THESIS

# MEASURING AND MANAGING PAIN AND STRESS ASSOCIATED WITH CASTRATION IN CULL BEEF BULLS

Submitted by

Paul English Repenning

Department of Animal Sciences

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2012

Master's Committee:

Advisor: Jason Ahola

Robert Callan
Jack Whittier
Terry Engle
J. Trent Fox

ABSTRACT

MEASURING AND MANAGING PAIN AND STRESS ASSOCIATED WITH CASTRATION IN CULL BEEF BULLS

The objectives of this research were to evaluate the effects of: 1) castration, 2) castration method (band vs. surgical) and 3) use of analgesia on measures of behavior, feedlot performance, and physiological responses in cull bulls.

In the first study, Angus, Hereford, and Angus crossbred bulls (n = 20; initial BW = 384 ± 59.3 kg; 336 ± 20.1 d old) were housed in feedlot pens equipped with the ability to measure individual daily feed intake. A balanced randomized block design using a 2 × 2 factorial arrangement of treatments was utilized. Factors included: 1) castration method (band vs. surgical), and 2) analgesia presence. A multimodal analgesia protocol (**MMA**) was used and consisted of subcutaneous ketamine-stun containing butorphanol (0.01 mg/kg), xylazine (0.02 mg/kg), ketamine (0.04 mg/kg), and a local 2% lidocaine hydrochloride anesthetic block of the spermatic cords (10 mL per cord) and scrotum (10 mL) on d 0. Flunixin meglumine (1.2 mg/kg) was also administered intravenously (**iv**) on d 0, 1, 2 and 3 to MMA cattle. Cattle were stratified to treatments based on breed, BW, age and a temperament score. Treatments included: 1) band castration without analgesia (**BAND)**, 2) band castration with analgesia (**BAND-MMA)**, 3) surgical castration without analgesia (**SURG**), and 4) surgical castration with analgesia (**SURG-MMA**). All castrations were performed on d 0. Chute exit velocity (**EV**) and time in chute (**TIC**) were collected on d -9, 0, 1, 2 and 13. Willingness-to-enter-chute (**WTE**) score, rectal temperature (**TEMP**), heart rate (**HR**), and respiration (**RESP**) were collected on d 0, 1, 2, 3 and 13. Cattle were weighed on d -9 and 13 while feeding behaviors were collected continuously for 57 d pre-castration and 28 d post-castration. There was a tendency ($P < 0.09$) for ADG to be

greater in cattle receiving analgesia. Both SURG treatments exhibited greater TEMP on d 1 ($P < 0.001$) and 2 ($P < 0.05$) compared to both BAND treatments. Mean DMI post-castration was greater ($P = 0.02$) in MMA treatments compared with non-medicated treatments. Meal duration was greater ($P < 0.05$) in both BAND treatments than in surgical castrates in wk 1 post-castration. Results suggest that pain mitigation reduces the impact of castration on ADG and DMI.

The second study was comprised of 2 experiments. In Exp. 1 Angus and Charolais-crossbred bull calves (n = 127; 309.8 ± 59.04 kg) and in Exp. 2 Hereford, Angus, and Hereford × Angus crossbred bulls (n = 30, 300.8 ± 4.96 kg), were stratified by BW and randomly assigned to 1 of 3 treatments: 1) band castration (**BAND**), 2) band castration with oral administration of meloxicam (**BAND-MEL**), and 3) sham castration (**SHAM**). The BAND and SHAM procedures were completed on d 0. The SHAM treatment consisted of all animal manipulations associated with band castration without band application. Meloxicam was administered on d -1, 0, and 1 (1.0 mg/kg, 0.5 mg/kg and 0.5 mg/kg, respectively) via an oral bolus. Body weight and a subjective chute score (**CS**) were collected on d -1, 0, 1, 7, 14, 21 and 28 (Exp. 1 only). In Exp. 1, jugular blood samples were collected immediately before castration and 24 hr post-castration for Substance P (**SP**) analysis. In both experiments, video documentation on d 0 was used to determine range of vertical head motion (**DIST**) on a subset of animals during treatment administration. In both experiments, ADG was similar ($P > 0.10$) between BAND and BAND-MEL, but ADG in SHAM cattle was greater ($P < 0.001$) and tended ($P = 0.07$) to be greater in castrates in Exp. 1 and 2, respectively. In Exp. 1, CS did not differ ($P > 0.10$) between BAND and BAND-MEL on any d, but castrates exhibited greater CS on d 1 and 28 than SHAM cattle. In Exp. 2, CS was not affected ($P > 0.10$) by castration or the presence of meloxicam. In Exp. 1,

