in cortisol, VAS scores, leg movements and vocalizations were greater in KN calves. Similar findings were described in a previous study where severe struggling and kicking of the back legs was reported at the time of surgical castration in 4 to 11 wk old calves, followed by rubber ring castration with less struggling and stamping of the hind legs at the time of castration in comparison to control calves which displayed normal behavior at the time of handling (Fell et al., 1986). These results suggest that calves actively react to knife castration by increasing leg movements and vocalizations as a response to acutely painful stimuli such as knife castration. As described in 1 wk old calves, changes in behavior could be due to the activation of mechanical nociceptors at the time of incision and the cutting and crushing of the spermatic cords. The VAS results indicate that band application causes discomfort. However, lack of differences in cortisol between CT and BA calves suggests that the discomfort experienced was not sufficient to generate a measurable activation of the pituitary-adrenal-axis. Although activation of chemical nociceptors would occur during the onset of hypoxia and anoxia (Gebhart and Ness, 1991) behavioral (with the exception of VAS) and physiological indicators of pain were undetectable minutes and days after castration.

A treatment × time interaction ($P = 0.03$) was also observed for SCT, where BA calves had lower SCT 60 min after castration compared to KN calves, and to KN and CT calves at 120 min after castration (Table 2.2). Scrotal temperature also presented a treatment × time interaction

($P < 0.01$) on d 7, where BA calves had lower temperatures compared to KN and CT calves (Table 2.3). Lack of differences in SCT between KN and CT calves is likely due to the release of inflammatory substances after tissue damage (Muir III and Woolf, 2001) which would increase scrotal temperature.

Two mo old calves had differences ($P \leq 0.05$) in behavioral observations for lying, standing, walking and eating durations from 2 to 4 h after castration. The KN calves spent less time eating and lying down and more time standing and walking compared to BA and CT calves. No treatment differences ($P > 0.10$) were seen in stride length immediately after castration and 120 min after castration, or in tail flicking, foot stamping and head turning 2 to 4 h after castration (Table 2.5). A treatment effect tendency ($P = 0.09$) was observed in behavioral observations for eating, where KN tended to eat for longer periods of time compared to BA and CT calves on d 1, 2, 3 and 5. A treatment × time interaction ($P = 0.06$) was also observed for head turning, where CT had a greater number of head turns than BA calves on d 3 after castration. No differences ($P > 0.10$) were seen in behavioral observations for lying, standing, walking, tail flicking, foot stamping, and lesion licking on d 1, 2, 3 and 5 after castration (Table 2.6).

Accelerometer recordings showed greater standing ($P < 0.01$) and lower lying ($P < 0.01$) percentages in KN calves compared to BA and CT calves. Standing and lying durations presented a treatment × time interaction tendency ($P = 0.06$; $P = 0.08$) where KN calves tended to have greater standing durations than CT calves on d 0, 1 and 5, and to CT and BA calves on d 2, while CT calves tended to have greater lying durations compared to BA and KN calves on d 1 after castration. No treatment differences ($P > 0.10$) were seen in accelerometer recordings for

standing and lying bouts up to 6 d after castration. In addition, there were no treatment effects ($P > 0.10$) for stride length on either d 0 or d 7 after castration (Table 2.6).

Inflammatory substances can reduce the pain threshold and activate mechanical and thermal nociceptors, that under normal circumstances wouldn't be activated (Tranquilli et al., 2013). Changes in behavior during the days following castration could be caused by the discomfort of reduced pain thresholds. These findings are similar to a previous study indicating an increase in standing time (82.2 %) after castration compared with prior to castration (37.9 %) during the first 24 h in 278.1 kg mixed beef calves (White et al., 2008).

Previous studies have reported elevated behavioral and physiological indicators of stress and pain in knife castrated compared to band and uncastrated calves at 2 mo of age (Fell et al., 1986; Stafford et al., 2002; Schwartzkopf-Genswein et al., 2005). Contrary to findings by Robertson et al. (1994) in 42 d old band castrated calves, in the present study, no physiological or behavioral indicators of pain, with the exception of VAS, were observed in BA calves compared to CT calves.

Overall, 2 mo old KN calves exhibited behavioral and physiological indicators of pain, while BA calves only differed from CT calves in the VAS score. The KN castrated calves had greater cortisol, SCT, VAS scores, standing percentage and walking duration as well as decreased eating and lying behaviors the day of castration, while greater standing and lower lying percentages were observed during the first 7 d after castration. These results suggest that knife castration is more painful than band castration in 2 mo old calves.

### 2.4.3 Experiment 3. Effect of band and knife castration on 4 mo old beef calves

A treatment × time interaction ($P < 0.01$; Fig. 2.1C) was seen for cortisol were each treatment differed from each other 60 and 120 min after castration. BA calves had greater cortisol concentrations, followed by KN calves and CT calves. This is the first study conducted in calves under 4 mo of age to find that cortisol was greatest in banded calves; all previous studies conducted with calves between 1 wk and 4 mo of age reported greater cortisol concentrations in surgically castrated compared to rubber ring, banded and uncastrated calves (Fell et al., 1986; Robertson et al., 1994; Molony et al., 1995). This could be explained by the use of a Calicrate bander in 4 mo old calves, in which the tension applied is mechanical, thus the ischemia, which can induce pain (Mellor and Stafford, 2000) may occur sooner than in younger calves.

A treatment × time interaction ($P < 0.01$) was observed for SCT where BA calves had lower SCT compared to CT and KN calves 60 and 120 min after castration (Table 2.2). A treatment × time interaction ($P < 0.01$) was observed for SCT, where BA calves had lower SCT on d 7 after castration compared to KN and CT calves (Table 2.3). This could be due to a greater vascularization of the testicles in older calves which could explain the lack of differences in SCT between KN and CT calves and the reason why the SCT was maintained in 4 mo old BA castrated calves.

No treatment differences ($P > 0.10$) were seen for substance P from 60 to 120 min after castration (Table 2.2). Average daily gain was greater ($P = 0.04$) in CT calves compared to KN calves, however no differences were seen between both groups and BA calves. No treatment differences ($P > 0.10$) were seen for cortisol, substance P, Temp, or CBC on d 0 and d 7 after castration (Table 2.3). Substance P is a neuroactive peptide responsible for modulating

nociception, emotional behavior and stress found across the central nervous system (DeVane, 2001). In the present study no differences were observed for this parameter across ages, however, to our knowledge substance P hasn't been previously assessed in calves at young ages after castration. Lack of differences could also be due to sample number, high individual variation or high inter and intra assay CV.

VAS scores were greater ($P < 0.01$) for KN calves, followed by BA calves with CT calves having the lowest score. Leg movements and vocalizations were greater ($P < 0.01$; $P = 0.01$) in KN compared to BA and CT calves. No differences ($P > 0.01$) were seen in urination or defecation at the time of castration (Table 2.4). This suggests that KN calves experienced more discomfort than BA calves, however BA calves still experienced some degree of discomfort during the application of the band as VAS scores are greater than CT calves.

A treatment × time interaction ($P = 0.02$) was observed for stride length. The KN calves had shorter stride length compared to BA and CT calves immediately after castration. In addition, KN calves tail flicked more ($P < 0.01$) and had a tendency to foot stamp ($P = 0.06$) more compared to BA and CT calves 2 to 4 h after castration. No treatment differences ($P > 0.10$) were observed for behavioral observations for lying, standing, walking, eating or head turning 2 to 4 h after castration (Table 2.5). The fact that greater VAS scores, leg movements, vocalizations, tail flicks, foot stamps and shorter stride length were observed in KN calves during and after castration suggests that KN calves experienced more discomfort than BA calves. Although band placement causes discomfort as seen by greater VAS scores than CT calves and greater cortisol concentrations 60 and 120 min after castration compared to KN and CT calves, it is likely that the discomfort experienced is not sufficient to generate a change in stride and may explain why there were no differences in stride between CT and BA calves. Contrary to our

findings, reduced hind stride length was reported in 210 d old band castrated calves compared to control calves (González et al., 2010). However caution should be taken when comparing results between different age groups as physiological and behavioral differences can vary considerably and experiments were performed at different times during the year.

Accelerometer recordings and behavioral observations showed a treatment × time interaction ($P \leq 0.05$) for standing and lying behavior, where KN calves had greater standing percentage (Fig. 2.2A) and standing duration (Fig. 2.2B) on d 1, 2 and 3 and greater mean lying duration on d 2, 3, 4 and 5 after castration. A greater ($P < 0.01$) number of standing (Fig. 2.2C) and lying bouts were seen in BA and KN calves than CT calves on d 0, while BA had greater number of standing and lying bouts on d 1 and 2 after castration. A treatment × time interaction ($P < 0.01$) was observed for tail flicking, foot stamping and lesion licking. Greater frequencies of tail flicks, foot stamps and lesion licks were observed in KN calves on d 1, 2 and 3 compared to BA and CT calves. The KN calves also had a greater number of tail flicks than CT calves and a greater number of foot stamps than BA calves on d 5, however no differences were seen in tail flicks between KN and CT calves compared to BA calves, or in foot stamps between KN and BA calves compared to CT calves. No differences ($P > 0.10$) were seen in behavioral observations for lying, walking, eating and head turning or for accelerometer recordings for lying percentage (Table 2.6).

The KN and BA calves presented significant changes in behavior. Independent of the method of castration, increased restless behavior (greater standing and lying bouts) is evidence of discomfort and is commonly seen after rubber ring castration (Robertson et al., 1994; Molony et al., 1995), however, the method of castration (band or knife) had a different effect on standing and lying duration. We speculate that BA calves reduced standing duration as a standing position

may be more likely to trigger nociceptors around the band causing discomfort as the weight of the testicles in a standing position could exert tension on the affected area surrounding the band, while tension could be reduced in a lying position. In addition, increased standing and lying bouts in BA calves could be due to discomfort experienced while standing and lying which would increase the number of transitions between postures, or the increase in bouts could be a coping mechanism, as motor fibers activate inhibitory neurons which can reduce the transmission of pain signals to the brain (Tranquilli et al., 2013). In contrast, KN calves increased standing duration, likely because the hind legs or the weight of the animal is not exerting pressure on the affected area as it would in a lying position. The KN calves stood and laid down for longer periods of time and tail flicked, foot stamped and lesion licked more on d 1 and d 2 after castration compared to BA and CT calves, suggesting the level of discomfort experienced by KN calves was greater. Standing and lying bouts were greater in BA calves compared to CT calves on d 2 indicating that BA castrated calves were still experiencing some level of discomfort. Unfortunately we could not compare our findings to previous studies as there is a lack of literature on behavior of 4 mo old calves after knife and band castration.

Overall, 4 mo old BA and KN calves had behavioral and physiological changes indicative of pain during and after castration. The BA calves presented greater cortisol concentrations 60 and 120 min after castration and greater restless behavior 2 to 3 d after castration; while KN calves had greater VAS scores, leg movement and vocalizations at the time of castration, shorter stride length immediately after castration, a greater number of tail flicks 2 to 4 h after castration, and greater standing activity for the first 5 d after castration. It is clear that both KN and BA castrations are painful methods, however behavioral responses associated with pain lasted for a longer period of time after KN castration.

### 2.4.4 Acute pain in 1 wk, 2 mo, and 4 mo calves

Acute pain is transient and is the result of the activation of pain pathways by noxious stimulus as an alarm signal in order to identify wounds or injuries (Serrie and Serviere, 2014). Acute pain has a protective role as it promotes healing and tissue repair (Muir III and Woolf, 2001), and depending on the type of tissue injury it can last for hours, days or weeks. In the present study acute pain was defined as the period of time from the start of castration until 7 d post castration.

Knife and band castration cause behavioral changes indicative of acute pain in all age groups assessed in this study, however, only 2 and 4 mo old calves presented physiological differences associated with pain. The only physiological parameters that differed between treatments were cortisol concentrations and SCT. Behavioral differences in 1 wk calves were detected up to d 3 after castration, while 2 mo and 4 mo calves presented differences up to d 6 and 5 respectively after castration. The KN castrated 4 mo old calves presented a greater number of behavioral indicators of pain compared to 2 mo old KN castrated calves however, comparison of the magnitude of differences between both age groups cannot be done, as behaviours and time points in which behavioral differences are observed vary between groups. The differences in behavior and physiology seen at different ages could also be due to the different methods of castration as 1 wk and 2 mo calves were banded with the same rubber rings which could cause more pressure in 2 mo old calves due to size of the scrotal neck. While 4-mo old calves were banded with a latex band using a Calicrate bander which can exert more pressure on the neck of the scrotum compared to applying a rubber ring with an elastrator. Incision of the scrotum in 1 wk old was done with a scalpel while 2 mo and 4 mo calves were castrated using a Newberry knife which could generate a different pain response as the Newberry knife requires cutting and

pulling at the same time while the scalpel is just cutting, and this may create a different sensation.

Identifying the most welfare friendly method of castration is challenging in 1 wk and 4 mo old calves because several behavioral and physiological changes were observed after BA and KN castration compared to CT calves. However, 1wk old KN castrated calves displayed differences in behaviors on the day of castration, while BA castrated calves displayed changes in behavior up to 2 and 3 d after castration suggesting that knife castration caused pain/stress for a shorter period of time compared to band castration. In contrast, 4 mo old KN castrated calves presented differences in behavior up to 5 d after castration, while BA calves exhibited differences up to 3 d after castration. Consequently, band castration in 4 mo old calves caused acute pain for a shorter period of time compared to knife castration. Interestingly, 2 mo old BA castrated calves did not present differences compared to CT calves with the exception of VAS. These results suggest that BA castration results in fewer indicators of pain and based on this may be a preferred method in 2 mo old calves compared to KN castration. It is important to clarify that the suggestions made are based on acute pain that occurs in the first 7 d after castration and it is essential to take into account the second part of this study which focuses on chronic pain (Marti et al., 2017) in order to be able to accurately select the most welfare friendly castration method.

This study shows that independent of age, band and knife castration produce acute pain in beef calves. Pain mitigation of painful husbandry procedures such as castration is of great importance in order to improve animal welfare. However, access to pain mitigation is limited in different parts of the world, therefore, based on our results, a non-therapeutic way to reduce pain associated with castration would be to band castrate calves younger than 2 mo of age. Future

studies are required to identify effective pain mitigation drugs specific to castration at young ages, as it is clear that this common husbandry practice causes pain and discomfort.

Table 2.1. Ethogram of behaviors recorded after castration modified from Molony et al. (1995).

| Behavior | Definition |
|---|---|
| Eating | Suckling from the udder or ingesting hay or straw from the ground or the feeder |
| Lying | Either lateral (laying with hip and shoulder on the ground with at least 3 limbs extended) or ventral (laying in sternal recumbency with legs folded under the body or one hind or front leg extended) lying |
| Walking | Walking forward more than 2 steps |
| Standing | Standing on all four legs |
| Not in sight | Calf was out of the observer's sight because it was inside the calf shelter, behind a cow or calf, or there is no visibility of the hindquarters |
| Foot stamping | Hind legs are lifted and forcefully placed on the ground or kicked outwards while standing |
| Head turning | When the head is turned and touches the side of the calf's body when standing, including head turning to groom |
| Tail flicking | Forceful tail movement beyond the widest part of the rump when standing, movement to one side is counted as one action |
| Lesion licking | Head turning to lick the lesion caused by castration while standing |

Table 2.2. Least square means (±SEM) of samples taken at T0, 60 and 120 min after castration for cortisol, substance P, and scrotal temperature (SCT) of non-castrated, band-castrated and knife-castrated Angus crossbred bulls castrated at 1 wk, 2 mo and 4 mo of age[1].

| | Treatment (T) | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Item | Control | Band | Knife | SEM[2] | T | Time | T × Time |
| ***1 wk*** | | | | | | | |
| Cortisol, nmol/L | 4.9 | 6.0 | 6.6 | 0.19 | 0.31 | 0.05 | 0.76 |
| Substance P, pg/mL | 92.6 | 108.7 | 100.6 | 0.01 | 0.12 | 0.07 | 0.47 |
| SCT, ºC | 31.5[a] | 29.4[b] | 29.5[b] | 0.07 | 0.03 | 0.16 | 0.22 |
| ***2 mo*** | | | | | | | |
| Cortisol, nmol/L | 3.3 | 3.9 | 4.9 | 0.18 | 0.36 | <0.01 | 0.01 |
| Substance P, pg/mL | 73.5 | 70.1 | 66.8 | 0.05 | 0.57 | 0.44 | 0.76 |
| SCT, ºC | 34.1[ab] | 33.0[b] | 34.8[a] | 0.32 | <0.01 | 0.50 | 0.03 |
| ***4 mo*** | | | | | | | |
| Cortisol, nmol/L | 2.9[c] | 9.1[a] | 6.8[b] | 0.09 | <0.01 | <0.01 | <0.01 |
| Substance P, pg/mL | 102.9 | 101.8 | 102.5 | 0.06 | 0.83 | <0.01 | 0.68 |
| SCT, ºC | 36.1[a] | 34.9[b] | 35.7[a] | 0.02 | <0.01 | <0.01 | <0.01 |

[a-c] Least square means within a row with differing superscripts differ ( $P \leq 0.05$).
[1]Values in table represent the mean of T0, 60 and 120-min samples, where T0 is immediately before castration.
[2]The values presented correspond to non-transformed means, however SEM and *P*-values correspond to ANOVA analysis using log or square root + 1 transformed data except for SCT for 2 mo old calves.

Table 2.3. Least square means (±SEM) of d 0 and d 7 for cortisol, substance P, body temperature (Temp), scrotal temperature (SCT), CBC (WBC, RBC, Platelets, and N:L ratio) and performance (initial BW, Final BW, and ADG) of non-castrated, band-castrated and knife-castrated Angus crossbred bulls castrated at 1 wk, 2mo and 4 mo of age[1].

| | Treatment (T) | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Item | Control | Band | Knife | SEM[2] | T | Time | T × Time |
| ***1 wk*** | | | | | | | |
| Cortisol, nmol/L | 4.0 | 4.6 | 4.2 | 0.16 | 0.86 | 0.63 | 0.35 |
| Substance P, pg/mL | 72.1 | 69.8 | 68.0 | 0.05 | 0.73 | 0.61 | 0.79 |
| Temp, ºC | 39.3 | 39.3 | 39.1 | 0.15 | 0.12 | 0.41 | 0.91 |
| SCT, ºC | 30.0[a] | 25.1[b] | 29.4[a] | 0.11 | <0.01 | <0.01 | <0.01 |
| CBC | | | | | | | |
| WBC, $10^9$/L | 10.1 | 9.3 | 10.1 | 0.10 | 0.67 | <0.01 | 0.17 |
| RBC, $10^{12}$/L | 7.9 | 8.4 | 8.1 | 0.21 | 0.24 | <0.01 | 0.67 |
| Platelets, $10^9$/L | 505.7 | 539.7 | 540.8 | 35.8 | 0.69 | 0.27 | 0.99 |
| N:L ratio | 0.8 | 0.7 | 0.9 | 0.05 | 0.56 | 0.07 | 0.95 |
| Performance | | | | | | | |
| Initial BW[1](d -1), kg | 41.2 | 44.4 | 43.6 | 1.59 | 0.35 | - | - |
| Final BW[2] (d 7), kg | 51.0 | 53.7 | 52.2 | 1.81 | 0.59 | - | - |
| ADG, kg/d | 1.3 | 1.4 | 1.3 | 0.07 | 0.71 | - | - |
| ***2 mo*** | | | | | | | |
| Cortisol, nmol/L | 1.9 | 1.5 | 1.7 | 0.15 | 0.16 | 0.52 | 0.81 |
| Substance P, pg/mL | 72.2 | 70.6 | 68.9 | 0.18 | 0.88 | 0.67 | 0.57 |
| Temp, ºC | 39.3 | 39.3 | 39.1 | 0.15 | 0.12 | 0.41 | 0.91 |
| SCT, ºC | 33.0[a] | 29.2[b] | 33.7[a] | 0.03 | <0.01 | <0.01 | <0.01 |
| CBC | | | | | | | |
| WBC, $10^9$/L | 11.7 | 11.8 | 11.4 | 0.05 | 0.66 | 0.95 | 0.21 |
| RBC, $10^{12}$/L | 11.3 | 11.6 | 10.9 | 0.002 | 0.41 | 0.73 | 0.99 |
| Platelets, $10^9$/L | 795.7 | 773.4 | 797.3 | 0.04 | 0.13 | 0.74 | 0.63 |
| N:L ratio | 0.7 | 0.6 | 0.6 | 0.02 | 0.88 | 0.01 | 0.09 |
| Performance | | | | | | | |
| Initial BW (d -1), kg | 90.9 | 92.5 | 91.1 | 1.73 | 0.79 | - | - |
| Final BW (d 7), kg | 94.3 | 95.1 | 94.1 | 1.81 | 0.93 | - | - |
| ADG, kg/d | 0.5 | 0.5 | 0.4 | 0.09 | 0.84 | - | - |
| ***4 mo*** | | | | | | | |
| Cortisol, nmol/L | 2.7 | 3.6 | 2.7 | 0.10 | 0.26 | 0.11 | 0.31 |
| Substance P, pg/mL | 103.5 | 101.5 | 101.1 | 6.61 | 0.96 | 0.76 | 0.65 |
| Temp, ºC | 39.3 | 39.3 | 39.1 | 0.15 | 0.12 | 0.41 | 0.91 |
| SCT, ºC | 36.2[a] | 33.9[b] | 36.5[a] | 0.02 | <0.01 | <0.01 | <0.01 |
| CBC | | | | | | | |
| WBC, $10^9$/L | 11.0 | 11.0 | 10.2 | 0.06 | 0.33 | 0.22 | 0.83 |
| RBC, $10^{12}$/L | 10.6 | 10.9 | 11.0 | 0.02 | 0.29 | 0.14 | 0.41 |
| Platelets, $10^9$/L | 612.0 | 616.8 | 660.2 | 0.03 | 0.81 | 0.26 | 0.44 |
| N:L ratio | 0.6 | 0.6 | 0.6 | 0.02 | 0.69 | <0.01 | 0.20 |
| Performance | | | | | | | |
| Initial BW (d -1), kg | 160.6 | 158.2 | 154.0 | 0.27 | 0.64 | - | - |
| Final BW (d 7), kg | 174.1 | 173.6 | 170.4 | 3.2 | 0.65 | - | - |
| ADG, kg/d | 1.9[a] | 1.6[ab] | 1.4[b] | 0.04 | 0.04 | - | - |

[a,b] Least square means within a row with differing superscripts differ ($P \leq 0.05$)

[1]Values in table represent the mean of d 0 and d 7 samples.

[2]CBC = complete blood cell count: WBC = white blood cell count; RBC = red blood cell count; N:L ratio = neutrophil-to-lymphocyte ratio.

[3]The values presented correspond to non-transformed means; however SEM and *P*-values correspond to ANOVA analysis using log or square root + 1 transformed data with the exception of Substance P for Exp. 3.

Table 2.4. Least square means (±SEM) at the time of castration for visual analog scale (VAS) scores and frequencies of urination, defecation, leg movement and vocalization of noncastrated, band-castrated and knife-castrated Angus crossbred bulls castrated at 1 wk, 2 mo and 4mo of age.

| | Treatment (T) | | | | *P*-value |
|---|---|---|---|---|---|
| Item | Control | Band | Knife | SEM[1] | T |
| ***1 wk*** | | | | | |
| VAS | 1.3[b] | 1.8[a] | 2.0[a] | 0.13 | <0.01 |
| Urination | 1.1 | 1.1 | 1.0 | 0.04 | 0.40 |
| Defecation | 1.0 | 1.0 | 1.0 | 0.01 | 0.32 |
| Leg movement | 1.0 | 1.0 | 1.0 | 0.01 | 0.35 |
| Vocalization | 1.0 | 1.0 | 1.0 | 0.02 | 0.74 |
| ***2 mo*** | | | | | |
| VAS | 1.2[c] | 1.4[b] | 2.2[a] | 0.12 | <0.01 |
| Urination | 1.1 | 1.0 | 1.0 | 0.25 | 0.26 |
| Defecation | 1.0 | 1.0 | 1.0 | 0.02 | 0.69 |
| Leg movement | 1.3[b] | 1.3[b] | 2.0[a] | 0.14 | <0.01 |
| Vocalization | 1.0[b] | 1.1[b] | 1.2[a] | 0.07 | 0.05 |
| ***4 mo*** | | | | | |
| VAS | 1.2[c] | 1.5[b] | 2.8[a] | 0.06 | <0.01 |
| Urination | 1.1 | 1.0 | 1.0 | 0.03 | 0.60 |
| Defecation | 1.0 | 1.0 | 1.0 | 0.01 | 0.33 |
| Leg movement | 1.4[b] | 1.7[b] | 3.6[a] | 0.14 | <0.01 |
| Vocalization | 1.0[b] | 1.1[b] | 1.4[a] | 0.11 | 0.01 |

[a-c] Least square means within a row with differing superscripts differ ($P \leq 0.05$).

[1]The values presented correspond to transformed means, SEM and *P*-value using square root + 1 transformation.

Table 2.5. Least square means (±SEM) of T0 and 120-min samples for stride length and behavioral observations (2 to 4 hours post-castration) of non-castrated, band-castrated and knife-castrated Angus crossbred bulls castrated at 1 wk, 2 mo and 4 mo of age[1].

| | Treatment (T) | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Item | Control | Band | Knife | SEM[3] | T | Time | T × Time |
| ***1 wk*** | | | | | | | |
| Stride Length, cm | 32.3 | 33.3 | 34.0 | 1.29 | 0.66 | 0.73 | <0.01 |
| Behavioral observations | | | | | | | |
| Lying | 94.3 | 99.7 | 89.0 | 0.47 | 0.41 | - | - |
| Standing | 21.0 | 17.5 | 19.0 | 0.36 | 0.67 | - | - |
| Walking | 5.0 | 3.1 | 4.0 | 0.16 | 0.20 | - | - |
| Eating | 9.7 | 10.0 | 9.1 | 0.33 | 0.50 | - | - |
| Tail flick | 21 [b] | 103 [ab] | 400 [a] | 2.61 | 0.02 | - | - |
| Foot stamp | 4.8 | 5.0 | 31.3 | 0.72 | 0.13 | - | - |
| Head turning | 5.7 | 3.6 | 8.8 | 0.33 | 0.22 | - | - |
| ***2 mo*** | | | | | | | |
| Stride Length, cm | 41.9 | 39.3 | 39.7 | 0.96 | 0.14 | 0.43 | 0.73 |
| Behavioral observations | | | | | | | |
| Lying | 95.2[a] | 102.9[a] | 81.5[b] | 0.21 | <0.01 | - | - |
| Standing | 21.1[b] | 16.6[b] | 32.7[a] | 0.33 | <0.01 | - | - |
| Walking | 1.6[b] | 1.4[b] | 3.1[a] | 0.13 | 0.05 | - | - |
| Eating | 9.8[a] | 10.2[a] | 9.0[b] | 0.29 | 0.02 | - | - |
| Tail flick | 46.6 | 62.6 | 116.6 | 1.69 | 0.51 | - | - |
| Foot stamp | 3.5 | 6.1 | 8.4 | 0.34 | 0.13 | - | - |
| Head turning | 8.4 | 8.4 | 4.4 | 0.35 | 0.52 | - | - |
| ***4 mo*** | | | | | | | |
| Stride Length, cm | 42.5 | 42.6 | 40.6 | 1.07 | 0.35 | 0.55 | 0.02 |
| Behavioral observations | | | | | | | |
| Lying | 87.0 | 80.6 | 80.6 | 0.60 | 0.68 | - | - |
| Standing | 30.8 | 30.5 | 36.4 | 0.52 | 0.68 | - | - |
| Walking | 2.6 | 3.9 | 3.4 | 0.20 | 0.88 | - | - |
| Eating | 9.3 | 8.6 | 8.8 | 0.55 | 0.27 | - | - |
| Tail flick | 146.5 [b] | 164.3[b] | 873.7 [a] | 2.85 | <0.01 | - | - |
| Foot stamp | 3.8 | 5.4 | 15.4 | 0.57 | 0.06 | - | - |
| Head turning | 3.8 | 5.1 | 13.1 | 0.47 | 0.24 | - | - |

[a,b] Least square means within a row with differing superscripts differ ($P \leq 0.05$).

[1]Values for stride length represent the mean of samples immediately after castration and after 120 min. Values for behavioral observations represent the mean of behaviors 2 to 4 hours after castration.

[2]The values presented correspond to nontransformed means, however SEM and *P*-values correspond to ANOVA analysis using square root +1 transformed data.

Table 2.6. Least square means (±SEM) during the first week after castration for stride length, behavioral observations (d 1, 2, 3 and 5) and accelerometer recordings of non-castrated, band and knife castrated Angus crossbred bulls castrated at 1 wk, 2 mo and 4 mo of age[1].

| | Treatment (T) | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Item | Control | Band | Knife | SEM[2] | T | Time | T × Time |
| ***1 wk*** | | | | | | | |
| Stride Length, cm | 33.7 | 34.8 | 34.0 | 0.84 | 0.68 | 0.07 | 0.97 |
| Behavioral observations | | | | | | | |
| Lying | 41.0 | 47.5 | 41.6 | 0.35 | 0.59 | <0.01 | 0.42 |
| Standing | 27.8 | 31.2 | 27.3 | 0.22 | 0.45 | <0.01 | 0.19 |
| Walking | 3.8 | 3.9 | 3.9 | 0.14 | 0.90 | <0.01 | 0.43 |
| Eating | 7.5 | 5.3 | 8.1 | 0.34 | 0.35 | 0.32 | 0.52 |
| Tail flick | 24.8 | 82.7 | 97.4 | 1.50 | 0.25 | 0.97 | 0.38 |
| Foot stamp | 24.5 | 24.2 | 19.7 | 0.92 | 0.98 | 0.43 | 0.39 |
| Head turning | 11.3 | 11.7 | 12.5 | 0.41 | 0.93 | <0.01 | 0.49 |
| Lesion licking | 0.39 | 0.69 | 0.11 | 0.13 | 0.70 | 0.10 | 0.55 |
| Accelerometer recordings | | | | | | | |
| Standing duration, min | 18.8 | 19.5 | 19.7 | 0.10 | 0.64 | <0.01 | 0.20 |
| Lying duration, min | 43.4 | 48.9 | 44.4 | 0.16 | 0.26 | <0.01 | 0.02 |
| Standing time, % | 27.6 | 27.1 | 28.1 | 0.01 | 0.79 | 0.17 | 0.68 |
| Lying time, % | 72.5 | 73.0 | 74.1 | 0.03 | 0.64 | 0.26 | 0.76 |
| Standing bouts | 22.7 | 21.2 | 21.8 | 0.11 | 0.54 | <0.01 | <0.01 |
| Lying bouts | 25.9 | 23.9 | 25.9 | 0.11 | 0.32 | <0.01 | 0.03 |
| ***2 mo*** | | | | | | | |
| Stride length, cm | 40.7 | 40.2 | 40.8 | 0.79 | 0.82 | 0.80 | 0.29 |
| Behavioral observations | | | | | | | |
| Lying | 40.0 | 38.2 | 37.1 | 0.29 | 0.41 | 0.76 | 0.55 |
| Standing | 28.5 | 31.6 | 33.9 | 0.31 | 0.11 | 0.90 | 0.67 |
| Walking | 3.6 | 2.3 | 3.3 | 0.97 | 0.63 | 0.04 | 0.53 |
| Eating | 13.5 | 14.0 | 21.5 | 0.51 | 0.09 | 0.47 | 0.46 |
| Tail flick | 86.6 | 78.4 | 191.3 | 2.40 | 0.16 | <0.01 | 0.58 |
| Foot stamp | 12.1 | 14.2 | 12.6 | 0.55 | 0.93 | 0.01 | 0.56 |
| Head turning | 16.8 | 11.8 | 11.2 | 0.36 | 0.50 | 0.14 | 0.06 |
| Lesion licking | 1.0 | 0.8 | 0.9 | 0.11 | 0.97 | 0.02 | 0.70 |
| Accelerometer recordings | | | | | | | |
| Standing duration, min | 41.6 | 40.6 | 46.4 | 0.60 | 0.22 | <0.01 | 0.06 |
| Lying duration, min | 60.9 | 56.3 | 55.7 | 0.21 | 0.38 | <0.01 | 0.08 |
| Standing time, % | 39.3[b] | 39.8[b] | 43.8[a] | 0.01 | <0.01 | <0.01 | 0.11 |
| Lying time, % | 60.7[a] | 60.2[a] | 56.2[b] | 0.01 | <0.01 | <0.01 | 0.11 |
| Standing bouts | 14.2 | 16.5 | 14.5 | 0.11 | 0.25 | <0.01 | 0.22 |
| Lying bouts | 14.9 | 17.6 | 15.7 | 0.11 | 0.19 | <0.01 | 0.35 |
| ***4 mo*** | | | | | | | |
| Stride Length, cm | 44.1 | 42.5 | 42.2 | 1.10 | 0.40 | 0.02 | 0.43 |
| Behavioral Observations | | | | | | | |
| Lying | 37.2 | 40.0 | 31.4 | 0.27 | 0.12 | <0.01 | 0.03 |
| Standing | 33.0 | 30.5 | 38.1 | 0.25 | 0.25 | 0.01 | 0.05 |
| Walking | 1.8 | 1.7 | 2.2 | 0.07 | 0.32 | 0.07 | 0.72 |
| Eating | 21.6 | 29.0 | 24.7 | 0.30 | 0.27 | 0.01 | 0.66 |
| Tail flick | 160.3[b] | 247.7[b] | 868.0[a] | 2.97 | <0.01 | <0.01 | <0.01 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Foot stamp | 15.6[b] | 15.8[b] | 45.2[a] | 0.55 | <0.01 | <0.01 | <0.01 |
| Head turning | 5.6[b] | 7.6[b] | 14.6[a] | 0.27 | <0.01 | 0.16 | 0.50 |
| Lesion licking | 1.8[b] | 1.4[b] | 4.5[a] | 0.14 | <0.01 | 0.04 | <0.01 |
| Accelerometer recordings | | | | | | | |
| Standing duration, min | 48.8[b] | 45.4[b] | 60.1[a] | 0.20 | 0.01 | 0.04 | <0.01 |
| Lying duration, min | 64.8 | 62.5 | 71.4 | 0.21 | 0.01 | <0.01 | <0.01 |
| Standing time, % | 39.8[b] | 41.0[b] | 44.6[a] | 0.01 | 0.01 | <0.01 | <0.01 |
| Lying time, % | 60.2[a] | 59.0[a] | 55.4[b] | 0.01 | 0.01 | <0.01 | 0.01 |
| Standing bouts | 12.6[ab] | 14.0[a] | 12.0[b] | 0.56 | 0.05 | <0.01 | <0.01 |
| Lying bouts | 14.1 | 14.8 | 12.5 | 0.66 | 0.07 | 0.01 | <0.01 |

[a,b] Least square means within a row with differing superscripts differ ( $P \leq 0.05$).

[1]Values for stride length represent the mean of d 0 and d 7 samples. Values for behavioral observations represent the mean of behavioral observations for d 1, 2, 3 and 5.Values for accelerometer recordings represent the mean of accelerometer recordings for d 0, 1, 2, 3, 4, 5 and 6.

[2]The values presented correspond to non-transformed means; however SEM and *P*-values correspond to ANOVA analysis using square root + 1 transformed data.

Table 2.7. Summary showing which parameters were greater (↑) or lower (↓) for band- and knife-castrated Angus crossbred bulls castrated at 1 wk, 2 mo and 4 mo of age in comparison with control calves.

| | Band | Knife |
|---|---|---|
| ***1 wk*** | | |
| | ↑ VAS | ↑VAS |
| | ↑Lying duration | |
| | ↓Standing and lying bouts | ↑Tail flicks |
| ***2 mo*** | | |
| | ↑VAS | ↑VAS |
| | ↓ST | ↑Cortisol |
| | | ↓Lying duration |
| | | ↑Walking duration |
| | | ↑Standing duration |
| | | ↓Eating duration |
| | | ↑Standing and ↓ lying percentage |
| ***4 mo*** | | |
| | ↑VAS | ↑VAS, leg movement, vocalizations |
| | ↑Cortisol | ↑Cortisol |
| | ↓ST | ↑Tail flicks, foot stamping, lesion licking |
| | ↑Standing bouts | ↓Stride length |
| | ↑Lying bouts | ↑Standing and lying duration |
| | | ↑Standing and ↓ lying percentage |

Figure 2.1. Least square means and SEM for salivary cortisol (nmol/L) immediately before castration and 60 and 120 min after castration of (A) 1-wk-old, (B) 2-mo-old, and (C) 4-mo-old calves. [a-c]Least square means with differing superscripts differ ($P \leq 0.05$).

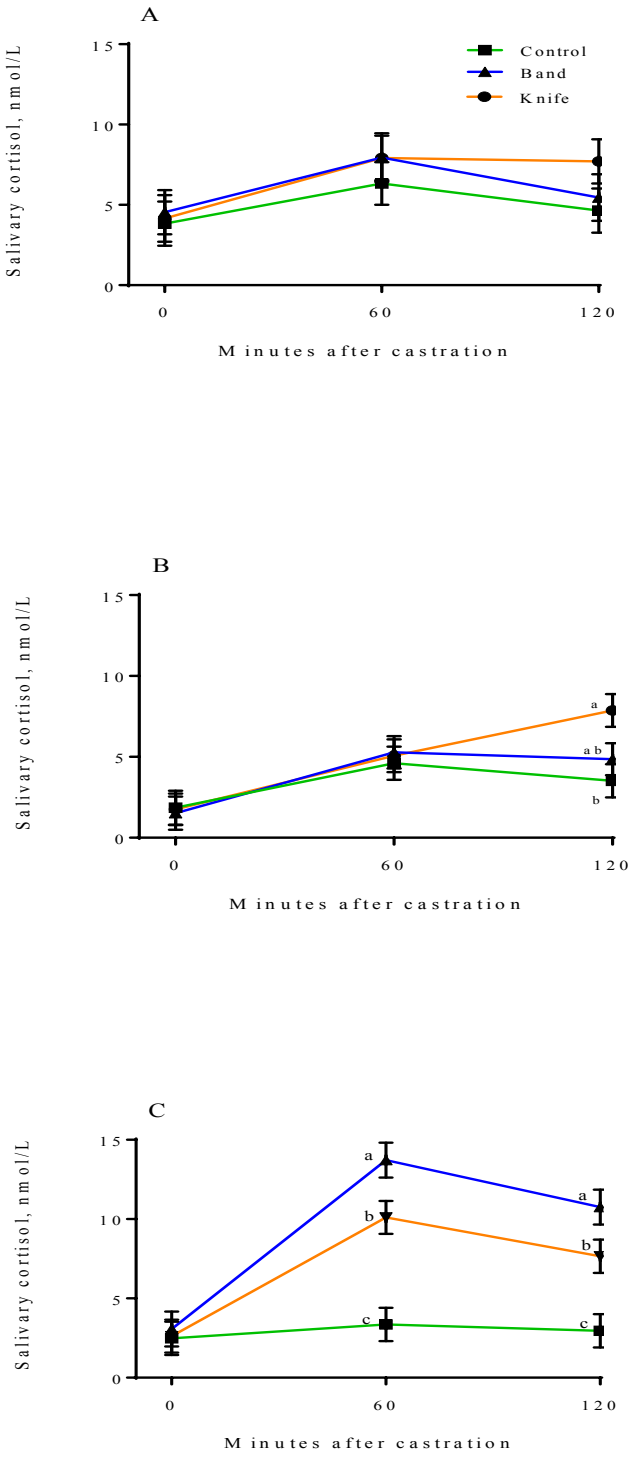
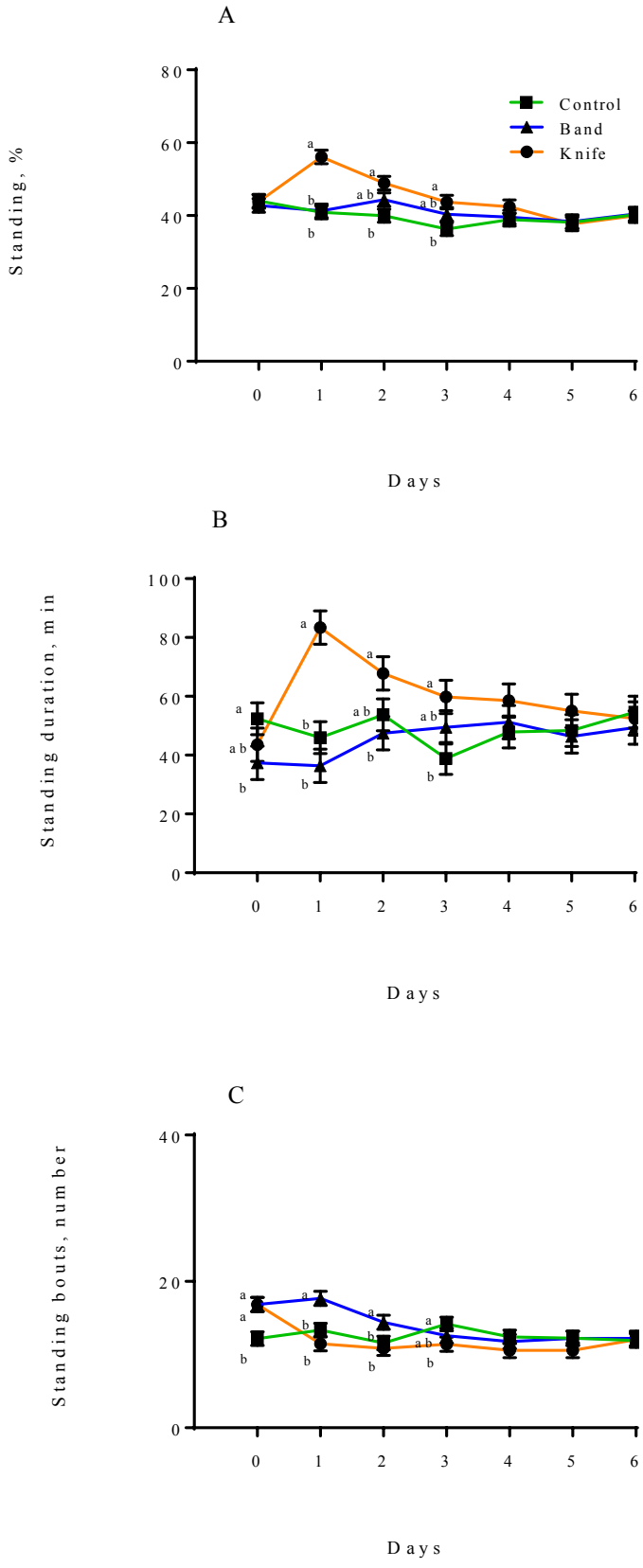


Figure 2.2. Least square means and SEM for (A) standing percentage, (B) mean standing duration, and, (C) standing bouts for 4-mo-old calves during 6 d after castration. [a,b]Least square means with differing superscripts differ (P ≤0.05).



# Chapter Three: Effect of a single dose of meloxicam prior to band and knife castration in 1 week old beef calves: I. Acute Pain

## 3.1 Abstract

In Western-Canada, approximately half of the bull calves produced are castrated before 1 wk of age. Therefore, it is important to identify effective analgesic drugs to mitigate pain associated with castration and consequently improve animal welfare. The aim of this study was to assess the efficacy of a single s.c. dose of meloxicam at mitigating pain associated with knife and band castration in 1 wk old calves. Seventy-two Angus crossbred bull calves (47.3 ± 6.70 kg of BW, 1 wk old) were used in a 3 × 2 factorial design where main factors included castration method−sham (CT), band (BA) or knife (KN) castration−and medication−lactate ringer's (NM) or 0.5 mg/kg BW of meloxicam (M). Measurements included different physiological and behavioral parameters. Samples were collected on d-1, immediately before castration (T0); and 60, 90 and 120 min and 1, 2, 3, and 7 d after castration except for VAS which was collected at the time of castration. The salivary cortisol concentrations were greater ($P$ = 0.04) in KN and BA calves than CT calves 60 min after castration, while 90 min after castration BA had greater concentrations than CT calves. Substance P concentrations were greater ($P$ = 0.04) in NM calves than M calves on d 3 and 7 after castration. The Serum amyloid-A (SAA) concentrations were greater ($P$ = 0.05) in KN calves than BA and CT calves on d 0, 2 and 3, while BA calves had greater SAA concentrations on d 7 than KN and CT calves. The visual analog scores were greater ($P$ <0.01) in KN calves than BA, and in BA compared to CT calves. The KN calves tail flicked more ($P$ < 0.01) than BA and CT calves, and NM calves tail flicked more ($P$ = 0.03) than M calves. No castration or medication effect ($P$ > 0.10) was observed for stride length, walking,

standing, lying ventral, eating, foot stamping, head turning, lying and standing percentage, performance, platelets or body temperature. Overall, knife castrated calves exhibited a greater acute pain response than band castrated calves. Meloxicam was able to reduce substance P concentrations, white blood cell counts and number of tail flicks after castration, suggesting that the drug was able to mitigate acute pain to some extent. However, meloxicam did not have an effect on the other physiological and behavioral parameters assessed.

## 3.2 Introduction

Castration is a painful husbandry procedure reported to have a negative impact on growth performance, especially when performed at older ages, therefore recommendations have been made to castrate calves as young as possible in order to reduce tissue damage and consequently reduce stress (Bretschneider, 2005). Nevertheless, independent of the method of castration, beef calves experience pain and discomfort at any age (Schwartzkopf-Genswein et al., 2012), as demonstrated by physiological and behavioral changes in calves as young as 1 wk of age (Robertson et al., 1994; Molony et al., 1995).

In Western Canada approximately 53 % of calves in cow-calf operations are castrated before 1 wk of age (Moggy et al., 2017), therefore it is of great importance to identify practical and effective pain mitigation strategies for this age group. Meloxicam is a non-steroidal anti-inflammatory drug (NSAID), approved for use in cattle in the EU and Canada, which has been reported to be effective at mitigating physiological indicators of pain after painful husbandry procedures such as dehorning and castration (Stewart et al., 2009; Roberts et al., 2015). Meloxicam is a practical analgesic option for producers due to its ease of administration (s.c) and long half-life (22 ± 3 h) (Stock and Coetzee, 2015), which makes it an attractive one time

administration analgesic. Although meloxicam is labeled for pain relief following debudding, it is not labeled for pain relief after castration.

To our knowledge there are no studies assessing the efficacy of injectable s.c. meloxicam in 1 wk old calves. Therefore, the objective of this project was to assess acute pain associated with different castration methods, and the efficacy of s.c. meloxicam administered immediately prior to castration in 1 wk old beef calves. We hypothesize that knife castration will have greater pain indicators and that meloxicam will be effective at reducing behavioral and physiological indicators of acute pain associated with castration.

## 3.3 Materials and Methods

This protocol was approved by Lethbridge Research Centre (ACC # 1410) and the University of Calgary (AC14-0159). All animals were cared for according to the Canadian Council of Animal Care guidelines (CCAC, 2009).

### 3.3.1 Animal housing and treatments

Seventy-two Angus crossbred bull calves (47.3 ± 6.70 kg of BW and 7-8 d old) were used in a 7 d experiment at the Agriculture and Agri-Food Canada Lethbridge Research Centre (LRC) (AB, Canada). Cow-calf pairs were transported from the ranch of origin to the LRC for 30 km in two separate groups of 36 calves each, as groups were castrated at 7-8 d of age on separate days 1 week apart. Upon arrival, animals were weighed and a small area (5 cm × 5 cm) was shaved on the neck of each calf in order to locate the jugular vein. The seventy-two cow-calf pairs were housed in 6 experimental pens (12 pairs per pen) three of which measured 36.7 m × 22.2 m, and the remaining three measured 40 m × 27 m. Each pen contained a calf shelter (2.4 m × 3.6 m × 1.4 m), a centrally located water system and straw bedding. The cows' diet consisted

of free choice alfalfa grass hay while the calves' diet consisted of milk from suckling and free choice salt blocks and loose minerals containing a coccidiostat (Diluted Rumensin Drug Premix 1100 (Medicated), HI-PRO FEEDS, Okotoks, Alberta, Canada) for the prevention of diarrhea caused by coccidiosis.

Calves were equally distributed by weight into pens and randomly assigned to treatments. Calves were the experimental unit as treatments were mixed within pens with 2 calves per treatment per pen. The experiment consisted of a 3 × 2 factorial design where main factors included castration method: sham (control calves, **CT**; *n* = 23), band (**BA**; *n* = 23) or knife (**KN**; *n* = 22) castration; and medication: single s.c. administration of lactated Ringer's (Lactated Ringer's Irrigation, Baxter Canada, Mississauga, Ontario, Canada) (**NM**; n = 33) or a single dose of 0.5 mg/kg of s.c. meloxicam (Metacam 20 mg/mL, Boheringer's Ingelhein, Burlington, Ontario, Canada) (**M**; n = 35), to yield: CT-NM (n = 12), CT-M (n = 11), BA-NM (n = 11), BA-M (n = 12), KN-NM (n = 10), KN-M (n = 12). A valid Veterinarian-Client-Patient relationship (VCPR) was established prior to the extra-label drug use of meloxicam, as this drug is not labeled for pain mitigation associated with castration. Calves were weighed in a portable chute and restrained, sampled and castrated on a tip table (Calf Roper, Ram-Bull Ltd, Barons, Alberta, Canada). Calves were castrated for a period of 2 to 3 min while tipped on the tip table and sampled for a period of 3 to 5 min while standing in the tip table. All calves were castrated between 7 and 8 d of age by the same experienced veterinarian. Band castration was performed by placing a band (Elastrator Pliers and Rings, Kane Veterinary Suppliers Ltd., Edmonton) using an elastrator on the neck of the scrotum above the testicles. Knife castration consisted of making a latero-lateral incision in the scrotum with a Newberry castration knife (Syrvet Inc., Waukee, IA) in order to externalize the testicles while crushing and cutting of the spermatic cords was

done using an emasculator. Sham castrated calves were handled the same way as knife and band castrated calves and the testicles were manipulated for a similar amount of time.

### 3.3.2 Measurements of acute pain and sample collection

Samples were collected during the first 7 d after castration, which was defined as the period of acute pain as previously reported by Meléndez et al. (2017b).

*Physiological parameters*

*Salivary cortisol*

Samples were collected on d-1, and immediately before castration (T0), 60, 90, 120 min and 1, 2, 3 and 7 d after castration. Saliva was collected by swabbing the oral cavity with a cotton swab placed in a plastic tube and frozen at -20 ºC for subsequent analysis. Samples were analyzed using an enzyme immunoassay kit (Salimetrics, LLC State College, PA). Inter-assay and intra-assay CV values were 16.1 % and 8.4 % respectively.

*Blood samples*

Blood samples were collected on d -1, and T0, 60, 90, 120 min and on 1, 2, 3 and 7 d after castration. Samples were obtained via jugular venipuncture into vacuum tubes (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ) for further analysis.

Samples for substance P were collected into 4-ml tubes containing EDTA (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ), where benzamidine hydrochloride was added to reduce substance P degradation. The sample was centrifuged for 15 min at 1.5 × *g* at 0 ºC, and the serum was decanted and frozen at -80 ºC. Samples were analyzed at Iowa State University, College of Veterinary Medicine (Ames, IA) with some modifications from the

previously described procedure by Van Engen et al. (2014). Non-extracted plasma samples were analyzed in duplicate with a double antibody radioimmuno-assay (RIA) using a purchased primary antibody, (Substance-P (3-11) Antibody for Immunohistochemistry Human, Rat, Mouse, Phoenix Pharmaceutical #H-061-05). The range of detection for substance P was between 5 to 320 pg/ml, with an average $R^2 = 0.99$. The coefficient of variation for intra-assay variability was 8.1 % and the inter-assay variability 15.3 %. The limit of detection was 10 pg/mL and the limit of quantitation was 20 pg/mL.

Blood samples for haptoglobin and serum amyloid-A (SAA) were collected into 6-ml non-additive tubes (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ), centrifuged for 15 min at 1.5 × *g* and the serum was decanted and frozen at -20 ºC for further analysis. Haptoglobin concentrations were analyzed using a Roche Cobas c501 biochemistry analyzer (Roche Diagnostics, Laval, QC, Canada) using a Tridelta bovine haptoglobin calibrator (TP801CAL, Tridelta, Maynooth, Ireland) and two levels of in-house controls (bovine serum pools) daily and two levels of Tridelta controls weekly. The inter-assay CV for haptoglobin was 7.6 %. SAA concentrations were analyzed using an enzyme linked immunosorbent assay (Tridelta Phase range SAA kit, TP 807, Tridelta development LTd, Maynooth, Ireland). The intra-assay and inter-assay CV were 13.7 % and 7.5 %, respectively.

Blood samples for CBC were collected into 4-ml EDTA tubes (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ) and a HemaTrueHematology Analyzer (Heska, Lobeland, Co) was used to measure red blood cells (RBC), white blood cells (WBC) and platelet counts. Hematocrit, hemoglobin, lymphocytes, monocytes, and granulocytes were also analyzed (data not shown).

*Scrotal and base temperatures (Scrotal temp and Base temp)*

Images of the scrotal area and the base of the scrotum were collected on d -1, T0 (before castration), 60, 90, 120 min and on d 1, 2, 3, and 7 after castration. Images were taken from behind the calves, approximately 1 m from the scrotum with a FLIR i60 infrared camera (FLIR Systems Ltd Burlington, Ontario, Canada) and analyzed with FLIR Tools v.5.1 (FLIR Systems Ltd Burlington, Ontario, Canada) using an emissivity coefficient of 0.98 to measure the Scrotal temp and the Base temp as indicators of inflammation.

*Rectal temperature* (Rectal temp)

Rectal temperature was obtained by inserting a digital thermometer (GLA M750 Livestock Thermometer, San Luis Obispo, CA) into the rectum of calves on d -1, T0 (before castration), 1, 2, 3 and 7 d after castration.

*Performance*

Animals were weighed on d -1, T0, and 1, 2, 3, and 7 d after castration in a portable scale. Weights collected on d- 1 and d 7 were used as initial and final body weight (BW). Average daily gain (ADG) was calculated by dividing the difference in weight from d -1 to d 7 by the number of days between weights (9).

*Behavioral parameters during castration*

Two experienced observers recorded visual analog scale (VAS), urination, leg movement and vocalization frequency during castration. The VAS consisted of placing a mark along a 10 cm continuum which served as an indicator of the observer's perception of the amount of discomfort that the animal experienced during castration. The mark was measured to the closest

0.5 cm and the measurements were analyzed. Frequency of urination, leg movement and vocalization were also recorded at the time of castration. Due to the experimental setting, observers could not be blind to the treatments.

*Behavioral parameters after castration*

*Behavioral observations*

To identify individual calves, penning tags were glued to the back of each calf on d – 1 using tag cement (Livestock Identification tag cement, W.J. Ruscoe Company, Akron, Ohio). Behavior was recorded with two cameras (2.0MP HD IR Bullet Camera, Avigilon, Vancouver, British Columbia, Canada) which were mounted on 6 m poles on the north and the south side of each experimental pen. Observer® XT (Noldus Information Technology, Wageningen, The Netherlands) was used by two experienced observers (blind to treatments) for scoring focal animal sampling from continuous recordings (Martin and Bateson, 2007) of 36 calves (6/treatment) to document the duration of standing, walking, lying, and suckling behavior, and frequency of tail flicking, head turning, lesion licking and foot stamping as previously described by Meléndez et al. (2017b). Behavioral observations were done continuously for a period of 2 h from 2 to 4 h after castration, and for 4 min every 10 min for a 3-h period 1, 2, 3 and 7 d after castration. Inter-rater and intra-rater reliability were 0.90 and 0.91 respectively.

*Stride length*

Stride length (SL) measurements were collected on d -1, immediately after castration and 120 min after castration. Stride length was videoed when the animals walked through a 1-m wide and 3-m long alley built with panels and placed immediately after the tip table. Stride length was collected and measured with some modifications from the previously described method of

Currah et al. (2009). Modifications included a different type of software for image analysis and no use of grid background at the time of video recording. GOM Player (GOM Lab, Gretech Corporation, Seoul, South Korea) was used to take two pictures of the calves when both of their hind legs were placed on the ground, and ImageJ (Bethesda, MD, USA) was used to measure the distance (cm) between the left and the right hind leg as they walked through the 3-m alley.

*Standing and lying behavior*

Standing and lying behavior was collected using hobo pendant G data loggers (Onset Computer Corporation, Bourne, MA). Hobo data loggers were covered in plastic film to protect them from moisture and wrapped in foam to protect the device from damage and reduce irritation due to rubbing on the legs of the calves. The data loggers were attached on d -1 to the left hind leg using a 4.6-m long latex flexible cohesive bandage (Latex Flexible Cohesive Bandage, Professional Preference, Calgary, Alberta, Canada). Data was used from days which had 24 h of data, therefore d -1 and 7 were excluded from the analysis. The data loggers were used to collect standing and lying bouts (number/day), total standing and lying duration (min/day) which was converted to a percentage (%), and mean standing and lying bout duration (min/day) (UBC AWP, 2013).

## 3.4 Calculation and statistical analysis

Data was analyzed using Mixed Models (SAS, version 9.4, SAS Inst. Inc., Cary, NC) to evaluate the effect of castration and medication on behavioral and physiological parameters. Fixed effects included castration, medication, time and their interactions, while random effects included pen and calf nested within pen. Data was analyzed for normality using Proc Univariate (SAS, version 9.4, SAS Inst. Inc., Cary, NC) and if data was not normally distributed it was

transformed. All physiological parameters were transformed using Naiperian log except for scrotal temperature, rectal temperature, total blood cell count, and weights. All behavioral parameters were square root + 1 transformed except for SL, and percentage data was arc-sin transformed. All data except for VAS, ADG, initial and final BW were analyzed using the repeated measure model (Proc Mixed of SAS). Covariance structures included compound symmetry, autoregressive order 1 and unstructured. The covariance structure with the lowest Schwarz's Bayesian criterion was selected as the analysis of preference. Physiological data from T0, 60, 90 and 120 min and behavioral data from 2 to 4 h after castration were analyzed separately from those obtained on d 1, 2, 3 and 7. Significance was established at $P \leq 0.05$ and a tendency between $0.05 < P \leq 0.10$. A post-hoc test was run when interactions were significant ($P \leq 0.05$) to separate the LS means using the PDIFF option in SAS. An intra-class correlation coefficient (ICC) with a 95 % CI was used to determine inter-rater and intra-rater reliability for behavior using IBM SPSS statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA).

## 3.5 Results

### 3.5.1 Physiology

*Minutes after castration*

Calves that received meloxicam tended ($P = 0.10$; Table 3.1) to have greater cortisol concentrations than NM calves (6.1 ± 0.66 vs 5.1 ± 0.66 nmol/L, respectively) 60 min after castration, however no differences ($P > 0.10$) were seen 90 and 120 min after castration. A castration × time interaction ($P = 0.04$) was observed for cortisol concentrations, where KN and BA calves had greater ($P = 0.01$; $P = 0.04$) cortisol concentrations than CT calves 60 min after

castration, while 90 min after castration only BA had greater ($P = 0.01$) cortisol concentrations than CT calves (Fig. 3.1A). No differences ($P > 0.10$) in cortisol concentrations were observed between BA and CT calves, compared to KN calves 90 min after castration and no differences ($P > 0.10$; $P > 0.10$) were observed between castration groups at T0 and 120 min after castration. Substance P tended to be greater ($P = 0.09$) in NM calves (102.7 ± 4.33 pg/mL) than M calves (94.2 ± 4.29 pg/mL) 120 min after castration. Base temp tended to be greater ($P = 0.08$) in BA (35.5 ± 0.57 °C) calves than KN (34.4 ± 0.57 °C) and CT (34.4 ± 0.57 °C) calves 90 min after castration, while BA (35.3 ± 0.55 °C) and CT (35.3 ± 0.58 °C) calves tended to have greater ($P = 0.08$) base temp than KN (33.5 ± 0.59 °C) calves 120 min after castration. Scrotal temp decreased (time effect; $P < 0.01$) over time in all treatments (Table 3.1).

*Days after castration*

Cortisol concentrations had a time effect ($P <0.01$), where greater ($P \leq 0.05$) concentrations were observed on d 1, 2, and 3 compared to d 7, and there was a tendency ($P \geq 0.06$) for concentrations to be greater on d 1 and 3 compared to d 2 after castration. Substance P concentrations were greater ($P = 0.04$) in NM calves than M calves 7 d after castration and tended to be greater ($P = 0.08$) on d 3 after castration (Fig. 3.2). Substance P concentrations also tended ($P = 0.06$) to be greater in CT-M, BA-NM, and KN-NM, calves than BA-M, and KN-M, while no differences were observed between CT-NM, and CT-M, BA-NM, BA-M, KN-NM, and KN-M calves. Haptoglobin concentrations increased (time effect; $P = 0.02$) up to d 2 and then returned to similar baseline levels on d 7 after castration. The SAA concentrations were greater ($P = 0.04$) in KN-NM, calves compared to CT-NM, CT-M, BA-NM and KN-M calves, while BA-M calves had greater ($P < 0.05$) SAA concentrations than CT-NM calves, however, no differences ($P > 0.10$) were observed between K-NM and B-M calves. Also, SAA concentrations

were greater ($P = 0.05$) in KN calves than BA and CT calves on d 0, 2 and 3, while BA (93 ± 12.5 µg/mL) calves had greater ($P \leq 0.05$) SAA concentrations than KN (64 ± 12.5 µg/mL) and CT (65 ± 11.9 µg/mL) calves on d 7.

White blood cell count was greater ($P = 0.01$; Table 3.1) in NM (11.0 ± 0.32 × $10^9$/L) calves than M (9.8 ± 0.30 × $10^9$/L) calves. The RBC count was greater ($P = 0.04$) in KN (8.1 ± 0.07 × $10^{12}$/L) calves and BA (8.1 ± 0.07 × $10^{12}$/L) calves than CT (7.9 ± 0.06 × $10^{12}$/L) calves. Platelets increased (time effect; $P = 0.01$) over time. Scrotal temp was lower ($P < 0.01$) in BA calves compared to CT and KN calves on d 2, 3 and 7 after castration (Fig. 3.1B). Base temp decreased (time effect; $P < 0.01$) during d 1, 2 and 3, while rectal temp increased (time effect; $P < 0.01$) and decreased over time (Table 3.1).

No castration × medication effect was observed for any of the physiological parameters with the exception of SAA, and no castration × medication × time effect was observed for any of the physiological parameters. No medication or castration differences ($P > 0.10$) were observed for initial and final BW or ADG (Table 3.2).

### 3.5.2 Behavior

A castration effect ($P < 0.01$) was observed for VAS scores, where KN (1.5 ± 0.06 cm) calves had the greatest scores, followed by BA (1.3 ± 0.06 cm), and then by CT (1.1 ± 0.06 cm) calves (Table 3.3). Leg movements were greater ($P < 0.01$) in KN (5.1 ± 0.16) and BA (4.2 ± 0.16) calves than CT (1.4 ± 0.16) calves, while KN (1.2 ± 0.04) calves vocalized more ($P = 0.02$) than BA (1.0 ± 0.04) and CT (1.0 ± 0.04) calves. Between 2 and 4 h after castration, suckling duration was greater (castration × medication interaction; $P = 0.02$) in KN-NM calves than CT-NM and BA-M calves, while CT-M calves had greater suckling duration than CT-NM and BA-

M calves. The NM calves tail flicked more ($P = 0.03$) than M calves. The KN (148 ± 2.0) calves tail flicked more ($P < 0.01$) than BA (40 ± 2.0) and CT (3 ± 2.0) calves. The KN-NM calves tended ($P = 0.06$) to lie down less than CT-NM and BA-M calves, while KN calves tended ($P = 0.09$) to walk more than BA and CT calves. The KN-NM calves tended ($P = 0.10$) to have greater standing duration than CT-NM, BA-M and KN-M calves, however no differences were observed between these groups and CT-M and BA-NM calves.

Standing bouts on d 0 were greater ($P = 0.02$) in BA-M (30 ± 1.7) calves than CT-NM (25 ±1.7), CT-M (23 ± 1.9), BA-NM (26 ± 1.8), KN-NM (27 ± 1.8) and KN-M (22 ± 1.6) calves, in KN-NM calves than CT-M and KN-M calves, and in CT-NM and BA-NM than KN-M calves. On d 1 after castration, CT-NM (23 ± 1.2), BA-NM (24 ± 1.3), BA-M (23.2 ± 1.2) and KN-NM (24 ± 1.3) calves had greater ($P < 0.05$) standing bouts than CT-M (18 ± 1.4) calves, while on d 4 BA-M (21 ± 1.1) calves had greater ($P < 0.05$) standing bouts than CT-NM (18 ± 1.1), BA-NM (17 ± 1.1) and KN-M (17 ± 1.0) calves. The NM (24 ± 0.7) calves tended ($P = 0.08$) to have greater ($P < 0.05$) standing bouts than M (21 ± 0.7) calves on d 1 after castration, while lying bouts tended to be greater ($P = 0.10$) in M (24 ± 1.3) calves compared to NM (21 ± 1.3) calves on d 3 after castration. Lying duration showed a medication effect ($P = 0.03$), where M calves tended to have greater ($P = 0.07$; $P = 0.08$) lying duration than NM calves on d 3, 4 and 5 after castration (Fig 3A). The NM (1.9 ± 0.01 min) calves had greater ($P = 0.03$) lateral lying than M (0.7 ± 0.01 min) calves, and NM (3 ± 0.8 min) calves tended ($P = 0.07$) to have greater ($P < 0.05$) lateral lying than M (0 ± 0.8 min) calves on d 3 after castration. Tail flicks were greater ($P = 0.03$) in M calves than NM calves on d 7 after castration (Fig. 3.3B). Lying lateral was greater ($P = 0.01$) in KN calves compared to BA calves on d 3, but no differences ($P > 0.10$) were observed between both KN and BA compared to CT calves, while KN calves had greater lateral

lying duration than BA and CT calves on d 7 (Fig. 3.3C). No castration × medication or castration × medication × time effects were observed for stride length, standing and lying behavior or for behavioral observations days after castration (Table 4).

## 3.6 Discussion

### 3.6.1 Physiology

The M calves had lower substance P concentrations than NM calves on d 3 and 7 after castration. Meloxicam is an NSAID which works by inhibiting COX enzymes which convert arachidonic acid into prostaglandins, which are pro-inflammatory substances (Ricciotti and FitzGerald, 2011). Prostaglandin E2 ($PGE_2$) has the ability to activate calcium channels in the sensory neurons which in turn stimulates the release of neurotransmitters, such as substance P (Nicol et al., 1992), a neuroactive peptide found across the central nervous system associated with pain, stress and anxiety (DeVane, 2001). Meloxicam administration has been previously reported to lower substance P concentrations in cases of acute synovitis in the horse and dehorning in cattle (Grauw et al., 2009; Coetzee et al., 2012). Surprisingly, differences in substance P concentrations in the present study were observed after the duration of action of meloxicam which has a half-life of 22 ± 3 h (Stock and Coetzee, 2015). Similar findings were reported by Allen et al. (2013) in 8 to 10 wk old cautery dehorned calves receiving oral meloxicam (half-life 27 h) (Stock and Coetzee, 2015), which showed reduced substance P levels 120 h after dehorning compared to calves receiving a placebo. Based on these findings, meloxicam is effective at decreasing substance P concentrations; however differences were only detectable well after the expected duration of the pharmacokinetic effect of meloxicam (up to 44 h post administration). Limiting sampling times to the specific duration of action of drugs could

lead to inaccurate conclusions, as present and previous studies have detected differences after this period of time.

Meloxicam did not have an effect on cortisol concentrations, contrary to previous studies which have shown a cortisol reduction in castrated (Roberts et al., 2015) and dehorned (Allen et al., 2013) calves receiving oral meloxicam compared to un-medicated calves. Differences in cortisol concentrations between studies could be due to differences in dose, as the dose for s.c. meloxicam is 0.5 mg/kg of BW, while oral meloxicam is 1 mg/kg of BW, due to injectable meloxicam not being labelled for castration (oral meloxicam is labelled for castration in Canada), or due to age as calves from the previously mentioned castration study were older calves weighing 227 kg. However, the castration effect observed in the present study is in accordance with previous findings in 1 wk old calves which reported greater cortisol concentrations in castrated compared to uncastrated calves (Robertson et al., 1994; Molony et al., 1995). A possible reason why differences were observed for substance P but not for cortisol concentrations could be because cortisol is an indicator of acute stress; therefore concentrations are likely to return to baseline levels the day of castration. Previous castration studies in 1 wk old calves have reported cortisol concentrations returning to baseline 2 h after castration (Robertson et al., 1994; Molony et al., 1995). Differences observed in substance P concentrations on d 3 and d 7 could have been the result of meloxicam's inhibitory effect on prostaglandin production during the first 2 d after castration, which could have consequently reduced $PGE_2$ concentrations and therefore reduced substance P concentration as previously mentioned.

Acute phase proteins (APP) increase in response to trauma, infection or inflammation (Hughes et al., 2014). In the present study, haptoglobin was not affected by castration or medication, which is contrary to the findings by Roberts et al. (2015) who reported a reduction in

haptoglobin concentrations after surgical castration in 227 kg calves receiving oral meloxicam compared to non-medicated calves. Lack of differences in haptoglobin concentrations could be explained by the difference in age at castration between the studies. Similar to our results, Brown et al. (2015), reported differences in haptoglobin concentrations between medicated (oral meloxicam) and non-medicated calves castrated near weaning but no differences were observed between medicated and non-medicated calves castrated near birth. Lack of differences in new born calves could be due to castration being an insufficient stimulus to initiate a haptoglobin response (Werling et al., 1996) and/or due to greater haptoglobin concentration near birth (Orro et al., 2008) which have reached a 'ceiling effect'.

Serum amyloid-A has been previously suggested to be a better indicator of acute inflammation than haptoglobin (Horadagoda et al., 1999), and could be one reason why castration methods affected SAA concentrations but not haptoglobin concentrations. An unexpected finding was greater SAA concentrations observed in KN calves at T0, a sample collected immediately before castration. A possible explanation could be that a number of calves in the KN group could have had subclinical inflammation (Karreman et al., 2000) during the first days after birth which could have increased SAA concentrations. However, this is unlikely as no differences were observed between castration groups on d - 1 and on d 1 after castration. The KN calves also had greater SAA concentrations than all other groups on d 2 and 3 after castration which could be associated with surgical trauma (Murata et al., 2004) and the subsequent inflammation caused by the scrotal incision and cutting and crushing of the cords after knife castration. In addition, greater SAA concentrations in BA calves on d 7 could be associated with tissue damage and inflammation caused by the band breaking through the skin which in turn can lead to secondary infections. It is common for APP concentrations to increase after a given

stimulus (Petersen et al., 2004), however, in the present study SAA concentrations decreased after castration. This could be due to greater APP concentrations near birth, possibly associated with parturition and/or colostrum intake, and the gradual reduction of concentrations during the first 3 weeks after birth (Orro et al., 2008).

Medicated calves had reduced WBC counts than non-medicated calves. Roberts et al. (2015) reported reduced concentrations in total WBC, neutrophils, eosinophils, monocytes, and RBC in castrated calves receiving oral meloxicam (1 mg/kg BW) compared to non-medicated calves. In the present study, castrated calves had greater RBC concentrations than uncastrated calves. Despite hematological differences between treatments, all calves had WBC and RBC concentrations within the normal range (4-12 $\times 10^3/\mu L$ and 5-10 $\times 10^6/L$, respectively) (Smith, 2008). It is possible that the effect observed with oral meloxicam was due to a greater dose and a different route of administration. Oral meloxicam has a prolonged analgesic and anti-inflammatory effect compared to s.c. meloxicam as it is absorbed and excreted from the body at a slower rate than s.c meloxicam. It is possible that the injectable meloxicam (0.5 mg/kg BW), used in the present study, is not labeled for castration because it is not as efficacious as oral meloxicam (1 mg/kg BW), which is labeled for its use to mitigate pain associated with castration. However, in a previous study, Brown et al. (2015) suggested that calves castrated near birth did not benefit from oral meloxicam based on a reduced effect on inflammation between medicated and non-medicated calves. Similar results were observed in the present study, in which substance P and WBC were the only physiological parameters influenced by meloxicam administration in 7 d old calves.

### 3.6.2 Behavior

As expected KN calves had the highest VAS scores (1.5 cm), leg movement and vocalization frequency at the time of castration, indicating that the scrotal incision and crushing and cutting of the cords was a noxious stimuli of sufficient intensity to produce nociceptive pain and behavioral changes (Woolf, 2010). Banded calves had high VAS scores (1.3 cm) and leg movement frequencies, likely due to the manipulation of the testicles in order to place the band; however this was not a stimuli of sufficient intensity to produce vocalizations. These findings are in accordance with previous findings in which knife calves reacted more actively to knife castration followed by banded calves and uncastrated control calves (Fell et al., 1986; Meléndez et al., 2017b). Meloxicam did not have an effect on VAS which can be explained by the fact that NSAID's do not have an effect on stimuli of high intensity (Malmberg and Yaksh, 1991), but work by decreasing peripheral and central sensitization by reducing prostaglandin production (Burian and Geisslinger, 2005).

Tail flicking is important as it was the only parameter that presented a castration and medication effect. An increase in tail flick number in KN calves could be due to pain/discomfort caused by an increase in the number of action potentials from the nociceptors surrounding the affected areas in the scrotum and spermatic chords, as inflammatory substances can reduce the pain threshold therefore increasing hyperalgesia and allodynia (Tranquilli et al., 2013). An increased number of tail flicks has been previously reported as an acute pain related behavior in farm animals (Molony and Kent, 1997). The M calves had a decreased number of tail flicks compared to NM calves 2 to 4 h after castration. Although not statistically significant, M calves had a lower number of tail flicks than NM calves on d 1 and 3 after castration, however the M calves had a significantly greater number of tail flicks than NM calves on d 7. Increase in the

number of tail flicks on d 7 was an unexpected finding; however differences in tail flicks observed on d 7 are likely due to the effect of meloxicam wearing off. These findings are of great importance as they show that meloxicam is effective 2 to 4 h after castration, however contrary to substance P results, the effect of meloxicam did not last beyond the duration of action period. Contrary to our results, Theurer et al. (2012), reported behavioral changes after the duration of action of oral meloxicam, where greater lying times were reported in medicated than un-medicated calves on d 0, 2, 3 and 4 after dehorning.

Lateral lying and restless behavior have been previously reported as pain related behaviors. Molony et al. (1993) hypothesized that lambs in pain spent more time in a lateral lying position in response to castration and tail docking, while increases in restlessness have been associated with pain/distress caused by ischemia after rubber ring castration in lambs (Dinniss et al., 1999). Interestingly, a study assessing different castration methods in calves did not find differences in lateral lying in 1wk old calves (Molony et al., 1995), while Robertson et al. (1994) reported that 6 d old calves spent significantly more time lateral lying than 21 and 42 d old calves. In the present study, KN calves spent more time in a lateral lying position than BA and CT calves on d 3 and d 7 after castration. Increased lateral lying could be a consequence of pain associated with wound inflammation in KN calves, and along with greater scrotal temp in KN and CT calves than BA calves on d 2, 3 and 7 after castration, indicates inflammation of the scrotal area for KN calves. The M calves spent less time in a lateral lying position than NM calves which could be due to the anti-inflammatory effects of meloxicam. In addition, the BA-M calves had a greater number of standing bouts which is in accordance with previous findings where 1 wk old band castrated calves had greater standing and lying bouts than knife castrated calves (Meléndez et al., 2017b). If restlessness is associated with ischemic pain we would expect

not only BA-M but also B-NM calves to have a greater number of restless bouts. Caution should be taken when interpreting both standing bouts and lateral lying as the numerical differences are small and may not be biologically significant.

Suckling duration was greater for KN-NM and CT-M calves the day of castration, which is contrary to expected. Interestingly, the CT-M calves had greater suckling durations than CT-NM calves. A possible explanation could be that meloxicam worked as an appetite stimulant, however, Todd et al. (2010) found that meloxicam does not act as an appetite stimulant in healthy calves, only in calves with diarrhea. More surprising was the increase in suckling duration in KN-NM calves, as we would expect M calves to benefit from the analgesic and anti-inflammatory properties of meloxicam, and consequently calves would be more likely to get up, walk and suckle compared to NM calves. A possible explanation for this finding is that calves increase suckling as a way of coping with pain, as suckling behavior increases oxytocin release (Lupoli et al., 2001), which has an anti-stress effect and the potential to increase nociceptive thresholds (Uvnäs-Moberg et al., 1998).

The NM calves tended to have greater substance P, lateral lying duration, lower number of lying bouts and greater number of standing bouts. The KN calves tended to walk more, while the KN-NM calves tended to stand more and lie less. These results are as expected and indicate that KN-NM calves experienced more acute pain/stress compared to other treatments. On the other hand, unexpected tendencies included salivary cortisol being greater in M calves 60 min after castration compared to NM calves, and BA-M calves presenting greater number of foot stamps than BA-NM calves. Although tendencies could be due to small sample size, small differences between treatments or high individual variability, we believe that the tendencies reported are relevant and that significance was not achieved mainly due to the small sample size.

### 3.6.3 Conclusion

Overall, SAA, scrotal temperature, VAS, tail flicks and lateral lying had a castration effect, where KN castration was the method which produced more pain related indicators. This could be due to the activation of the mechanical nociceptors at the time of the incision and cutting and crushing of the spermatic chords (VAS). Consequently, the tissue damage caused by knife castration leads to an inflammatory reaction surrounding the affected area (SAA and scrotal temperature). Inflammation can also lead to pain related behaviors, such as tail flicking, which has been previously associated with skin irritation in cattle (Kiley-Worthington, 1976) and lateral lying which has been associated with pain in lambs after castration (Molony et al., 1993).

In addition, a medication effect was observed for substance P, WBC and tail flicks. It is likely that meloxicam was effective at reducing these variables due to the association between these parameters and prostaglandins, as prostaglandins have the ability to reduce the pain threshold (Tranquilli et al., 2013), release substance P (Nicol et al., 1992) and modulate immune cells (Tilley et al., 2001). However, meloxicam did not have an effect on the other physiological and behavioral parameters measured in this study.

Table 3.1. Least square means (± SEM) of samples collected at T0, 60, 90 and 120 min after castration for cortisol, substance P, base scrotal temperature (Base temp) and scrotal temperature (Scrotal temp) and on d 1, 2, 3 and 7after castration of cortisol, substance P, haptoglobin, serum amyloid-A (SAA), complete blood count (CBC), base scrotal temperature (Base temp), scrotal temperature (Scrotal temp) and rectal temperature (Rectal Temp) of non-castrated (CT), band (BA) and knife (KN) castrated 1-wk-old Angus crossbred bull calves with (M) or without (NM) a single s.c. meloxicam administration[1]

| | Treatment[2] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CT | | BA | | KN | | | *P-Value*[3] | | | | |
| Item | NM | M | NM | M | NM | M | *SEM*[4] | CAS | MED | T | CAS ×T | MED ×T |
| *Minutes after castration* | | | | | | | | | | | | |
| Cortisol, nmol/L | 3.9 | 4.5 | 4.8 | 6.0 | 6.2 | 4.6 | 0.12 | 0.08 | 0.62 | <0.01 | 0.04 | 0.10 |
| Substance P, pg/mL | 97.1 | 103.1 | 101.7 | 93.4 | 102.7 | 99.5 | 0.04 | 0.90 | 0.78 | 0.02 | 0.18 | 0.09 |
| Base temp, ºC | 34.4 | 35.2 | 35.5 | 35.7 | 34.5 | 34.7 | 0.55 | 0.04 | 0.29 | <0.01 | 0.08 | 0.93 |
| Scrotal temp, ºC | 34.5 | 34.7 | 34.1 | 34.1 | 33.7 | 34.0 | 0.59 | 0.18 | 0.58 | <0.01 | 0.67 | 0.49 |
| *Days after castration* | | | | | | | | | | | | |
| Cortisol, nmol/L | 4.2 | 4.0 | 3.3 | 3.8 | 3.5 | 3.2 | 0.11 | 0.78 | 0.84 | <0.01 | 0.98 | 0.56 |
| Substance P, pg/mL | 92.3 | 99.1 | 100.2 | 91.7 | 99.1 | 90.1 | 0.05 | 0.83 | 0.14 | <0.01 | 0.68 | 0.04 |
| Haptoglobin, g/L | 0.3 | 0.2 | 0.2 | 0.3 | 0.3 | 0.3 | 0.10 | 0.31 | 0.92 | 0.02 | 0.72 | 0.19 |
| SAA, µg/mL | 102.6[c] | 102.8[bc] | 103.1[bc] | 114.0[ab] | 148.7[a] | 108.2[b] | 0.14 | 0.01 | 0.80 | <0.01 | 0.05 | 0.57 |
| *CBC* | | | | | | | | | | | | |
| WBC, × $10^9$/L | 11.4 | 9.8 | 10.3 | 9.9 | 11.4 | 9.8 | 0.54 | 0.59 | 0.01 | 0.09 | 0.62 | 0.19 |
| RBC, × $10^{12}$/L | 8.0 | 7.8 | 8.1 | 8.0 | 8.0 | 8.2 | 0.09 | 0.04 | 0.64 | <0.01 | 0.30 | 0.46 |
| Platelets, × $10^9$/L | 521.6 | 496.3 | 486.9 | 480.5 | 467.3 | 481.2 | 23.24 | 0.31 | 0.76 | 0.01 | 0.83 | 0.84 |
| Base temp, ºC | 33.7 | 33.9 | 33.9 | 33.9 | 34.3 | 33.8 | 0.42 | 0.83 | 0.76 | <0.01 | 0.13 | 0.50 |
| Scrotal temp, ºC | 32.7 | 33.1 | 31.1 | 31.4 | 34.0 | 33.3 | 0.42 | <0.01 | 0.96 | <0.01 | <0.01 | 0.32 |
| Rectal temp, ºC | 39.4 | 39.4 | 39.3 | 39.5 | 39.4 | 39.4 | 0.11 | 0.71 | 0.26 | <0.01 | 0.97 | 0.26 |

[a-c] Least square means within a row with different superscripts differ ($P$ <0.05)

[1] Values represent the mean of T0, 60, 90 and 120 min and d 1, 2, 3 and 7 after castration.

[2]CT: sham non-castrated calves; BA: band castrated calves; KN: knife castrated calves; NM: single s.c. injection of lactated Ringer's immediately before castration; M: single injection of s.c. meloxicam (0.5 mg/Kg) immediately before castration.

[3]CAS: castration effect; MED: medication effect, T: time effect.

[4]The values presented correspond to non-transformed means, however SEM and P-values correspond to ANOVA analysis using log or square root + 1 transformed data.

Table 3.2. Least square means (± SEM) of performance (initial and final BW and ADG) of the first week post castration of non-castrated (CT), band (BA) and knife (KN) castrated 1-wk-old Angus crossbred bull calves with (M) or without (NM) a single s.c. meloxicam administration.

| | Treatment[1] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CT | | BA | | KN | | | *P-Value*[2] | | |
| Item | NM | M | NM | M | NM | M | *SEM*[3] | CAS | MED | CAS × MED |
| Performance | | | | | | | | | | |
| Initial BW (d-1), kg | 46.2 | 46.7 | 46.9 | 47.2 | 49.0 | 47.8 | 2.09 | 0.65 | 0.95 | 0.91 |
| Final BW (d7), kg | 55.2 | 56.1 | 55.4 | 57.2 | 58.1 | 56.6 | 2.41 | 0.78 | 0.84 | 0.77 |
| ADG, kg/d | 1.0 | 1.0 | 0.9 | 1.1 | 1.0 | 1.0 | 0.10 | 0.93 | 0.32 | 0.46 |

[1]CT: sham non-castrated calves; BA: band castrated calves; KN: knife castrated calves; NM: single s.c. injection of lactated Ringer's immediately before castration; M: single injection of s.c. meloxicam (0.5 mg/Kg) immediately before castration.
[2] CAS: castration effect; MED: medication effect.
[3]The values presented correspond to non-transformed means, SEM and *P*-values correspond to ANOVA analysis.