DIST did not differ ($P > 0.10$) between BAND and BAND-MEL, but when pooled, castrates exhibited greater ($P = 0.04$) DIST than SHAM. In Exp. 1, plasma SP concentrations did not differ ($P > 0.10$) across treatments. Results indicate no impact of meloxicam administration on performance or behavioral and physiological responses to band castration. However, there was an impact of castration on ADG and CS.

**Keywords:** Beef bulls, Behavior, Castration, Pain mitigation

ACKNOWLEDGEMENTS

I have been so fortunate to have a diverse group of mentors that served on my committee, I would like to thank all of you for the tremendous amount of time and faith you put in me. Jack Whittier, thank you for sharing your years of knowledge in animal science research. Robert Callan, you have been instrumental in facilitating my growth in veterinary medicine. Trent Fox, like Dr. Callan you have provided me much guidance in understanding production animal medicine. I look to both you as examples for the veterinarian I strive to become. Terry Engle, you are an excellent teacher and a great mentor, thank you for helping guide me through the nebulous area of animal welfare research. Jason Ahola, you have been tolerant and understanding of me, and dedicated to my success as a scientist. You allowed me to take ownership in my research and find a subject I am passionate about, all while helping me work through my weakness and expand on my strengths. You have been an outstanding advisor and your compassion for your students is second-to-none. I am blessed to have been able to learn from you.

None of these projects would have been possible without the excellent husbandry provided by the Agricultural Research, Development and Education Center. I must thank Clayton Shonk and the employees at ARDEC their efforts and the use of the cattle.

Dr. George Seidel so generously allowed me the use of his cattle. His willingness to help in so many ways speaks to his passion for the betterment of the beef industry and science. Furthermore, Dr. Seidel provided valuable insight to our design and interpretation. I am glad he takes retirement so lightly.

Kraig Peel, you provide industry experience and a deep knowledge of the technical skills I will cherish in my future career. I appreciate the guidance you gave me over the past few years.

I would not have survived without the fellowship and hard work from Jesse French, Ryan Giles and Bo Bigler. I relied so heavily on their help and cooperation through my graduate career. I am proud to call these gentlemen my friends.

More graduate and undergraduate students than I can mention helped this research come to fruition. I would like to thank Marco Dib, James Damone and the brothers of Alpha Gamma Rho Fraternity for their continued support.

My parents, Harriett and Tom, have been the encouragement and support I needed when I thought I was in over my head. Your love has always been so encouraging. Thank you for having confidence in my abilities and guided me along the way.

My sister, Asta, was always there to listen when I thought it all was too much. I admire her work ethic and compassion for the people around you. I was so fortunate to have her only a phone call away. Thank you for always being there.

Finally, I want to thank my best friend and love of my life, Emily Schou. I was no pleasure to be with for the past 2 years. Your endless love was all the motivation I needed to make it. I am sorry you have had to become accustom to my unusual hours and less-than-pleasant demeanor. I only hope to be able to provide a fraction of the support you afford me over the rest of our lives. You are my guiding light and I am blessed to have you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# CHAPTER I

# REVIEW OF LITERATURE

## Introduction

Animal husbandry practices perceived, or known, to be painful or stressful have entered a realm of criticism and scrutiny. As society grows more aware of these practices and their implications on pain and stress (Rollin, 2004), scientific investigations must keep a body of objective data to guide consumers and potentially legislation.

The European Union (**EU**) formed the Treaty on the Functioning of the European Union (**TFEU**) in 2009. Within Title II, Article 13 of this treaty states,

> *"In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the Member States relating in particular to religious rites, cultural traditions and regional heritage"* (European Union, 2009).