Table 3.3. Least square means (± SEM) at the time of castration for visual analog scale (VAS) scores and frequencies of urination, leg movement and vocalization and for a 2 h period 2 to 4 h after castration of behavioral observations of non-castrated (CT), band (BA) and knife (KN) castrated 1-wk-old Angus crossbred bull calves with (M) or without (NM) a single s.c. meloxicam administration[1]

| | Treatment[2] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CT | | BA | | KN | | | *P-Value*[3] | | |
| Item | NM | M | NM | M | NM | M | *SEM* | CAS | MED | CAS × MED |
| VAS[4], cm | 1.1$^{c}$ | 1.1$^{c}$ | 1.2$^{b}$ | 1.4$^{b}$ | 1.6$^{a}$ | 1.5$^{a}$ | 0.08 | <0.01 | 0.54 | 0.23 |
| Urination[4], n | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 0.03 | 0.55 | 0.41 | 0.56 |
| Leg movement[4], n | 1.5$^{b}$ | 1.2$^{b}$ | 3.1$^{a}$ | 5.4$^{a}$ | 5.0$^{a}$ | 5.3$^{a}$ | 0.23 | <0.01 | 0.49 | 0.44 |
| Vocalization[4], n | 1.0$^{b}$ | 1.0$^{b}$ | 1.1$^{a}$ | 1.0$^{a}$ | 1.2$^{a}$ | 1.1$^{a}$ | 0.05 | 0.02 | 0.49 | 0.59 |
| Behavioral obs.[5] | | | | | | | | | | |
| Walking, min | 2.0 | 5.2 | 8.3 | 2.3 | 11.81 | 8.8 | 0.61 | 0.09 | 0.25 | 0.33 |
| Standing, min | 22.3 | 43.7 | 39.5 | 21.5 | 61.6 | 35.9 | 0.96 | 0.14 | 0.24 | 0.10 |
| Lying, min | 95.8 | 71.4 | 70.6 | 96.1 | 46.2 | 74.5 | 1.41 | 0.15 | 0.55 | 0.06 |
| Suckling, min | 3.0$^{b}$ | 34.6$^{a}$ | 13.6$^{ab}$ | 6.5$^{b}$ | 38.0$^{a}$ | 12.3$^{ab}$ | 1.12 | 0.36 | 0.77 | 0.02 |
| Tail flick, n | 14.5 | 0.0 | 55.3 | 26.7 | 209.6 | 88.0 | 2.31 | <0.01 | 0.03 | 0.25 |
| Foot stamping, n | 1.6 | 6.5 | 11.2 | 2.5 | 27.2 | 6.6 | 0.90 | 0.24 | 0.26 | 0.33 |
| Head turning, n | 2.0 | 8.8 | 11.1 | 2.6 | 12.0 | 3.8 | 0.59 | 0.73 | 0.39 | 0.13 |

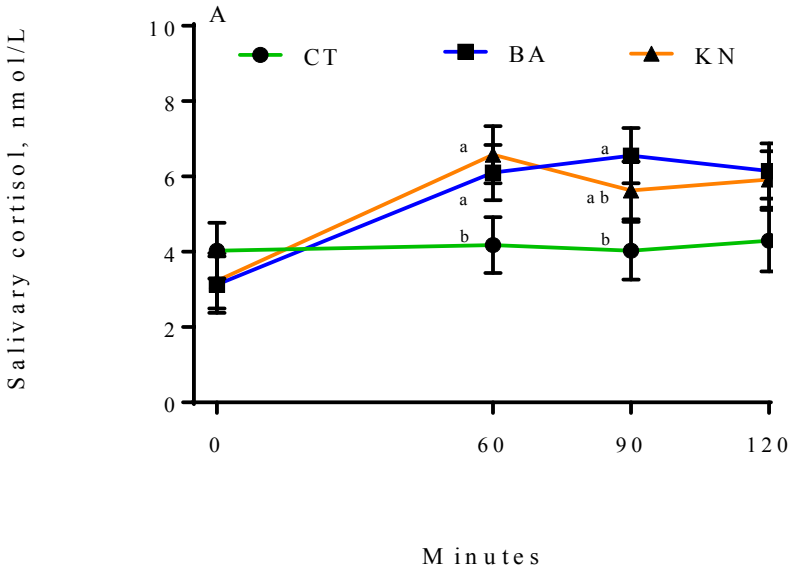
[a-c] Least square means within a row with differing superscripts differ ($P < 0.05$).
[1]Values represent the mean of time 2 to 4 h after castration.
[2]CT: sham non-castrated calves; BA: band castrated calves; KN: knife castrated calves; NM: single s.c. injection of lactated Ringer's immediately before castration; M: single injection of s.c. meloxicam (0.5 mg/Kg) immediately before castration.
[3]CAS: castration effect; MED: medication effect, T: time effect.
[4]The values presented correspond to transformed means, SEM and *P*-value using square root +1 transformation.
[5]The values presented correspond to non-transformed means; however SEM and *P*-values correspond to ANOVA analysis using square root + 1 transformation.

Table 3.4. Least square means (± SEM) of samples taken on d 1, 2, 3 and 7 for stride length and behavioral observations, and for samples taken on d 0, 1, 2, 3, 4, 5, and 6 of standing and lying behavior of non-castrated (CT), band (BA) and knife (KN) castrated 1-wk-old Angus crossbred calves with (M) or without (NM) a single s.c. meloxicam administration.

| | Treatment[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CT | | BA | | KN | | | *P-Value*[2] | | | | |
| Item | NM | M | NM | M | NM | M | *SEM*[3] | CAS | MED | T | CAS ×T | MED ×T |
| Stride length[4], cm | 37.0 | 38.6 | 36.5 | 35.6 | 38.7 | 38.1 | 1.48 | 0.27 | 0.97 | 0.83 | 0.77 | 0.31 |
| Standing and lying | | | | | | | | | | | | |
| Standing, % | 27.8 | 26.1 | 28.0 | 27.2 | 27.7 | 28.0 | 1.05 | 0.80 | 0.39 | < 0.01 | 0.67 | 0.90 |
| Lying, % | 72.2 | 74.0 | 72.0 | 72.8 | 72.3 | 72.0 | 1.06 | 0.70 | 0.39 | <0.01 | 0.64 | 0.90 |
| Standing duration, min | 20.7 | 21.2 | 21.1 | 19.3 | 20.4 | 22.2 | 1.30 | 0.68 | 0.85 | <0.01 | 0.54 | 0.39 |
| Lying duration, min | 45.0 | 46.8 | 48.0 | 41.0 | 45.6 | 44.5 | 2.51 | 0.84 | 0.31 | <0.01 | 0.35 | 0.03 |
| Standing bouts, n | 20.4 | 18.7 | 20.1 | 21.5 | 20.6 | 19.1 | 0.70 | 0.19 | 0.30 | <0.01 | 0.15 | 0.08 |
| Lying bouts, n | 24.1 | 23.7 | 23.2 | 28.1 | 25.4 | 25.5 | 1.73 | 0.58 | 0.30 | <0.01 | 0.34 | 0.10 |
| Behavioral ob. | | | | | | | | | | | | |
| Walking, min | 2.8 | 2.4 | 2.3 | 3.4 | 2.7 | 2.5 | 0.16 | 0.99 | 0.97 | 0.23 | 0.73 | 0.30 |
| Standing, min | 29.7 | 29.7 | 24.1 | 34.1 | 23.6 | 26.4 | 0.42 | 0.43 | 0.31 | 0.28 | 0.66 | 0.69 |
| Lying Lateral, min | 0.9 | 0.7 | 1.1 | 0.0 | 3.7 | 1.3 | 0.15 | <0.01 | 0.03 | 0.04 | 0.01 | 0.07 |
| Lying Ventral, min | 39.5 | 43.3 | 45.2 | 35.2 | 42.6 | 43.9 | 0.50 | 0.70 | 0.48 | 0.36 | 0.67 | 0.67 |
| Eating, min | 7.9 | 9.6 | 6.1 | 8.0 | 8.9 | 8.6 | 0.35 | 0.68 | 0.71 | 0.18 | 0.22 | 0.28 |
| Tail flick, n | 42.5 | 55.2 | 49.0 | 29.8 | 44.5 | 75.5 | 1.10 | 0.49 | 0.56 | <0.01 | 0.24 | 0.03 |
| Foot stamp, n | 5.4 | 0.1 | 3.2 | 2.8 | 1.0 | 2.9 | 0.33 | 0.80 | 0.43 | 0.04 | 0.55 | 0.43 |
| Head turning, n | 6.3 | 9.8 | 4.7 | 4.6 | 7.3 | 6.9 | 0.33 | 0.28 | 0.64 | 0.83 | 0.23 | 0.94 |

[1]CT: sham non-castrated calves; BA: band castrated calves; KN: knife castrated calves; NM: single s.c. injection of lactated Ringer's immediately before castration; M: single injection of s.c. meloxicam (0.5 mg/Kg) immediately before castration.
[2] CAS: castration effect; MED: medication effect.
[3]The values presented correspond to non-transformed means, SEM and *P*-values correspond to ANOVA analysis.
[4]Stride length: stride length mean of T0 and 120 min after castration.

Figure 3.1. Least square means and SEM for (A) cortisol (nmol/L) concentrations of T0, 60, 90 and 120 min and (B) scrotal temperature (ºC) of d 1, 2, 3, and 7 after control (CT), band (BA), and knife (KN) castration of 1-wk-old Angus crossbred bull calves. Least square means with differing superscripts differ ($P \leq 0.05$).



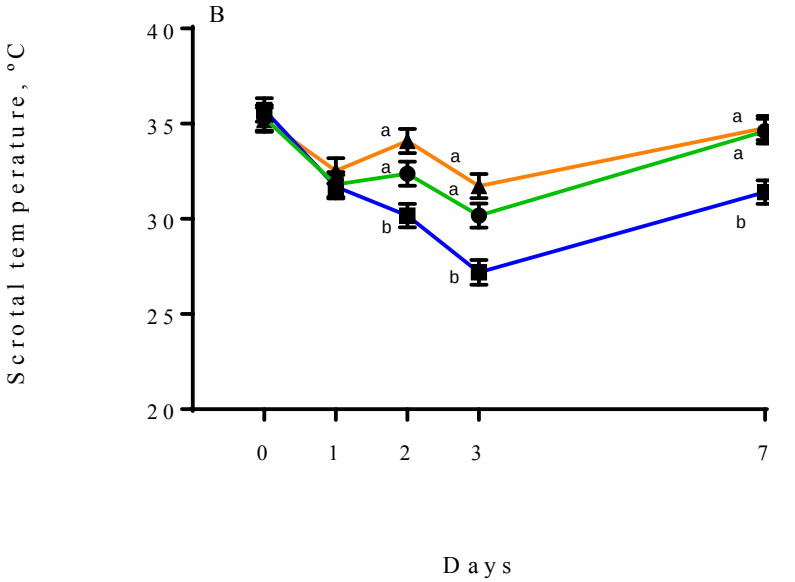



Figure 3.2. Least square means and SEM for substance P (pg/mL) concentrations of d 1, 2, 3 and 7 after band and knife castration of medicated (M) and non-medicated (NM)1-wk-old Angus crossbred bull calves. * $P \leq 0.10$. ** $P \leq 0.05$.

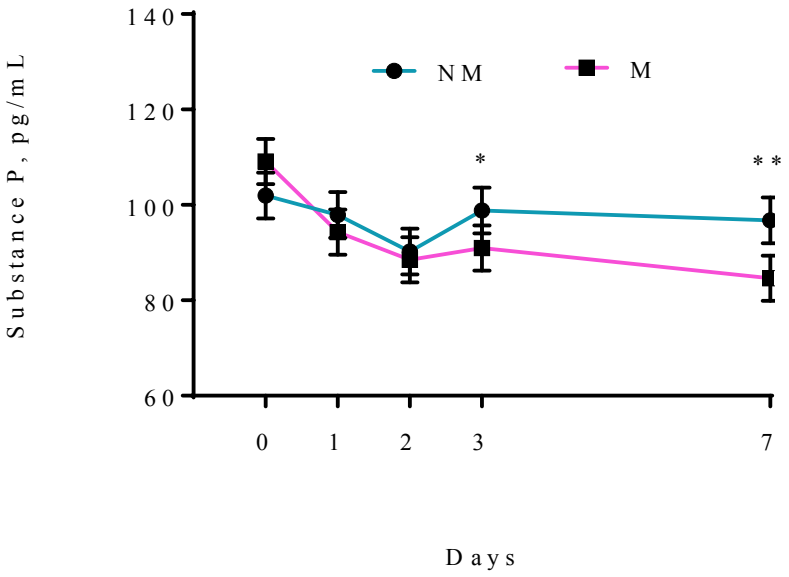
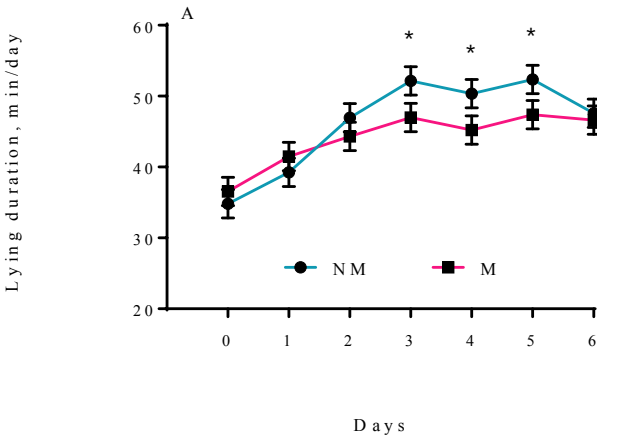
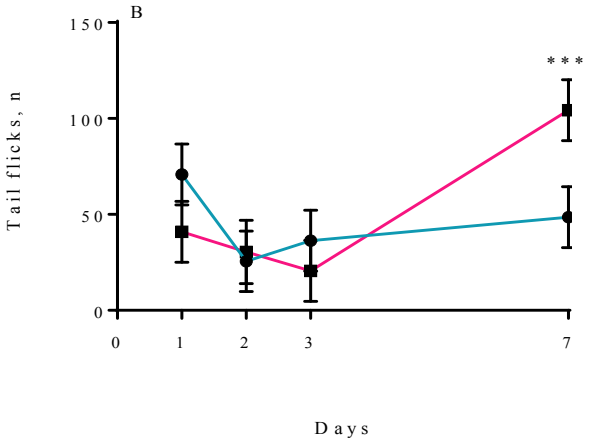
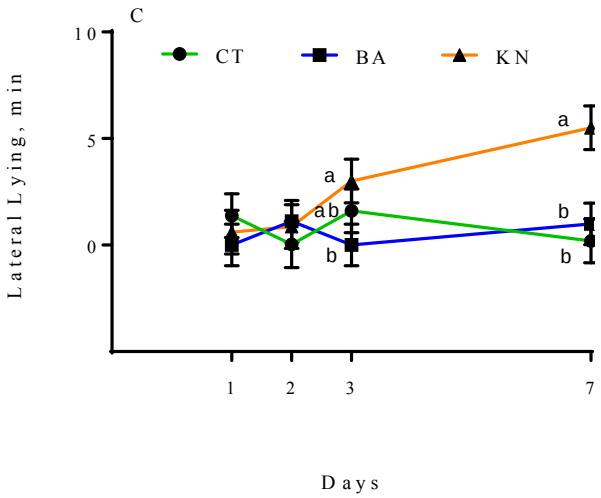


Figure 3.3. Least square means and SEM for (A) lying duration (min/day) of d 1, 2, 3, 4, 5 and 6, (B) tail flicks (n/observation period) of d 1, 2, 3 and 7 and for (C) lateral lying (min/observation period) d 1, 2, 3, and 7 after control (CT), band (BA) and knife (KN) castration of medicated (M) and non-medicated (NM) 1-wk-old Angus crossbred bull calves. Least square means with differing superscripts differ (P ≤ 0.05). * *P* ≤ 0.10. ** *P* ≤ 0.05. ***P ≤ 0.01.



# Chapter Four: Effect of subcutaneous Meloxicam on indicators of acute pain and distress after castration and branding in 2 mo old beef calves

## 4.1 Abstract

The aim of this study was to assess the effect of a single dose of s.c. meloxicam at mitigating pain associated with knife castration alone and knife castration + branding in 2-mo old calves. Seventy-two Angus crossbred bull calves (128 ± 18.5 kg of BW) were used in a 3 × 2 factorial design where main factors included procedure: sham (control calves, **CT**; *n* = 24), knife (**KN**; *n* = 24) or knife + branding (**BK**; *n* = 24) and medication: single s.c. administration of lactated ringer's solution (**NM**; n = 36) or a single dose of 0.5 mg/kg of s.c. meloxicam (**M**; n = 36). Different physiological and behavioral parameters were collected as indicators of acute pain. No medication effects ($P > 0.10$) were observed for salivary cortisol, substance P and scrotal temperature min after the procedure or for cortisol, substance P, serum amyloid A and platelets d after the procedure. Haptoglobin had a procedure × medication × time interaction ($P = 0.05$), where BK-NM calves had greater haptoglobin concentrations than BK-M, KN-M, CT-M and CT-NM calves on d 1 and 3 after procedure, while BK-NM and KN-NM calves had greater haptoglobin concentrations than BK-M, KN-M, CT-NM and CT-M calves on d 2 after the procedure. A procedure × medication interaction ($P < 0.01$) was observed for ADG, where CT-M, KN-NM and BK-M calves had greater ADG than KN-M and BK-NM calves, while CT-NM calves had greater ADG than BK-NM calves. Walking duration had a procedure × medication interaction ($P < 0.01$), where it was greater ($P < 0.05$) in BK-NM and KN-NM calves than CT-NM, CT-M, KN-M and BK-M calves 3 to 5 h after the procedure. Lying duration had a medication effect ($P = 0.04$) where M calves had greater ($P < 0.05$) lying duration than NM

calves 3 to 5 h after castration. A medication effect ($P < 0.01$) was observed for tail flicks, the NM calves had a greater a number of tail flicks than M calves 3 to 5 h after the procedure. No medication effects ($P > 0.10$) were observed for stride length, standing and lying behavior and behavioral observations. Overall, BK calves presented greater physiological and behavioral indicators of acute pain than KN calves, while meloxicam was effective at reducing physiological and behavioral indicators of acute pain associated with knife castration and knife castration + branding.

## 4.2 Introduction

Castration is a common husbandry procedure done in order to reduce aggressive behavior, improve meat quality and increase on farm safety (Jacobs et al., 1977; Stafford and Mellor, 2005). Common castration methods include band, knife and Burdizzo castration (Weaver et al., 2008) with knife castration being reported as the most common method conducted by veterinarians in the USA (Coetzee et al., 2010). In addition, multiple procedures such as ear tagging, vaccination, dehorning and branding are typically done in combination with castration in order to reduce the number of times calves must be handled.

Hot-iron branding is a common method of permanent identification in beef cattle. In North America, branding is done to establish ownership and in Canada it is also done to meet the requirements for exporting cattle into the USA (Schwartzkopf-Genswein et al., 2012). A Western Canadian survey reported that over half of the calves (54 %) were branded and only 4 % of the respondents used pain mitigation (Moggy et al., 2017).

Both castration and branding are painful procedures (Schwartzkopf-Genswein et al., 1997ab; Stafford and Mellor, 2005; Pang et al., 2006) usually performed without the use of analgesia or anesthesia in North America. Meloxicam is a non-steroidal anti-inflammatory drug

(NSAID) and a practical option for producers due to its ease of administration (s.c.) and long lasting half-life (22 ± 3 h) (Stock and Coetzee, 2015).

Therefore, the aim of this study was to assess acute pain indicators associated with castration alone and the combination of castration + branding, and to assess the effect of meloxicam at mitigating these indicators in 2-mo beef old calves. Our hypothesis was that the combination of multiple stressors would elicit a greater stress/pain response than castration alone, and that a single s.c. dose of meloxicam would reduce pain indicators due meloxicam's analgesic and anti-inflammatory properties.

## 4.3 Materials and Methods

This protocol was approved by the Animal Care Committees of the Lethbridge Research Centre (ACC number 1410) and the University of Calgary (AC14- 0159) and animals were cared for in accordance with the Canadian Council of Animal Care (CCAC, 2009).

### 4.3.1 Animal Housing and Management

Seventy-two Angus crossbred beef calves (128 ± 18.5 kg of BW) were brought to the Lethbridge Research Centre (LRC) from a neighbouring ranch located 30 km from the LRC. Calves were separated into two groups of 36 calves as animals were castrated on different days 1 wk apart. Cow-calf pairs were housed in 6 experimental pens containing a calf shelter (2.4 m × 3.6 m × 1.4 m), straw bedding and a centrally located water system. Three of the pens measured 36.7 m × 22.2 m, and three pens measured 40 m × 27 m. Free choice alfalfa grass was available for the cows, while the calves diet consisted of free choice alfalfa grass hay, milk from suckling and free choice salt blocks and loose minerals containing a coccidiostat (Diluted Rumensin Drug Premix 1100 (Medicated), HI-PRO FEEDS, Okotoks, Alberta, Canada) to prevent diarrhea caused by coccidiosis.

Calves were weighed in a portable chute (Pearsons Livestock Equipment, Thedford, Nebraska) and sampled while standing in a tipping table (Calf Roper, Ram-Bull Ltd, Barons, Alberta, Canada). All calves were castrated and branded on a tipping table (Hi-Qual Manufacturing Canada Ltd., MB, Canada) while lying on their left side. Castration was performed first and consisted of making an incision in the scrotum with a Newberry knife (Syrvet Inc., Waukee, IA) and crushing and cutting of the cords with an emasculator. All castrations were done by the same experienced veterinarian. Branding was done with the use of an electric hot-iron based on 3 combined marks: a number, a symbol and a letter (3 = M) placed on the right rib cage when calves were tipped. Sham calves were handled in the same way as castrated and branded calves. The testicles were manipulated for a similar amount of time and the same iron used to make the brand but unheated was placed on the calves simulating the pressure exerted with the hot-iron. Branding was done by the same experienced person. Calves were castrated for an average time of 1.1 ± 0.19 min, branded for 0.5 ± 0.18 min and sampled for 3.1 ± 2.75 min.

Calves were equally distributed by weight into treatments and pens, and randomly assigned to treatments. The experiment consisted of a 3 × 2 factorial design where main factors included procedure**:** sham (control calves, **CT**; *n* = 23), knife castration (**KN**; *n* = 24) or branding and knife castration (**BK**; *n* = 24) and medication: single dose of 0.5 mg/kg of s.c. meloxicam (Metacam 20 mg/mL, Boheringer's Ingelhein, Burlington, Ontario, Canada) (**M**; n = 36) or the corresponding volume of a single s.c. administration of lactated ringer's solution (Lactated Ringer's Irrigation, Baxter Canada, Mississauga, Ontario, Canada) (**NM**; n = 35), to yield: CT-NM (n = 11), CT-M (n = 12), KN-NM (n = 12), KN-M (n = 12), BK-NM (n = 12), BK-M (n = 12).

### 4.3.2 Measurements of Acute pain and Sample Collection

***Cortisol.*** Salivary samples were collected 24 h before castration (d -1), immediately before castration (**T0**), 60, 90, 120, 180 min and on d 1, 2, 3 and 7 after castration. Saliva was collected, stored and analyzed as described by Meléndez et al. (2017b). The inter-assay CV was 13.2 % while the intra-assay CV was 9.9 %.

***Substance P, Serum Amyloid-A, Haptoglobin and Complete Blood Count.*** Blood samples were collected from all calves through jugular venipuncture on d -1, immediately before castration (T0), 60, 90, 120, 180 min and on d 1, 2, 3 and 7 after castration. Samples for substance P were collected, centrifuged for 15 min at 1.5 × *g* at 0 °C, stored and analyzed as previously described by Meléndez et al. (2017b). Briefly, samples were collected into a 6-ml tubes containing EDTA (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ), where benzamidine hydrochloride was added to reduce substance P degradation. Samples were analyzed at Iowa State University, College of Veterinary Medicine (Ames, IA) with some modifications from the previously described procedure by Van Engen et al. (2014). The intra-assay CV was 11.9 % and the inter-assay CV was calculated at 24.2 %.

Blood samples for serum amyloid-A (SAA) and haptoglobin were collected on d 1, 2, 3 and 7, stored and analyzed as previously described by Meléndez et al. (2017a). Briefly samples were collected into a 10-ml non-additive tube (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ), centrifuged for 15 min at 1.5 × *g* at 4 °C and the serum was decanted and frozen at -80 ºC for further analysis. The inter-assay CV for haptoglobin was 7.6 %, while SAA intra-assay and inter-assay CV were 5.7 % and 13.5 %, respectively.

Blood samples for CBC were collected into a 6-ml EDTA tube (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ) on d 1, 2, 3 and 7 and red blood cells (RBC), white blood cells (WBC), platelets (PLT) and neutrophil: lymphocyte ratio (N:L) were measured using a HemaTrueHematology Analyzer (Heska, Lobeland, Co).

***Scrotal Area Temperature (SCT).*** Images of the area of the scrotum were collected on d -1, immediately before castration (T0), 60, 90, 120, 180 min and on d 1, 2, 3 and 7 after castration. Images were collected and analyzed as previously described by Meléndez et al. (2017b). Briefly, a FLIR i60 infrared camera (FLIR Systems Ltd., Burlington, ON, Canada) was used to take infrared images of the scrotal area and FLIR Tools version 5.1 (FLIR Systems Ltd.) was used to delineate the scrotal area and to record the maximum temperature.

***Rectal temperature (Rectal temp).*** A digital thermometer (M750 Livestock Thermometer, GLA Agricultural Electronics, San Luis Obispo, CA) was used to collect rectal temperature on d -1, immediately before castration (T0), and on d 1, 2, 3 and 7 after castration.

***Performance.*** A portable scale (Pearsons Livestock Equipment, Thedford, Nebraska) was used to obtain the initial (average of d -1 and d 0) and final (d 7) BW. The ADG (kg/d) was calculated by subtracting the weights on d 7 from the average of d -1 and 0 and dividing the result by the number of days in the experiment (7 d).

***Behavioral frequencies and Visual Analog Scale (VAS).*** Behavioral scoring during castration was collected as previously described by Meléndez et al. (2017b). Briefly, two experienced observers marked a line along a 10 cm continuum of their perception of the amount of pain calves were experiencing during castration and recorded the frequency of urination,

defecation, leg movement and vocalizations. Due to the experimental setting, observers could not be blind to the treatments.

***Electronic reactivity measurements (ERM).*** The tipping table was equipped with one 3 dimension accelerometer and the three forces were added to obtain an overall force during castration and branding procedures. Analog signals (V) from the accelerometer were sent to a computer at a rate of 100 samples/ s. Data from control calves collected during sham castration and sham branding was used as the baseline for calves that were castrated and branded. Variables included number of peaks between 1 and 2 SD, 2 and 3 SD, and above or below 3 SD above and below the mean (Fig. 4.1A) and total area between the mean ± 1 SD, mean ± 2 SD, and mean ± 3 SD (Fig. 4.1B).

***Stride length.*** Stride length was collected as previously described by Meléndez et al. (2017b). Briefly, calves were recorded when walking through an alley on d-1, immediately after castration, 180 min and on d 1, 2, 3 and 7 after castration. Pictures of the back legs were taken with GOM player (GOM Lab, Gretech Corporation, Seoul, South Korea), while stride length was measured using Image J (National Institutes of Health Image, Bethesda, MD).

***Behavioral observations.*** Half of the animals of each treatment were recorded for behavioral observations and focal animal sampling from continuous recordings (Martin and Bateson, 2007) were done for frequency and duration behaviors as described by Meléndez et al. (2017b). Two experienced observers scored behavior for a 2 h period on d 0 between 3 to 5 h relative to treatment application and for 4 min every 10 min for a 4 h period on d 1, 2, 3 and 7 for a subset of 6 animals per treatment. Inter-rater and intra-rater reliability were 0.95 and 0.91 respectively.

***Standing and lying behavior.*** Animals were equipped with accelerometers (Hobo pendant G, Onset Computer Corporation, Bourne, MA) in order to measure standing and lying time, duration and bouts (UBC AWP, 2013) as previously described by Meléndez et al. (2017b). Briefly, accelerometers were placed on d -1 with Vet Wrap (Professional Preference, Calgary, Canada) and removed on d 7. Only days with 24 h of information were included in the analysis (d 0 to d 6).

### *Statistical analysis*

Salivary cortisol, substance P, SAA, haptoglobin, CBC, stride length and behavior the days post castration were analyzed using the MIXED procedure in SAS (SAS, version 9.4, SAS Inst. Inc., Cary, NC) to evaluate the effect of procedure, medication and time on all variables. Fixed effects included procedure, medication, time and their interactions, while random effects included pen and calf within pen. Animals were the experimental unit as treatments were mixed within pen. All data was analyzed using the mixed repeated models with the exception of behavior during castration. Behavior during castration (VAS, frequency of leg movement, urination, defecation, vocalizations and ERM) and performance was analyzed as described above without time effect (as there were no repeated measures). Data was tested for normal distribution with PROC UNIVARIATE (SAS, version 9.4, SAS Inst. Inc., Cary, NC) and physiological data that did not follow a normal distribution was log transformed while behavioral data was square root + 1 transformed. The data collected on d-1 was used as a covariate for all physiological parameters and stride length. Electronic reactivity measurements collected for sham calves at the time of castration and branding were used as the mean for ERM for KN and BK calves. Urination and defecation were not analyzed as these behaviors were not present during castration or branding. The analysis with the covariance structure (unstructured, compound symmetry and

autoregressive order one) with the lowest Schwarz's Bayesian criterion was selected as the analysis of choice. Data from the day of castration was analyzed separately from the data the days after castration. Least squares means differences were determined using the PDIFF option in SAS. Effect of procedure, medication and time were statistically significant when $P \leq 0.05$ and considered a tendency when $0.05 < P \leq 0.10$. An intra-class correlation coefficient with a 95 % CI was used to calculate intra and inter observer reliability of two experienced observers using IBM SPSS statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA).

## 4.4 Results and Discussion

### 4.4.1 Physiology

***Cortisol.*** A procedure × time effect ($P < 0.01$) was observed for cortisol (Fig. 4.2A), where KN and BK calves had greater ($P \leq 0.01$ ) cortisol concentrations than CT calves 60 min after the procedure, while BK calves had the greatest ($P < 0.05$) cortisol concentrations, KN calves had intermediate, and CT calves had the lowest concentrations 90, 120 and 180 min after the procedure. No medication effect ($P > 0.10$) was observed for cortisol min or d after the procedure, and no procedure effect ($P > 0.10$) was observed d after castration.

Contrary to our findings, previous studies have reported a reduction in cortisol concentrations in castrated calves receiving NSAID's, such as surgically castrated calves receiving oral meloxicam compared to un-medicated surgically castrated 227 kg calves (Roberts et al., 2015), carprofen, in band castrated compared to un-medicated band castrated 5.5 mo old calves (Pang et al., 2006) and Burdizzo castrated calves receiving ketoprofen compared to un-medicated Burdizzo castrated 11 mo old calves (Ting et al., 2003). However, in the previous studies, carprofen and ketoprofen were administered intravenously 20 min before castration, while oral meloxicam was given concurrently to castrated animals as a bolus administered

directly into the rumen. A possible reason for lack of medication effect in the current study may be that meloxicam administered s.c. immediately before the time of the procedure does not block pain, therefore it would not have an effect on the acute response to castration or branding. However, differences in physiological parameters were expected after castration as NSAID's have peripheral and central anti-nociceptive mechanisms (Burian and Geisslinger, 2005). It is possible that administering s.c. meloxicam immediately before castration did not give enough time for the drug to be absorbed to reach therapeutic levels in the tissues, which could have made a difference in the physiological parameters collected 3 h after castration. However, according to our results, a study assessing pre-emptive analgesia with subcutaneous meloxicam at three different time points (6, 3, and 0 h) prior to knife castration did not find treatment differences in physiological (WBC, RBC, PLT, SAA, haptoglobin, rectal temperature, salivary and hair cortisol) indicators of stress and inflammation up to 4 hours after castration (Meléndez et al., 2017a). On the other hand, therapeutic levels might not be as important as the overall effect of medication on specific metabolic and physiological pathways triggered by pain/stress, which could explain the differences observed in physiological responses across studies.

Similar to our results, Sutherland et al. (2013) did not see differences in cortisol concentrations between surgically castrated, dehorned, or surgically castrated + dehorned 3 mo old calves 0, 24 and 72 h after treatment. The author suggested that lack of differences in cortisol concentrations could be due to a potential ceiling effect of the cortisol response to either castration or dehorning, however cortisol AUC in castrated + dehorned calves was greater than only castrated or only dehorned calves up to 6 h after the procedure, providing some evidence that the combination of procedures is more painful. Similar results were reported by Mosher et al. (2013) who found a tendency for cortisol to be greater 60 min after castration in surgically

castrated + dehorned 3 to 4 mo old calves than those that were only castrated. Although, different castration methods and painful procedures such as dehorning and branding can cause different physiological responses, both procedures are painful and stressful and therefore likely to increase cortisol concentrations.

***Substance P.*** No procedure or medication effects ($P > 0.10$) were observed for substance P min or d after procedure (Table 4.1). These findings are similar to results reporting no differences in substance P levels 60 and 120 min and on d 7 after different castration methods (control, band and knife) in 1 wk old calves (Meléndez et al., 2017b), and on d 0, 1 and 7 after band castraion in medicated or un-medicated (oral meloxicam) weaned calves (Repenning et al., 2013). However caution should be taken when comparing results as the age of the calves differ between experiments. In addition, lack of differences could be a result of other factors (alone or in combination) including, high inter-assay CV, high individual animal variation in the measurements taken which could mask treatment effects, sampling times being inadequate to detect differences among treatments, variables collected were not sensitive enough to detect differences among treatments or that calves did no experience pain the days following castration and branding.

***Serum Amyloid-A and Haptoglobin.*** A procedure × time interaction ($P < 0.01$) was observed for SAA (Fig. 4.2B), where KN and BK calves had greater ($P < 0.01$) SAA concentrations than CT calves on d 1, 2 and 3, while no differences ($P > 0.10$) were observed between procedures on d 0 and 7. No medication effects ($P > 0.10$) were observed for SAA the days after castration (Table 4.1).

A procedure × medication × time effect ($P = 0.05$) was observed for haptoglobin (Fig. 4.2C), where BK-M calves had greater ($P = 0.04$) concentrations than BK-NM calves on d 0. On

d 1, BK-NM calves had greater ($P < 0.05$) concentrations than BK-M, KN-M, CT-NM, and CT-M calves. The KN-NM calves had greater ($P < 0.05$) haptoglobin concentrations than CT-NM, CT-M, K-M calves and tended ($P = 0.09$) to have greater haptoglobin concentrations than BK-M calves. The KN-M calves had greater ($P < 0.05$) haptoglobin concentrations than CT-M calves and tended ($P = 0.06$) to have greater haptoglobin concentrations than CT-NM calves. On d 2, BK-NM and KN-NM calves had greater ($P < 0.05$) haptoglobin concentrations than CT-NM, CT-M, KN-M and BK-M calves, while BK-M calves had greater ($P < 0.05$) concentrations than CT-M calves and tended ($P = 0.07$) to have greater haptoglobin concentrations than CT-NM calves. On d 3, BK-NM calves had greater ($P < 0.05$) haptoglobin concentrations than BK-M, KN-M, CT-M, and CT-NM calves, while KN-NM calves had greater ($P < 0.05$) haptoglobin concentrations than CT-M and CT-NM calves. On d 7 after castration KN-M calves had greater ($P < 0.05$) haptoglobin concentrations than BK-M calves, and tended ($P = 0.07$; $P = 0.09$) to have greater haptoglobin concentrations than CT-NM and BK-NM calves.

Both haptoglobin and SAA concentrations were above the normal range for healthy bovines (Haptoglobin: < 0.1 g/L and SAA: 1.3 ± 0.4 μg/mL) (Ceciliani et al., 2012) and followed the normal acute phase protein response which increases 24 to 48 h after a challenge and returns to baseline levels approximately 4 to 7 d after (Petersen et al., 2004). Medication effects have been previously described for haptoglobin concentrations, where ketoprofen administration reduced haptoglobin concentrations 1 d after Burdizzo castration in 13 mo old calves (Ting et al., 2003) and up to 3 d after surgical castration in 5.5 mo old calves (Earley and Crowe, 2002). Oral meloxicam has also been reported to decrease haptoglobin concentrations after surgical castration in calves at weaning weighing between 216 to 228 kg (Brown et al., 2015) and in 227 kg calves (Roberts et al., 2015). In contrast, there is a lack of literature evaluating the response of

SAA after castration and pain mitigation. A study in 7 to 8 mo old beef calves reported greater SAA concentrations than baseline levels after surgical castration, but no effect of time of s.c. meloxicam administration (6, 3 and 0 h before castration) on SAA concentrations (Meléndez et al., 2017a). Lack of differences in the previous study could be due to the fact that all treatments received meloxicam, however no medication effect was observed for SAA in the present study which assessed both medicated and un-medicated calves. A possible explanation could be that NSAID's do not have the same effect of reducing the production of different APP's, which could explain the medication effect observed for haptoglobin but not for SAA.

***Complete Blood Count.*** A medication × time effect ($P < 0.01$) was observed for WBC count (Fig. 4.3A), M calves had a greater ($P < 0.05$) WBC count than NM calves on d 0, while NM calves had a greater ($P < 0.05$) WBC count than M calves on d 1 and 2. No differences ($P > 0.10$) were observed on d 3 and 7. A procedure × time effect ($P = 0.04$) was also observed for WBC counts, where KN and BK calves had a greater ($P < 0.05$) WBC count than CT calves on d 1. The BK calves tended ($P = 0.09$) to have a greater WBC count than CT calves, but no differences were observed between both groups and KN calves on d 2. The BK calves tended ($P = 0.09$) to have a greater WBC count than CT calves while KN calves had a greater ($P < 0.05$) WBC count than CT calves on d 3.

A medication × procedure effect ($P = 0.04$) was observed for RBC count, where the BK-NM calves had a greater RBC count than CT-NM calves on d 0, 1, 2, 3 and 7, however no differences were observed between both groups and CT-M, KN-NM, KN-M, and BK-NM calves. The RBC count also had a medication × time effect ($P = 0.02$), where M ($9.7 \pm 0.15 \times 10^{12}$/L) calves had a greater ($P < 0.05$) RBC count than NM ($9.3 \pm 0.15 \times 10^{12}$/L) calves on d 7 after treatment, however no differences ($P > 0.10$) were observed on d 1, 2, and 3.

A medication × time effect ($P = 0.02$) was observed for the N:L ratio (Fig. 4.3B). The NM calves had a greater ($P < 0.05$) N:L ratio than M calves on d 2 after procedure, while NM calves tended ($P = 0.07$) to have a greater N:L ratio than M calves on d 1. A procedure × time effect ($P < 0.01$) was also observed for the N:L ratio, where KN (0.6 ± 0.04) and BK (0.7 ± 0.04) calves had a greater ($P < 0.05$) N:L ratio than CT (0.4 ± 0.04) calves on d 1. The KN (0.5 ± 0.04) calves had a greater ($P < 0.05$) N:L ratio than CT (0.4 ± 0.04) calves, while BK (0.5 ± 0.04) calves tended ($P = 0.06$) to have greater N:L ratio than CT calves on d 2. The KN (0.6 ± 0.04) calves tended to have greater ($P = 0.10$) N:L ratio than CT (0.5 ± 0.04) calves on d 3, while BK (0.4 ± 0.04) calves tended to have greater ($P = 0.06$) N:L ratio on d 7. No differences ($P > 0.10$) were observed on d 0 after treatment. The N:L ratio had a procedure × medication × time effect ($P = 0.08$) tendency where KB-NM, KB-M and KN-NM calves tended to have greater N:L ratios than CT-NM, CT-M and KN-M calves on d 1 after procedure. No procedure or medication effects ($P > 0.10$) were observed for PLT.

Similar to our findings, Ballou et al. (2013) reported an increase in N:L ratio and total leukocytes in surgically castrated calves compared to non-castrated calves 6 h after castration, and a reduction in leucocytes and N:L ratio following the administration of lidocaine and flunixin meglumine. Total WBC concentrations were lower in calves given lidocaine + flunixin meglumine before dehorning compared to calves dehorned without pain relief, but no differences were observed for calves castrated or castrated + dehorned with or without pain relief (Sutherland et al., 2013). In contrast previous studies have reported no effect of NSAID's on blood parameters after castration (Pang et al., 2006; Moya et al., 2014). Although levels of WBC, RBC and N:L differed between treatments, levels were within the normal range (Smith, 2008) meaning that calves were not immunocompromised by castration or branding.

***Scrotal and rectal temperature.*** No procedure or medication effects ($P > 0.10$) were observed for SCT min after procedure (Table 4.1). A medication effect ($P = 0.04$) was observed for SCT, where M (36.6 ± 0.46 °C) calves had lower ($P < 0.05$) SCT than NM (36.9 ± 0.46 °C) calves on d 1, 2, 3, and 7. A procedure effect ($P = 0.01$) was also observed where BK (36.9 ± 0.46 °C) and KN (36.9 ± 0.46 °C) calves had greater SCT than CT (36.5 ± 0.46 °C) calves on d 1, 2 , 3 and 7. A medication × time interaction ($P = 0.01$) was observed for rectal temperature, where NM (39.4 ± 0.05 °C) calves had greater ($P < 0.05$) rectal temperature than M (39.2 ± 0.05 °C) calves on d 1 after treatment. A procedure × time interaction ($P = 0.03$) was observed for rectal temperature, where KN (39.4 ± 0.06 °C) and BK (39.3 ± 0.06 °C) calves had greater ($P < 0.05$) rectal temperature than CT (39.1 ± 0.06 °C) calves on d 1, while on d 7, KN (39.2 ± 0.06 °C) calves had greater ($P < 0.05$) rectal temperatures than BK (39.0 ± 0.06 °C) calves, however no differences were observed between both groups and CT (39.1 ± 0.06 °C) calves. No differences ($P > 0.10$) were observed for rectal temperature on d 0, 2 and 3 after treatment.

Although NSAIDS are used in veterinary medicine to reduce body temperature in animals with fever (Lees et al., 2004), the animals in the present study did not present a fever (≥ 40 °C) and the differences in rectal temperature and SCT between M and NM calves and CT, KN and BK calves was so small that differences likely lack biological significance.

***Weight and ADG.*** A procedure × medication interaction ($P = 0.01$) was observed for ADG, where CT-M, KN-NM and BK-M calves had greater ($P < 0.05$) ADG than KN-M and BK-NM calves, while CT-NM calves had greater ($P < 0.05$) ADG than BK-NM calves, but no differences ($P > 0.10$) were observed between CT-NM, CT-M, KN-NM and BK-M calves, nor between CT-NM and KN-M calves (Table 4.2). No medication or procedure effects ($P > 0.10$) were observed for initial and final BW.

Mosher et al. (2013) reported no differences in ADG between castrated, dehorned, and castrated + dehorned 3 to 4 mo old calves. The ADG was greater in CT-NM and CT-M calves as expected as the animals did not experience the trauma associated with surgery or burn. However, the BK-M calves had greater ADG than BK-NM calves, which may be due to the reduced pain which would motivate the calves to get up, walk and suckle, however, we would also expect to see a greater ADG in KN-M calves compared to KN-NM calves. It could be possible that meloxicam is more effective at alleviating pain caused by branding (somatic pain) than pain caused by knife castration (somatic and visceral pain). However, the application of flunixin meglumine did not have any effect on wound healing or pain response associated with branding (Tucker et al., 2014) and studies in cancer patients show that NSAIDS are effective at mitigating both somatic and visceral pain (Mercadante et al., 1999).

### 4.4.2 Behavior

***Behavioral frequencies and VAS.*** A procedure × medication interaction ($P = 0.04$) was observed for leg movements, where the BK-M calves had a greater ($P < 0.05$) number of leg movements than CT-NM, CT-M, KN-NM and KN-M calves during the procedures, but no differences ($P > 0.10$) were observed between BK-M and BK-NM calves (Table 4.3). The KN-M calves had greater ($P < 0.05$) leg movements than CT-NM, CT-M, and KN-NM calves, however no differences ($P > 0.10$) were observed between KN-M and BK-NM calves. No differences ($P > 0.10$) were observed between CT-NM and CT-M calves. A tendency on procedure × medication interaction ($P = 0.10$) was observed for vocalizations, where BK-NM and BK-M calves tended ($P = 0.10$) to have greater vocalization numbers than CT-NM, CT-M, KN-NM and KN-M calves during treatment. A medication effect ($P = 0.08$) was observed for VAS where M (3.0 ± 0.07 cm) calves tended to have greater scores than NM (2.6 ± 0.07 cm) calves. A

procedure effect ($P < 0.01$) was also observed for VAS where BK (5.5 ± 0.07 cm) calves had greater ($P < 0.05$) VAS scores, followed by KN (2.6 ± 0.07 cm) calves, and then by CT (0.4 ± 0.07 cm) calves.