Individual states within the EU have the right to further mandate animal welfare laws beyond what is universally mandated across the EU. The Commission's Scientific Committee on Animal Health and Animal Welfare in 2001 recommended that hot-iron branding, castration and dehorning be eliminated from farming operations (European Commission, 2001). Furthermore, the EU is subject to the Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes, which does state that animals should not undergo unnecessary suffering, pain or injury. Conclusions from the committee on castration state:

> "*Castration causes severe pain and distress. According to some studies surgical castration seems to be less acceptable from a welfare point of view than Burdizzo or rubber rings. However those last two techniques can only easily be done on young*

*calves. Local anaesthesia or local anaesthetic plus systemic analgesia act to reduce the pain"* (European Commission, 2001).

In the United Kingdom, law prohibits laymen (non-veterinarians) from performing surgical castration or Burdizzo castration on calves older than 2 mo of age without analgesia (MAFF, 1992). This policy suggests a concern among British citizens (or legislators) associated with castration and ensuring that an animal does not suffer unnecessarily.

New Zealand adopted policy on castration and dehorning in 1960 and has since renewed this law in the Animals Protection Act of 1999 in which section 201 of the latter Act makes it an offense for any person to (a) castrate, any bovine animal, sheep, goat or pig, over the age of 9 mo, unless performed under veterinary supervision; and (b) except for veterinarians, for any person to dehorn, or cause an animal to be dehorned, over the age of 20 mo unless pain is prevented (NAWAC, 2005).

Some American grocery retailers have begun to adopt animal welfare auditing systems which could impact the profitability of beef producers. The Global Animal Partnership (**GAP**) has enacted a 5-step animal welfare rating standard for beef cattle (GAP, 2009). Within the castration section of these standards, a note is made that calves are ideally castrated before 7 d of age with an emasculator band. At the highest level (Step 5+) cattle are not to be castrated at all. That said, at the lowest level (Step 1) calves are to be castrated by 6 mo of age (GAP, 2009). Currently, GAP standards are only being used by 1 beef retailer, Whole Foods, but are pursuing adoption from other retail grocers (GAP, 2012). Despite the small market share that undergoes GAP's standards, the idea that non-governmental organization (**NGO**) welfare standards are beginning to penetrate the retail market suggests a potential for more consumer-driven welfare verification.

## Castration

*Methods.* There are 3 methods of castration noted by the American Veterinary Medical Association (**AVMA**): physical, chemical, and hormonal (AVMA, 2009). Physical methods are by far the most common technique, especially in American production settings (Coetzee et al., 2010). Though chemical and hormonal techniques may show promise in a clinical setting, a trivial amount of producers and veterinarians utilize them.

Physical castration methods include: external crushing of the spermatic cord, surgical castration and band castration. When surveyed in 2010, American veterinarians responded that surgical removal of the testis was the most common method used in their practices (Coetzee et al., 2010). That said, nearly 7 million male cattle are castrated annually in America (USDA, 2009), with likely a great portion of these being completed by producers, not veterinarians. Therefore, it is difficult to determine which method is applied most commonly in all beef cattle domestically.

Crushing of the spermatic cord to interrupt blood flow to the testis and cause subsequent atrophy can be used to castrate. Typically, a Burdizzo emaculatome is applied to each spermatic cord individually and clamped on each spermatic cord in order to crush each individually. In theory, the testis will atrophy, but the scrotum will remain intact, so long as the scrotum was not crushed in its entirety at any given point during the procedure (Gilbert and Fubini, 2004). When surveyed in 2007, only 3.5% of American cow-calf operations that responded to a survey claimed to use Burdizzo or clamp methods of castration (USDA, 2008).

In 2007, 49.2% of cow-calf operations in America surveyed responded that surgical castration (remove testicles with a blade) was the method of castration used (USDA, 2007).

When feedlot operations were surveyed in 1999, 48.4% of operations claimed to use surgical castration at arrival of intact males (USDA, 2000). Surgical castration can be completed in a variety a ways. Excision of the scrotum can be completed with a knife, scalpel or Newberry Knife. The Newberry knife allows a simultaneous bilateral excision of medial aspects of the scrotum. Excision of the distal aspect of the scrotum can be accomplished readily with a knife or scalpel, though some producers and veterinarians use scalpels or knives to excise the distal aspects, particularly in heavy bulls (Gilbert and Fubini, 2004).

Once the testes are exposed, external testicular fascia are stripped by knife, scalpel or gauze pad. Exposing the spermatic cord yields its own challenges in ligating the cord. Ligation of the spermatic cord can be accomplished by using an emasculator which crushes and cuts the cord. Emasculator followed by suturing the cord, ligating the cord with suture then cutting the cord, or by a Henderson Castration Tool (Stone Manufacturing, Kansas City, MO) are all means of addressing cord ligation. The Henderson Castration Tool is typically attached to a 14-volt cordless electric drill and is clamped to each spermatic cord individually. At this point, the drill is engaged and the cord twisted until the testicle is released while ligating the cord (Stone Manufacturing, 2005).

In lighter, younger calves, ligation is often overlooked with the testes being pulled away from the body cavity until connective tissue is torn. This traction will stretch the spermatic cord until the vasculature ruptures (Gilbert and Fubini, 2004). Often a knife may be used to cut the cord ventral to the testicle.

Band castration, typically categorized as “bloodless castration,” can be accomplished in a variety of ways in various ages and weights of cattle. Rubber ring castration typically occurs in younger calves with small scrotal circumference with a rubber ring applicator or elastrator. In

larger cattle, a ratchet-style applicator tightly is available to secure a latex band around the neck of the scrotum (No Bull Enterprise, St. Francis, KS). All band emasculator techniques are aimed to eliminate blood supply to the scrotum and testis to cause subsequent necrosis and sloughing within approximately 3 wk (Gilbert and Fubini, 2004). In 2007, 39.5% of American cow-calf producers who responded to a survey claimed to band castrate calves at less than 3 mo of age, while 7.8% of respondents claimed to band castrate bulls older than 3 mo of age (USDA, 2008). In 1999 when feedlot operations were surveyed, 65.3% claimed to use band castration on arrival of intact males (USDA, 2000).

The nature of modern beef production systems makes castration of bull calves intended for beef production advantageous with an array of managerial benefits. Castration reduces the number of unwanted pregnancies (Stafford and Mellor, 2005), reduces sexual activity, and reduces aggressiveness (Kent et al., 1996). Meat quality issues arise with intact bulls as shown by fewer incidents of dark cutting carcasses in steers than bulls (Worrell et al., 1987).

Although the benefits of castration for producers clearly outweigh whatever adverse impacts are associated with castration as evident in the myriad of castrations completed annually in America (USDA, 2009), a previous study showed that intact males exhibited greater ADG and feed efficiency (Seidemen et al., 1982). However this does not account for carcass discounts intact bulls are subject to compared to steers (Faulkner et al., 1992).

Castration of bull calves yields an array of adverse effects on animal behavior, health and performance. When compared to intact males or previously castrated bulls, animals recently castrated exhibited reduced ADG and DMI (Fisher et al., 1996), reduced hind leg step length (Gonzalez et al., 2010), increased mean plasma cortisol (Fisher et al. 1996) and increased mean plasma haptoglobin concentration (Fisher et al., 2001).

*Comparing Methods of Castration.* Fisher et al. (2001) compared the effects of surgical castration and band castration on 14 mo-old beef bulls. The authors reported no differences in time spent recumbent or ambulatory between surgical castrates, band castrates and intact bulls. Surgical castrates spent less time grazing than band castrates, as well intact bulls exhibited greater time spent grazing than both surgical and band castrates. Plasma concentration of haptoglobulin was greater in surgical castrates than band and intact bulls on d 1, 2 and 3 post-castration.

## Pain in the bovine

The main societal issue with animal welfare associated with castration is typically associated with pain (Rollin, 2004). All humans have most certainly experienced some form of pain beginning early in life, thus it is readily translated across all age groups and demographics.

The International Association for the Study of Pain (**IASP**) defines pain in humans as: "*An unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage"* (IASP, 1979).

Previous studies in animal sciences have defined pain in the animal as:

> "*Animal pain is an aversive, sensory experience representing awareness by the animal of damage or threat to the integrity of its tissues; (note that there might not be any damage). It changes the animal's physiology and behaviour to reduce or avoid the damage, to reduce the likelihood of its recurrence and to promote recovery. Non-functional (non-useful) pain occurs when the intensity or duration of the experience is not appropriate for damage sustained (especially if none exists) and when physiological and behavioural responses are unsuccessful in alleviating it"* (Molony, 1997).