These results demonstrate that surgical castration and hot iron branding are painful procedures as observed by greater VAS scores and vocalizations compared to CT calves, however, branding elicits more vigorous behavioral responses than surgical castration at the time of the procedure. This could be due to the differences in pain, as somatic pain is localized and allows for rapid motor reflexes, while visceral pain is poorly localized and leads to muscle contraction and autonomic and emotional responses (Gebhart and Ness, 1991). Similar behavioral results for hot-iron branding have been previously reported in a study comparing hot-iron branding and freeze branding, where hot-iron branded calves vocalized more and had greater exertion forces than freeze or sham calves (Schwartzkopf-Genswein et al, 1997b). Greater VAS scores have also been reported in surgically castrated calves compared to band and control calves (Fell et al., 1986; Meléndez et al., 2017b). An unexpected finding was the tendency for VAS scores to be greater in NM calves than M calves. Although the difference is small, we would not expect meloxicam to have an effect at the time of castration as NSAID's have little effect on behavioral responses induced by intense stimuli (Malmberg and Yaksh, 1991).

***Electronic reactivity measurements.*** During branding, a procedure effect ($P < 0.01$) was observed for number of accelerometer peaks between 2 and 3 SD above and below the mean (baseline of control calves) and greater or lower than 3 SD above or below the mean, where BK calves had a greater number of peaks than KN calves (Table 4.4). However, no differences ($P > 0.10$) were observed for number of peaks above and below the mean between 1 to 2 SD at the

time of branding (Table 4.4). A procedure effect ($P < 0.05$) was also observed for total area, where BK calves had greater ($P < 0.05$) total area than KN calves between the mean ± 1 SD, the mean ± 2 SD and the mean ± 3 SD. During castration, no medication or procedure effects ($P > 0.10$) were observed for number of peaks between 1 to 2 SD, 2 to 3 SD, and greater or lower than 3 SD, and total area between the mean and ± 1 SD, ± 2 SD and ± 3SD above and below the mean.

As expected no differences were observed for accelerometer movement at the time of castration, as both groups of calves were surgically castrated. However, differences were observed for branding, as one group was branded with a hot-iron while the other group was sham branded. This is in agreement with the results observed for VAS scores.

***Stride length.*** No medication or procedure effects ($P > 0.10$) were observed for stride length immediately after or 180 min after castration (Table 4.5). However, a procedure effect ($P < 0.01$) was observed for stride length, where KN (43 ± 1.1 cm) and BK (43 ± 1.0 cm) calves had greater stride length than CT (40 ± 1.0 cm) calves on d 1, 2, 3 and 7. No medication effect ($P > 0.10$) was observed for stride length on d 1, 2, 3, and 7.

Similar results were observed by Meléndez et al. (2017b) who reported no differences in stride length immediately after and 120 min after castration in control, band and knife castrated 2 mo old calves. Contrary to our findings, control, band and knife castrated calves at 2-mo of age did not present differences in stride length on d 1, 2, 3 and 5 after castration (Meléndez et al., 2017b). This finding is difficult to explain, as we would expect KN and BK calves to have a shorter stride length than CT calves. Currah et al. (2009) suggested shortening of the stride length as a behavioral indicator of pain associated with surgical castration after observing longer stride lengths in 3 mo old calves receiving flunixin meglumine and a lidocaine epidural than

calves receiving a lidocaine epidural or no medication. Differences between studies could be due to the time of sampling as differences in the previous study were observed 4 and 8 h after castration, while in the present study calves were sampled immediately after and 4 h after castration. In addition, the previous study used the combination of an analgesic and a local anesthetic which has been previously reported to be more effective at mitigating the cortisol (Stafford et al., 2002; Webster et al., 2013) and the leukocyte response (Ballou et al., 2013) associated with castration.

***Behavioral observations.*** A procedure × medication interaction ($P < 0.01$) was observed for walking duration (Table 4.3). The BK-NM and KN-NM calves had greater ($P < 0.05$) walking duration than CT-NM, CT-M, KN-M and BK-M calves 3 to 5 h after treatment. Lying duration had a medication effect ($P = 0.04$) where M (87 ± 0.4 min) calves had greater ($P < 0.05$) lying duration than NM (66 ± 0.4 min) calves 3 to 5 h after treatment. A procedure effect ($P = 0.03$) was also observed for lying duration 3 to 5 h after treatment, the KN (66 ± 0.5 min) and BK (64 ± 0.5 min) calves had lower ($P < 0.05$) lying durations than CT (97 ± 0.5 min) calves. A medication effect ($P < 0.01$) was observed for tail flicks, the NM (1449 ± 2.4) calves had greater ($P < 0.05$) number of tail flicks than M (608 ± 2.4) calves 3 to 5 h after treatment. A procedure effect ($P < 0.01$) was also observed for tail flicks, the KN (1346 ± 3.0) and BK (1711 ± 3.0) calves had a greater ($P < 0.05$) number of tail flicks than CT (29 ± 3.0) calves 3 to 5 h after treatment. A procedure effect ($P = 0.01$; $P < 0.01$) was observed for standing and foot stamping, where the KN (55 ± 0.5 min) and BK (58 ± 0.5) calves had greater ($P < 0.05$) standing duration than CT (29 ± 0.5 min) calves and the BK (30 ± 0.5) calves had greater foot stamping than CT (2 ± 0.5) and KN (9 ± 0.5) calves 3 to 5 h after treatment. A procedure effect ($P = 0.08$) was observed for head turning, BK (24 ± 0.6) calves tended to have greater ($P = 0.08$) head turning

than CT (3 ± 0.6) calves, however, no differences ($P > 0.10$) were observed between both groups and KN (12 ± 0.6) calves 3 to 5 h after treatment. No medication or procedure effects ($P > 0.10$) were observed for eating or lesion licking 3 to 5 h after treatment (Table 4.1).

Meloxicam reduced pain related behaviors as seen by a reduction in walking and tail flicking, and an increase in lying duration in M calves compared to NM calves. Contrary to our findings, Sutherland et al. (2013) did not see differences in tail flicking between castrated, dehorned and castrated + dehorned 3 mo old calves either receiving pain relief or no pain relief 3 h after castration. Discrepancies between studies could be due to the difference in painful procedures (dehorning vs branding), which can elicit different behavioral responses and/or to differences in medication (lidocaine + flunixin meglumine vs meloxicam). Although no differences were observed for tail flicks and head turns between BK and KN calves, BK calves had numerically greater number of tail flicks and head turns 3 to 5 h after castration, suggesting that BK calves experienced more pain.

When behaviors related to pain were evaluated the days after treatment, a procedure × medication interaction ($P = 0.01$) was observed for head turning, where KN-NM calves had greater ($P < 0.05$) head turns than CT-NM, CT-M, KN-M and BK-M calves, but no differences ($P > 0.10$) were observed between KN-NM and BK-NM calves (Table 4.5). Head turning was greater ($P < 0.05$) in BK-NM calves than CT-NM and KN-M calves, but no differences ($P > 0.10$) were observed between BK-NM calves and CT-M and BK-M calves. No differences ($P > 0.10$) were observed between CT-NM, CT-M, KN-M and BK-M. A procedure × time tendency ($P = 0.06$) was observed for head turning (Table 4.5), where BK (9.7 ± 2.25) calves had greater ($P < 0.05$) head turns and KN (9.3 ± 2.25) calves tended ($P = 0.09$) to have greater head turns than CT (5.0 ± 2.36) calves on d 1. The BK (12.1 ± 1.91) and KN (6.0 ± 1.91) calves had

greater ($P < 0.05$) head turns than CT (4.0 ± 2.00) calves on d 2 after castration, while no differences ($P > 0.10$) were observed between treatments on d 3 and 7.

Head turning numbers were reduced by meloxicam as seen by a lower number of head turns in BK-M and KN-M calves. However, KN calves had a lower number of head turns than BK calves suggesting that BK calves experienced more discomfort than KN calves, which is in accordance with head turning 2 to 4 h after castration.

A procedure × medication × time effect ($P = 0.03$) was observed for foot stamping (Fig. 4.4A), where BK-NM calves had greater ($P < 0.05$) foot stamping than CT-NM, KN-M, and BK-M calves, and tended ($P = 0.06$) to be greater than CT-M calves on d 1 after treatment. On d 2 after treatment, BK-NM calves had greater ($P < 0.05$) foot stamping than CT-NM, CT-M, KN-NM, KN-M and BK-M calves. No differences ($P > 0.10$) were observed on d 3 and 7 after treatment (Table 4.5). Contrary to our findings, a previous study reported no differences in time spent foot stamping between castrated + dehorned calves receiving lidocaine and flunixin meglumine and non-medicated calves 3 h after treatment (Sutherland et al., 2013). Differences between studies could be due to differences in procedures, medication and sampling times.

A procedure × time effect ($P = 0.01$) was observed for tail flicks (Fig. 4.4B), where KN and BK calves had a greater ($P < 0.05$) number of tail flicks than CT calves on d 1 and 3 after treatment, while BK calves had the greatest ($P < 0.05$) number of tail flicks, followed by KN calves, and then by CT calves on d 2 after castration. These results suggest that branding in combination with castration is more painful than surgical castration alone, as seen by a greater number of tail flicks on d 2. Although not significant, a previous study reported greater number of tail flicks in knife (191) than band (78) and control (86) 2 mo old calves on d 1, 2, 3 and 5

after castration (Meléndez et al., 2017b). Tail flicks were also greater at the time of hot-iron branding than freeze or sham branding in 320 kg calves (Schwartzkopf-Genswein et al., 1997b).

A procedure effect ($P = 0.01$) was observed for eating, where CT (27 ± 0.4 min) calves had greater eating duration than BK (16 ± 0.4 min) calves, however no differences ($P > 0.10$) were observed between both groups and KN (21 ± 0.4 min) calves. Although there were no differences between CT and KN calves, it is likely that greater eating duration leads to greater ADG as CT calves had greater ADG than KN and BK calves. However, values for eating could be different if these were scored for 24 h compared to 4 h. Contrary to our results, castrated, dehorned and castrated + dehorned calves receiving lidocaine and meglumine flunixin had greater eating times than un-medicated castrated, dehorned and castrated + dehorned calves (Sutherland et al., 2013). Differences between studies could be due to the added effect of the anesthetic which could temporarily block the pain associated with the procedures and consequently calves would be more likely to eat compared to calves experiencing pain.

***Standing and lying behavior.*** Standing percentage tended (procedure × medication interaction; $P = 0.06$) to be greater while lying percentage tended (procedure × medication interaction; $P = 0.06$) to be lower in BK-NM calves than KN-NM and BK-M calves, however no differences ($P > 0.10$) were observed between these groups and CT-NM, CT-M and KN-NM calves (Table 4.5). Lying duration was greater (procedure × time interaction; $P < 0.01$) in CT calves than KN and BK calves on d 0, 1 and 2 after treatment (data not shown). No differences were observed on d 3, 4, 5, or 6 after treatment (data not shown), suggesting that animals in pain lie for less time than animals that are not in pain.

A procedure × time effect ($P < 0.01$) was observed for stranding bouts, where KN and BK calves had greater ($P < 0.05$) standing bouts than CT calves on d 1 and 2, while BK calves

had greater ($P < 0.01$) standing bouts than CT calves, and there was a tendency ($P = 0.09$) for KN calves to have greater standing bouts than CT calves on d 0. No differences ($P > 0.10$) were observed on d 3, 4, 5 and 6. A procedure × time effect ($P < 0.01$) was also observed for lying bouts, BK calves had greater ($P < 0.05$) lying bouts than CT calves, and KN calves tended ($P = 0.09$) to have greater lying bouts than CT calves on d 0. Lying bouts were greater ($P < 0.05$) in KN and BK calves than CT calves on d 1. The KN calves had greater ($P < 0.05$) lying bouts than CT calves on d 2, but no differences ($P > 0.10$) were observed between both groups and BK claves.

Lying and standing bouts are an indicator of restless behavior which is associated with pain caused by ischemia (Dinniss et al., 1999). A previous study reported a decrease in standing and lying bouts in band castrated 1-wk old calves while, an increase in standing and lying bouts in 4 mo old band castrated calves, but no differences in 2 mo old band castrated calves (Meléndez et al., 2017b). It seems that restlessness is not only linked to pain caused by ischemia but it might be to linked with general discomfort as calves that were surgically castrated, and branded + castrated presented greater standing bouts than CT calves.

No medication effects ($P > 0.10$) were observed for stride length, standing and lying behavior for standing and lying bouts, and behavioral observation for walking, standing, lying, eating, tail flicks and lesion licking. No procedure effects ($P > 0.10$) were observed for standing duration, walking, standing, lying and lesion licking. Lack of effects could be due to a reduced sample size, lack of sensitivity of parameters collected, high individual variability or suboptimal sampling time.

### 4.4.3 Conclusion

Overall, the combination of procedures elicits a greater physiological and behavioral response than performing knife castration alone. Meloxicam did not have an effect on cortisol, substance P, SAA, PLT, stride length, standing and lying behavior (standing and lying duration, standing and lying bouts) and behavioral observation for eating and lesion licking. However, meloxicam was effective at reducing the haptoglobin response, RBC and WBC counts, N:L ratio, scrotal and rectal temperature, tail flicks, head turning, and walking and lying duration. No differences were observed between KN-M and BK-M calves for the previously mentioned parameters, suggesting that meloxicam was equally effective at mitigating pain caused by knife castration alone and the combination of knife castration + branding. Meloxicam can be used as a drug to mitigate pain associated with castration and branding, however more studies are needed to identify the most effective drug, route of administration and dose for painful husbandry procedures.

Table 4.1. Least square means (± SEM) of samples collected at T0, 60, 90 and 120 min after procedure for cortisol, substance P and scrotal temperature (SCT); and at d 1, 2, 3 and 7 after procedure for cortisol, substance P, serum amyloid-A (SAA), haptoglobin, CBC (WB WBC, RBC, PLT, and N:L ratio), scrotal temperature (SCT), and rectal temperature (Rectal temp) of non-castrated (CT), knife (KN) and branded and knife (BK) castrated 2-mo-old Angus crossbred calves with (M) or without (NM) a single s.c. meloxicam administration[1]

| | Treatment (T)[2] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CT | | KN | | BK | | | *P-Value* | | | | | | |
| Item | NM | M | NM | M | NM | M | *SEM* [3] | PRD | MED | PRD×MED | Time | PRD × T | MED × T | PRD×MED×T |
| Minutes after castration | | | | | | | | | | | | | | |
| Cortisol, nmol/L | 3.2 | 3.8 | 7.4 | 5.9 | 7.7 | 7.5 | 0.13 | <0.01 | 0.48 | 0.49 | <0.01 | <0.01 | 0.35 | 0.36 |
| Substance P, pg/mL | 85.3 | 78.3 | 80.1 | 79.4 | 82.6 | 78.0 | 0.06 | 0.63 | 0.35 | 0.76 | 0.09 | 0.54 | 0.45 | 0.24 |
| SCT, ºC | 36.5 | 36.7 | 36.5 | 36.5 | 36.7 | 36.3 | 0.24 | 0.74 | 0.60 | 0.27 | 0.03 | 0.42 | 0.32 | 0.17 |
| Days after castration | | | | | | | | | | | | | | |
| Cortisol, nmol/L | 3.1 | 2.0 | 2.5 | 3.7 | 2.9 | 2.3 | 0.13 | 0.17 | 0.47 | 0.11 | 0.13 | 0.38 | 0.87 | 0.31 |
| Substance P, pg/mL | 83.8 | 80.6 | 78.7 | 75.8 | 84.5 | 81.4 | 0.07 | 0.25 | 0.64 | 0.88 | 0.15 | 0.29 | 0.15 | 0.31 |
| SAA, µg/mL | 71.3 | 66.1 | 119.8 | 109.5 | 115.6 | 120.6 | 0.09 | <0.01 | 0.54 | 0.79 | <0.01 | <0.01 | 0.15 | 0.28 |
| Haptoglobin, g/L | 0.2 | 0.2 | 0.4 | 0.3 | 0.5 | 0.3 | 0.08 | <0.01 | 0.02 | 0.26 | <0.01 | <0.01 | <0.01 | 0.05 |
| CBC[4] | | | | | | | | | | | | | | |
| WBC, × $10^9$/L | 10.1 | 9.8 | 10.5 | 10.4 | 10.8 | 10.2 | 0.27 | 0.17 | 0.12 | 0.69 | 0.13 | 0.04 | <0.01 | 0.26 |
| RBC, × $10^{12}$/L | 9.4$^{b}$ | 9.8$^{ab}$ | 9.6$^{ab}$ | 9.7$^{ab}$ | 9.9$^{a}$ | 9.6$^{ab}$ | 0.14 | 0.74 | 0.54 | 0.04 | <0.01 | 0.19 | 0.02 | 0.40 |
| PLT, × $10^9$/L | 504 | 500 | 493 | 491 | 491 | 497 | 10.5 | 0.54 | 0.98 | 0.67 | <0.01 | 0.77 | 0.23 | 0.82 |
| N:L ratio | 0.4 | 0.5 | 0.6 | 0.5 | 0.6 | 0.5 | 0.02 | 0.02 | 0.18 | 0.34 | 0.02 | <0.01 | 0.02 | 0.08 |
| SCT, ºC | 36.6 | 36.4 | 37.2 | 36.7 | 36.9 | 36.8 | 0.48 | 0.01 | 0.04 | 0.36 | <0.01 | 0.31 | 0.66 | 0.89 |
| Rectal temp, ºC | 39.2 | 39.2 | 39.3 | 39.3 | 39.3 | 39.3 | 0.06 | 0.06 | 0.53 | 0.80 | <0.01 | 0.03 | 0.01 | 0.24 |

[a-b]Least square means within a row with differing superscripts differ ($P \leq 0.05$)

[1] Values in the table represent the mean of T0, 60, 90 and 120 min and the mean of d 1, 2, 3 and 7.

[2]CT: sham non-castrated calves; KN: knife castrated calves; BK: branded and knife castrated calves; NM: single s.c. injection of lactate ring immediately before procedure; M: single injection of s.c. meloxicam (0.5 mg/Kg) immediately before procedure.

[3]The values correspond to nontransformed means; however, the SEM and the *P*-values correspond to ANOVA analysis using log transformed data.

[4]CBC = complete blood count; WBC = white blood cell count; RBC = red blood cell count; N:L ratio = neutrophil-to-lymphocyte ratio.

Table 4.2. Least square means (± SEM) of initial BW, final BW and ADG of 2-mo-old Angus crossbred calves during the first week after castration of non-castrated (CT), knife (KN) and branded and knife (BK) castrated calves with (M) or without (NM) a single s.c. meloxicam administration.

| | Treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CT | | KN | | BK | | | *P-Value* | | |
| Item | NM | M | NM | M | NM | M | *SEM*[1] | PRD | MED | PRD × MED |
| Performance | | | | | | | | | | |
| Initial BW (d-1), kg | 124.9 | 124.0 | 133.8 | 129.2 | 127.9 | 129.7 | 5.42 | 0.43 | 0.77 | 0.83 |
| Final BW (d7), kg | 133.6 | 133.4 | 142.1 | 135.7 | 133.5 | 138.8 | 5.61 | 0.63 | 0.93 | 0.58 |
| ADG, kg/d | 1.2$^{ab}$ | 1.3$^{a}$ | 1.1$^{bc}$ | 0.9$^{c}$ | 0.9$^{c}$ | 1.3$^{a}$ | 0.07 | <0.01 | 0.09 | 0.01 |

[a-c]Least square means within a row with differing superscripts differ ($P \leq 0.05$)
[1]The values correspond to non-transformed means, SEM and *P*-values.

Table 4.3. Least square means (± SEM) at the time of procedure of visual analog scale (VAS), leg movement, and vocalizations; immediately after and 180 min after procedure of stride length; and 2 to 4 h after procedure for a 2 h period of behavioral observations of non-castrated (CT), knife (KN) and branded and knife (BK) castrated 2-mo-old Angus crossbred calves with (M) or without (NM) a single s.c. meloxicam administration

| | Treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CT | | KN | | BK | | | *P-Value* | | |
| Item | NM | M | NM | M | NM | M | *SEM*[1] | PRD | MED | PRD × MED |
| VAS, cm | 0.4 | 0.3 | 2.2 | 2.9 | 5.1 | 5.8 | 0.08 | <0.01 | 0.08 | 0.37 |
| Leg movement, n | 2.6[d] | 2.1[d] | 5.2[c] | 7.5[b] | 9.1[ab] | 10.8[a] | 0.13 | <0.01 | 0.03 | 0.04 |
| Vocalization, n | 2.0 | 2.6 | 2.2 | 1.5 | 6.8 | 9.9 | 0.17 | <0.01 | 0.29 | 0.10 |
| Behavioral obs. | | | | | | | | | | |
| Walking, min | 2.1[b] | 2.9[b] | 5.2[a] | 2.5[b] | 7.0[a] | 3.3[b] | 0.16 | <0.01 | <0.01 | <0.01 |
| Standing, min | 24.8 | 32.2 | 66.1 | 43.7 | 75.3 | 40.8 | 0.70 | 0.01 | 0.07 | 0.14 |
| Lying, min | 101.8 | 94.1 | 53.4 | 82.1 | 43.4 | 85.0 | 0.77 | 0.03 | 0.04 | 0.12 |
| Eating, min | 13.0 | 15.8 | 15.1 | 14.4 | 12.3 | 11.6 | 0.62 | 0.60 | 0.81 | 0.91 |
| Tail flick, n | 41 | 17 | 1840 | 851 | 2465 | 957 | 4.2 | <0.01 | <0.01 | 0.12 |
| Head turning, n | 4.6 | 1.8 | 17.6 | 6.6 | 16.7 | 31.5 | 0.96 | 0.08 | 0.76 | 0.34 |
| Foot stamping, n | 2.1 | 1.0 | 12.9 | 5.4 | 28.3 | 31.7 | 0.75 | <0.01 | 0.37 | 0.64 |
| Lesion licking, n | 0.8 | 0.3 | 0.5 | 0.6 | 1.3 | 1.1 | 0.20 | 0.58 | 0.77 | 0.85 |

[a-d]Least square means within a row with differing superscripts differ ($P \leq 0.05$)
[1]The values correspond to nontransformed means; however, the SEM and the *P*-values correspond to ANOVA analysis using square root + 1 transformation.

Table 4.4. Least square means (±SEM) for electronic reactivity measurements at the time of castration and branding of knife (KN) and branded and knife (BK) castrated calves 2-mo-old Angus crossbred calves with (M) or without (NM) a single s.c. meloxicam administration.

| | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | BA | | KN | | | *P-Value* | | |
| Item | NM | M | NM | M | *SEM*[1] | PRD | MED | PRD × MED |
| *Castration* | | | | | | | | |
| Peaks between 1-2 SD, n | 971 | 890 | 1017 | 955 | 1.7 | 0.43 | 0.34 | 0.86 |
| Peaks between 2-3 SD, n | 119 | 110 | 127 | 141 | 0.7 | 0.46 | 0.86 | 0.46 |
| Peaks above and below 3 SD, n | 143 | 160 | 131 | 134 | 0.9 | 0.20 | 0.80 | 0.64 |
| TA between ±1 SD, V × s | 18.7 | 21.1 | 17.6 | 18.4 | 0.30 | 0.38 | 0.64 | 0.81 |
| TA between ± 2 SD, V × s | 10.5 | 12.9 | 9.2 | 9.8 | 0.29 | 0.23 | 0.61 | 0.71 |
| TA between ± 3 SD, V × s | 7.5 | 9.6 | 6.5 | 7.0 | 0.27 | 0.23 | 0.59 | 0.71 |
| *Branding* | | | | | | | | |
| Peaks between 1-2 SD, n | 979 | 927 | 1002 | 915 | 1.6 | 0.69 | 0.64 | 0.88 |
| Peaks between 2-3 SD, n | 119 | 120 | 200 | 193 | 1.2 | 0.01 | 0.79 | 0.96 |
| Peaks above and below 3 SD, n | 97 | 102 | 166 | 188 | 1.4 | 0.01 | 0.81 | 0.96 |
| TA between ±1 SD, V × s | 2.6 | 4.6 | 16.9 | 23.4 | 0.41 | <0.01 | 0.12 | 0.58 |
| TA between ± 2 SD, V × s | 0.1 | 1.2 | 9.7 | 17.5 | 0.36 | <0.01 | 0.15 | 0.48 |
| TA between ± 3 SD, V × s | 0.14 | 0.08 | 3.4 | 5.6 | 0.26 | <0.01 | 0.55 | 0.46 |

Table 4.5. Least square means (± SEM) immediately after and 180 min after treatment, on d 1, 2, 3 and 7 after castration of stride length, behavioral observations and standing and lying behavior of non-castrated (CT), knife (KN) and branded and knife (BK) castrated 2 –mo-old Angus crossbred calves with (M) or without (NM) a single s.c. meloxicam administration[1]

| | Treatment (T) | | | | | | | *P-Value* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CT | | KN | | BK | | | | | | | | | |
| Item | NM | M | NM | M | NM | M | *SEM*[2] | PRD | MED | PRD × MED | Time | PRD ×T | MED ×T | PRD × MED ×T |
| Stride length[3], cm | 38.8 | 41.5 | 42.7 | 42.1 | 42.1 | 42.1 | 1.25 | 0.15 | 0.49 | 0.40 | <0.01 | 0.36 | 0.18 | 0.68 |
| Stride length[4], cm | 39.0 | 41.9 | 43.8 | 42.8 | 42.8 | 42.9 | 1.22 | 0.01 | 0.42 | 0.64 | <0.01 | 0.64 | 0.60 | 0.29 |
| Behavioral obs. | | | | | | | | | | | | | | |
| Walking, min | 1.8 | 2.2 | 2.0 | 2.6 | 1.8 | 2.0 | 0.10 | 0.88 | 0.24 | 0.89 | 0.18 | 0.61 | 0.99 | 0.79 |
| Standing, min | 29.6 | 28.7 | 33.3 | 29.5 | 35.5 | 34.6 | 0.55 | 0.53 | 0.85 | 0.82 | 0.19 | 0.28 | 0.53 | 0.57 |
| Lying, min | 64.5 | 65.1 | 60.1 | 63.9 | 58.7 | 59.3 | 0.42 | 0.44 | 0.51 | 0.96 | 0.13 | 0.40 | 0.49 | 0.81 |
| Eating, min | 25.5 | 29.1 | 19.7 | 22.3 | 14.5 | 17.6 | 0.47 | 0.01 | 0.16 | 0.91 | <0.01 | 0.98 | 0.66 | 0.63 |
| Tail flick, n | 36 | 80 | 330 | 159 | 535 | 324 | 2.6 | <0.01 | 0.21 | 0.34 | <0.01 | <0.01 | 0.85 | 0.41 |
| Head turning, n | 4.1$^{c}$ | 6.5$^{bc}$ | 11.8$^{a}$ | 4.9$^{c}$ | 10.7$^{ab}$ | 6.9$^{bc}$ | 0.26 | 0.08 | 0.08 | 0.01 | 0.60 | 0.06 | 0.80 | 0.24 |
| Foot stamp, n | 2.0 | 4.0 | 6.2 | 4.2 | 11.6 | 3.6 | 0.32 | 0.11 | 0.19 | 0.12 | <0.01 | 0.03 | 0.25 | 0.02 |
| Lesion licking, n | 0.7 | 0.9 | 1.6 | 0.8 | 1.6 | 0.8 | 0.12 | 0.42 | 0.11 | 0.38 | 0.01 | 0.87 | 0.88 | 0.50 |
| Standing and lying beh. | | | | | | | | | | | | | | |
| Standing, % | 39.3 | 39.2 | 38.6 | 40.8 | 41.3 | 38.8 | 0.01 | 0.74 | 0.86 | 0.06 | < 0.01 | 0.11 | 0.94 | 0.91 |
| Lying, % | 60.7 | 60.8 | 61.4 | 59.2 | 58.7 | 61.2 | 0.01 | 0.74 | 0.86 | 0.06 | <0.01 | 0.11 | 0.94 | 0.91 |
| Standing duration, min | 41.8 | 45.0 | 39.5 | 42.0 | 43.3 | 38.5 | 0.17 | 0.39 | 0.86 | 0.21 | <0.01 | 0.12 | 0.85 | 0.90 |
| Lying duration, min | 59.7 | 62.8 | 58.9 | 58.0 | 60.1 | 56.3 | 0.18 | 0.39 | 0.87 | 0.57 | <0.01 | <0.01 | 0.21 | 0.66 |
| Standing bouts, n | 14.4 | 13.5 | 15.6 | 15.2 | 15.1 | 15.7 | 0.09 | 0.08 | 0.70 | 0.57 | <0.01 | <0.01 | 0.78 | 0.51 |
| Lying bouts, n | 15.4 | 14.7 | 16.4 | 15.9 | 15.5 | 16.6 | 0.48 | 0.38 | 0.95 | 0.38 | <0.01 | <0.01 | 0.20 | 0.73 |

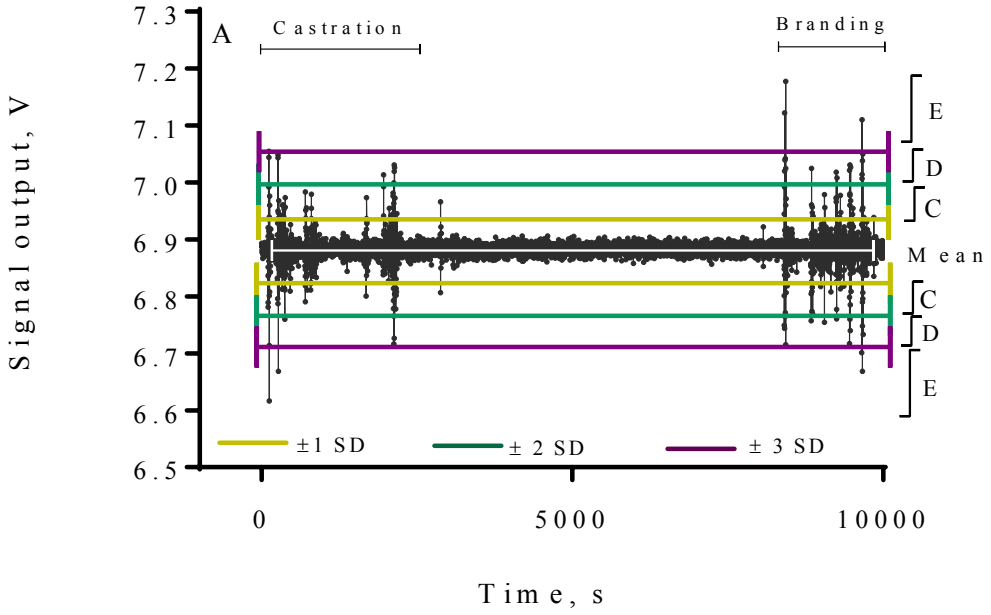
[a-c]Least square means within a row with differing superscripts differ ($P \leq 0.05$).

[1]Values for stride length represent the mean of the samples immediately after and 180 min after procedure. Values for standing and lying represent the mean of d 1, 2, 3, 4, 5, and 6 after procedure. Values for behavioral observations and stride length represent the mean of d 1, 2, 3 and 7 after procedure.

[2]The values represented correspond to non-transformed means; however, SEM and *P*-values correspond to ANOVA analysis using square root + 1 transformed data for behavioral observations.

[3]Stride length immediately after and 180 min after procedure.
[4] Stride length of d 1, 2, 3 and 7 after procedure.

Figure 4.1. Signal output in volts of the addition of three forces of a three dimensional accelerometer indicating movement of the tipping table by a calf (#74) during knife castration and branding. (A) C = number of peaks between 1 and 2 SD above and below the mean, D = number of peaks between 2 and 3 SD above and below the mean, and E = number of peaks above or below 3 SD above or below the mean. (B) F= total area between ± 1 SD, G = total area between ± 2 SD and H = total area between ± 3 SD.



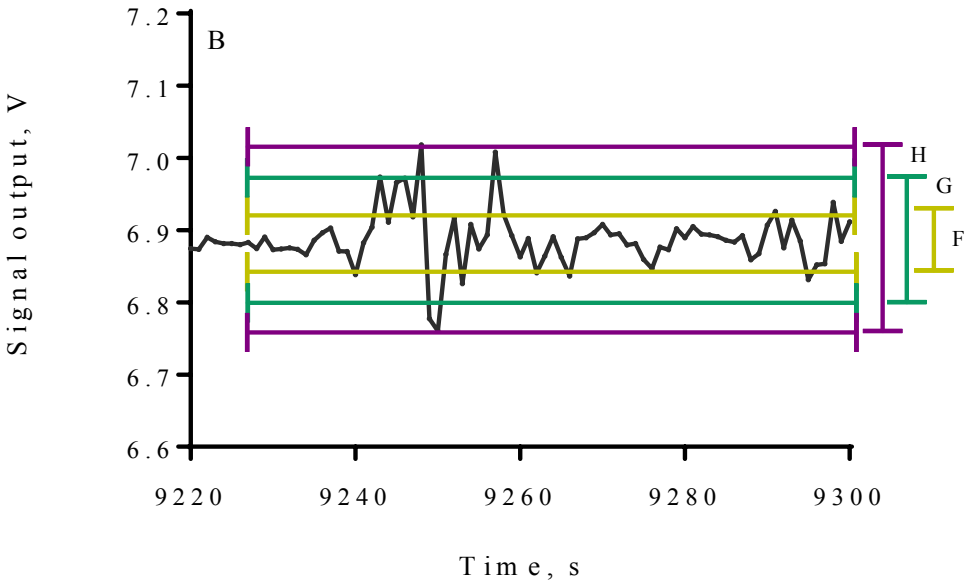



Figure 4.2. Least square means and SEM for (A) salivary cortisol (nmol/L) immediately before treatment (T0), 60, 90, 120 and 180 min after treatment and (B) serum amyloid-A (µg/mL) and (C) haptoglobin on d 0, 1, 2, 3 and 7 after castration of non-castrated (CT), knife (KN) and branded and knife (BK) castrated 2 mo old Angus crossbred calves with (M) or without (NM) a single s.c. meloxicam administration.

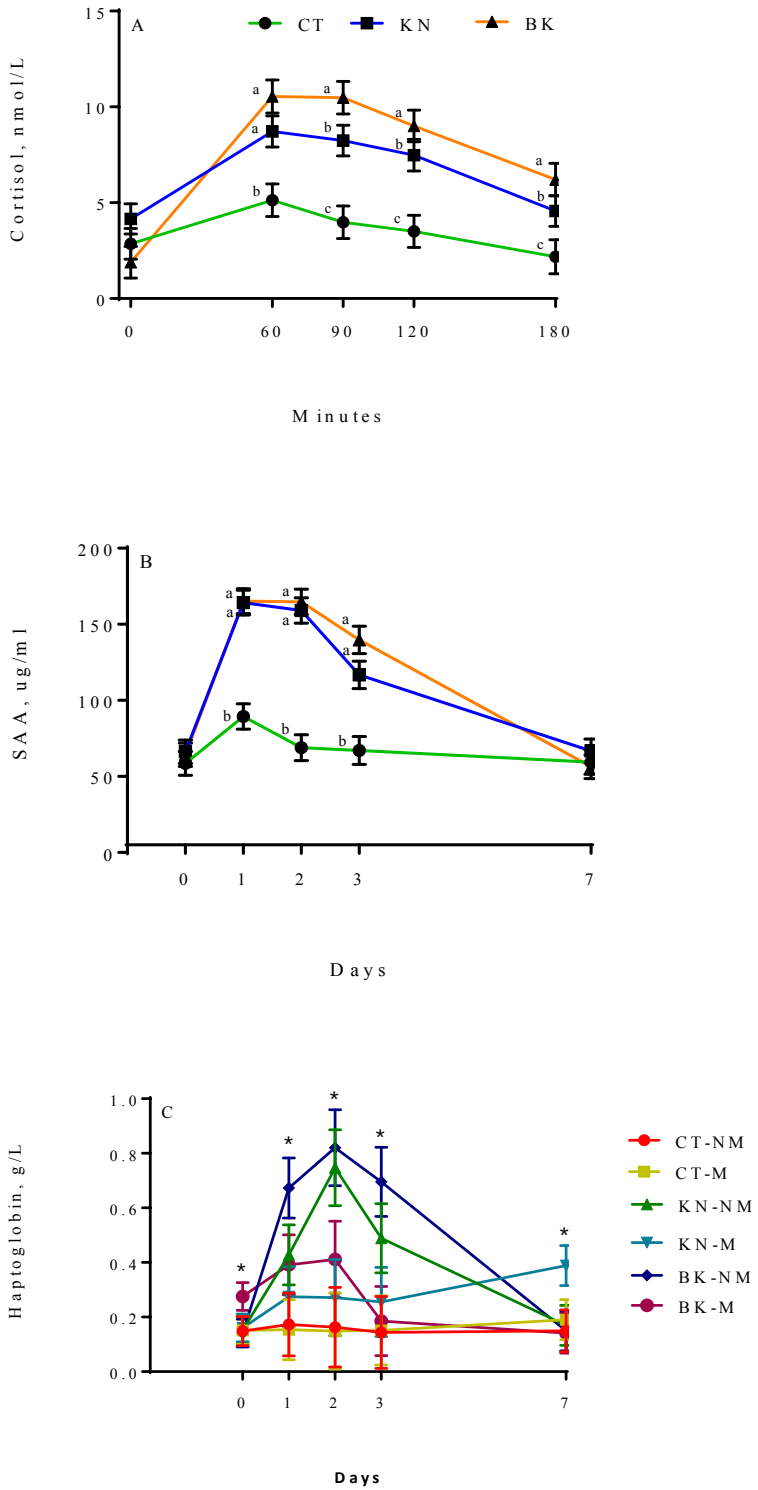


Figure 4.3. Least square means and SEM for (A) WBC count (× 109/L), (B) N:L ratio, and (C) RBC count (× 1012/L) on d 1, 2, 3 and 7 of non-castrated (CT), knife (KN) and branded and knife (BK) castrated 2 mo old Angus crossbred calves with (M) or without (NM) a single s.c. meloxicam administration.

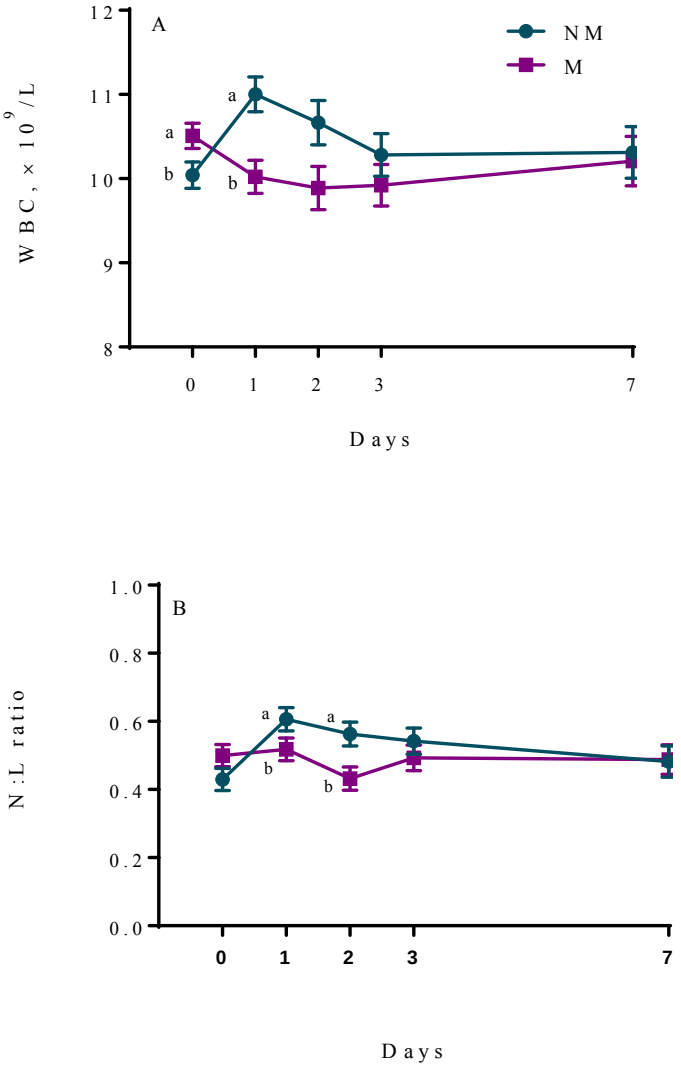
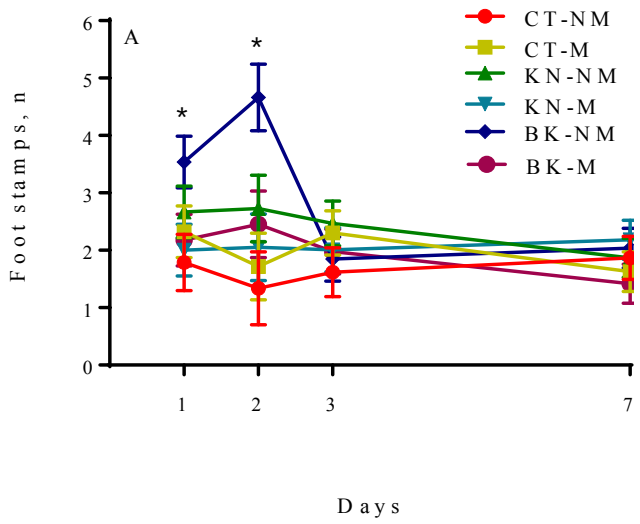
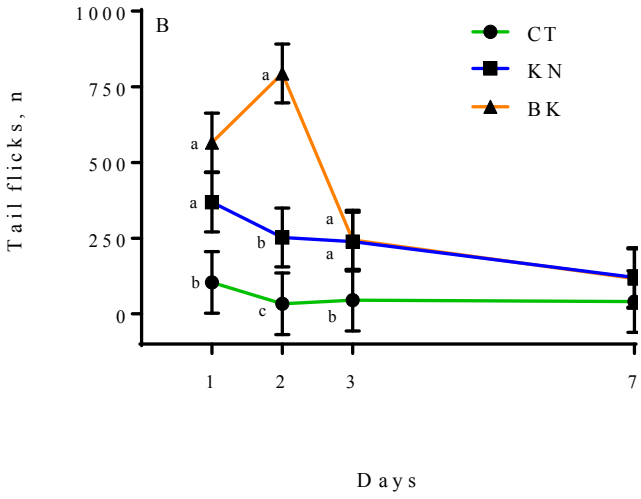


Figure 4.4. Least square means and SEM for (A) foot stamps and (B) tail flicks on d 1, 2, 3 and 7 of non-castrated (CT), knife (KN) and branded and knife (BK) castrated 2 mo old Angus crossbred calves with (M) or without (NM) a single s.c. meloxicam administration.



# Chapter Five: Effect of timing of subcutaneous meloxicam administration on indicators of pain after knife castration of weaned calves

## 5.1 Abstract

The newly revised Canadian Codes of Practice for the management of beef cattle requires that as of 2018, calves older than 6 months of age be castrated using pain control. Castration is a husbandry procedure commonly done without pain control, and there is a lack of agreement on an effective pain mitigation strategy specific to castration. The aim of this study was to identify the optimal time of administration of meloxicam prior to castration. Thirty-four Angus and Angus crossbred bull calves (282 ± 4.8 kg BW, 7 to 8 mo old) were randomly assigned to one of three treatments receiving a single s.c. injection of meloxicam (0.5 mg/kg BW): 6 h (**6H**; n = 11); 3 h (**3H**; n = 12); or immediately (**0H**; n = 11) before knife castration. Measurements included visual analog scale (VAS), head movement (HM), accelerometer movement (AM) and strain gauge exertion force (EF) on the squeeze chute, stride length (SL), lying and standing behavior, salivary cortisol (SC), haptoglobin (H), serum amyloid A (SAA), substance P (SP), and scrotal temperature (ST). Samples were collected on d -7, -5, -2, -1, and immediately before castration (T0); and 30, 60, 120, and 240 min and 1, 2, 5, 7, 14, 21 and 28 d after castration, except for VAS, AM, EF and HM which were obtained at the time of castration. A time × treatment effect ($P = 0.01$) was observed for SP, where 0H had lower concentrations than 3H and 6H calves 1 d after castration, while 3H calves tended to have greater levels than 6H calves 5 d after castration. Mean ST was greater ($P < 0.01$) in 6H calves compared to 0H and 3H calves 120 min after castration, while 6H and 3H calves had greater ST compared to 0H calves 240 min after castration. On d 1 after castration, 6H calves had greater ST than 0H and 3H calves, while 0H

calves had greater ST compared to 3H and 6H calves on d 28 after castration. The SL tended ($P$ = 0.09) to be shorter in 3H and 6H calves than 0H calves 30, 60, 120, and 240 min after castration. Number of peaks from the AM between 2 to 3 standard deviations (SD) above or below the mean were greater ($P$ = 0.03) in 3H and 6H calves than 0H calves. No treatment differences ($P$ > 0.10) were observed for the number of peaks and area for AM and EF, VAS, HM, SC or H. Based on these results, the optimal time to administer s.c. meloxicam in 7 to 8 mo old knife castrated calves is immediately before castration (0H) as evidenced by fewer indicators of pain and inflammation compared to 3H and 6H.

## 5.2 Introduction

In Western Canada, approximately 1 % of ranch calves are castrated at 6 mo of age or older (Moggy et al., 2017). While in the United States bulls arriving at feedlots account for approximately 0.9 % and the majority (91.2 %) of feedlots castrate these animals (USDA. 2013). Castration at weaning without pain control is an animal welfare issue as older animals show greater behavioral and physiological changes associated with pain and distress (Bretschneider, 2005). As of January, 2018 the Canadian Beef Codes of Practice (NFACC, 2013) requires pain control for castrating calves older than 6 mo of age (Schwartzkopf-Genswein et al., 2012), however, no standard pain mitigation protocols have been established.

An effective way to manage the pain of castration could be to provide preventive analgesia. Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) and an attractive analgesic option for post-operative pain in livestock due to the ease of administration (s.c.) and long lasting effect (half-life 22 h) (Stock and Coetzee, 2015). Oral meloxicam has been shown to reduce the acute inflammatory response in yearling bulls after surgical castration (Roberts et al., 2015). Alternative routes of administration, such as s.c., may be more practical than oral or i.v.

administration, but to our knowledge there are no studies examining when s.c. meloxicam should be administered relative to castration in order to produce optimal analgesia. A pharmacokinetic study showed that peak plasma levels of meloxicam are achieved 12 h after oral administration (Coetzee et al., 2009).

Therefore, the objective of this study was to compare the effect of pre-emptive meloxicam given s.c. at three time points prior to surgical castration on indicators of pain associated with inflammation in 7 to 8 mo old beef calves. We hypothesise that the administration of s.c. meloxicam 6 h prior to castration would be more effective at reducing pain indicators after castration compared to 3 and 0 h prior to castration.

## 5.3 Materials and Methods

This protocol was approved by the Lethbridge Research Centre Animal Care Committee (ACC # 1428), all animals were cared for in accordance to the Canadian Council of Animal Care guidelines (CCAC, 2009).

### 5.3.1 Animal housing and management

Thirty-four 7 to 8 mo old Angus and Angus crossbred bull calves (282 ± 28.0 kg BW) were used in a 28 d experiment. Cows and calves were transported separately to the Agriculture and Agri-Food Canada Lethbridge Research Centre (LRC) (Lethbridge, Alberta, Canada) during the first week after birth. Calves used in the present experiment were control calves from a previous castration experiment. Prior to weaning, calves were housed in a 76.9 ha pasture adjacent to the LRC feedlot facility with a perennial grass pasture consisting of brome grass, orchard and creeping red fescue and annual cereal crops including oats, and spring and fall triticale. Calves were weaned at 7 to 8 mo of age, 3 wk prior to the start of the trial in order to

adapt the calves to the experimental pens. Calves were vaccinated with a 7-way clostridial vaccine (Ultrabac®/Somubac®, Zoetis Canada Inc., Kirkland, Canada) and housed in 2 experimental pens (40.2 m × 27.4 m) with a 24.4 m × 2.45 m concrete apron in front of the feed bunk. Calves were fed once a day at 0900 a total mixed ration consisting of 80 % barley silage, 17 % dry-rolled barley and 3 % supplement with vitamins and minerals to meet beef cattle requirements (NRC, 2016). Water was available *ad libitum* in a centrally located trough (80 cm × 40 cm × 50 cm). Animals weights of d -1 were used to equally distribute animals across pens and treatments, and were randomly assigned to one of 3 treatments receiving a single s.c. injection of meloxicam (0.5 mg/kg BW): 6 h (**6H**; n = 11); 3 h (**3H**; n = 12); or immediately (**0H**; n = 11) before surgical castration.

Calves were restrained in a hydraulic squeeze chute (Cattlelac Cattle, Reg Cox Feedmixers Ltd, Lethbridge, Alberta, Canada) where they were sampled and castrated. In order to avoid castrating calves at different time points during the day, 6H calves and 3H calves were run through the chute 6 h and 3 h prior to castration in order to administer the corresponding s.c. meloxicam dose with a 16 gauge, 1 inch needle in the neck of the calves, while the 0H group received s.c. meloxicam immediately before castration. The same veterinarian performed surgical castration on all the calves by making a latero-lateral incision on the scrotum with a Newberry castration knife (Syrvet Inc., Waukee, IA). Testicles were then externalised one at a time from the scrotum and an emasculator was used to crush and cut the spermatic cords.

### 5.3.2 Physiological parameters

*Blood samples*

Blood samples were collected via jugular vein-puncture into vacuum tubes (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ) on d -7, -5, -2, -1, immediately before castration (T0), 30, 60, 120, and 240 min and 1, 2, 5, 7, 14, 21 and 28 d after castration. Additional samples were collected 60 and 240 min and on d 1, 2 and 5 after castration to determine plasma concentrations of meloxicam. These samples were collected into 10-ml lithium heparin tubes (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ), centrifuged for 15 min at 1.5 *g* at 0 °C and the serum was decanted and stored at -80 ºC for further analysis using high-pressure liquid chromatography (Agilent 1100 Pump, Column Compartment, and Autosampler, Santa Clara, CA, USA) with mass spectrometry detection (LTQ, Thermo Scientific, San Jose, CA, USA) at Iowa State University, College of Veterinary Medicine (Ames, IA). The limit of quantitation (LOQ) of the analysis was 1.0 ng/mL with a limit of detection (LOD) of 0.3 ng/ml.

Samples to determine concentrations of substance P (SP) were collected on d -1, T0, 30, 60, 120, 240 min and on d 1, 2, 5, 7, 14, 21 and 28 into a 6-ml vacuum tube with EDTA (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ). The sample was then centrifuged for 15 min at 1.5 *g* at 0°C and the serum was decanted and frozen at -80 ºC for further SP analysis which was previously described by Van Engen et al. (2014) with some modifications at Iowa State University, College of Veterinary Medicine (Ames, IA). Non-extracted plasma samples were analyzed in duplicate with a double antibody radioimmuno- assay (RIA) using a purchased primary antibody, (Substance-P (3-11) Antibody for Immunohistochemistry Human, Rat, Mouse, Phoenix Pharmaceutical #H-061-05). The range of detection for SP was between 5 to 320 pg/ml, with an average $R^2 = 0.98$. The coefficient of variation for intra-assay variability was 8.2 % and

the inter assay variability was calculated at 20.6 %. The limit of detection was 10 pg/mL and limit of quantitation was 20 pg/mL.

Samples to determine complete blood cell count (CBC) were collected on d-7, -5, -2, -1 (T0), 30, 60, 120, and 240 min and on d 1, 2, 5, 7, 14, 21 and 28 after castration. The sample was collected in a 6-mL EDTA tube (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ) for complete blood count using a HemaTrueHematology Analyzer (Heska, Lobeland, Co) which measured white blood cells (WBC), red blood cells (RBC), platelet count and the neutrophil:lymphocyte (N: L) ratio.

Blood samples to measure haptoblogin were collected on d-1, 1, 2, 5, 7, 14, 21 and 28 while SAA was collected on d-1, 1, 2, 5, and 7. The sample was collected into 10-mL non-additive tubes (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ), which were centrifuged for 15 min at 1.5 *g* at 4 °C and the serum was decanted and frozen at -80 ºC for further analysis. Haptoglobin concentrations were analyzed using a Roche Cobas c501 biochemistry analyzer (Roche Diagnostics, Laval, QC, Canada) using a Tridelta bovine haptoglobin calibrator (TP801CAL, Tridelta, Maynooth, Ireland) and two levels of in-house controls (bovine serum pools) daily and two levels of Tridelta controls weekly. Samples to determine SAA concentrations were collected on d-1, 1, 2, 5 and 7 and analyzed using an enzyme linked immune sorbent assay (Tridelta Phase range SAA kit, TP 807, Tridelta development LTd, Maynooth, Ireland).

*Salivary Cortisol*

Saliva samples to determine cortisol concentration were collected on d -7, -5, -2, and -1 before castration, immediately before castration (T0), 30, 60, 120, and 240 min and 1, 2, 5, 7, 14,

21 and 28 d after castration. Samples were collected with a cotton swab and immediately stored in a plastic tube and frozen at -20 °C for further cortisol analysis using an enzyme immunoassay kit (Salimetrics LLC, State College, PA). Inter-assay and intra-assay variability values were 14.1 % and 5.9 % respectively.

*Hair cortisol*

Hair from the forehead was clipped and stored in plastic bags at room temperature on d-1 and on d 28 after castration for further cortisol analysis. Hair samples were processed as described in detail by Moya et al. (2013) and cortisol quantified using an enzyme-linked immunosorbent assay (Salimetrics LLC, State College, PA). Values for inter-assay variability were 8.3 % and 5.8 % for intra-assay variability.

*Scrotal temperature (ST)*

Thermographic images of the scrotal area were collected on d -7, -5, -2, immediately before castration (T0), 30, 60, 120, 240 min and on d 1, 2, 5, 7, 14, 21 and 28 after castration. Images were taken from behind the calves 1 m from the scrotal area using a FLIR I60 infrared camera (FLIR Systems Ltd Burlington, Ontario, Canada) and analyzed with FLIR Tools v.5.1 (FLIR Systems Ltd Burlington, Ontario, Canada) using an emissivity coefficient of 0.98 to measure scrotal temperature.

*Rectal temperature (RT)*

Rectal temperature was measured on d -7, -5, -2, -1, 0, 1, 2, 5, 7, 14, 21 and 28 after castration using a digital thermometer (GLA Agricultural Electronics, M750 Livestock Thermometer, San Luis Obispo, CA).

*Performance*

Animals were weighed on d -1, 1, 2, 5, 7, 14, 21 and 28 after castration in the squeeze chute. Initial body weight was the weight collected on d -1 and the final body weight was the weight collected on d 28.

*Lesion score*

Scrotal swelling was evaluated using an 11 point scale as described by Molony et al. (1995) and then collapsed into a 5 score scale. The scores consisted of: 4) Presence of supurative discharge with inflammatory response that required intervention, 3) Presence of pus with increasing inflammatory response, 2) Increasing degree of swelling with obvious erythema but without pus, 1) Increasing degree of swelling without obvious erythema and 0) No swelling, inflammation or infection visible or palpable.

### 5.3.3 Behavioral parameters

*Behavior scored and observed during castration*

The visual analog scale (VAS) consisted of a 10-cm line (the far right indicating extreme pain and the far left no pain) in which two experienced observers marked their perception of the amount of pain the calf was exhibiting during castration. The distance from the start of the line to the mark was measured to the nearest 0.5 cm and was used as an indicator of pain response to castration. In addition, the frequency of urination, defecation, vocalization and leg movement were recorded. Observers were blind to the treatments.

Head movement (HM) was recorded using a video camera placed in front and to the right side of the head gate in order to record head movement during castration. Videos were imported into Kinovea (General Public License) version 2; motion tracking software and the middle of the hairline of the muzzle was used as a reference point to track total head movement distance.

In addition, the squeeze chute was equipped with three one dimension accelerometers each measuring a different movement (vertical, horizontal and lateral) and the right and the left head gate were equipped as previously described by (Schwartzkopf-Genswein et al., 1997b) with two strain gauges to measure the force exerted when the animals pushed against or pulled away from the head gate. Baseline measurements were collected for 20 s for all calves on d -1 when animals were restrained in the squeeze chute and head gate. The force from the three accelerometers were added to obtain the overall accelerometer force, and the force measured from the right and left strain gauges were also added to obtain an overall force. The variables evaluated included number of peaks (defined as change in direction of the analog signal) and total area (TA) (total area under peaks). Accelerometer and strain gauge forces were summarized to obtain the total number of peaks between 1 and 2 SD, 2 and 3 SD and 3 SD above and below the mean, as well as total area above and below 1, 2, and 3 SD from the mean.

*Stride Length (SL)*

Calves were recorded walking along a 1 m × 3 m alley on d -7, -5, -2, and -1, immediately after castration, 30, 60, 120, 180 and 240 min after castration as well as on d 1, 2, 5, 7 and weekly thereafter until d 28. The software GOM Player (GOM Lab, Gretech Corporation, Seoul, South Korea) was then used to take 2 pictures of the hind legs when both feet were flat on the ground, one with the right foot in front of the left and another picture with the left foot in front of the right. The distance between both hind feet was measured using a modified method previously described by (Currah et al., 2009) using ImageJ (Bethesda, MD) and the average was used for further analysis.

*Behavioral observations in experimental pens*

Behavior was recorded using two Avigilon cameras (2.0MP HD IR Bullet Camera, Avigilon®, Vancouver, BC, Canada) located on the north and south side of the experimental pens mounted on 6 m poles. Numbered penning tags were placed on the back of the calves using tag cement (Livestock Identification tag cement, W.J. Ruscoe Company, Akron, Ohio) in order to identify calves. Two experienced observers did focal sampling from continuous recordings (Martin and Bateson, 2007) of standing, lying, walking, eating, tail flicking, foot stamping and head turning (Table 5.1) using The Observer® XT (Noldus Information Technology, Wageningen, The Netherlands). These behaviors were scored on the day of castration for a period of 3 h, between 4 to 7 h after castration, and during the same time of day on d 2 and 3 after castration for 6 calves per treatment. Due to technical problems the behavior for d 1 was not recorded. Inter-rater and intra-rater reliability was 0.99 and 0.98 for the two experienced observers that were blinded to treatments.

*Standing and lying behavior*

On d -7 calves were fitted with Hobo data loggers (Onset Computer Corporation, Bourne, MA) which were covered in plastic film to protect the device from rain and attached to the left hind leg immediately below the hocks with 10.2 cm wide latex flexible cohesive bandage (Latex Flexible Cohesive Bandage, Professional Preference, Calgary, Alberta, Canada) to automatically measure the duration of lying and standing. Hobos were changed every 7 d and placed on the alternate hind limb. Information obtained from the Hobos was summarized into standing and lying percentage (%), mean standing and lying bout duration (min/day), as well as standing and lying bouts (number/day) (UBC AWP, 2013).

*Feeding behavior*

All calves were fitted with a radio frequency eat tag and each pen was equipped with a GrowSafe feed bunk monitoring system (GrowSafe Systems, Airdrie, Alberta, Canada) with 5 feeding tubs which recorded feeding behavior for each individual calf 24 h a day during a 28 d period. The GrowSafe system consists of two antenna's located in the feed bunk which detect individual ear tags when calves are within 50 cm of the feed bunk and records feeding behavior information (Schwartzkopf-Genswein et al., 1999). The electronic monitoring system records feed intake (kg/d), bunk attendance frequency (visits/d) and bunk attendance duration (min/d) which can be used to calculate feeding time (min/d), dry matter intake (kg/day), feeding rate (g/min), meal frequency (number/d), meal duration (min/meal) and meal size (kg/meal). For this study a meal criterion of 300 s was selected as it has been previously used for a wide range of cattle sizes (Sowell et al., 1998; Schwartzkopf-Genswein et al., 2003).

***Statistical Analysis***

A power analysis was conducted for the primary outcomes (salivary cortisol and stride length) with the SD of each parameter observed in a previous study under the same experimental conditions (Moya et al., 2014) and an $\alpha$ of 0.05 and a power of 0.08. The power analysis indicated that at least 7-11 calves per treatment were necessary to detected expected differences among treatments. Mixed Models (SAS, version 9.4, SAS Inst. Inc., Cary, NC) was used to assess the effects of time of meloxicam administration on all variables. The mean baseline for variables measured before castration were used as a covariate for variables measured after castration. Calves were the experimental unit as treatments were mixed within the pen. All parameters, except for VAS, HM, ADG, initial and final BW were analysed using a mixed repeated measures model Proc Mixed (SAS, version 9.4, SAS Inst. Inc., Cary, NC). Covariance structures included compound symmetry, unstructured, and autoregressive order 1. Time and

treatment were considered as fixed effects and were tested for interactions, while pen, and calves nested within pen, were considered random effects. Physiological parameters were log transformed, percentage data was arcsine transformed, and behavioral parameters were square-root transformed when data did not follow a normal distribution. Due to significant differences in baseline levels for SAA, the values for the different time points were subtracted from the baseline values. Significance was established at a $P \leq 0.05$ and tendencies between $0.05 < P \leq 0.10$. When interactions were significant ($P \leq 0.05$) treatment means were separated with a post-hoc test using the PDIFF option in SAS. The LS means were generated after adjusting for covariates and normality was tested using Proc Univariate (SAS, version 9.4, SAS Inst. Inc., Cary, NC). The feeding information was collapsed from days to weeks for analysis. The days in which the different treatment groups presented a particular swelling score were analyzed using a Wilcoxon-Mann-Whitney test (SAS 9.4, SAS Inst. Inc. Cary, NC) and medians and 95 % distributions free confidence limits were calculated using the univariate procedure (SAS 9.4, SAS Inst. Inc. Cary, NC). IBM SPSS statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA) was used to calculate the intra-class correlation coefficient (ICC) with a 95% CI to determine behavior inter-rater and intra-rater reliability. Reliability was calculated by comparing scores of students and an experienced observer (inter) of 8 2-h-long videos, and comparing scores of students of the same video but evaluated at different time points (intra).

## 5.4 Results

### 5.4.1 Day of castration

*Physiology*

Meloxicam concentration presented a treatment × time interaction ($P$ <0.01), where 6H and 3H calves had greater meloxicam levels (1796 ± 112.7 ng/ml and 1572 ± 112.7 ng/ml,

respectively) than 0H calves (858 ± 112.7 ng/ml) 60 min after castration, but no differences ($P \geq 0.10$) were seen between treatments at 240 min after castration (6H: 1519 ± 72.6 ng/ml; 3H: 1630 ± 72.6 ng/ml; 0H:1623 ± 72.6 ng/ml). No treatment or interaction effects ($P > 0.10$) were observed for SP or salivary cortisol concentrations 30, 60, 120 and 240 min after castration, however, there was an overall increase (time effect: $P < 0.01$) in SP and salivary cortisol concentrations. A treatment × time interaction ($P < 0.01$; Fig. 5.1) was observed for ST, where 6H and 0H calves had greater ST than 3H calves at T0 and 30 min after castration. Sixty minutes after castration 6H calves had greater ST than 3H, however, no differences were observed between both groups and 0H calves. The 6H calves had greater scrotal temperatures compared to 0H and 3H calves 120 min after castration, while 6H and 3H calves had greater temperatures than that of 0H calves 240 min after castration (Table 5.2).

*Behavior*

No treatment effects ($P > 0.10$) were observed for VAS, urination, defecation, leg movement, vocalizations or HM during castration. Stride length increased (time effect; $P < 0.01$) during the minutes following castration regardless of treatment and tended ($P = 0.09$) to be shorter in 3H and 6H calves than 0H calves 30, 60, 120, and 240 min after castration. Number of peaks between 2 to 3 SD recorded by the squeeze chute accelerometers were greater ($P = 0.03$) in 3H and 6H calves than 0H calves and tended to be greater ($P = 0.07$) above 3 SD in 3H and 6H calves compared to 0H calves. No treatment differences ($P > 0.10$) were observed for total number of peaks and total area for accelerometers and strain gauges.

### 5.4.2 Day 1 to 28 after castration

*Physiology*

Meloxicam concentrations decreased ($P < 0.01$) on d 1 (566 ± 26.5 ng/mL), 2 (197 ± 26.5 ng/mL) and 5 (9.6 ± 26.57 ng/mL) after castration, however no effects or interactions ($P > 0.10$) were observed. Substance P had a treatment × time interaction ($P < 0.01$; Fig. 5.2), where the 6H and 3H calves presented greater SP concentrations than 0H calves on d 1 after castration, while 3H calves tended to have greater SP concentrations than 6H calves on d 5 after castration. The N:L ratio was greater ($P = 0.05$) in 0H calves compared to 3H and 6H calves on d 1, 2, 5, 7, 14 and 28 after castration. The N:L ratio and WBC increased (time effect; $P < 0.01$) up to d 2 regardless of treatment and returned to baseline levels after d 2. Platelets decreased (time effect; $P < 0.01$) for all treatments after castration until d 2, and then increased reaching the highest concentrations on d 21, while RBC didn't present an obvious pattern. Haptoglobin, SAA and salivary cortisol concentrations increased (time effect; $P < 0.01$) after castration regardless of treatment reaching the highest concentrations on d 5 for haptoglobin and on d 2 for SAA and salivary cortisol.

Scrotal temperature had a treatment × time interaction ($P < 0.01$; Fig. 5.3) were 6H calves had greater temperatures than 0H and 3H calves on d 1 after castration, while 0H calves had greater temperatures compared to 3H and 6H calves on d 28 after castration. Body temperature increased (time effect; $P < 0.01$) up to d 2 for all treatments, and then went back to baseline levels, spiking again on d 28. No treatment or interaction effects ($P > 0.10$) were observed for performance (Table 5.3) or lesion scores (Table 5.4).

*Behavior*

Stride length, standing and walking increased (time effect; $P < 0.01$) while lying decreased after castration for all treatments. Foot stamping presented a treatment × time interaction ($P < 0.01$; Fig. 5.4), where 3H calves had a greater number of foot stamps on d 0

compared to 0H and 6H calves, and a lower number of foot stamps on d 3 compared to 6H calves but no differences were seen between both groups and 0H calves. Tail flicks tended ($P = 0.09$) to be greater for 0H calves compared to 3H calves on d 0, 2 and 3, however, no differences were seen between both groups and 6H calves. Standing and lying behavior as well as feeding behavior had a time effect ($P < 0.01$). Standing and lying duration increased during the first week after castration and followed an inconsistent pattern during subsequent weeks for all treatments. Standing percentages increased while lying percentages decreased during the first days following castration regardless of treatment, while standing and lying bouts followed an inconsistent pattern. Dry matter intake increased for all treatments over time but no obvious pattern was observed for feeding time, feeding rate, meal frequency or meal size (Table 5.5). No adverse event happened during the trial.

## 5.5 Discussion

Tissue damage can cause central sensitization which is a heightened response of the spinal cord neurons and can lead to increased pain from either painful (hyperalgesia) or non-painful (allodynia) stimuli (Ochroch et al., 2003). Abram and Yaksh (1993) demonstrated that central sensitization or 'wind up' could not be prevented by inhalation anesthesia alone, highlighting the importance of using pre-emptive analgesia which has the potential to eliminate central sensitization. If pre-emptive analgesia is effective, it would be a valuable tool to put into practice before painful husbandry procedures, as it would reduce post-operative pain, consequently improving animal welfare.

Previous studies have reported lack of considerable behavioral changes after Burdizzo castration at the time of weaning (Lambertz et al., 2014; Lambertz et al., 2015), suggesting that weaning and castration should be done at the same time to reduce labour costs (Lambertz et al.,

2014). Although castrating and weaning calves at the same time would optimize management, caution should be taken when interpreting the results, as the previously mentioned study assessed behavioral changes in Burdizzo castrated calves while clamp castration has been reported to be the method that causes the least amount of pain and distress compared to ring, band and surgical castration (Stafford et al., 2002). In the present study, castration was performed 3 weeks after weaning in order to eliminate potential stressors which could mask the effect of castration on physiological and behavioral indicators. One limitation of this study may be that breed effect on the behavioral and physiological parameters measured could not be accounted for because the breed of the calf sires was unknown.

In the present study, a lack of differences in VAS scores, urination, defecation, leg movement, vocalization, strain gauge exertion force and HM during castration were expected, as calves did not receive an anesthetic to block the pain during the procedure, therefore, (despite individual pain tolerance differences) all calves experienced a similar amount of pain. However, we expected to see treatment differences in physiological and behavioral parameters after castration, as we predicted that meloxicam concentrations in plasma would be greater in the 6H calves at the time of castration and therefore it would be more effective at reducing pain and inflammation compared to the 3H and 0H calves. Greater escape behavior (number of peaks measured from the accelerometers on the squeeze chute) observed in 3H and 6H calves was likely due to an increase in agitation as these animals had to go through the chute twice; once in order to receive the medication and a second time to be castrated. It is not likely that the time of meloxicam administration had an effect on the amount of escape behavior occurring in the squeeze chute because meloxicam does not block the pain experienced at the time of castration but works by decreasing peripheral and central sensitization by reducing prostaglandin

production (Burian and Geisslinger, 2005). Behavioral differences were limited to foot stamping while a tendency was observed for tail flicks. Lack of differences in feeding behavior have been previously reported in 213 d old calves receiving ketoprofen alone or after multiple i.m. injections post sham, band and surgical castration (Moya et al., 2014).

Coetzee et al. (2008) suggested substance P may be a good measurement to differentiate between stress caused by nociception, and stress caused by handling, based on a study in which greater substance P concentrations were reported in 4 to 6 mo old surgically castrated calves compared to a non-castrated group, while no differences were observed in plasma cortisol concentrations. Similar results were obtained in the present study, in which substance P concentrations differed across treatments on d 1 after castration, and no differences were observed for salivary cortisol across treatments, although sampling times vary between studies. A possible explanation for the difference observed between treatments on d 1 could be that 6H and 3H calves had to go through the chute twice before castration. The 0H calves only went through the chute once at the time of castration, therefore the extra handling could have increased substance P levels in 6H and 3H calves as it is also associated with stress. Another possible explanation is that 0H calves benefited from receiving the drug immediately before castration which is in accordance with findings from a previous study assessing the effectiveness of administering oral meloxicam 12 h or immediately before dehorning (Allen et al., 2013). Calves that received meloxicam 12 h prior to dehorning presented low prostaglandin $E_2$ ($PGE_2$) concentrations up to 12 h post-dehorning, while calves that received meloxicam immediately before dehorning had reduced $PGE_2$ up to 3 d post procedure. The author suggested this could have been due to a longer duration of action of meloxicam when administered immediately before dehorning, and could be the reason why 0H calves had lower substance P concentrations.

Scrotal temperature was greatest in the 6H calves between T0 to 240 min post castration and d 1, suggesting that this group presented greater scrotal inflammation up to 1 d after castration. Although scrotal temperature results were not expected, the findings were similar to the substance P results, suggesting that 0H and 3H calves could have benefited from a longer exposure to meloxicam after castration. A similar finding was reported in a human study evaluating the effects of morphine administered 1 h prior (i.m.) to surgery, at the time of induction (i.v), or at the time of incision closure (i.v) in females undergoing hysterectomy (Richmond et al., 1993). Twenty-four hours post-surgery the induction group requested less morphine than the incision closure group, however, the induction group had greater VAS pain scores on movement than the incision group 48 h post-surgery, suggesting that the 'extra morphine' was effective at controlling pain 2 d post-surgery (Katz, 1995). These findings are in agreement with SL and foot stamping results, which indicate that 0H calves experienced less pain/distress compared to 3H and 6H calves, but contrary to tail flicking numbers, which although were not significantly different they are numerically greater in the 0H calves compared to 3H and 6H calves.

The N:L ratio was the only parameter which was elevated in the 0H calves, however, all calves presented values above the normal range (0.3 to 0.6) (Smith, 2008). A possible explanation for the increased N:L ratio for all calves during the first 28 d after castration could be due to stress associated with surgical castration. A typical stress leucogram is characterized by neutrophilia and lymphopenia caused by endogenous or exogenous steroid release (Jones and Allison, 2007). However, lymphocyte values were within the normal range (2.5 to 7.5 $\times 10^3$/ µL) (Smith, 2008), while the neutrophil values were above the normal range for all treatments, therefore affecting the N:L ratio. Even though 0H calves presented greater levels, it is unlikely

for this finding to be associated with the time of meloxicam administration as meloxicam's effect should only last for 44 ± 3 h (Stock and Coetzee, 2015), while differences in N:L ratio were observed up to 28 d after castration. We speculate that the neutrophilia seen across treatments could be due to non-infective inflammation or subclinical secondary infections to castration (Roland et al., 2014).

Inflammation, infection and trauma can increase acute phase proteins as bacterial toxins and tissue injury secret cytokins which have the potential of activating hepatocites and initiating acute phase protein production (Petersen et al., 2004). Although acute phase protein response varies between individual animals (Jacobsen et al., 2004), haptoglobin and SAA have been suggested to be accurate indicators of inflammatory conditions in cattle (Horadagoda et al., 1999). In the present study no differences were detected in haptoglobin or SAA levels, however, all treatment groups had greater haptoglobin and SAA concentrations than normal values (HP: <0.1 g/l; SAA: 1.3 ± 0.4 mg/l) (Ceciliani et al., 2012), indicating that meloxicam did not eliminate the inflammatory response mediated by acute phase proteins. Based on previous findings from Brown et al. (2015), who reported a reduction in haptoglobin levels in weaned surgically castrated calves receiving oral meloxicam compared to non-medicated calves, we speculate that s.c. meloxicam reduced the acute phase inflammatory response, however, we cannot be certain of this as a control group not receiving meloxicam would be necessary in order to be able to make this comparison.

Our hypothesis for this study was based on the prediction that absorption of subcutaneous meloxicam would be faster than oral administration, which has been reported to take 12 h to reach peak plasma concentrations (Coetzee et al., 2009). Based on this information, we estimated that peak plasma levels would be reached 6 h after administration. However, meloxicam

concentrations did not differ between treatments at 240 min sampling, therefore, assuming that peak plasma levels were reached before 240 min, and that peak plasma levels are equivalent to peak tissue levels, we would not expect to see differences between treatments after 240 min. Conversely, it is possible that peak meloxicam levels could have occurred after 240 min sampling or that differences seen between treatments could be related to the total time the calves were exposed to the drug after the onset of tissue damage which would explain the differences in physiological and behavioral parameters observed hours and 1 d after castration. Lack of differences in the majority of physiological and behavioral parameters could be due to underpowered sample sizes, as sample size was not calculated for all outcomes in addition to a large individual animal variation. Other possibilities include times of meloxicam administration not being substantially different from one another, response variables might not have been sensitive enough or that time of s.c. administration has no effect.

Based on our results, there is little evidence that providing meloxicam subcutaneously before castration had any additional benefit, and contrary to our hypothesis, 0H calves presented fewer indicators of pain and distress compared to 6H and 3H calves probably because of the increased exposure to meloxicam. Administering s.c. meloxicam immediately before castration has the added benefit of increasing the exposure time to meloxicam, handling the calves once, consequently reducing extra labour costs for the producer and more importantly, reducing unnecessary stress to the calves. However, there is a need for a pharmacokinetic study using subcutaneous meloxicam to determine when peak plasma levels are reached.

Table 5.1. Ethogram of behaviors recorded modified from Molony et al. (1995).

| Behavior | Definition |
|---|---|
| Eating | When the head of the calf was in the feeder |
| Lying | Either lateral (laying with hip and shoulder on the ground with at least 3 limbs extended) or ventral (laying in sternal recumbency with legs folded under the body or one hind or front leg extended) lying |
| Walking | Walking forward more than 2 steps |
| Standing | Standing on all four legs |
| Foot stamping | Hind legs are lifted and forcefully placed on the ground or kicked outwards while standing. One leg lift and one lowering was counted as one |
| Head turning | When the head is turned and touches the side of the calf's body, including head turning to groom |
| Tail flicking | Forceful tail movement beyond the widest part of the rump, movement to one side is counted as one action |
| Kneeling | Front legs are bent on the ground while back legs are extended |

Table 5.2. Least square means (±SEM) of samples collected during or at T0, 30, 60, 120 and 240 min after castration for meloxicam, substance P (SP), salivary cortisol, scrotal temperature (ST), visual analog scale (VAS), head movement (HM), accelerometer movement and strain gauge exertion force peak number and total area (TA), and stride length (SL) of surgically castrated Angus and Angus crossbred bulls receiving s.c. meloxicam 6, 3 and 0 h before castration[1].

| | Treatment (T) | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Item | 0H | 3H | 6H | SEM[2] | T | Time | T × Time |
| *Physiology* | | | | | | | |
| Meloxicam, ng/mL[3] | 1241.2[b] | 1601.5[a] | 1657.7[a] | 0.07 | <0.01 | <0.01 | <0.01 |
| SP, pg/mL | 125.7 | 129.7 | 129.6 | 0.04 | 0.74 | <0.01 | 0.10 |
| Salivary Cortisol, nmol/L | 5.3 | 5.4 | 5.4 | 0.12 | 0.98 | <0.01 | 0.25 |
| ST, ºC | 29.7[b] | 29.2[b] | 31.3[a] | 0.60 | 0.04 | 0.34 | <0.01 |
| *Behavior* | | | | | | | |
| VAS,[4] cm | 2.7 | 2.5 | 3.3 | 0.08 | 0.09 | - | - |
| Urination, n | 1.0 | 1.0 | 1.0 | 0.02 | 0.99 | - | - |
| Defecation, n | 1.0 | 1.0 | 1.0 | 0.01 | 0.35 | - | - |
| Leg movements, n | 2.4 | 2.6 | 2.7 | 0.14 | 0.54 | - | - |
| Vocalizations, n | 1.0 | 1.0 | 1.1 | 0.07 | 0.18 | - | - |
| HM[5], cm | 23.0 | 27.1 | 24.1 | 3.42 | 0.70 | - | - |
| *Accelerometers[6]* | | | | | | | |
| Peaks above and below the mean between 1-2 SD, n | 1698.5 | 2722.0 | 2001.8 | 9.50 | 0.19 | - | - |
| Peaks above and below the mean between 2-3 SD, n | 484.4[b] | 1209.7[a] | 1485.7[a] | 9.12 | 0.03 | - | - |
| Peaks above and below the mean 3 SD, n | 413.4 | 1759.1 | 1420.8 | 7.38 | 0.07 | - | - |
| TA above and below 1 SD, V × s | 148.3 | 180.9 | 172.9 | 1.44 | 0.15 | - | - |
| TA above and below 2 SD, V × s | 27.1 | 43.0 | 44.0 | 0.94 | 0.41 | - | - |
| TA above and below 3SD, V × s | 21.0 | 26.5 | 24.9 | 0.74 | 0.58 | - | - |
| *Strain Gauges[7]* | | | | | | | |
| Peaks above and below the mean between 1-2 SD, n | 4000.3 | 2451.1 | 3591.8 | 9.20 | 0.58 | - | - |
| Peaks above and below the mean between 2-3 SD, n | 2015.7 | 1401.9 | 1572.7 | 7.79 | 0.75 | - | - |
| Peaks above and below the mean 3 SD, n | 2930.5 | 3409.5 | 1237.6 | 13.3 | 0.23 | - | - |
| TA above and below 1 SD, V × s | 1672.7 | 1486.8 | 1094.8 | 4.28 | 0.50 | - | - |
| TA above and below 2 SD, V × s | 589.4 | 861.7 | 442.6 | 5.18 | 0.47 | - | - |
| TA above and below 3SD, V × s | 301.1 | 596.7 | 240.7 | 4.33 | 0.37 | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SL[8], cm | 49.1 | 47.6 | 47.7 | 0.84 | 0.09 | <0.01 | 0.44 |

[a,b] Least square means within a row with differing superscripts differ ($P \leq 0.05$).
[1]Values in table represent the mean of the corresponding sampling times.
[2]The values presented correspond to non-transformed means, however SEM and *P*-values correspond to ANOVA analysis using Napierian logarithm or square root + 1 transformed data.
[3]Meloxicam = mean of 60 and 240 min sample plasma meloxicam concentrations.
[4]VAS = VAS collected during castration.
[5]HM = head movement collected during castration.
[6]Accelerometer = accelerometer peak number and total area collected during castration.
[7]Strain Gauges = Strain gauges peak number and total area collected during castration.
[8]SL = stride length collected immediately after, and 30, 60, 120 and 240 minutes after castration.

Table 5.3. Least square means (±SEM) of samples taken the days after castration for meloxicam, substance P (SP), complete blood count (CBC), haptoglobin, serum amyloid A (SAA), salivary cortisol, hair cortisol, scrotal temperature (ST), body temperature (Temp) and performance (initial BW, final BW and ADG) of surgically castrated Angus and Angus crossbred bulls receiving subcutaneous meloxicam 6, 3 and 0 h before castration[1].

| | Treatment (T) | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Item | 0H | 3H | 6H | SEM[2] | T | Time | T × Time |
| Meloxicam[3], ng/mL | 296.2 | 225.4 | 255.9 | 0.14 | 0.88 | <0.01 | 0.38 |
| SP[4], pg/mL | 116.9 | 119.9 | 120.2 | 0.03 | 0.52 | <0.01 | 0.01 |
| CBC[5] | | | | | | | |
| N:L ratio | 1.2[a] | 0.9[b] | 0.9[b] | 0.03 | 0.05 | <0.01 | 0.78 |
| WBC, $10^9$/L | 11.6 | 11.1 | 11.0 | 0.05 | 0.36 | <0.01 | 0.97 |
| RBC, $10^{12}$/L | 8.2 | 8.4 | 8.1 | 0.17 | 0.16 | <0.01 | 0.45 |
| Platelets, $10^9$/L | 542.2 | 483.4 | 520.4 | 0.43 | 0.14 | <0.01 | 0.33 |
| Haptoglobin[6], g/L | 1.8 | 1.9 | 2.1 | 0.14 | 0.47 | <0.01 | 0.12 |
| SAA[7], ug/mL | 19.2 | 23.7 | 27.0 | 0.80 | 0.68 | <0.01 | 0.69 |
| Salivary Cortisol[8], nmol/L | 3.5 | 3.4 | 3.3 | 0.04 | 0.97 | <0.01 | 0.96 |
| Hair Cortisol[9], nmol/L | 1.9 | 2.0 | 1.7 | 0.08 | 0.14 | 0.74 | 0.21 |
| ST[10], ºC | 34.9 | 34.8 | 35.2 | 0.30 | 0.65 | <0.01 | <0.01 |
| Temp[11], ºC | 39.7 | 39.6 | 39.6 | 0.05 | 0.61 | <0.01 | 0.66 |
| Performance | | | | | | | |
| Initial BW (day -1), kg | 283.3 | 280.3 | 283.1 | 8.72 | 0.96 | - | - |
| Final BW (day 28), kg | 303.6 | 301.3 | 309.8 | 8.46 | 0.76 | - | - |
| ADG, kg/d | 0.7 | 0.7 | 0.9 | 0.18 | 0.12 | - | - |

[a,b] Least square means within a row with differing superscripts differ ($P \leq 0.05$).
[1]Values in table represent the mean of the corresponding sampling days.
[2]The values presented correspond to non-transformed means, however SEM and *P*-values correspond to ANOVA analysis using Napierian logarithm or square root + 1 transformed data.
[3]Meloxicam= plasma meloxicam concentration collected on on d 1, 2 and 5 after castration.
[4]Substance P= substance P collected d 1, 2, 5, 7, 14, 21 and 28 after castration.
[5]CBC= CBC collected on d 1, 2, 5, 7, 14, 21 and 28 after castration.
[6]Haptoglobin= haptoglobin collected d 1, 2, 5 and 7 after castration.
[7]SAA = serum amyloid A collected d 1, 2, 5 and 7 after castration.
[8]Salivary Cortisol = salivary cortisol collected on d 1, 2, 5, 7, 14, 21 and 28 after castration
[9]Hair Cortisol = hair cortisol collected on d -8 and 28.
[10]ST= scrotal temperature collected on d 0, 1, 2, 5, 7, 14, 21, and 28 after castration.
[11]Temp= rectal body temperature collected on d 0, 1, 2, 5, 7, 14, 21, and 28 after castration.

Table 5.4. Lesion scores of surgically castrated Angus and Angus crossbred bulls receiving subcutaneous meloxicam 6, 3 and 0 h before castration from d 1 to d 28 post castration.

| Scores | Treatment[1] | | | | | | | | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0H | | | 3H | | | 6H | | | |
| | N | Median | 95% FCL | N | Median | 95% FCL | N | Median | 95% FCL | |
| 4 | 8 | 1 | 1-14 | 7 | 1 | 1-14 | 7 | 2 | 1-14 | 0.54 |
| 3 | 10 | 2 | 1-28 | 10 | 5 | 1-21 | 13 | 5 | 1-28 | 0.80 |
| 2 | 6 | 5 | 5-14 | 10 | 14 | 5-28 | 8 | 14 | 2-28 | 0.13 |
| 1 | 4 | 18 | 14-28 | 4 | 18 | 14-28 | 5 | 14 | 14-28 | 0.96 |

[1]FCL: 95% confidence limits distribution free.