Pain perception, or nociception, is the transduction, transmission, modulation, projection and perception of some sort of painful stimulus (Anderson and Muir, 2005). Using the Anderson

and Muir (2005) explanation of nociception, in the example of castration, the physical stimulus of castration is transduced into action potentials by pain receptors on C and A delta nerve fibers. The electrical signal created from the nerve action potentials are then transmitted to the dorsal horn of the spinal cord. It is within the spinal cord that the electrical signal is modulated by neurons and projected to the brain (Anderson and Muir, 2005).

Tissue damage induces an inflammatory response which causes the release of chemicals like prostaglandins (**$PGE_2$**), histamine, cyclooxygenase (**COX**) and cytokines. These chemicals, among others, induce peripheral sensitization (Woolf and Slater, 2000).

Chronic peripheral sensitization causes the release of an influx of neurotransmitters like Substance P (**SP**) and glutamate which removes the barrier of $Mg^{2+}$ to N-methyl-D-aspartate (**NMDA**) receptors which induces further pain response (Woolf and Slater, 2000).

*Pain Mitigation.* Local anaesthetics are commonly used as a pre-emptive analgesia (Anderson and Muir, 2005). Given what we know about transduction of pain, local anaesthetics block open $Na^+$ channels to prevent nerve impulses in response to a painful stimulus (Webb and Pablo, 2009; Coetzee et al., 2011). Local anaesthetics (specifically lidocaine) have relatively quick action with a sustained period of desensitization (Wilcke et al., 1983).

Opioids are a class of analgesics with a sedative effect that block $Ca^+$ channels to prevent the activation of nerve response to pain. Butorphanol is classified as an opioid and schedule IV narcotic, therefore is can only to be administered by a Drug Enforcement Agency (**DEA**)-licensed veterinarian. Butorphanol is primarily a κ-receptor agonist and μ-receptor antagonist (Branson and Gross, 2001). The agonist effect on κ-receptors is what allows for sedation and dysphoria in patients administered butorphanol (Millan, 1990)

The NMDA receptor antagonists block central sensitization of pain modulation. A commonly used NMDA antagonist is ketamine which is also controlled by the DEA as a Schedule III drug (Branson and Gross, 2001). Ketamine is a rapid-acting anaesthetic, whose action is to disrupt the central nervous system (Plumb, 2005), but maintain consciousness (Branson and Gross, 2001). In cattle, it is often recommended that another drug, like xylazine, is used as premedication then ketamine can be administered at 2.0 mg/kg intravenously (Thurmon and Benson, 1986). There are negative side-effects associated with ketamine, including: hypertension, cardiac arrest, depressed respiration and soreness at the injection site due to its low pH of 3.5 to 5.5 (Plumb, 2005).

Alpha-2-adrenergic agonists are considered sedative-analgesics drugs. A common α2-adrenergic agonist is xylazine which is commonly used in ruminants as a sedative-analgesic, though ruminants are exceptionally sensitive with the lethal dose being 0.3 mg/kg (Hopkins, 1972). Typically, a ruminant dose (which varies greatly from other species) is 0.05 to 0.15 mg/kg intravenously. In ruminants, xylazine's adverse effects include: bloat, regurgitation, ataxia, and excessive salivation (Plumb, 2005).

Non-steroidal ant-inflammatory drugs (**NSAID**) inhibit one or more steps in the conversion of arachidonic acid to prostaglandins by inhibiting cyclooxygenase (**COX**) enzymes (Boynton et al., 1988). These drugs act on the site of tissue damage to prevent peripheral sensitization induced by inflammatory mediators (Anderson and Muir, 2005). Importantly, COX enzymes are divided into COX 1 and 2. Cyclooxygenases 1 induce the production of prostaglandins necessary for normal physiological function [i.e. act to maintain homeostasis of gastrointestinal cells (Massferrer and Kulkarni, 1997)]. It is for this reason, that non-specific COX-inhibitors are known to cause gastrointestinal bleeding and ulcers (Coetzee, 2011).