Table 5.5. Least square means1 (±SEM) of stride length (SL), behavioral observations, standing and lying behavior and feeding behavior of surgically castrated Angus and Angus crossbred bulls receiving subcutaneous meloxicam 6, 3 and 0 h before castration[1].

| | Treatment (T) | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|
| Item | 0H | 3H | 6H | SEM[2] | T | Time | T × Time |
| SL[3], cm | 48.1 | 48.0 | 47.6 | 0.69 | 0.48 | <0.01 | 0.22 |
| Behavioral observations[4] | | | | | | | |
| Lying | 32.1 | 43.7 | 25.6 | 1.32 | 0.81 | <0.01 | 0.20 |
| Standing | 121.8 | 115.3 | 114.8 | 0.70 | 0.85 | <0.01 | 0.16 |
| Walking | 12.3 | 8.0 | 10.1 | 0.21 | 0.12 | <0.01 | 0.29 |
| Eating | 42.4 | 40.3 | 35.5 | 0.65 | 0.47 | 0.20 | 0.38 |
| Tail flick | 311.4 | 61.3 | 186.4 | 2.69 | 0.09 | 0.45 | 0.16 |
| Foot stamp | 4.4 | 8.1 | 7.1 | 0.33 | 0.64 | 0.99 | <0.01 |
| Head turning | 10.2 | 7.0 | 5.9 | 0.29 | 0.18 | 0.57 | 0.13 |
| Standing/Lying behavior[5] | | | | | | | |
| Standing duration, min | 182.0 | 196.1 | 176.2 | 0.41 | 0.82 | <0.01 | 0.47 |
| Lying duration, min | 75.0 | 76.9 | 78.7 | 0.57 | 0.53 | <0.01 | 0.93 |
| Standing time, % | 52.5 | 51.5 | 53.1 | 0.01 | 0.60 | <0.01 | 0.77 |
| Lying time, % | 47.5 | 48.5 | 46.9 | 0.01 | 0.60 | <0.01 | 0.77 |
| Standing bouts | 9.7 | 9.1 | 9.1 | 0.06 | 0.64 | <0.01 | 0.49 |
| Lying bouts | 12.4 | 11.9 | 10.7 | 0.08 | 0.19 | <0.01 | 0.63 |
| Feeding behavior[6] | | | | | | | |
| Dry matter feed intake, kg/d | 8.7 | 8.5 | 8.6 | 0.20 | 0.76 | <0.01 | 1.00 |
| Feeding time, min/d | 203.7 | 199.6 | 203.4 | 5.48 | 0.80 | <0.01 | 0.99 |
| Feeding rate, g/min | 43.1 | 44.5 | 43.4 | 1.26 | 0.43 | <0.01 | 0.55 |
| Meal frequency, meal/d | 9.6 | 8.8 | 9.2 | 0.49 | 0.15 | <0.01 | 0.53 |
| Meal duration, min/meal | 32.5 | 26.0 | 26.2 | 5.74 | 0.41 | 0.47 | 0.53 |
| Meal size, kg/meal | 1.0 | 1.1 | 1.1 | 0.04 | 0.29 | <0.01 | 0.36 |

[1]Values in table represent the mean of the corresponding sampling days.
[2]The values presented correspond to non-transformed means, however SEM and *P*-values correspond to ANOVA analysis using square root + 1 transformed data for behavioral observations and standing and lying behavior.
[3]SL = stride length collected at d 1, 2, 5, 7, 14, 21, and 28 after castration.
[4]Behavioral observations = behavioral observation collected on d 0, 2 and 3 after castration.
[5]Standing and lying behavior = standing and lying behavior were collected every day, however data from sampling days (d-1, 7, 14, 21, and 28) were excluded due to incomplete data.
[6]Feeding behavior= feeding behavior information collected daily throughout the experiment.

Figure 5.1. Least square means and SEM of scrotal temperature of T0, 30, 60, 120 and 240 min after surgical castration of Angus and Angus crossbred bulls receiving subcutaneous meloxicam 6, 3 and 0 h before castration. Least square means with differing superscripts differ ($P \leq 0.05$).

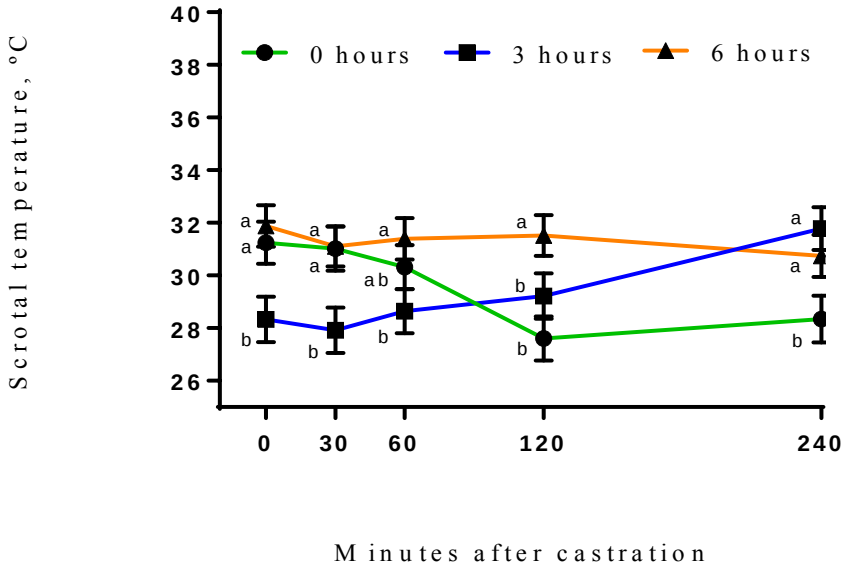


Figure 5.2. Least square means and SEM for substance P concentrations of d 1, 2, 5, 7 14, 21 and 28 after surgical castration of Angus and Angus crossbred bulls receiving subcutaneous meloxicam 6, 3 and 0 h before castration. Least square means with differing superscripts differ ($P \leq 0.05$).

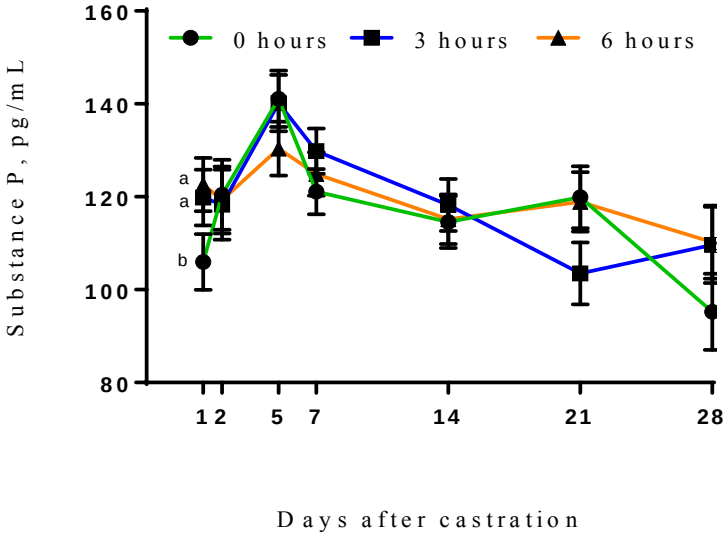


Figure 5.3. Least square means and SEM of scrotal temperature of d 1, 2, 5, 7 14, 21 and 28 after surgical castration of Angus and Angus crossbred bulls receiving subcutaneous meloxicam 6, 3 and 0 h before castration. Least square means with differing superscripts differ ($P \leq 0.05$).

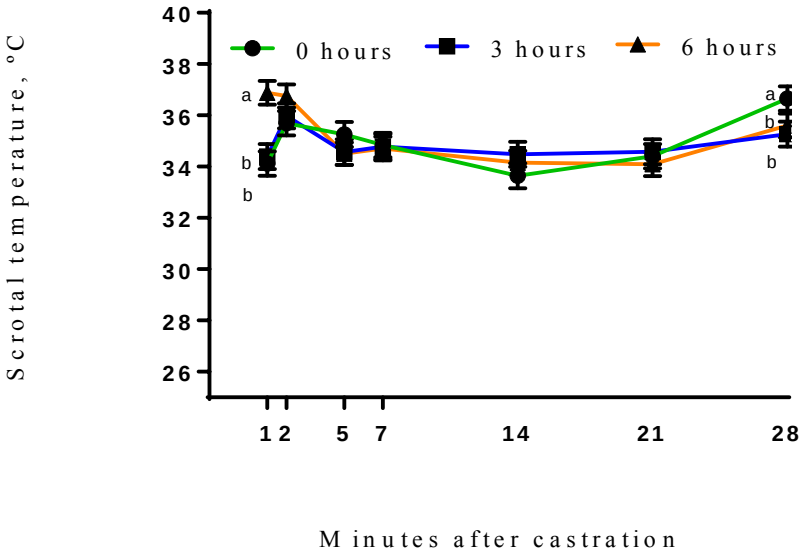
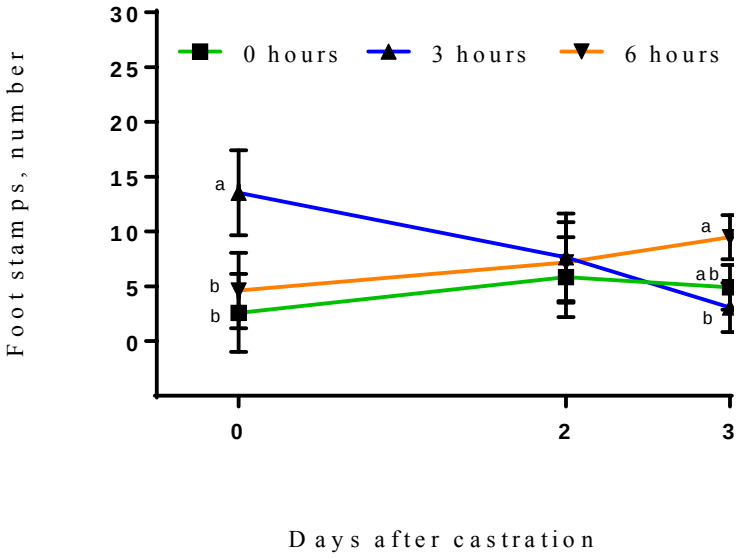


Figure 5.4. Least square means and SEM of foot stamping of d 0, 2 and 3 after surgical castration of Angus and Angus crossbred bulls receiving subcutaneous meloxicam 6, 3 and 0 h before castration. Least square means with differing superscripts differ ($P \leq 0.05$).



# Chapter Six: Effect of meloxicam and lidocaine administered alone or in combination on indicators of pain and distress during and after knife castration in weaned beef calves

## 6.1 Abstract

To assess the effect of meloxicam and lidocaine on indicators of pain associated with castration, forty-eight Angus crossbred beef calves (304 ± 40.5 kg of BW) were used in a 28 d experiment. The experiment consisted of a 2 × 2 factorial design where main factors included meloxicam: non-medicated (**N**; *n* = 24) single s.c. administration of lactated ringer's solution and medicated (**M**; *n* = 24) single dose of 0.5 mg/kg of s.c. meloxicam or lidocaine: non-medicated (**R**; n = 24) ring block administration of lactated ringer's solution or medicated (**L**; *n* = 24) ring block administration of lidocaine, to yield no meloxicam + no lidocaine (**N-R**; *n* = 12), no meloxicam + lidocaine (**N-L**; *n* =12), meloxicam + no lidocaine (**M-R**; *n* = 12) and meloxicam + lidocaine (**M-L**; *n* = 12). A lidocaine × time effect ($P < 0.01$) was observed for salivary cortisol, where L calves had lower ($P < 0.05$) cortisol concentrations than R calves 30 and 60 min after castration. A meloxicam effect ($P = 0.03$) was observed, where M calves had lower ($P < 0.05$) cortisol concentrations than N calves during 240 min after castration A lidocaine × time effect ($P < 0.01$) was observed for SAA concentrations, where R calves had greater SAA concentrations than L calves on d 1, 3, 21 and 28 after castration. A meloxicam × time effect ($P = 0.01$) was observed for haptoglobin concentrations, where M calves had lower haptoglobin concentrations than N calves on d 1 and 2 after castration. A lidocaine effect ($P < 0.01$) was observed for VAS, leg movement and head movement distances, where L calves had lower ($P < 0.05$) VAS scores, lower ($P < 0.05$) number of leg movements, and lower ($P < 0.05$) head movement distance than

R calves at the time of castration. Overall, lidocaine and meloxicam had an effect on physiological and behavioral parameters. Although there was no clear drug interaction, lidocaine and meloxicam reduced physiological and behavioral parameters at different time points, therefore the combination of drugs is likely to be more effective at mitigating pain during and after castration than either drug on its own.

## 6.2 Introduction

Routine husbandry procedures, such as castration, have been reported to cause physiological and behavioral changes indicative of pain and distress (Coetzee, 2011). However, castration is a practice commonly done without the use of pain control. As of January 2018, it is a requirement of the Canadian Beef Codes of Practice (NFACC, 2013) to castrate calves (6 mo of age or older) with the use of pain mitigation.

Drugs commonly used to mitigate pain at the time of castration include local anaesthetics and analgesics. Lidocaine, a local anaesthetic that is frequently used in veterinary medicine due to its fast onset of action and low toxicity (compared to other local anaesthetics), works by blocking sodium channels, therefore interrupting the transmission of action potentials in the neurons (Egger et al., 2013). Meloxicam, a non-steroidal anti-inflammatory drug (NSAID), reduces inflammation and pain by inhibiting COX enzymes, which convert arachidonic acid into prostaglandins, which are pro-inflammatory substances (Ochroch et al., 2003).

Previous studies assessing the effect of lidocaine after surgical castration have reported a reduction in the cortisol response (Fisher et al., 1996), while a reduction in haptoglobin concentrations have been reported after oral meloxicam administration in surgically castrated calves (Brown et al., 2015; Roberts et al., 2015). A review assessing cortisol levels associated

with castration, suggested that an analgesic and an anaesthetic administered together were more effective at mitigating pain than when either drug was administered alone (Coetzee, 2011).

Currently, there is a lack of research assessing the combination of a lidocaine ring block and s.c. meloxicam to mitigate pain associated with castration. Therefore, the aim of the study was to evaluate the effect of meloxicam, lidocaine, and the combination of meloxicam and lidocaine on indicators of pain in 7-8 mo old beef calves. We hypothesised that the combination of drugs would be more effective at mitigating knife castration related pain than either drug administered alone.

## 6.3 Materials and Methods

This protocol was approved by the Animal Care Committees of the Lethbridge Research Centre (ACC number 1522) and the University of Calgary (AC15- 0138). Animals were cared for in accordance with the Canadian Council of Animal Care (CCAC, 2009).

### 6.3.1 Animal Housing and Management

Forty-eight Angus crossbred beef calves (304 ± 40.5 kg of BW), 7 to 8 mo of age, were used in a 28 d experiment. Calves were divided into two groups of 24 animals each and castrated on different days, 7 d apart. Calves were vaccinated with a 7-way clostridial vaccine (Ultrabac®/Somubac®, Zoetis Canada Inc., Kirkland, Canada) and weaned 3 wk prior to the start of the trial, in order to adapt the calves to the experimental pens. Calves were housed in 4 experimental pens (40.2 m × 27.4 m) containing straw bedding and ad libitum water was provided through a centrally located water system. The diet consisted of a total mixed ration consisting of 80 % barley silage, 17 % dry-rolled barley and 3 % supplement with vitamins and minerals to meet beef cattle nutrition requirements (NRC, 2016).

Calves were restrained in a hydraulic squeeze chute (Cattlelac Cattle, Reg Cox Feedmixers Ltd, Lethbridge, Alberta, Canada) where they were sampled and castrated. Calves were equally distributed by weight into pens, and randomly assigned to treatments. The experiment consisted of a 2 × 2 factorial design where main factors included meloxicam: non-medicated (**N**; *n* = 24) single s.c. administration of lactated ringer's solution (Lactated Ringer's Irrigation, Baxter Canada, Mississauga, Ontario, Canada) and medicated (**M**; *n* = 24) single dose of 0.5 mg/kg of s.c. meloxicam (Metacam 20 mg/mL, Boheringer's Ingelhein, Burlington, Ontario, Canada) or lidocaine: non-medicated (**R**; n = 24) ring block administration of lactated ringer's solution (Lactated Ringer's Irrigation, Baxter Canada, Mississauga, Ontario, Canada) or medicated (**L**; n = 24) ring block administration of lidocaine (lidocaine hydrochloride 20 mg/mL and epinephrine 0.01 mg/mL, Bimeda, Ontario, Canada) to yield no meloxicam + no lidocaine (**N-R**; *n* = 12), no meloxicam + lidocaine (**N-L**; *n* =12), meloxicam + no lidocaine (**M-R**; *n* = 12) and meloxicam + lidocaine (**M-L**; *n* = 12). The lidocaine with epinephrine block and the sham block were administered 30 min prior to castration to allow time for the lidocaine to be absorbed. The ring block consisted of administering 5 ml into each spermatic cord and 20 ml subcutaneously around the neck of the scrotum. Meloxicam and the sham injection were administered s.c. on the neck of the calves. The same veterinarian performed the scrotal lidocaine block, and the surgical castration on all the calves by making a latero-lateral incision on the scrotum with a Newberry castration knife (Syrvet Inc., Waukee, IA) and an emasculator was used to crush and cut the spermatic cords.

### 6.3.2 Measurements of pain indicators and Sample Collection

***Saliva and hair cortisol.*** Saliva was collected, stored and analyzed as described by Meléndez et al. (2017b). Salivary samples were collected 24 h before castration (d -1),

immediately before castration (**T0**), 30, 60, 120, 240 min and on d 1, 2, 3, 6, 14, 21 and 28 after castration. Salivary cortisol concentrations were quantified using an enzyme immunoassay kit (Salimetrics, State College, PA). The inter-assay CV was 10.3 % while the intra-assay CV was 9.2 %. Hair cortisol was collected as previously described by Meléndez et al. (2017a). Briefly, hair from the forehead of the calves was clipped on d – 1 and d 28 and stored in plastic bags at room temperature. Samples were handled as described by Moya et al. (2013) and cortisol was quantified using an enzyme-immunosorbent assay (Salimetrics, State College, PA). The intra-assay and the inter-assay's CV were 9.7 % and 12.1% respectively.

***Serum Amyloid-A, Haptoglobin, Meloxicam and Complete Blood Count.*** Blood samples were collected from all calves through jugular venipuncture on d -1, immediately before castration (T0), 30, 60, 120, 240 min and on d 1, 2, 3, 6, 14, 21 and 28 after castration.

Blood samples for serum amyloid-A (SAA) and haptoglobin were collected, stored and analyzed as previously described by Meléndez et al. (2017b). Briefly samples were collected into a 10-ml non-additive tube (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ), centrifuged for 15 min at 1.5 × *g* at 4 °C and the serum was decanted and frozen at -80 ºC for further analysis. The inter-assay CV for haptoglobin was 7.6 %, while SAA intra-assay and inter-assay CV were 8.8 % and 10.3 %, respectively.

Blood samples for CBC were collected into a 6-ml EDTA tube (BD vacutainer; Becton Dickinson Co., Franklin Lakes, NJ) and red blood cells (RBC), white blood cells (WBC) were measured using a HemaTrueHematology Analyzer (Heska, Lobeland, Co).

***Scrotal Area Temperature (SCT).*** Images of and around the area of the scrotum were collected and analyzed as previously described by Meléndez et al. (2017b). Images were

collected on d -1, immediately before castration (T0), 30, 60, 120, 240 min and on d 1, 2, 3, 6, 14, 21 and 28 after castration. A FLIR i60 infrared camera (FLIR Systems Ltd., Burlington, ON, Canada) was used to capture infrared images of the scrotal area and FLIR Tools version 5.1 (FLIR Systems Ltd.) was used to delimit the scrotal area and to record the maximum temperature.

***Rectal temperature (Rectal temp).*** A digital thermometer (M750 Livestock Thermometer, GLA Agricultural Electronics, San Luis Obispo, CA) was used to collect rectal temperature on d -1, immediately before castration (T0), 30, 60, 120, 240 min and on d 1, 2, 3, 6, 14, 21 and 28 after castration.

***Performance.*** Calves were weighed in a hydraulic squeeze chute (Cattlelac Cattle, Reg Cox Feedmixers Ltd, Lethbridge, Alberta, Canada) to obtain the initial (d -1) and final (d 28) BW. The ADG (kg/d) was calculated by subtracting the weights on d 28 from the weight obtained on d -1, and dividing the result by 29 which was the number of days in the experiment.

***Behavioral frequencies and Visual Analog Scale (VAS).*** Behavioral scoring during castration was collected as previously described by Meléndez et al. (2017b). Two experienced observers which were blind to treatments, placed a mark along a 10 cm line (far left indicating no pain and far right extreme pain) as an indicator of their perception of the amount of pain calves were experiencing during castration. In addition, observers recorded the frequency of urination, defecation, leg movement and vocalizations.

***Electronic reactivity measurements (ERM).*** Electronic reactivity measurements were collected as previously described by Meléndez et al. (2017a). The right and left head gate were equipped with load cells to measure force caused by pressure on the head gate while cattle

pushed or pulled against them while the chute was equipped with three one dimension accelerometers measuring lateral, vertical and horizontal movement. The analog signals (V) from the accelerometer and load cells were sent to a computer at a rate of 100 samples/ s. Data from the accelerometer was added to obtain an overall accelerometer force and the data from the left and right head gate was added to obtain an overall head gate force. Data from d -1 was used as a baseline for each calf. Variables included the number of peaks between 1 and 2 SD, 2 and 3 SD, and above or below 3 SD above and below the mean and total area between the mean ± 1 SD, mean ± 2 SD, and mean ± 3 SD.

***Head movement (HM).*** Head movement was collected and analyzed as previously described by Meléndez et al. (2017a). A video camera was placed in front of the head gate during castration to record head movement. Kinovea (General Public License) version 2 was used to track the total head movement distance (cm) during castration. The middle of the hairline of the muzzle was used as a reference point.

***Stride length.*** Stride length was collected as previously described by Meléndez et al., 2017b). Briefly, calves were recorded when walking through an alley on d-1, immediately after castration, 30, 60, 120, 240 min and on d 1, 2, 3, 6, 14, 21 and 28 after castration. Pictures of the back legs were taken with GOM player (GOM Lab, Gretech Corporation, Seoul, South Korea), and where measured using Image J (National Institutes of Health Image, Bethesda, MD) to obtain stride length (cm).

***Behavioral observations.*** Half of the animals of each treatment (6 animals/ treatment) were recorded for behavioral observations and focal animal sampling from continuous recordings (Martin and Bateson, 2007) to score frequency and duration behaviors as described by

Meléndez et al. (2017b). Two experienced observers scored behavior between 5 to 7 h relative to castration for a 2 h period on d 0, 1 and 2. Inter-rater and intra-rater reliability were 0.93 and 0.95 respectively.

***Standing and lying behavior.*** Standing and lying duration was measured as previously described in Meléndez et al. (2017b). Accelerometers (Hobo pendant G, Onset Computer Corporation, Bourne, MA) were used to measure standing and lying percentage, and average duration and bouts (UBC AWP, 2013). Accelerometers were placed on the calves on d -1 using Vet Wrap (Professional Preference, Calgary, Canada) and changed weekly. The days in which the accelerometers were changed were excluded from the analysis due to incomplete data collection.

***Feeding behavior.*** Feeding behavior was collected as previously described by Meléndez et al. (2017a). Briefly, calves were fitted with a radio frequency ear tags and each pen was equipped with a GrowSafe feed bunk monitoring system (GrowSafe Systems, Airdrie, Alberta, Canada) with 5 feeding tubs which recorded feeding behavior for each individual calf 24 h a day over a 28 d period. The following variables were calculated from the feeding behavior data recorded: feeding duration (min/d), dry matter intake (kg/day), feeding rate (g/min), meal frequency (number/d), meal duration (min/meal) and meal size (kg/meal). As in the previous study, a meal criterion of 300 s was selected as it has been previously used in cattle (Sowell et al., 1998; Schwartzkopf-Genswein et al., 2003).

### *Statistical analysis*

Data was analyzed using the MIXED procedure in SAS (SAS, version 9.4, SAS Inst. Inc., Cary, NC) to evaluate the effect of lidocaine, meloxicam and time on all variables. Fixed effects

included meloxicam, lidocaine, time and their interactions, while random effects included pen and calf within pen. Animals were the experimental unit as treatments were mixed within pen. All data, with the exception of behavior during castration (VAS, frequency of leg movement, vocalizations, ERM) hair cortisol and performance, was analyzed using mixed repeated models. Data was tested for normal distribution with PROC UNIVARIATE (SAS, version 9.4, SAS Inst. Inc., Cary, NC) and physiological data that did not follow a normal distribution was log transformed while behavioral data was square root + 1 transformed and percentage data from behavioral data was arcsin transformed. The data collected on d-1 was used as a covariate for all physiological parameters and stride length, while data collected 1 week before castration was used as the baseline for feeding behavior. Electronic reactivity measurements collected on d-1 were used as a baseline for each calf. Urination and defecation were not analyzed as these behaviors were not present during castration. Covariance structures included unstructured, compound symmetry and autoregressive order one. The structure with the lowest Schwarz's Bayesian criterion was selected as the analysis of choice. Data from the day of castration was analyzed separately from the data the days after castration, with the exception of CBC counts. Least square mean differences were determined using the PDIFF option in SAS. Effect of lidocaine, meloxicam and time were statistically significant when $P \leq 0.05$ and considered a tendency when $0.05 < P \leq 0.10$.

## 6.4 Results and Discussion

### 6.4.1 Physiology

A lidocaine × time effect ($P < 0.01$) was observed for salivary cortisol, where L calves had lower ($P < 0.05$) cortisol concentrations than R calves 30 and 60 min after castration, however no differences ($P > 0.10$) were observed at T0, 120 and 240 min after castration (Fig.

6.1A). A meloxicam effect ($P = 0.03$) was observed, where NM (6.2 ± 0.10 nmol/L) calves had greater ($P < 0.05$) cortisol concentrations than M (4.9 ± 0.10 nmol/L) calves min after castration. No meloxicam or lidocaine differences ($P > 0.10$) were observed for salivary cortisol concentrations the days after castration. A lidocaine tendency ($P = 0.09$) was observed for hair cortisol, where L (3.0 ± 0.10 nmol/L) calves had lower ($P < 0.05$) hair cortisol concentrations than R (3.4 ± 0.10 nmol/L) calves on d 28.

Lidocaine has an onset of action of 5 to 10 min after administration and a duration of action of 60 to 120 min, which can be prolonged if combined with epinephrine (Egger et al., 2013). Although lidocaine with epinephrine was used in the present study, a reduction in cortisol concentrations were only observed up to 60 min after castration. Similar findings have been reported by Earley and Crowe (2002) who found a reduction in cortisol concentrations up to 75 min post castration, when intra-testicular lidocaine was injected 20 min before surgical castration in 5.5 mo old calves. Likewise, Ballou et al. (2013) reported that an intra-testicular and scrotal lidocaine injection, administered immediately before castration, reduced the peak cortisol response in 3 mo old dairy calves. Contrary to our findings, a previous study reported that intra-testicular lidocaine administered 15 min before castration, eliminated the cortisol response for band and ring castration, but had little effect on surgical pull or surgical cut castration in 2 to 4 mo old calves (Stafford et al., 2002). Another study reported that lidocaine applied subcutaneously as a ring block 20 min before castration, had no effect on the cortisol response and was associated with a second increase in cortisol 120 min after surgical castration in 2 to 3 mo old calves (Webster et al., 2013). Differences between the findings of our study and those that reported no lidocaine effect could be due to differences in route of administration, as calves in the present study received lidocaine into each spermatic cord and around the neck of the

scrotum, while the others received an intra-testicular or subcutaneous injection around the neck of the scrotum. In addition, calves in the present study were older, therefore the cortisol response would be greater than in younger calves due to greater tissue damage at the time of castration (Bretschneider, 2005) and it might be possible that the application of lidocaine with epinephrine would have slowed down the lidocaine absorption and therefore would have had a longer duration of action (Egger et al., 2013).

Meloxicam was able to reduce the cortisol response min after castration, but was not effective at reducing the duration of the cortisol response which was back to baseline levels 120 min after castration. Previous studies have reported flunixin meglumine, administered immediately before castration and lidocaine, reduced the cortisol response at 0.5 and 1.5 h after surgical castration in 3 mo old calves (Ballou et al., 2013), and in 2 to 3 mo old calves (Webster et al., 2013), while ketoprofen was able to reduce cortisol (area under the curve) in surgically castrated 5.5 mo old calves (Earley and Crowe, 2002). Contrary to previous studies where the combination of an analgesic and an anaesthetics were more effective at reducing the cortisol response than either drug alone (Stafford et al., 2002; Ballou et al., 2013), in the present study we did not observer a lidocaine and meloxicam interaction for cortisol.

A lidocaine × time effect ($P < 0.01$) was observed for SAA concentrations, where R calves had greater SAA concentrations than L calves on d 1, 3, 21 and 28 after castration (Fig. 6.1B). A meloxicam × time effect ($P = 0.01$) was observed for haptoglobin concentrations, where M calves had lower haptoglobin concentrations than N calves on d 1 and 2 after castration (Fig. 6.1C). A lidocaine × time tendency ($P = 0.07$) was observed for haptoglobin, where L calves had greater ($P < 0.05$) haptoglobin concentrations than R calves on d 0, while L calves

tended ($P = 0.08$) to have lower haptoglobin levels than R calves on d 3 after castration (data not shown).

Pro-inflammatory cytokines stimulate the production of acute phase proteins in response to inflammation, infection, trauma or stress (Murata et al., 2004). SAA has been previously reported to increase after inflammatory diseases (Alsemgeest et al., 1994), viral (Gånheim et al., 2003) and bacterial infections (Horadagoda et al., 1994) in cattle; however few studies have assessed the SAA response after castration (Meléndez et al., 2017a). Lidocaine has been reported to inhibit pro-inflammatory cytokines, while stimulating the production of anti-inflammatory cytokines (Lahav et al., 2002), which could explain the reduction in SAA concentrations observed in calves receiving lidocaine. The ability of lidocaine to block nerve impulses is short, however, it seems that the anti-inflammatory effect lidocaine had on cytokines during this period of time was sufficient to produce differences in SAA concentrations up to 28 d after castration.

Meloxicam had an effect on haptoglobin concentrations, which was in contrast to SAA concentrations. Several studies have reported a reduction in the haptoglobin response after Burdizzo and surgical castration in calves receiving an NSAID (Earley and Crowe, 2002; Ting et al., 2003; Brown et al., 2015; Roberts et al., 2015). This is an interesting finding as we would expect both APP proteins to be affected in the same way by both lidocaine and meloxicam. Similar findings were reported in a previous study where meloxicam was able to reduce the haptoglobin response but not the SAA response to castration and branding (Meléndez et al., 2018). The author speculated that NSAID's might not have the same effect on the response of different APP's, but based on our findings local anaesthetics might also have different effects on the APP response. Different analgesic and anaesthetic agents might affect the production of

cytokines and glucocorticoids to a greater or lesser extent (Baumann et al., 1989) which ultimately affects APP production.

No meloxicam or lidocaine effects ($P > 0.10$) were observed for scrotal temperature min after castration. A meloxicam × time tendency ($P = 0.06$) was observed for scrotal temperature where N (35.7 ± 0.29 °C) calves tended to have greater ($P = 0.06$) scrotal temperature than M (35.1 ± 0.29 °C) calves on d 1 after castration. A lidocaine × time tendency ($P = 0.09$) was observed for scrotal temperature where L (33.6 ± 0.44 °C) calves tended to have lower ($P = 0.09$) scrotal temperature than R (34.6 ± 0.44 °C) calves on d 14 after castration. A meloxicam effect ($P = 0.04$) was observed for rectal temperature, where M (39.8 ± 0.05 °C) calves had greater ($P < 0.05$) rectal temperature than N (39.7 ± 0.05 °C) calves min after castration. No meloxicam or lidocaine effects ($P > 0.10$) were observed for rectal temperature d after castration. Although differences in temperature were observed, these might likely lack biological significance as differences between treatments were very small.

A lidocaine × time effect ($P < 0.01$) was observed for WBC, where L calves had lower ($P < 0.05$) WBC counts 120 min and on d 21, and tended to have lower ($P = 0.06$) counts than R calves on d 2 and 6 after castration (data not shown). A meloxicam × time effect ($P < 0.01$) was also observed for WBC, where M (11.5 ± 0.41 × $10^9$/L) calves had lower ($P < 0.05$) WBC counts than N (13.6 ± 0.41 × $10^9$/L) calves on d 1, while the M (11.3 ± 0.30 × $10^9$/L) calves tended to have lower ($P = 0.07$) counts than N (12.1 ± 0.30 × $10^9$/L) calves at 240 min after castration and the N (9.1 ± 0.13 × $10^9$/L) calves tended to have lower WBC counts at T0 than M (9.5 ± 0.41 × $10^9$/L) calves. A lidocaine × time effect ($P < 0.01$) was observed for N:L ratio, where L (0.7 ± 0.08; 1.0 ± 0.08) calves had a lower ($P < 0.05$) N:L ratio than R (1.1 ± 0.08; 1.2 ± 0.08) calves 120 and 240 min after castration. A meloxicam × time effect ($P < 0.01$) was also observed for

N:L ratio, where M (0.9 ± 0.08; 1.1 ± 0.08) calves had a lower ($P < 0.05$) N:L ratio than L (1.0 ± 0.08; 1.4 ± 0.08) calves 240 min and on d 1, after castration.

Both lidocaine and meloxicam were able to reduce the leukocyte response at different time points after castration, similar to the results observed for haptoglobin and SAA. This is in agreement with a previous study reporting a reduction in leukocytosis and neutrophilia in 3 mo old calves receiving lidocaine and flunixin meglumine before surgical castration (Ballou et al., 2013).

### 6.4.2 Behavior

A lidocaine effect ($P < 0.01$) was observed for VAS, leg movement and head movement distance, where L (3.1 ± 0.09 cm; 8.6 ± 0.15; 1263 ± 2.1 cm) calves had lower ($P < 0.05$) VAS scores, fewer ($P < 0.05$) leg movements and lower ($P < 0.05$) head movement distances than R (6.8 ± 0.09 cm; 20.2 ± 0.15; 2181 ± 2.1 cm) calves at the time of castration. Head movement had a meloxicam × lidocaine tendency ($P = 0.07$) where M-L and N-L calves and had lower ($P < 0.05$) head movement distance than M-R calves, however no differences were observed between N-R and N-L, M-L and M-R calves (Table 6. 3). A lidocaine effect ($P < 0.05$) was observed for accelerometer and head gate TA ± 1SD, ± 2 SD, or ± 3 SD, where L (head gate: 131 ± 1.7; 51 ± 1.7; 25 ± 1.7 V × s; accelerometer: 6 ± 0.3; 3 ± 0.3; 2 ± 0.3 V × s) calves had lower ($P < 0.05$) area than R (head gate: 346 ± 1.7; 173 ± 1.7; 111 ± 1.7 V × s; accelerometer: 21 ± 0.3; 14 ± 0.3; 11 ± 0.3 V × s) calves. A lidocaine effect ($P < 0.05$) was observed for the number of accelerometer peaks above and below 3 SD, where L (55 ± 1.2) calves had a lower ($P < 0.05$) number of peaks than R (191 ± 1.2) calves. A lidocaine tendency ($P = 0.10$; $P = 0.08$) was observed, where L (101 ± 1.4; 156 ± 2.3) calves had a lower ($P < 0.05$) number of accelerometer

and head gate peaks than R (151 ± 1.4; 232 ± 2.3) calves between 2 to 3 SD above and below the mean.

Similar results were reported by Stafford et al. (2002) who found a reduction in pain related behaviors during clamp and surgical castration in 2 to 4 mo old calves receiving lidocaine compared to un-medicated calves. Lack of a meloxicam effect at the time of castration could also be due to the route of administration, as s.c. meloxicam administered immediately before castration is unlikely to have a central analgesic effect at the time of castration due to the time it would take to be absorbed. Moya et al. (2014) also reported a lack of differences in VAS scores and movement in the chute at the time of band and surgical castration with or without an i.m. injection of ketoprofen 30 min before castration.

A meloxicam effect ($P = 0.08$; $P = 0.07$) was observed for eating and foot stamping, M (26 ± 0.7 min; 2 ± 0.4 n) calves tended to have greater ($P = 0.08$) eating duration and lower ($P = 0.07$) foot stamp number than N (18 ± 0.7 min; 5 ± 0.4 n) calves on d 0, 1 and 2 after castration. A lidocaine effect ($P = 0.07$) was observed for tail flicks, where L (260 ± 3.4) calves tended to have lower ($P = 0.08$) number of tail flicks than R (642 ± 3.4) calves on d 0, 1 and 2 after castration. No differences ($P > 0.10$) were observed for head turning, lesion licking, walking, standing or lying ventral (Table 6.4). The tendencies observed are expected findings, however lack of differences in behaviour are likely due to the delayed behavioral scoring. We would expect to see clear differences in behaviour after castration; however, it was not possible to assess behaviour before 5 hours due to the timing of the physiological samples.

A lidocaine × time effect ($P = 0.04$) was observed for stride length, where L (51 ± 1.0 cm) calves had greater SL than R (47 ± 1.0 cm) calves on d 28 after castration. No meloxicam or

lidocaine effects ($P > 0.10$) were observed for stride length minutes after castration. Although we would expect calves experiencing less pain to have a greater stride length (Currah et al., 2009), we would also expect to see differences in stride length during the duration of action of lidocaine or meloxicam and not on d 28 after castration. Although previous studies have reported differences in physiological and behavioral parameters 2 to 5 days after the duration of action of meloxicam (Theurer et al., 2012; Allen et al., 2013; Meléndez et al., 2018), is unlikely that lidocaine would have an effect on stride length 28 d after castration.

A lidocaine × time effect was observed for standing percentage, L (49.3 ± 2.29 %) calves tended to have greater ($P = 0.08$) standing percentage than R (44.5 ± 2.19 %) calves on d 5 after castration. No differences were observed for lying percentage. A lidocaine × time effect ($P < 0.01$) was observed for standing duration. On d 1 and 5 L (465 ± 31.7 min; 162 ± 29.0 min) calves had greater standing duration than R (292 ± 31.7 min; 90 ± 29.0 min) calves, while on d 3 and 7 L (184 ± 30.0 min; 156 ± 26.5 min) calves had lower standing duration than R (275 ± 30.0 min; 293 ± 26.5 min) calves. A lidocaine × time effect ($P < 0.01$) was observed for lying duration, where R (134 ± 7.7 min; 129 ± 7.1 min; 78 ± 6.7 min) calves had greater ($P < 0.05$) lying duration than L (89 ± 7.7 min; 96 ± 7.1 min; 54 ± 6.7 min) calves on d 3, 4 and 7, and tended to have greater ($0.05 < P \leq 0.10$) lying duration on d 9, 12 and 13 after castration (Table 6.4).

Previous studies have reported an increase in standing behavior following surgical castration compared to prior to castration (White et al., 2008) and in surgically castrated calves compared to sham calves (Webster et al., 2013). Therefore, we would expect un-medicated calves to have a greater standing percentage than all other treatments; however L calves had a greater standing percentage and a lower lying percentage than R calves. A possible reason could

be that the lidocaine injection caused local tissue irritation (Egger et al., 2013) which could have increased discomfort causing calves to spend more time standing and less time lying. A previous study reported calves treated with lidocaine had higher head turning frequency, statue standing, tail flicking and changes in posture (Webster et al., 2013).

### Conclusion

Overall, lidocaine was effective at reducing physiological and behavioral indicators of pain (salivary cortisol, SAA, WBC, VAS, leg movement, head distance, electronic reactivity measurement) while meloxicam only reduced physiological indicators of pain (salivary cortisol, haptoglobin and WBC). Parameters that weren't affected by either drug included performance and feeding behavior. Meloxicam and lidocaine had a different effect on the APP response, and the effect observed for salivary cortisol and WBC was observed at different time points after castration, therefore although we did not see a meloxicam and lidocaine interaction, administering lidocaine and meloxicam together is more effective at mitigating pain indicators after surgical castration for a longer period of time. Further studies are needed to identify the most effective pain mitigation protocol.

Table 6.1. Least squares means (± SEM) of samples collected at T0, 30, 60, 120 and 240 min after procedure for salivary cortisol, meloxicam, scrotal temperature (SCT) and rectal temperature (Temp); and at d 1, 2, 3 and 7 after procedure for salivary cortisol, hair cortisol, meloxicam, scrotal temperature (SCT), rectal temperature (Temp), serum amyloid-A (SAA), haptoglobin and CBC (WBC, RBC, PLT, and N:L ratio) of surgically castrated weaned Angus crossbred calves with (M) or without (N) a single s.c. meloxicam injection and with (L) or without (R) a lidocaine ring block[1]

| | Treatment | | | | | *P-Value* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LI | | ME | | | | | | | | | |
| Item | N-R | N-L | M-R | M-L | *SEM* [2] | MEL | LID | MEL×LID | T | MEL× T | LID × T | MEL×LID×T |
| Minutes after castration | | | | | | | | | | | | |
| Cortisol, nmol/L | 7.3 | 5.1 | 5.1 | 4.7 | 0.13 | 0.03 | 0.20 | 0.77 | <0.01 | 0.17 | <0.01 | 0.16 |
| SCT, ºC | 33.7 | 33.8 | 34.8 | 33.6 | 0.38 | 0.57 | 0.52 | 0.28 | 0.03 | 0.17 | 0.77 | 0.87 |
| Temp, ºC | 39.7 | 39.7 | 39.8 | 39.9 | 0.07 | 0.04 | 0.71 | 0.35 | <0.01 | 0.70 | 0.67 | 0.17 |
| Days after castration | | | | | | | | | | | | |
| Cortisol, nmol/L | 4.1 | 4.5 | 3.7 | 3.9 | 0.09 | 0.18 | 0.17 | 0.81 | <0.01 | 0.51 | 0.84 | 0.31 |
| Hair cortisol, nmol/L | 3.5 | 3.1 | 3.3 | 2.9 | 0.14 | 0.72 | 0.09 | 0.89 | - | - | - | - |
| Meloxicam, | $0^{c}$ | $0^{c}$ | $429^{b}$ | $651^{a}$ | 0.88 | <0.01 | 0.01 | 0.01 | <0.01 | <0.01 | 0.40 | 0.40 |
| SCT, ºC | 35.2 | 35.2 | 35.4 | 34.9 | 0.28 | 0.86 | 0.17 | 0.24 | <0.01 | 0.06 | 0.08 | 0.33 |
| Temp, ºC | 39.8 | 39.7 | 39.8 | 39.8 | 0.07 | 0.49 | 0.31 | 0.42 | <0.01 | 0.61 | 0.43 | 0.54 |
| SAA, µg/mL | 153 | 138 | 147 | 122 | 0.1 | 0.94 | <0.01 | 0.52 | <0.01 | 0.80 | <0.01 | 0.19 |
| Haptoglobin, g/L | 1.5 | 1.6 | 1.4 | 1.1 | 0.09 | 0.17 | 0.16 | 0.12 | <0.01 | 0.01 | 0.07 | 0.42 |
| CBC[4] | | | | | | | | | | | | |
| WBC, $\times 10^{9}$/L | $10.8^{a}$ | $10.8^{a}$ | $11.3^{a}$ | $9.9^{b}$ | 0.27 | 0.40 | 0.01 | 0.02 | <0.01 | <0.01 | <0.01 | 0.69 |
| RBC, $\times 10^{12}$/L | 8.5 | 8.4 | 8.5 | 8.4 | 0.15 | 0.97 | 0.48 | 0.97 | <0.01 | 0.10 | 0.10 | 0.01 |

a-b Least square means within a row with differing superscripts differ ( $P \leq 0.05$)

[1]Values in the table represent the mean of T0, 30, 60, 120 and 240 min and the mean of d 1, 2, 3 and 7, except for meloxicam were values were T0, 60 and 120 min and on d 0, 1, 2 and 3.

[2]The values correspond to nontransformed means; however, the SEM and the P-values correspond to ANOVA analysis using log transformed data.

[4]CBC = complete blood count; WBC = white blood cell count; RBC = red blood cell count; N:L ratio = neutrophil-to- lymphocyte ratio.

Table 6.2. Least squares means (± SEM) of initial BW, final BW and ADG of weaned Angus crossbred calves during the first 28 d after surgical castration with (M) or without (N) a single s.c. meloxicam injection and with (L) or without (R) a lidocaine ring block[1]

| | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LI | | ME | | | *P-Value* | | |
| Item | N-R | N-L | M-R | M-L | *SEM*[1] | MEL | LID | MEL × LID |
| Performance | | | | | | | | |
| Initial BW (d-1), kg | 300.1 | 301.7 | 303.1 | 300.6 | 12.27 | 0.94 | 0.97 | 0.87 |
| Final BW (d7), kg | 320.7 | 320.6 | 322.2 | 318.2 | 12.65 | 0.98 | 0.87 | 0.88 |
| ADG, kg/d | 0.73 | 0.65 | 0.66 | 0.61 | 0.12 | 0.51 | 0.46 | 0.85 |

[1]The values correspond to non-transformed means, SEM and *P*-values.

Table 6.3. Least squares means (± SEM) of visual analog scale (VAS), leg movement, vocalizations, head movement and electronic reactivity measurements of weaned Angus crossbred calves during surgical castration with (M) or without (N) a single s.c. meloxicam injection and with (L) or without (R) a lidocaine ring block1

| | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LID | | MEL | | | | *P-Value* | |
| Item | N-R | N-L | M-R | M-L | *SEM*[1] | MEL | LID | MEL × LID |
| VAS, cm | 6.6 | 2.8 | 7.0 | 3.5 | 0.12 | 0.26 | <0.01 | 0.60 |
| Leg movement, n | 20.4 | 8.1 | 20.0 | 9.0 | 0.21 | 0.78 | <0.01 | 0.50 |
| Vocalization, n | 0.2 | 0.0 | 0.33 | 0.21 | 0.06 | 0.39 | 0.34 | 0.69 |
| Head movement, cm | 2017 | 1604 | 2346 | 922 | 3.8 | 0.45 | <0.01 | 0.07 |
| *Accelerometers* | | | | | | | | |
| Peaks between 1-2 SD, n | 498 | 430 | 614 | 408 | 2.9 | 0.84 | 0.28 | 0.81 |
| Peaks between 2-3 SD, n | 156 | 76 | 148 | 126 | 1.9 | 0.58 | 0.10 | 0.55 |
| Peaks above 3 SD, n | 190 | 41 | 191 | 71 | 1.6 | 0.70 | <0.01 | 0.57 |
| TA above and below 1 SD, V × s | 19.0 | 6.1 | 23.0 | 7.1 | 0.40 | 0.56 | <0.01 | 0.80 |
| TA above and below 2 SD, V × s | 12.9 | 3.4 | 15.6 | 3.7 | 0.36 | 0.70 | <0.01 | 0.81 |
| TA above and below 3SD, V × s | 10.2 | 2.6 | 12.2 | 2.5 | 0.34 | 0.84 | <0.01 | 0.75 |
| *Head Gate* | | | | | | | | |
| Peaks between 1-2 SD, n | 703 | 435 | 1147 | 771 | 4.1 | 0.14 | 0.81 | 0.36 |
| Peaks between 2-3 SD, n | 157 | 142 | 308 | 171 | 2.9 | 0.19 | 0.08 | 0.52 |
| Peaks above 3 SD, n | 235 | 159 | 492 | 177 | 4.5 | 0.79 | 0.76 | 0.60 |
| TA above and below 1 SD, V × s | 242 | 133 | 451 | 129 | 0.2 | 0.12 | <0.01 | 0.29 |
| TA above and below 2 SD, V × s | 135 | 53 | 212 | 49 | 2.1 | 0.32 | <0.01 | 0.60 |
| TA above and below 3SD, V × s | 92 | 26 | 130 | 24 | 1.9 | 0.51 | 0.01 | 0.79 |

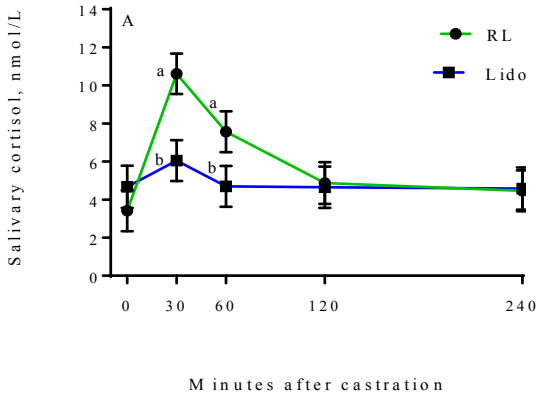
Table 6.4. Least squares means (± SEM) immediately after and 30, 60, 120 and 240 min and on d 1, 2, 3 and 7 after castration of stride length, 5 to 8 h after castration on d 0 and d2standing and lying behavior and feeding behavior of 28 days after surgical castration of weaned Angus crossbred calves with (M) or without (N) a single s.c. meloxicam injection and with (L) or without (R) a lidocaine ring block

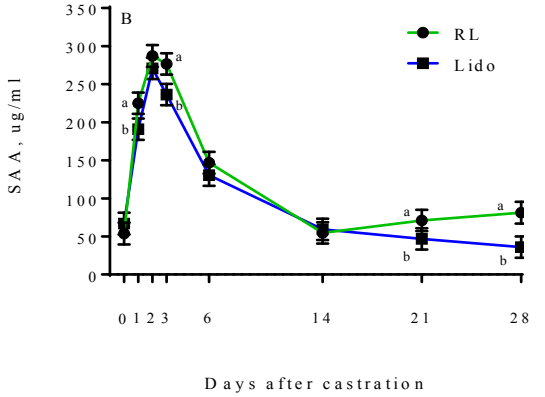
| | Medication | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LID | | MEL | | | *P-Value* | | | | | | |
| Item | N-R | N-L | M-R | M-L | *SEM*[1] | MEL | LIDO | MEL × LID | Time | MEL ×T | LID ×T | MEL × LID ×T |
| Stride length[2], cm | 46.9 | 47.4 | 46.2 | 46.0 | 0.93 | 0.27 | 0.83 | 0.70 | 0.04 | 0.60 | 0.50 | 0.74 |
| Stride length[3], cm | 46.5 | 47.6 | 46.2 | 46.2 | 0.99 | 0.39 | 0.54 | 0.59 | <0.01 | 0.86 | 0.03 | 0.85 |
| Standing and lying beh. | | | | | | | | | | | | |
| Standing, % | 47.9 | 47.0 | 48.2 | 45.6 | 0.06 | 0.62 | 0.79 | 0.18 | < 0.01 | 0.34 | 0.02 | 0.88 |
| Lying, % | 52.4 | 53.0 | 51.9 | 53.0 | 0.14 | 0.87 | 0.79 | 0.14 | 0.01 | 0.32 | 0.38 | 0.91 |
| Standing duration, min | 125.4 | 98.7 | 95.7 | 117.4 | 0.50 | 0.54 | 0.66 | 0.12 | <0.01 | 0.51 | <0.01 | 0.29 |
| Lying duration, min | 66.3 | 59.8 | 65.8 | 57.7 | 0.23 | 0.73 | 0.06 | 0.51 | <0.01 | 0.89 | <0.01 | 0.74 |
| Behavioral obs. | | | | | | | | | | | | |
| Standing, min | 94.6 | 81.9 | 83.1 | 89.1 | 0.76 | 0.64 | 0.81 | 0.14 | 0.14 | 0.54 | 0.50 | 0.32 |
| Lying, min | 16.2 | 27.8 | 27.4 | 20.7 | 1.24 | 0.80 | 0.64 | 0.18 | <0.01 | 0.40 | 0.47 | 0.62 |
| Eating, min | 18.8 | 15.4 | 26.8 | 25.3 | 0.73 | 0.08 | 0.56 | 0.68 | 0.08 | 0.51 | 0.43 | 0.43 |
| Tail flicks, n | 630 | 309 | 654 | 210 | 4.4 | 0.97 | 0.07 | 0.88 | 0.05 | 0.43 | 0.13 | 0.64 |
| Foot stamping, n | 4.1 | 5.2 | 2.6 | 1.8 | 0.43 | 0.07 | 0.84 | 0.47 | 0.79 | 0.64 | 0.91 | 0.19 |
| Head turning, n | 5.4 | 4.7 | 4.8 | 5.8 | 0.22 | 0.76 | 0.90 | 0.35 | 0.06 | 0.51 | 0.57 | 0.69 |
| Lesion licking, n | 0.6 | 0.7 | 0.8 | 0.8 | 0.16 | 0.99 | 0.78 | 0.84 | 0.02 | 0.54 | 0.63 | 0.83 |
| Feeding behavior[4] | | | | | | | | | | | | |
| Dry matter intake, kg/d | 8.4 | 8.5 | 7.9 | 8.7 | 0.27 | 0.65 | 0.10 | 0.22 | <0.01 | 0.92 | 0.16 | 0.43 |
| Feeding time, min/d | 196 | 197 | 191 | 203 | 6.2 | 0.92 | 0.29 | 0.35 | <0.01 | 0.77 | 0.10 | 0.80 |
| Feeding rate, g/min | 44.8 | 45.4 | 44.1 | 45.3 | 1.21 | 0.74 | 0.47 | 0.79 | <0.01 | 0.43 | 0.93 | 0.22 |
| Meal frequency, meal/d | 10.7 | 11.4 | 11.3 | 11.5 | 0.37 | 0.33 | 0.09 | 0.56 | <0.01 | 0.69 | 0.76 | 0.47 |

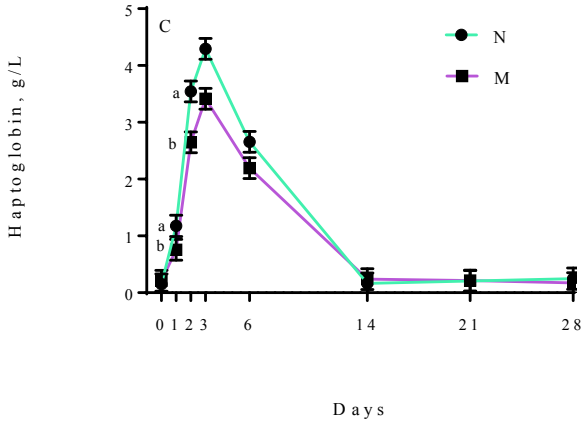
| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meal duration, min/meal | 20.3 | 18.1 | 18.8 | 19.2 | 0.88 | 0.80 | 0.34 | 0.08 | <0.01 | 0.74 | 0.62 | 0.91 |
| Meal size, kg/meal | 0.9 | 0.8 | 0.8 | 0.8 | 0.04 | 0.62 | 0.80 | 0.14 | <0.01 | 0.88 | 0.93 | 0.63 |

[1]The values represented correspond to non-transformed means; however , SEM and *P*-values correspond to ANOVA analysis using square root + 1 transformed data for standing and lying behavior.
[2]Stride length values represent the means of sampling immediately after and 30, 60, 120 and 240 min after castration.
[3] Stride length values represent the means of d 1, 2, 3, 6, 14, 21 and 28 after castration.

Figure 6.1. Least square means and SEM for (A) salivary cortisol (nmol/L) immediately before treatment (T0), 30, 60, 120 and 240 min after treatment and (B) serum amyloid-A (µg/mL) and (C) haptoglobin on d 0, 1, 2, 3, 6, 14, 21 and 28 after surgical castration surgical castration surgical castration of weaned Angus crossbred calves with (M) or without (N) a single s.c. meloxicam injection and with (L) or without (R) a lidocaine ring block.



# Chapter Seven: General Discussion and Conclusions

## 7.1 Summary of results

The aim of this thesis was to assess pain associated with castration in young (1 week, 2 month and 4 month old calves) and weaned beef calves (7 to 8 month old calves). The first part of the study focused on assessing acute pain, which was defined as pain occurring in the first 7 days after castration. Indicators of acute pain were evaluated to assess a) the effect of band and knife castration in 1 week, 2 month and 4 month old calves, b) the effect of a single dose of subcutaneous meloxicam administered immediately before band and knife castration in 1 week old calves, and c) the effect of a single dose of subcutaneous meloxicam administered immediately before knife castration or the combination of knife castration and branding in 2 month old calves. The second part of the study consisted of assessing pain in weaned beef calves up to 28 days after knife castration. Indicators of pain were evaluated to assess the effect of a) a single subcutaneous injection of meloxicam administered 6, 3 and 0 hours prior to knife castration and b) administration of lidocaine or meloxicam alone or in combination prior to knife castration in weaned calves.

In the first experiment, Chapter 2, the aim of the study was to identify which method (band or knife) of castration produces less acute pain at three relevant industry ages (1 week, 2 month and 4 months). Independent of age, band and knife castration caused acute pain, however a greater number of physiological and behavioural parameters showed differences among treatments in older calves (4 months of age) than in young calves (2 months of age and less). Behavioural but no physiological indicators were observed after knife and band castration in 1 week old calves.

In Chapter 3, the aim of the study was to assess acute pain indicators associated with different castration methods and if a single dose of subcutaneous meloxicam would reduce acute pain indicators associated with band and knife castration in 1 week old calves. Contrary to findings in Chapter 2, some physiological parameters and a greater number of behavioural parameters associated with pain were observed after knife and band castration in 1 week old calves. Findings observed in Chapter 3 include a greater number of pain related indicators in knife than band castrated calves, which included greater VAS scores, SAA concentrations, scrotal temperatures, frequencies of tail flicks and lateral lying durations. In addition, meloxicam was effective at reducing some physiological and behavioural parameters associated with pain such as substance P, SAA, WBC, tail flicks and lateral lying.

In Chapter 4 the aim of the study was to assess acute pain indicators associated with different painful procedures and if a single dose of subcutaneous meloxicam would reduce acute pain indicators associated with multiple (knife castration + branding) and single (knife castration) painful procedures in 2 month old calves. Overall, calves exposed to both multiple and single painful procedures had greater physiological (cortisol, SAA, WBC, SCT, and rectal temperature) and behavioural (VAS, vocalization, tail flicks, standing and lying) responses than control calves. Calves that experienced multiple painful procedures had greater cortisol concentrations, VAS scores, vocalizations and tail flick frequencies than those that received a single painful procedure, suggesting that the combination of procedures is more painful than a single procedure. In addition, meloxicam was able to reduce some of the physiological and behavioural pain related indicators such as haptoglobin, WBC, rectal temperature, walking, lying, head turning and tail flicking.

In Chapter 5, the aim of the study was to assess the effect of timing of meloxicam administration 6, 3 or 0 hours prior to knife castration in weaned calves. There was little evidence that administering meloxicam before castration had any additional benefit as seen by a lack of differences between treatments in the majority of physiological and behavioural parameters. Contrary to our hypothesis 0 h calves had fewer indicators of pain/distress compared to 3 and 6 h calves.

In Chapter 6, the aim of the study was to assess the effect of lidocaine or meloxicam alone or in combination on indicators of pain during and after knife castration in weaned calves. Lidocaine reduced physiological (SAA and WBC) and behavioural (VAS, head movement distance, movement in the chute and leg movement) indicators of pain, while meloxicam reduced only physiological (haptoglobin and WBC) indicators of pain. No lidocaine and meloxicam interactions were observed.

## 7.2 Thesis relevance

The overall objective of this thesis was to assess physiological and behavioural indicators associated with pain during and after band and knife castration and to assess pain mitigation strategies in beef calves at different ages. The findings of this thesis are relevant to the beef industry, veterinarians, the advancement of scientific knowledge and addressing societal concerns regarding pain management in farm animals.

*Beef Industry and veterinarians*. The studies conducted as part of this thesis help to bring awareness to producers that castration is a painful procedure independent of the age it is conducted. Indicators of pain post-castration were still observed in 1 week old calves. This highlights the importance of identifying effective pain mitigation strategies for both knife and band castration methods in beef calves at all ages.

*Science.* The studies conducted as part of this thesis help to further identify physiological and behavioural indicators that can be used to assess pain during and after castration in beef calves. This can be useful for futures studies assessing different castration methods and possibly different painful procedures and well as pain mitigation drugs. In addition, gaps of knowledge were identified in the literature such as the pharmacokinetics of subcutaneous meloxicam, efficacy of pre-emptive analgesia and therapeutic levels of meloxicam in cattle.

*Societal Concern.* The public is increasingly concerned about the conditions in which livestock are raised (Rollin, 2004) including routine painful husbandry procedures such as castration, dehorning and branding (Weary and Fraser, 2004). Retailers have been key in improving animal welfare standards on the farm and in the slaughter plants, as pressure from activists has led to the reinforcement of animal welfare standards such as the incorporation of on-farm and slaughter plant audits (Grandin, 2014). This thesis is relevant to the public as it is a science-based assessment of pain with the objective of identifying non-therapeutic and therapeutic strategies to mitigate pain.

## 7.3 Physiological and behavioural parameters

In this thesis physiological and behavioural parameters are generally described as pain indicators. These can be further categorized into indicators associated with stress (salivary and hair cortisol and CBC), inflammation (CBC, acute phase proteins and infrared thermography), and pain/discomfort (cortisol, substance P, VAS, ERM, standing and lying behaviour, stride length, tail flicks, foot stamps, head turning and lesion licking).

Pain is a subjective state (Fink, 2000) and currently there is no single indicator available to the scientific community to determine its presence or severity in animals. The majority of previous studies assessing castration pain and mitigation strategies have typically assessed only a

few physiological pain indicators with a greater number of behavioural parameters, or vice versa, while studies assessing a combination of several physiological and behavioural outcomes are rare (Stafford et al., 2002; Bretschneider, 2005; Coetzee, 2011). A total of 6 to 8 physiological and 16 to 19 behavioural parameters were used to assess the pain of castration as part of the five studies conducted within this thesis. The large number of indicators used to assess pain within each experiment was to provide a more complete assessment of pain to gain a better understanding of what the calves might be experiencing during and after castration. Collection of both behavioural and physiological samples is challenging and has the disadvantage that one or the other will be compromised when timing of expected responses overlap. For example, changes in salivary cortisol were observed 30 and 240 minutes after castration, which is the same time period when the greatest number of pain related behaviours are observed. A way to solve this problem would be to focus only on behavioural or physiology parameters, however when assessing pain, similar to when assessing animal welfare, it is necessary to evaluate a range of measures (Broom and Johnson, 1993).

Parameters that showed differences in the majority of experiments include: salivary cortisol, acute phase proteins, scrotal temperature, tail flicks, ERM and VAS. Furthermore, tail flicks, lateral and ventral lying, WBC, scrotal and rectal temperature showed differences after procedures (castration methods and multiple and single painful procedures) and medication (meloxicam or lidocaine). Differences in some of these parameters were very small and may lack biological significance, however until specific cut-off's for each particular parameter are established, biological significance is difficult to determine. A difference of 1°C might seem biologically insignificant but it has been previously reported to affect performance in cattle suffering from heat stress (Silanikove, 2000).

## 7.4 Best age and method of castration

Based on findings from Chapter 2, independent of age, band and knife castration caused acute pain, however a greater number of physiological and behavioural parameters were observed in older calves (4 months of age) than in young calves (2 months of age and less) which is in agreement with previous findings (Robertson et al., 1994; Bretschneider, 2005; Brown et al., 2015). These findings are also in agreement with a recommendation by the OIE Terrestrial Health Code (OIE, 2017) stating 'when practical animals should be castrated younger than 3 months of age'. In addition, it is a requirement within the Canadian Beef Codes of Practice (NFACC, 2013) that calves be castrated 'as young as possible'.

The castration method which presented the least indicators of acute pain for each age of castration was band castration in 1 week and 2 month old calves. It is difficult to decide on a castration method for knife and band castration in 4 month old calves because both methods generated changes in a similar number of physiological and behavioural indicators. Band castration is a common castration method as it is relatively easy to perform especially in young calves, however possible side effects include tetanus and iatrogenic cryptorchidism when the band is not properly placed above both testicles (Weaver et al., 2008), therefore it is very important that all castration methods be performed by competent personnel (NFACC, 2013).

Overall, a non-therapeutic way to reduce acute pain associated with castration is to band castrate 1 week old calves. The assessment of castration pain lasting longer than 7 days showed swelling and inflammation beyond 7 days after knife and band castration in 1 week, 2 month and 4 month old calves (Marti et al., 2017). Therefore, caution should be taken when interpreting results for young calves as physiological and behavioural parameters were only evaluated for

acute pain and it is necessary to take into consideration chronic pain before more concrete conclusions and recommendations can be made.

## 7.5 Effect of meloxicam on pain indicators

Painful husbandry procedures such as ear tagging, and branding are commonly done in combination with castration and all of these procedures are commonly done without pain mitigation (Coetzee et al., 2010; Moggy et al., 2017). A survey done in Western Canada reported that 90 % of the beef calves are castrated at 3 months of age or younger however only 10% of the respondents used pain control (Moggy et al., 2017). In Canada, pain mitigation is currently a requirement in calves older than 6 months of age (NFACC, 2013). However, young calves could also benefit from analgesia as both physiological and behavioural changes indicative of pain/discomfort have been previously reported in 1 week old calves after castration (Robertson et al., 1994; Molony et al., 1995; Brown et al., 2015).

The results obtained from three different studies in which the effect of meloxicam was assessed in 1 week, 2 month and 7 to 8 month old calves showed that meloxicam was effective at reducing pain associated with castration. Findings from Chapter 3 showed that 1 week old calves that received meloxicam had lower substance P concentrations, WBC counts, lateral lying duration and tail flick frequencies than un-medicated calves. Results from Chapter 4 showed lower haptoglobin concentrations, scrotal and rectal temperature, WBC counts, tail flicking and head turning frequencies, walking duration and increased RBC counts and lying duration in calves that received meloxicam than un-medicated 2 month old calves. While, Chapter 6 showed that 7 to 8 month old calves treated with meloxicam had lower salivary cortisol, haptoglobin concentrations and WBC counts.

Therefore, meloxicam was effective at mitigating pain associated with band and knife castration in 1 week old calves, knife castration and the combination of knife castration and branding in 2 month old calves, and knife castration in 7 to 8 month old calves. These findings suggest that subcutaneous meloxicam provides some pain relief to both knife and band castrated calves at varying ages.

## 7.6 Improvement of pain mitigation

Meloxicam is a NSAID which works by inhibiting COX-2 enzymes which convert arachidonic acid into prostaglandins (Ricciotti and FitzGerald, 2011). Meloxicam is an attractive analgesic and anti-inflammatory drug due to its long half-life in comparison to other NSAIDS like ketoprofen and flunixin meglumine and ease of administration (s.c). In Canada, oral meloxicam is labelled for pain mitigation associated with band and knife castration, while injectable meloxicam is labelled to mitigate pain associated with dehorning, but not for castration. Oral meloxicam has the advantage of a longer duration of action than subcutaneous meloxicam, as it is absorbed and excreted from the body at a slower rate. Although the duration of action of subcutaneous meloxicam is shorter than oral meloxicam, a faster absorption could be beneficial when administering analgesia immediately before castration.

Post-surgical pain hypersensitivity is a consequence of central sensitization caused by tissue damage, which can be reduced with the administration of pre-emptive analgesia (Woolf and Chong, 1993). We wouldn't expect pre-emptive analgesia to have an effect on operative pain, as NSAIDS do not have an effect on high intensity stimuli (Malmberg and Yaksh, 1991), but we would expect a reduction on central and peripheral sensitization in the post-operative period. If pre-emptive analgesia is effective in beef cattle, this could be a strategy incorporated into pain mitigation protocols to reduce the pain associated with castration. Results from Chapter

5 did not show differences in pain indicators after castration when meloxicam was administered 6, 3 or 0 hours prior to knife castration in 7 to 8 month old calves. Greater substance P concentrations and movement in the chute in the 6 and 3 h calves were likely due to handling the animals twice, once to receive the medication and a second time for castration, which could have caused a greater stress response than the 0 h group that was only handled once. Lower scrotal temperatures observed in 3 and 0 h calves could have been a result of increased exposure to the drug in comparison with 6 h calves. Similar findings were reported by Allen et al. (2013) who observed lower $PGE_2$ concentrations when oral meloxicam was administered immediately prior to dehorning than 12 hours before. There is a need for a pharmacokinetic study of subcutaneous meloxicam as well as a study to identify therapeutic concentrations of meloxicam in cattle.

The combination of an anesthetic and an analgesic has been reported to be more effective at mitigating the cortisol response associated with castration than either drug on its own (Coetzee, 2011). Although in Chapter 6 the combination of lidocaine and meloxicam did not show a statistical interaction, further studies should assess this again as meloxicam and lidocaine had an effect on different indicators and at different time points which can be more effective at reducing pain indicators when the drugs are used in combination than either drug on its own.

## 7.7 Limitations of pain assessment

As previously mentioned we saw differences in the majority of physiological and behavioural parameters collected when taking into consideration all of the experiments, but this wasn't the case within experiments. Reasons why certain pain indicators showed differences while others didn't could be due to a small sample size, high individual variability, inadequate sampling time, or that the indicators collected were not adequate to assess pain or nociception. Reasons why differences in indicators vary across experiments is likely due to differences in

castration age, castration methods, combination of painful procedures, and pain mitigation drugs. For example, in Chapter 2 comparison across ages could not be done because calves were castrated at different time points and were handled differently at each age. A possible solution would have been to handle all ages in a similar way; however it was important that the handling of the calves (during castration) at each age group, reflect how castration in calves is currently managed by the majority of producers so as to make the findings as relevant to the beef industry as possible. One week old calves were castrated while lying on a straw bale, 2 month old calves were castrated on a tip table, and 4 month old calves were castrated while standing in a squeeze chute. Within each age group all calves were handled in a similar way consequently reducing the effect of handling stress. Comparing physiological and behavioural parameters across ages could have some limitations as physiological and behavioural responses can vary across ages (Mellor et al., 2000).

A potential bias exists for VAS scores in Chapters 2, 3, 4 and 5, as observers were not blind to treatments due to the experimental conditions. However, in all of the chapters, observers were blind to treatments for behavioural scoring of duration and frequency behaviours and stride length assessment.

## 7.8 Future research

There are many areas for future research in assessing and mitigating pain in beef cattle which are discussed below. The sampling time in which physiological and behavioural parameters are collected is crucial in the detection of differences between treatments. Therefore, improvement of sampling times for each physiological and behavioural parameter should be studied. Sampling times of previous studies can be used as a reference for when to expect differences, however it is important to further assess if increasing the number of samples

collected or adjusting the time of sample collection could be more effective at detecting changes between treatments. This is of great importance because although we observed differences in physiological and behavioural parameters at the expected time points, we also observed differences at unexpected time points. Furthermore, lack of differences in physiological or behavioural parameters observed across studies could be due to incorrect sampling times.

Identification of physiological and behavioural parameters associated with pain should also be identified for different ages. As observed in this thesis, differences in parameters vary across ages but are more evident in older ages. Research assessing the indicators which are relevant for each age is paramount for studies evaluating pain in beef cattle with limited resources.

Future research should take into account both acute and chronic pain, in order to identify the period of time in which animals are likely to be in pain after castration or other painful husbandry procedures. This will also include identification of physiological and behavioural parameters specific to acute pain, like cortisol and VAS, and specific to chronic pain such as inflammation and lesion scores, or both such as behaviour. Evaluation of chronic pain is important in order to make accurate recommendations, as different castration methods have been shown to cause inflammation for different periods of time after the procedure (Molony et al., 1995; Stafford et al., 2002).

Pharmacokinetic information of analgesic or anaesthetic drugs in combination with the therapeutic concentrations for each drug can be helpful in recognizing if there is a need for one or multiple analgesic treatments. It is important to reduce pain for the period of time when animals are expected to be in pain, which can vary across ages and methods. In addition, different routes of administration and different dosages should also be evaluated to pinpoint the

most effective drug and route of administration. This could be done by comparing different drugs and routes of administration to positive and/or negative control groups.

If implementing analgesic protocols is not feasible, alternatives to castration should be studied such as the practicality of implementing immuno-castration for older calves or the adoption of management practices for intact bulls.

## 7.9 Conclusion

Band castration caused fewer indicators of pain compared to knife castrated animals in 1 week and 2 month old calves, while similar numbers of indicators were observed in 4 month old calves. Knife castrated calves had a greater number of pain indicators than banded calves and a single subcutaneous dose of meloxicam was effective at mitigating some physiological and some behavioural parameters after knife and band castration in 1 week old calves. Multiple painful procedures produced a greater response in indicators associated with pain and a single subcutaneous dose of meloxicam was effective at mitigating some physiological and some behavioural indictors in 2 month old calves. There was no added benefit observed of administering meloxicam 6 and 3 hours prior to castration in weaned calves and lidocaine was effective at mitigating some physiological and some behavioural indicators of pain while meloxicam was effective at mitigating some physiological parameters, however there was no lidocaine and meloxicam interaction.

Based on these findings it is recommended that producers castrate calves as young as possible, administer meloxicam regardless of age to reduce pain and inflammation associated with castration, and we observed that lidocaine is effective in blocking procedural pain.

This thesis is the first to utilize numerous physiological and behavioural indicators to assess the effect of knife and band castration in young beef calves and to assess the effect of

subcutaneous meloxicam in pre-emptive analgesia, as a multimodal approach in combination with lidocaine, and as an analgesic and anti-inflammatory to mitigate pain associated with castration.

# References


Abram, S. E., and T. L. Yaksh. 1993. Morphine, but not inhalation anesthesia, blocks post-injury facilitation. the role of preemptive suppression of afferent transmission. Anesthesiology. 78(4): 713-721.

Allen, K.A., J.F. Coetzee, L.N. Edwards-Callaway, H. Glynn, J. Dockweiler, B. KuKanich, H. Lin, C. Wang, E. Fraccaro, M. Jones and L. Bergamasco. 2013. The effect of timing of oral meloxicam administration on physiological responses in calves after cautery dehorning with local anesthesia. J. Dairy Sci. 96(8): 5194-5205. doi: 10.3168/jds.2012-6251

Alsemgeest, S.P.M., H.C. Kalsbeek, T. Wensing, J.P. Koeman, A.M. Van Ederen, and E. Gruys. 1994. Concentrations of serum amyloid-a (SAA) and haptoglobin (HP) as parameters of inflammatory diseases in cattle. Vet. Q. 16(1): 21-23. doi: 10.1080/01652176.1994.9694410

Baldridge, S. L., J. F. Coetzee, S. S. Dritz, J. B. Reinbold, R. Gehring, J. Havel, and B. Kukanich. 2011. Pharmacokinetics and physiologic effects of intramuscularly administered xylazine hydrochloride-ketamine hydrochloride-butorphanol tartrate alone or in combination with orally administered sodium salicylate on biomarkers of pain in holstein calves following castration and dehorning. Am. J. Vet. Res. 72(10): 1305-1317. doi: 10.2460/ajvr.72.10.1305

Ballou, M. A., M. A. Sutherland, T. A. Brooks, L. E. Hulbert, B. L. Davis, and C. J. Cobb. 2013. Administration of anesthetic and analgesic prevent the suppression of many leukocyte responses following surgical castration and physical dehorning. Vet. Immunol. Immunopathol. 151(3-4): 285-293. doi: 10.1016/j.vetimm.2012.11.018

Baumann, H., K. R. Prowse, S. Marinković, K. A. Won, and G. P. Jahreis. 1989. Stimulation of hepatic acute phase response by cytokines and glucocorticoids. Ann N Y Acad Sci 557(1): 280-296. doi: 10.1111/j.1749-6632.1989.tb24021.x

Besson, J. M., and A. Chaouch. 1987. Peripheral and spinal mechanisms of nociception. Physiol. Rev. 67(1): 67-186. doi: 10.1152/physrev.1987.67.1.67

Brambell, F. W. R. 1965. Report of the technical committee to enquire into the welfare of animals kept under intensive livestock husbandry systems, The Brambell Report. December, London.

Bretschneider, G. 2005. Effects of age and method of castration on performance and stress response of beef male cattle: A review. Livest. Prod. Sci. 97(2): 89-100. doi: 10.1016/j.livprodsci.2005.04.006

Brondani, J. T., K. R. Mama, S. P. Luna, B. D. Wright, S. Niyom, J. Ambrosio, P. R. Vogel, and C. R. Padovani. 2013. Validation of the english version of the UNESP-Botucatu

multidimensional composite pain scale for assessing postoperative pain in cats. BMC Vet. Res. 9(1): 143. doi: 10.1186/1746-6148-9-143

Broom, D. M. 1991. Animal welfare: Concepts and measurement. J. Anim. Sci. 69(10): 4167-4175. doi: 10.2527/1991.69104167x

Broom, D. M. 1986. Indicators of poor welfare. Br. Vet. J. 142(6): 524-526.

Broom, D. M., and K. G. Johnson. 1993. Assessing Welfare: Short-Term Responses. In Stress and Animal Welfare. Springer's, Dordrecht. p. 87-110

Brown, A. C., J. G. Powell, E. B. Kegley, M. S. Gadberry, J. L. Reynolds, H. D. Hughes, J. A. Carroll, N. C. Burdick Sanchez, Y. V. Thaxton, E. A. Backes, and J. T. Richeson. 2015. Effect of castration timing and oral meloxicam administration on growth performance, inflammation, behavior, and carcass quality of beef calves. J. Anim. Sci. 93(5): 2460-2470. doi: 10.2527/jas.2014-8695

Burian, M., and G. Geisslinger. 2005. COX-dependent mechanisms involved in the antinociceptive action of NSAIDs at central and peripheral sites. Pharmacol. Ther. 107(2): 139-154. doi: 10.1016/j.pharmthera.2005.02.004

Canada Council of Animal Care. 2009. CCAC guidelines on: Animal use protocol review (2009). Can. Counc. Anim. Care, Ottawa, ON. URL: https://www.ccac.ca/Documents/Standards/Guidelines/Protocol_Review.pdf

Ceciliani, F., J. J. Ceron, P. D. Eckersall, and H. Sauerwein. 2012. Acute phase proteins in ruminants. J Proteomics. 75(14): 4207-4231. doi: 10.1016/j.jprot.2012.04.004

Coetzee, J. F. 2011. A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: Practical implications for cattle production within the United States. Appl. Anim. Behav. Sci. 135(3): 192-213. doi: 10.1016/j.applanim.2011.10.016

Coetzee, J. F., L. N. Edwards, R. A. Mosher, N. M. Bello, A. M. O'Connor, B. Wang, B. Kukanich, and D. A. Blasi. 2012. Effect of oral meloxicam on health and performance of beef steers relative to bulls castrated on arrival at the feedlot. J. Anim. Sci. 90(3): 1026-1039. doi: 10.2527/jas.2011-4068

Coetzee, J. F., B. KuKanich, R. A. Mosher, and P. S. Allen. 2009. Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. Vet Ther. 10(4): E1-E8.

Coetzee, J. F., B. V. Lubbers, S. E. Toerber, R. Gehring, D. U. Thomson, B. J. White, and M. D. Apley. 2008. Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. Am. J. Vet. Res. 69(6): 751-762. doi: 10.2460/ajvr.69.6.751

Coetzee, J. F., R. A. Mosher, B. KuKanich, R. Gehring, B. Robert, J. B. Reinbold, and B. J. White. 2012. Pharmacokinetics and effect of intravenous meloxicam in weaned holstein calves following scoop dehorning without local anesthesia. BMC Vet. Res. 8(1): 153. doi: 10.1186/1746-6148-8-153

Coetzee, J. F., A. L. Nutsch, L. A. Barbur, and R. M. Bradburn. 2010. A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. BMC Vet. Res. 6(1): 12. doi: 10.1186/1746-6148-6-12

Criminal Code. 2017. (R.S.C., 1985, c. C-46). http://laws-lois.justice.gc.ca/eng/acts/C-46/ (Accessed 19 February 2018.)

Currah, J. M., S. H. Hendrick, and J. M. Stookey. 2009. The behavioral assessment and alleviation of pain associated with castration in beef calves treated with flunixin meglumine and caudal lidocaine epidural anesthesia with epinephrine. Can. Vet. J. 50(4): 375-382.

de Oliveira, F. A., S. P. L. Luna, J. B. do Amaral, K. A. Rodrigues, A. C. Sant'Anna, M. Daolio, and J. T. Brondani. 2014. Validation of the UNESP-botucatu unidimensional composite pain scale for assessing postoperative pain in cattle. BMC Vet. Res. 10(1): 1. doi: 10.1186/s12917-014-0200-0

DeVane, C. L. 2001. Substance P: A new era, a new role. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 21(9): 1061-1069. doi: 10.1592/phco.21.13.1061.34612

Dinniss, A. S., K. J. Stafford, D. J. Mellor, R. Bruce, and R. Ward. 1999. The behaviour pattern of lambs after castration using a rubber ring and/or castrating clamp with or without local anaesthetic. N. Z. Vet. J. 47: 198-203. doi: 10.1080/00480169.1999.36143

Dockweiler, J. C., J. F. Coetzee, L. N. Edwards-Callaway, N. M. Bello, H. D. Glynn, K. A. Allen, M. E. Theurer, M. L. Jones, K. A. Miller, and L. Bergamasco. 2013. Effect of castration method on neurohormonal and electroencephalographic stress indicators in holstein calves of different ages. J. Dairy Sci. 96(7): 4340-4354. doi: 10.3168/jds.2012-6274

Dowling, J. 1982. Autonomic indices and reactive pain reports on the McGill pain questionnaire. Pain. 14(4): 387-392. doi: 10.1016/0304-3959(82)90146-4

Duncan, I. J. H. 2005. Science-based assessment of animal welfare: Farm animals. Rev. –Off. Int. Epizoot. 24(2): 483-492.

Eadie, E. N. 2012. Understanding Animal Welfare: An Integrated Approach. Springer's Science and Business Media. p. 22.

Earley, B., and M. A. Crowe. 2002. Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. J. Anim. Sci. 80(4): 1044-1052. doi: 10.2527/2002.8041044x

Egger, C. M., L. Love, and T. Doherty. 2013. Pain management in veterinary practice. John Wiley & Sons. p. 85-91.

Fell, L. R., R. Wells, and D. A. Shutt. 1986. Stress in calves castrated surgically or by the application of rubber rings. Aust. Vet. J. 63(1): 16-18. doi: 10.1111/j.1751-0813.1986.tb02864.x

Fink, R. 2000. Pain assessment: The cornerstone to optimal pain management. Proc (Bay Univ Med Cent). 13(3): 236-239. doi: 10.1080/08998280.2000.11927681

Fisher, A. D., M. A. Crowe, M. E. Alonso De La Varga, and W. J. Enright. 1996. Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. J. Anim. Sci. 74(10): 2336-2343. doi: 10.2527/1996.74102336x

Fisher, A. D., T. W. Knight, G. P. Cosgrove, A. F. Death, C. B. Anderson, D. M. Duganzich, and L. R. Matthews. 2001. Effects of surgical or banding castration on stress responses and behaviour of bulls. Aust. Vet. J. 79(4): 279-284. doi: 10.1111/j.1751-0813.2001.tb11981.x

Fraser, D., D. M. Weary, E. A. Pajor, and B. N. Milligan. 1997. A scientific conception of animal welfare that reflects ethical concerns. Animal welfare. 6:187-205.

Gånheim, C., C. Hulten, U. Carlsson, H. Kindahl, R. Niskanen, and K. P. Waller. 2003. The acute phase response in calves experimentally infected with bovine viral diarrhoea virus and/or mannheimia haemolytica. Zoonoses Public Health. 50(4): 183-190. doi: 10.1046/j.1439-0450.2003.00658.x

Gebhart, G. F., and T. J. Ness. 1991. Central mechanisms of visceral pain. Can. J. Physiol. Pharmacol. 69(5): 627-634. doi: 10.1139/y91-093

Gleerup, K. B., B. Forkman, C. Lindegaard, and P. H. Andersen. 2015a. An equine pain face. Vet Anaesth Analg. 42(1): 103-114. doi: 10.1111/vaa.12212

Gleerup, K. B., P. H. Andersen, L. Munksgaard, and B. Forkman. 2015b. Pain evaluation in dairy cattle. Appl. Anim. Behav. Sci. 171: 25-32. doi: 10.1016/j.applanim.2015.08.023

González, L. A., K. S. Schwartzkopf-Genswein, N. A. Caulkett, E. D. Janzen, T. A. McAllister, E. Fierheller, A. L. Schaefer, D. B. Haley, J. M. Stookey, and S. Hendrick. 2010. Pain mitigation after band castration of beef calves and its effects on performance, behavior, and salivary cortisol. J. Anim. Sci. 88(2): 802-810. doi: 10.2527/jas.2008-1752

Grandin, T. 2014. Animal welfare and society concerns finding the missing link. Meat Sci. 98(3): 461-469. doi: 10.1016/j.meatsci.2014.05.011

Grauw, J. C. d., C. H. A. Lest, P. Brama, B. P. B. Rambags, and P. R. v. Weeren. 2009. In vivo effects of meloxicam on inflammatory mediators, MMP activity and cartilage biomarkers in

equine joints with acute synovitis. Equine Vet. J. 41(7): 693-699. doi: 10.2746/042516409X436286

Ha, H., and C. N. Liu. 1966. Organization of the spino-cervico-thalamic system. J. Comp. Neurol. 127(4): 445-469. doi: 10.1002/cne.901270403

Handwerker, H. O., S. Kilo, and P. W. Reeh. 1991. Unresponsive afferent nerve fibres in the sural nerve of the rat. J. Physiol. 435(1): 229-242. doi: 10.1113/jphysiol.1991.sp018507

Harrison, R. 1964. Animal machines: The new factory farming industry. Stuart.

Health of Animals Act. 2015. (S.C. 1990, c. 21). http://laws-lois.justice.gc.ca/eng/acts/H-3.3/ (Accessed 19 February 2018.)

Horadagoda, A., P. D. Eckersall, J. C. Hodgson, H. A. Gibbs, and G. M. Moon. 1994. Immediate responses in serum TNFα and acute phase protein concentrations to infection with pasteurella haemolytica A1 in calves. Res. Vet. Sci. 57(1): 129-132. doi: 10.1016/0034-5288(94)90094-9

Horadagoda, N. U., K. M. Knox, H. A. Gibbs, S. W. Reid, A. Horadagoda, S. E. Edwards, and P. D. Eckersall. 1999. Acute phase proteins in cattle: Discrimination between acute and chronic inflammation. Vet. Rec. 144(16): 437-441. doi: 10.1136/vr.144.16.437

Hughes, H. D., J. A. Carroll, N. C. B. Sanchez, and J. T. Richeson. 2014. Natural variations in the stress and acute phase responses of cattle. Innate Immunity. 20(8): 888-896. doi: 10.1177/1753425913508993

Jacobs, J. A., J. C. Miller, E. A. Sauter, A. D. Howes, A. A. Araji, T. L. Gregory, and C. E. Hurst. 1977. Bulls steers. II. palatability and retail acceptance. J. Anim. Sci. 45(4): 699-702. doi: 10.2527/jas1977.454699x

Jacobsen, S., P. H. Andersen, T. Toelboell, and P. M. H. Heegaard. 2004. Dose dependency and individual variability of the lipopolysaccharide-induced bovine acute phase protein response. J. Dairy Sci. 87(10): 3330-3339. doi: 10.3168/jds.S0022-0302(04)73469-4

Jones, M. L., and R. W. Allison. 2007. Evaluation of the ruminant complete blood cell count. Vet. Clin. North Am. Food Anim. Pract. 23(3): 377-402. doi: 10.1016/j.cvfa.2007.07.002

Karreman, H. J., G. H. Wentink, and T. Wensing. 2000. Using serum amyloid a to screen dairy cows for sub-clinical inflammation. Vet. Q. 22(3): 175-178. doi: 10.1080/01652176.2000.9695051

Katz, J. 1995. Pre-emptive analgesia: Evidence, current status and future directions. Eur J Anaesthesiol Suppl. 10, 8-13.

Kiley-Worthington, M. 1976. The tail movements of ungulates, canids and felids with particular reference to their causation and function as displays. Behaviour. 56(1): 69-114. doi: 10.1163/156853976X00307

Kennard, M. A. 1954. The course of ascending fibers in the spinal cord of the cat essential to the recognition of painful stimuli. J. Comp. Neurol. 100(3): 511-524. doi: 10.1002/cne.901000304

Kiley-Worthington, M. 1989. Ecological, ethological, and ethically sound environments for animals: Toward symbiosis. J Agric Ethics. 2(4): 323-347.

Lahav, M., M. Levite, L. Bassani, A. Lang, H. Fidder, R. Tal, S. Bar-Meir, L. Mayer, and Y. Chowers. 2002. Lidocaine inhibits secretion of IL-8 and IL-1β and stimulates secretion of IL-1 receptor antagonist by epithelial cells. Clin. & Exp. Immunol. 127(2): 226-233. doi: 10.1046/j.1365-2249.2002.01747.x

Lambertz, C., A. Farke-Röver, E. Moors, and M. Gauly. 2015. Effects of castration and weaning conducted concurrently or consecutively on behaviour, blood traits and performance in beef calves. Animal. 9(1): 122-129. doi: 10.1017/S1751731114002080

Lambertz, C., A. Farke-Röver, E. Moors, and M. Gauly. 2014. Comparison of the effects of weaning and castration when conducted separately or in combination on the behaviour of crossbred beef cattle. Appl. Anim. Behav. Sci. 161: 28-33. doi: 10.1016/j.applanim.2014.09.003

Lees, P., M. F. Landoni, J. Giraudel, and P. L. Toutain. 2004. Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest. J. Vet. Pharmacol. Ther. 27(6): 479-490. doi: 10.1111/j.1365-2885.2004.00617.x

Lehmann, H. S., G. C. Musk, M. Laurence, T. H. Hyndman, J. Tuke, T. Collins, K. B. Gleerup, and C. B. Johnson. 2017. Mitigation of electroencephalographic and cardiovascular responses to castration in bos indicus bulls following the administration of either lidocaine or meloxicam. Vet Anaesth Analg. 44(6): 1341-1352. doi: 10.1016/j.vaa.2017.04.009

Lomax, S., and P. A. Windsor. 2013. Topical anesthesia mitigates the pain of castration in beef calves. J. Anim. Sci. 91(10): 4945-4952. doi: 10.2527/jas.2012-5984

Ludington, E., and F. Dexter. 1998. Statistical analysis of total labor pain using the visual analog scale and application to studies of analgesic effectiveness during childbirth. Anesth. Analg. 87(3): 723-727. doi: 10.1213/00000539-199809000-00045

Lupoli, B., B. Johansson, K. Uvnäs-Moberg, and K. Svennersten-Sjuanja. 2001. Effect of suckling on the release of oxytocin, prolactin, cortisol, gastrin, cholecystokinin, somatostatin and insulin in dairy cows and their calves. J. Dairy Res. 68(2): 175-187. doi: 10.1017/S0022029901004721

Malmberg, A. B., and T. L. Yaksh. 1991. Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. Science. 257(5074):1276-1279. doi: 10.1126/science.1381521

Marti, S., D. M. Meléndez, E. A. Pajor, D. Moya, C. E. M. Heuston, D. Gellatly, E. D. Janzen, and K. S. Schwartzkopf-Genswein. 2017. Effect of band and knife castration of beef calves on welfare indicators of pain at three relevant industry ages: II. chronic pain. J. Anim. Sci. 95(10): 4367-4380. doi: 10.2527/jas.2017.1763

Martin, P., and P. Bateson. 2007. Recording Methods. In Measuring Behaviour: An Introductory Guide. Third ed. Cambridge University Press. p. 48-54.

McGlone, J. J. 1993. What is animal welfare? J Agric Environ Ethics, 6(2), 26-36.

McLennan, K. M., C. J. Rebelo, M. J. Corke, M. A. Holmes, M. C. Leach, and F. Constantino-Casas. 2016. Development of a facial expression scale using footrot and mastitis as models of pain in sheep. Appl. Anim. Behav. Sci. 176: 19-26. doi: doi.org/10.1016/j.applanim.2016.01.007

Meat Inspection Act. 2015. (R.S.C., 1985, c. 25 (1st Supp.)). http://laws-lois.justice.gc.ca/eng/acts/M-3.2/ (Accessed 19 February 2018.)

Meléndez, D. M., S. Marti, E. A. Pajor, D. Moya, D. Gellatly, E. D. Janzen, and K. S. Schwartzkopf-Genswein. 2018. Effect of a single dose of meloxicam prior to band or knife castration in 1 week old beef calves: Acute pain. J. Anim. Sci. *In press*.

Meléndez, D. M., S. Marti, E. A. Pajor, D. Moya, D. Gellatly, E. D. Janzen, and K. S. Schwartzkopf-Genswein. 2017a. Effect of timing of subcutaneous meloxicam administration on indicators of pain after knife castration of weaned calves. J. Anim. Sci. 95(12): 5218-5229. doi: 10.2527/jas2017.1978

Meléndez, D. M., S. Marti, E. A. Pajor, D. Moya, C. E. M. Heuston, D. Gellatly, E. D. Janzen, and K. S. Schwartzkopf-Genswein. 2017b. Effect of band and knife castration of beef calves on welfare indicators of pain at three relevant industry ages: I. acute pain. J. Anim. Sci. 95 (10): 4352-4366. doi: 10.2527/jas.2017.1762

Mellor, D. J. 2016. Updating animal welfare thinking: Moving beyond the “Five freedoms” towards “a life worth living”. Animals. 6(3): 21. doi: 10.3390/ani6030021

Mellor, D. J., C. J. Cook, and K. J. Stafford. 2000. Quantifying some responses to pain as a stressor. Wallingford, UK: CABI Publishing. p. 171-198.

Mellor, D. J., V. Molony, and I. S. Robertson. 1991. Effects of castration on behaviour and plasma cortisol concentrations in young lambs, kids and calves. Res. Vet. Sci. 51(2): 149-154. doi: 10.1016/0034-5288(91)90005-9

Mellor, D. J., and C. S.W. Reid. 1994. Concepts of animal well-being and predicting the impact of procedures on experimental animals. Improving the Well-being of Animals in the Research Environment. p. 3-18.

Mellor, D. J., and K. J. Stafford. 2000. Acute castration and/or tailing distress and its alleviation in lambs. N. Z. Vet. J. 48(2): 33-43. doi: 10.1080/00480169.2000.36156

Mercadante, S., A. Casuccio, A. Agnello, S. Pumo, J. Kargar, and S. Garofalo. 1999. Analgesic effects of nonsteroidal anti-inflammatory drugs in cancer pain due to somatic or visceral mechanisms. J. Pain Symptom Manage. 17(5): 351-356. doi: 10.1016/S0885-3924(98)00141-9

Merskey, H. 1986. Pain terms: a current list with definitions and notes on usage. Pain. 3: S215-S221.

Milne, R. J., R. D. Foreman, G. J. Giesler Jr, and W. D. Willis. 1981. Convergence of cutaneous and pelvic visceral nociceptive inputs onto primate spinothalamic neurons. Pain. 11(2): 163-183. doi: 10.1016/0304-3959(81)90003-8

Moggy, M. A., E. A. Pajor, W. E. Thurston, S. Parker, A. M. Greter, K. S. Schwartzkopf-Genswein, J. R. Campbell, and M. C. Windeyer. 2017. Management practices associated with pain in cattle on western canadian cow–calf operations: A mixed methods study. J. Anim. Sci. 95(2): 958-969. doi: 10.2527/jas.2016.0949

Molony, V., and J. E. Kent. 1997. Assessment of acute pain in farm animals using behavioral and physiological measurements. J. Anim. Sci. 75(1): 266-272. doi: 10.2527/1997.751266x

Molony, V., J. Kent, and I. S. Robertson. 1995. Assessment of acute and chronic pain after different methods of castration of calves. Appl. Anim. Behav. Sci. 46(1): 33-48. doi: 10.1016/0168-1591(95)00635-4

Molony, V., J. E. Kent, and I. S. Robertson. 1993. Behavioural responses of lambs of three ages in the first three hours after three methods of castration and tail docking. Res. Vet. Sci. 55(2): 236-245. doi: 10.1016/0034-5288(93)90087-V

Mosher, R. A., C. Wang, P. S. Allen, and J. F. Coetzee. 2013. Comparative effects of castration and dehorning in series or concurrent castration and dehorning procedures on stress responses and production in holstein calves. J. Anim. Sci. 91(9): 4133-4145. doi: 10.2527/jas.2012-6007

Moya, D., K. S. Schwartzkopf-Genswein, and D. M. Veira. 2013. Standardization of a non-invasive methodology to measure cortisol in hair of beef cattle. Livest Sci. 158(1): 138-144. doi: 10.1016/j.livsci.2013.10.007

Moya, D., L. A. González, E. Janzen, N. A. Caulkett, E. Fireheller, and K. S. Schwartzkopf-Genswein. 2014. Effects of castration method and frequency of intramuscular injections of

ketoprofen on behavioral and physiological indicators of pain in beef cattle. J. Anim. Sci. 92(4): 1686-1697. doi: 10.2527/jas.2013-7298

Muir III, W. W., and C. J. Woolf. 2001. Mechanisms of pain and their therapeutic implications. J. Am. Vet. Med. Assoc. 219(10): 1346-1356. doi: 10.2460/javma.2001.219.1346

Murata, H., N. Shimada, and M. Yoshioka. 2004. Current research on acute phase proteins in veterinary diagnosis: An overview. Vet. J. 168(1): 28-40. doi: 10.1016/S1090-0233(03)00119-9

National Farm Animal Care Council. 2013. Code of Practice for the Care and Handling of Beef Cattle. (Accessed 26 November 2016.)

Nicol, G. D., D. K. Klingberg, and M. R. Vasko. 1992. Prostaglandin E2 increases calcium conductance and stimulates release of substance P in avian sensory neurons. J. Neurosci. 12(5): 1917-1927.

NRC. 2016. Nutrient requirements of beef cattle. 8th rev. ed. Natl. Acad. Press, Washington, DC.

Ochroch, E. A., I. A. Mardini, and A. Gottschalk. 2003. What is the role of NSAIDs in pre-emptive analgesia? Drugs. 63(24): 2709-2723. doi: 10.2165/00003495-200363240-00002

OIE. 2017. Article 7.9.5. Recommendations for painful animal procedures, in chapter 7.9 animal welfare and beef cattle production systems. Terrestrial Animal Health Code, OIE, Paris, France.

Orro, T., S. Jacobsen, J. LePage, T. Niewold, S. Alasuutari, and T. Soveri. 2008. Temporal changes in serum concentrations of acute phase proteins in newborn dairy calves. The Veterinary Journal. 176(2): 182-187. doi: 10.1016/j.tvjl.2007.02.010

Pang, W. Y., B. Earley, T. Sweeney, and M. A. Crowe. 2006. Effect of carprofen administration during banding or Burdizzo castration of bulls on plasma cortisol, in vitro interferon-γ production, acute-phase proteins, feed intake, and growth. J. Anim. Sci. 84(2): 351-359. doi: 10.2527/2006.842351x

Petersen, H. H., J. P. Nielsen, and P. M. H. Heegaard. 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res. 35(2): 163-187. doi: 10.1051/vetres:2004002

Pieler, D. , W. Peinhopf, A. C. Becher, J. E. Aurich, S. Rose-Meierhöfer, R. Erber, E. Möstl, and C. Aurich. 2013. Physiological and behavioral stress parameters in calves in response to partial scrotal resection, orchidectomy, and Burdizzo castration. J. Dairy Sci. 96(10): 6378-6389. doi: 10.3168/jds.2013-6683

Price, E. O., T. E. Adams, C. C. Huxsoll, and R. E. Borgwardt. 2003. Aggressive behavior is reduced in bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 81(2): 411-415. doi: 10.2527/2003.812411x

Reid, J., A. M. Nolan, J. M. L. Hughes, D. Lascelles, P. Pawson, and E. M. Scott. 2007. Development of the short-form glasgow composite measure pain scale (CMPS-SF) and derivation of an analgesic intervention score. Anim Welf. 16: 97.

Repenning, P. E., J. K. Ahola, R. J. Callan, J. T. French, R. L. Giles, B. J. Bigler, J. F. Coetzee, L. W. Wulf, R. K. Peel, and J. C. Whittier. 2013. Impact of oral meloxicam administration before and after band castration on feedlot performance and behavioral response in weanling beef bulls. J. Anim. Sci. 91(10): 4965-4974. doi: 10.2527/jas.2012-6070

Ricciotti, E., and G. A. FitzGerald. 2011. Prostaglandins and inflammation. Arterioscler. Thromb. Vasc. Biol. 31(5): 986-1000. doi: 10.1161/ATVBAHA.110.207449

Richmond, C. E., L. M. Bromley, and C. J. Woolf. 1993. Preoperative morphine pre-empts postoperative pain. The Lancet. 342(8863): 73-75. doi: 10.1016/0140-6736(93)91284-S

Roberts, S. L., H. D. Hughes, N. C. Burdick Sanchez, J. A. Carroll, J. G. Powell, D. S. Hubbell, and J. T. Richeson. 2015. Effect of surgical castration with or without oral meloxicam on the acute inflammatory response in yearling beef bulls. J. Anim. Sci. 93(8): 4123-4131. doi: 10.2527/jas.2015-9160

Robertson, I. S., J. E. Kent, and V. Molony. 1994. Effect of different methods of castration on behaviour and plasma cortisol in calves of three ages. Res. Vet. Sci. 56(1): 8-17. doi: 10.1016/0034-5288(94)90189-9

Roland, L., M. Drillich, and M. Iwersen. 2014. Hematology as a diagnostic tool in bovine medicine. J. Vet. Diagn. Invest. 26(5): 592-598. doi: 10.1177/1040638714546490

Rollin, B. E. 2004. Annual meeting keynote address: Animal agriculture and emerging social ethics for animals. J. Anim. Sci. 82(3): 955-964. doi: 10.2527/2004.823955x

Schaible, H. G., and R. F. Schmidt. 1988. Time course of mechanosensitivity changes in articular afferents during a developing experimental arthritis. J. Neurophysiol. 60(6): 2180-2195. doi: 10.1152/jn.1988.60.6.2180

Schwartzkopf-Genswein, K. S., J. M. Stookey, J. Berg, J. Campbell, D. B. Haley, E. Pajor, and I. McKillop. 2012. Code of practice for the care and handling of beef cattle: Review of scientific research on priority issues. National Farm Animal Care Council. Canada.

Schwartzkopf-Genswein, K. S., M. E. Booth-McLean, T. A. McAllister, and G. J. Mears. 2005. Physiological and behavioural changes in holstein calves during and after dehorning or castration. Can. J. Anim. Sci. 85(2): 131-138. doi: 10.4141/A04-051

Schwartzkopf-Genswein, K. S., C. Huisma, and T. A. McAllister. 1999. Validation of a radio frequency identification system for monitoring the feeding patterns of feedlot cattle. Livest. Prod. Sci. 60(1): 27-31. doi: 10.1016/S0301-6226(99)00047-0

Schwartzkopf-Genswein, K. S., R. Silasi, and T. A. McAllister. 2003. Use of remote bunk monitoring to record effects of breed, feeding regime and weather on feeding behavior and growth performance of cattle. Can. J. Anim. Sci. 83(1): 29-38. doi: 10.4141/A02-027

Schwartzkopf-Genswein, K. S., J. M. Stookey, A. M. d. Passillé, and J. Rushen. 1997. Comparison of hot-iron and freeze branding on cortisol levels and pain sensitivity in beef cattle. Can. J. Anim. Sci. 77(3): 369-374. doi: 10.4141/A96-127

Schwartzkopf-Genswein, K. S., J. M. Stookey, and R. Welford. 1997. Behavior of cattle during hot-iron and freeze branding and the effects on subsequent handling ease. J. Anim. Sci. 75(8): 2064-2072. doi: 10.2527/1997.7582064x

Serrie, A., and J. Serviere. 2014. Pain: Definitions, concepts and mechanisms in humans and farm animals. Advances in Animal Biosciences. 5(3): 297-309. doi: 10.1017/S2040470014000181

Silanikove, N. 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. Livest. Prod. Sci. 67(1): 1-18. doi: 10.1016/S0301-6226(00)00162-7

Smith, B. P. 2008. Large animal internal medicine. 4th ed. St. Louis, US, Mosby. p. 265.

Sneddon, L. U. 2009. Pain perception in fish: Indicators and endpoints. Ilar J. 50(4): 338-342. doi: 10.1093/ilar.50.4.338

Sowell, B F., J. G. P. Bowman, M. E. Branine, and M. E. Hubbert. 1998. Radio frequency technology to measure feeding behavior and health of feedlot steers. Appl. Anim. Behav. Sci. 59(4): 277-284. doi: 10.1016/S0168-1591(98)00110-5

Stafford, K. J., and D. J. Mellor. 2005. The welfare significance of the castration of cattle: A review. N Z Vet J. 53(5): 271-278. doi: 10.1080/00480169.2005.36560

Stafford, K. J., D. J. Mellor, S. E. Todd, R. A. Bruce, and R. N. Ward. 2002. Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. Res. Vet. Sci. 73(1): 61-70. doi: 10.1016/S0034-5288(02)00045-0

Stewart, M., J. M. Stookey, K. J. Stafford, C. B. Tucker, A. R. Rogers, S. K. Dowling, G. A. Verkerk, A. L. Schaefer, and J. R. Webster. 2009. Effects of local anesthetic and a nonsteroidal antiinflammatory drug on pain responses of dairy calves to hot-iron dehorning. J. Dairy Sci. 92(4): 1512-1519. doi: 10.3168/jds.2008-1578

Stewart, M., G. A. Verkerk, K. J. Stafford, A. L. Schaefer, and J. R. Webster. 2010. Noninvasive assessment of autonomic activity for evaluation of pain in calves, using surgical castration as a model. J. Dairy Sci. 93(8): 3602-3609. doi: 10.3168/jds.2010-3114

Stock, M. L., and J. F. Coetzee. 2015. Clinical pharmacology of analgesic drugs in cattle. Vet. Clin. North Am. Food Anim. Pract. 31(1): 113-138. doi: 10.1016/j.cvfa.2014.11.002

Sutherland, M. A., M. A. Ballou, B. L. Davis, and T. A. Brooks. 2013. Effect of castration and dehorning singularly or combined on the behavior and physiology of holstein calves. J. Anim. Sci. 91(2): 935-942. doi: 10.2527/jas.2012-5190

Theurer, M. E., B. J. White, J. F. Coetzee, L. N. Edwards, R. A. Mosher, and C. A. Cull. 2012. Assessment of behavioral changes associated with oral meloxicam administration at time of dehorning in calves using a remote triangulation device and accelerometers. BMC Vet. Res. 8(1): 48. doi: 10.1186/1746-6148-8-48

Tilley, S. L., T. M. Coffman, and B. H. Koller. 2001. Mixed messages: Modulation of inflammation and immune responses by prostaglandins and thromboxanes. J. Clin. Invest. 108(1): 15-23. doi: 10.1172/JCI13416

Ting, S. T. L., B. Earley, J. M. L. Hughes, and M. A. Crowe. 2003. Effect of ketoprofen, lidocaine local anesthesia, and combined xylazine and lidocaine caudal epidural anesthesia during castration of beef cattle on stress responses, immunity, growth, and behavior. J. Anim. Sci. 81(5): 1281-1293. doi: 10.2527/2003.8151281x

Todd, C. G, S. T. Millman, D.R. McKnight, T. F. Duffield, and K. E. Leslie. 2010. Nonsteroidal anti-inflammatory drug therapy for neonatal calf diarrhea complex: Effects on calf performance. J. Anim. Sci. 88(6): 2019-2028. doi: 10.2527/jas.2009-2340

Torebjork, E. 1985. Nociceptor activation and pain. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 308(1136): 227-234. doi: 10.1098/rstb.1985.0023

Tranquilli, W. J., J. C. Thurmon, and K. A. Grimm. 2013. Pain and its Management In Lumb and Jones' Veterinary Anesthesia and Analgesia. Tranquilli, W. J., J. C. Thurmon, and K. A. Grimm, eds. 4th ed. John Wiley & Sons.

Tucker, C. B., E. M. Mintline, J. Banuelos, K. A. Walker, B. Hoar, A. Varga, D. Drake, and D. M. Weary. 2014. Pain sensitivity and healing of hot-iron cattle brands. J. Anim. Sci. 92(12): 5674-5682. doi: 10.2527/jas.2014-7887

University of British Columbia Animal Welfare Program. 2013. UBC Animal Welfare Program: SOP - HOBO data loggers. Univ. of British Columbia, Vancouver, BC, Canada. p.1 -23.

USDA. 2013. Feedlot 2011, part I: Management practices on U.S. feedlots with a capacity of 1,000 or more head. No. 626.03137-12. USDA, Anim. Plant Health Inspection Serv., Vet. Serv., Cent. Epidemiol. Anim. Health, Fort Collins, CO.

Uvnäs-Moberg, K., P. Alster, M. Petersson, A. Sohlström, and E. Björkstrand. 1998. Postnatal oxytocin injections cause sustained weight gain and increased nociceptive thresholds in male and female rats. Pediatr. Res. 43(3): 344-348. doi: 10.1203/00006450-199803000-00006

Van Engen, N. K., M. L. Stock, T. Engelken, R. C. Vann, L. W. Wulf, L. A. Karriker, W. D. Busby, J. Lakritz, A. J. Carpenter, B. J. Bradford, W. H. Hsu, C. Wang, and J. F. Coetzee. 2014. Impact of oral meloxicam on circulating physiological biomarkers of stress and inflammation in beef steers after long-distance transportation. J. Anim. Sci. 92(2): 498-510. doi: 10.2527/jas.2013-6857

Von Borell, E., J. Langbein, G. Després, S. Hansen, C. Leterrier, J. Marchant-Forde, R. Marchant-Forde, M. Minero, E. Mohr, A. Prunier, D. Valance, and I. Veissier. 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals—a review. Physiol. Behav. 92(3): 293-316. doi: 10.1016/j.physbeh.2007.01.007

Weary, D. M., and D. Fraser. 2004. Rethinking painful management practices. The Wellbeing of Farm Animals: Challenges and Solutions. p. 325-338.

Weaver, A. D., G. St Jean, and A. Steiner. 2008. Castration. In Bovine Surgery and Lameness. 2nd ed. John Wiley & Sons, Incorporated. p. 191-197.

Webster, H. B., D. Morin, V. Jarrell, C. Shipley, L. Brown, A. Green, R. Wallace, and P. D. Constable. 2013. Effects of local anesthesia and flunixin meglumine on the acute cortisol response, behavior, and performance of young dairy calves undergoing surgical castration. J. Dairy Sci. 96(10): 6285-6300. doi: 10.3168/jds.2012-6238

Webster, J. 2016. Animal welfare: Freedoms, dominions and “a life worth living”. Animals. 6(6): 35. doi:10.3390/ani6060035

Werling, D., F. Sutter, M. Arnold, G. Kun, P. C. J. Tooten, E. Gruys, M. Kreuzer, and W. Langhans. 1996. Characterisation of the acute phase response of heifers to a prolonged low dose infusion of lipopolysaccharide. Res. Vet. Sci. 61(3): 252-257. doi: 10.1016/S0034-5288(96)90073-9

White, B. J., J. F. Coetzee, D. G. Renter, A. H. Babcock, D. U. Thomson, and D. Andresen. 2008. Evaluation of two-dimensional accelerometers to monitor behavior of beef calves after castration. Am. J. Vet. Res. 69(8): 1005-1012. doi: 10.2460/ajvr.69.8.1005

Woolf, C. J. 2010. What is this thing called pain? J. Clin. Invest. 120(11): 3742-3744. doi: 10.1172/JCI45178

Woolf, C. J., and M. S. Chong. 1993. Preemptive analgesia-treating postoperative pain by preventing the establishment of central sensitization. Anesth. Analg. 77(2): 362-379.

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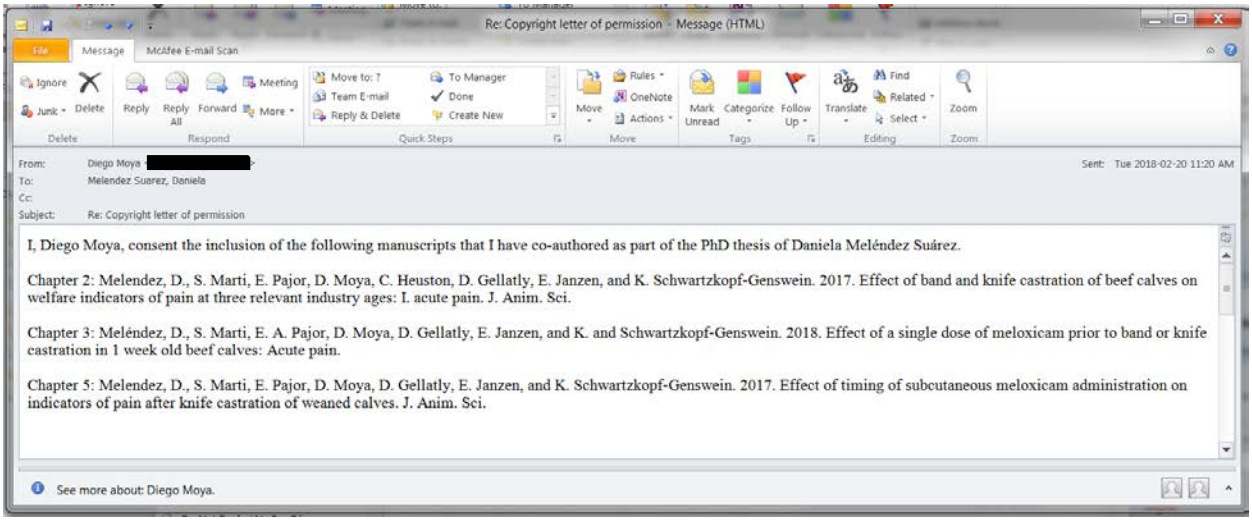


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I, Courtney Heuston, consent the inclusion of the following manuscripts that I have co-authored as part of the PhD thesis of Daniela Meléndez Suárez.
Chapter 2: Melendez, D., S. Marti, E. Pajor, D. Moya, C. Heuston, D. Gellatly, E. Janzen, and K. Schwartzkopf-Genswein. 2017. Effect of band and knife castration of beef calves on welfare indicators of pain at three relevant industry ages: I. acute pain. J. Anim. Sci.
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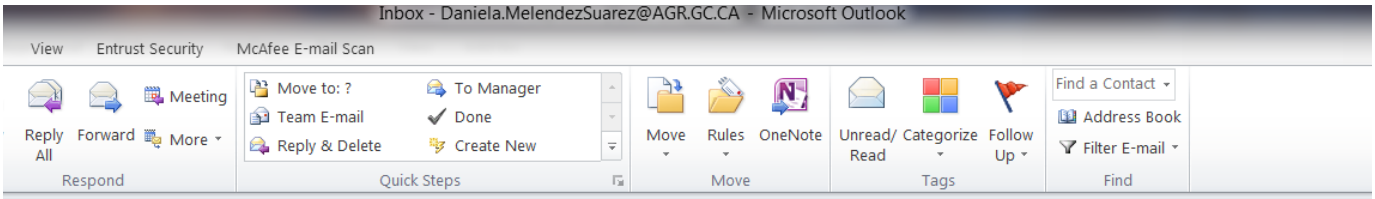


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Schwartzkopf-Genswein, Karen

Sent: Tue 2018-02-20 11:40 AM

To: Melendez Suarez, Daniela; Marti, Sonia; Edmond Anthony Pajor; Diego Moya; janzene@ucalgary.ca; Désirée Gellatly; Courtney Heuston [redacted]

I, Karen Schwartzkopf-Genswein, consent the inclusion of the following manuscripts that I have co-authored as part of the PhD thesis of

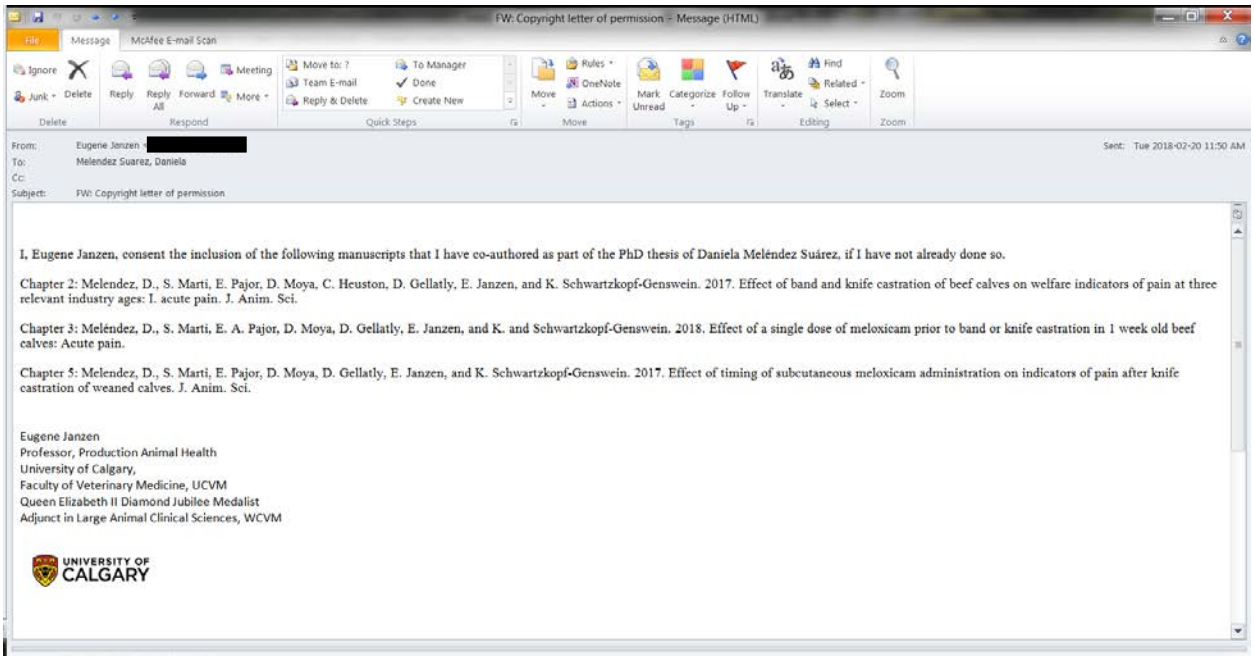
Daniela Meléndez Suárez.

Chapter 2: Melendez, D., S. Marti, E. Pajor, D. Moya, C. Heuston, D. Gellatly, E. Janzen, and K. Schwartzkopf-Genswein. 2017. Effect of band and knife castration of beef calves on welfare indicators of pain at three relevant industry ages: I. acute pain. J. Anim. Sci.

Chapter 3: Meléndez, D., S. Marti, E. A. Pajor, D. Moya, D. Gellatly, E. Janzen, and K. and Schwartzkopf-Genswein. 2018. Effect of a single dose of meloxicam prior to band or knife castration in 1 week old beef calves: Acute pain.

Chapter 5: Melendez, D., S. Marti, E. Pajor, D. Moya, D. Gellatly, E. Janzen, and K. Schwartzkopf-Genswein. 2017. Effect of timing of subcutaneous meloxicam administration on indicators of pain after knife castration of weaned calves. J. Anim. Sci.

*Dr. Karen Schwartzkopf-Genswein*
*Senior Research Scientist*
*Beef Welfare and Behaviour*



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I, Eugene Janzen, consent the inclusion of the following manuscripts that I have co-authored as part of the PhD thesis of Daniela Meléndez Suárez, if I have not already done so.

Chapter 2: Melendez, D., S. Marti, E. Pajor, D. Moya, C. Heuston, D. Gellatly, E. Janzen, and K. Schwartzkopf-Genswein. 2017. Effect of band and knife castration of beef calves on welfare indicators of pain at three relevant industry ages: I. acute pain. J. Anim. Sci.

Chapter 3: Meléndez, D., S. Marti, E. A. Pajor, D. Moya, D. Gellatly, E. Janzen, and K. and Schwartzkopf-Genswein. 2018. Effect of a single dose of meloxicam prior to band or knife castration in 1 week old beef calves: Acute pain.

Chapter 5: Melendez, D., S. Marti, E. Pajor, D. Moya, D. Gellatly, E. Janzen, and K. Schwartzkopf-Genswein. 2017. Effect of timing of subcutaneous meloxicam administration on indicators of pain after knife castration of weaned calves. J. Anim. Sci.

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Queen Elizabeth II Diamond Jubilee Medalist
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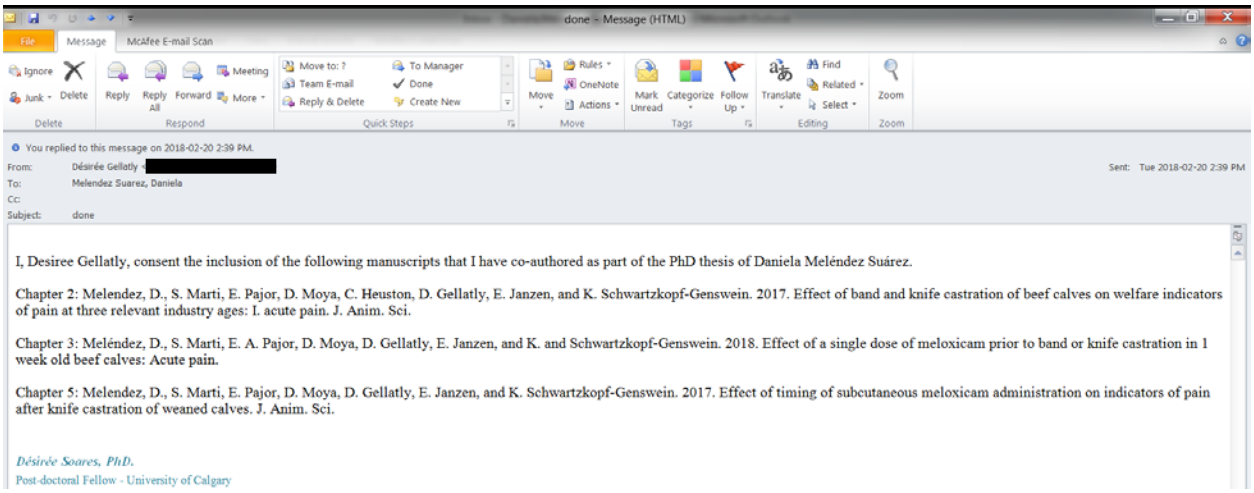


I, Desiree Gellatly, consent the inclusion of the following manuscripts that I have co-authored as part of the PhD thesis of Daniela Meléndez Suárez.

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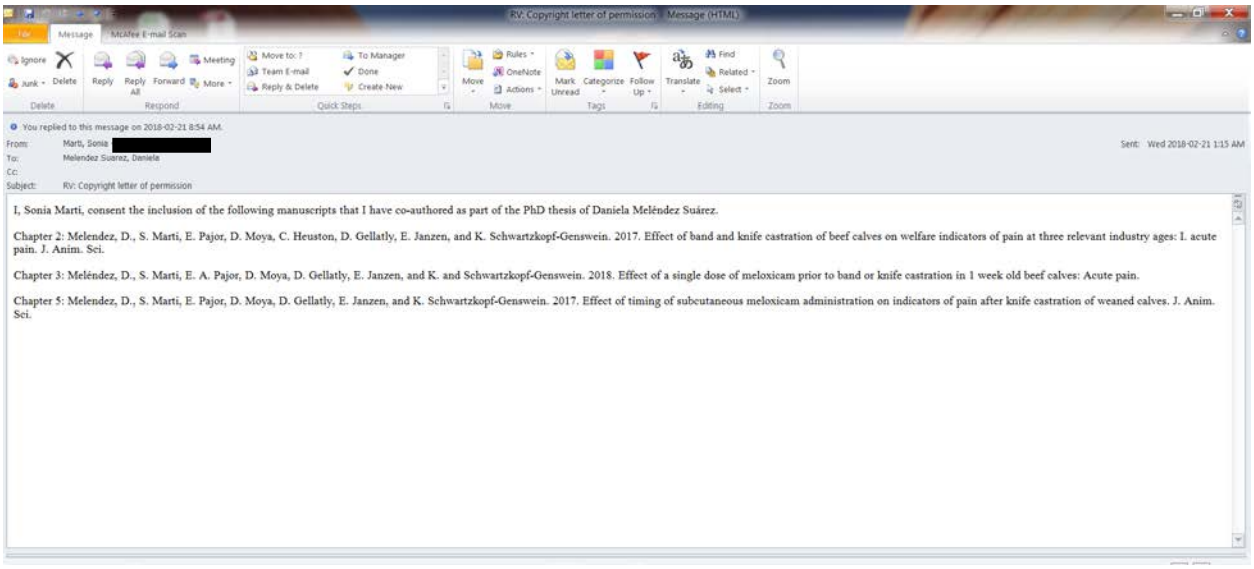
*Désirée Soares, PhD.*
Post-doctoral Fellow - University of Calgary



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I, Sonia Marti, consent the inclusion of the following manuscripts that I have co-authored as part of the PhD thesis of Daniela Meléndez Suárez.

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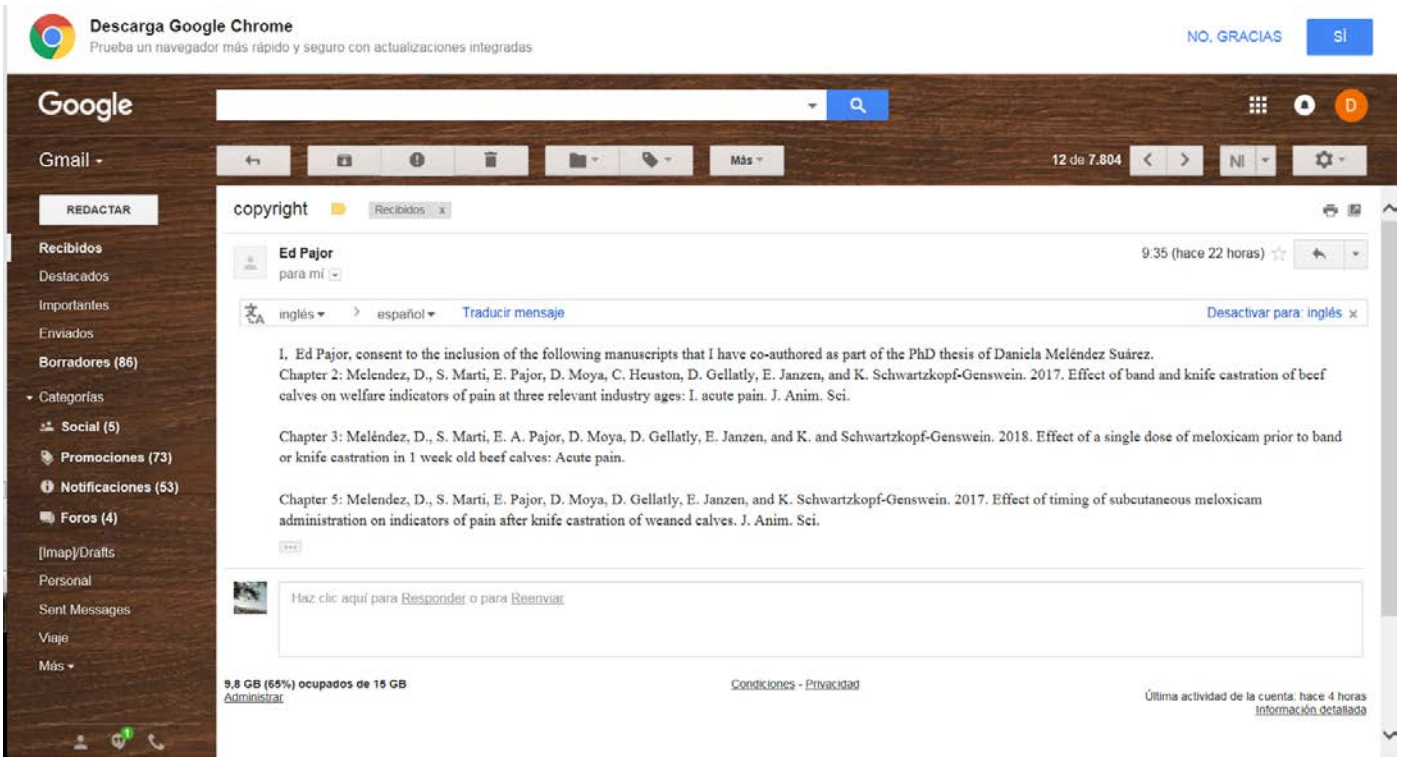
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February 22, 2018

University of Calgary
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Re: Permission to cite publications in Ph.D. thesis

The following papers have, or will be published in Journal of Animal Science, during Daniela Meléndez's visiting worker term at the Lethbridge Research and Development Centre. Copyright interest of AAFC employees in publications is owned by Canada, in accordance with Section 12 of the Copyright Act.

- Melendez, D., S. Marti, E. Pajor, D. Moya, C. Heuston, D. Gellatly, E. Janzen, and K. Schwartzkopf-Genswein. 2017. Effect of band and knife castration of beef calves on welfare indicators of pain at three relevant industry ages: I. acute pain. J. Anim. Sci.

- Meléndez, D., S. Marti, E. A. Pajor, D. Moya, D. Gellatly, E. Janzen, and K. and Schwartzkopf-Genswein. 2018. Effect of a single dose of meloxicam prior to band or knife castration in 1 week old beef calves: Acute pain. J. Anim. Sci.

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AAFC grants consent to Daniela Meléndez, co-author, to include these papers in chapters 2, 3 and 5, respectively, in her Ph.D. thesis.

Please contact me if you have any questions.

Sincerely,

[redacted]

Shannan Little
Acting ADRDT
Lethbridge Research and Development Centre

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