Cyclooxygenases 2 production is initiated post-tissue damage (Smith and Langenbach, 2001) and induces the release of inflammatory mediators like $PGE_2$ which act on central pain patterns (Svensson and Yaksh, 2002). Drugs considered NSAID's include, but are not limited to: flunixin meglumine, ketoprofen, phenylbutazone, acetylsalicylic acid, carprofen, and meloxicam (Plumb, 2005). Anderson and Muir (2005) consider NSAID's to be one of the most important means of pain mitigation as often tissue damage needs to be dealt with for a prolonged period time. Upon reviewing the literature, we found no evidence that NSAID's disrupt homeostatic central nervous system function, thus NSAID's can be delivered without the side effects of a sedative drug as listed above. Each NSAID has its own adverse effects associated with it. As noted earlier, common adverse effects of NSAID's are gastrointestinal bleeding or ulcers (Plumb, 2005; Coetzee, 2011)

*Multimodal Analgesia.* There are a series of pathways that contribute to the nociception of pain. Multimodal analgesia approach (**MMA**) combines multiple analgesic drugs which creates a synergy to treat pain in multiple pathways (Lamont, 2008). A common example of a multimodal analgesia combines an opioid (butorphanol), an α2-adrenergic agonist (xylazine), and an NMDA-antagonist (ketamine). This combination of drugs has been commonly dubbed as a "ketamine stun" (Abrahamsen, 2008). From a practical standpoint, recumbence at surgery of ruminants is not always advantageous, especially at castration in a production setting. By incorporating an MMA into the regimen of sedation and analgesia, lower doses of each drug can be used to allow for adequate analgesia and sedation, while keeping the animal from lying. The ketamine stun can be modified to allow for standing stun with a subcutaneous injection (Abrahamsen, 2008).

The ketamine stun accounts for 3 separate pain pathways, but does not address acute pain associated at the site of surgery or painful stimulus or chronic tissue damage releasing inflammatory mediators. To this end, a local anaesthetic and an NSAID can be incorporated to address an additional 2 pathways for pain transmission.

*Analgesics and Anaesthetics.* Currently, there are no analgesic drugs approved by the United States Food and Drug Administration (**FDA**) for the mitigation of pain in food animals (Compendium of Veterinary Products, 2010). Flunixin meglumine is the only NSAID labelled for use in beef and dairy cattle for the treatment of pyrexia associated with respiratory disease and mastitis, as well as inflammation associated with endotoxemia (Smith et al., 2008). Cost of approval and difficulties in identifying and quantifying pain objectively are likely the reasons the private sector and FDA are reluctant in pursuing means of approving drugs for pain mitigation. Despite this rationale, it is difficult to understand how many European countries have already mandated the use of local anaesthetics and analgesics at the time of painful husbandry procedures, while American producers do not even have a clear legal pathway to administer pain mitigation in food animals.

*Extralabel Drug Use.* Currently in the United States, veterinarians do have the ability to prescribe a drug not labelled for food animals if there is not an approved alternative under the American Medicinal Drug Use Clarification Act (AMDUCA; FDA, 1994). There are a plethora of regulations associated with the prescription of extralabel (drug is not labelled for desired purpose) drug use, one of which is that a licensed veterinarian must supervise all extralabel drug use (AVMA, 2007). The AVMA has created a guide for veterinarians to properly follow the legal pathways to extralabel drug use. A drug approved for food animals not labelled for the desired use, or a drug approved for non-food animals, or a drug approved for humans can be

used if there is not a drug approved for food animals labelled for the desired use. This can only happen if alternatives are entertained in the order presented above (AVMA, 2007).

Withdraw time to avoid harmful residues in animals destined for food production is something to be heeded vigilantly with extralabel drug use. Labelled withdraw times on food-animal-approved products must be respected, while established, though not labelled, withdrawal times on non-food-animal-approved products must be respected. This all must be documented clearly with individual animal records (AVMA, 2007).

## Behavioral Measurements

*Procedure Response Score.* Literature focusing on the impact of castration on behavior often incorporates a procedure response score to indicate the amount of resistance to the procedure. A study examining the effects of sub-anaesthetic ketamine stun (xylazine and ketamine) on 4 to 6 mo-old beef calves (n = 22) at castration revealed that calves receiving the sub-anaesthetic ketamine stun and uncastrated control calves exhibited a lower response score than castrates that received a placebo or xylazine alone (Coetzee et al., 2010). A study examining the effects of a local anaesthetic at the time of band or Burdizzo castration on 5.5 mo-old dairy calves (n = 56), found that animals receiving local anaesthesia exhibited less of a response to castration than calves that did not receive local anaesthesia (Fell et al., 1986). The authors noted calves that underwent surgical castration exhibited greater struggling and kicking at castration than calves that were banded. The AVMA defines these behaviours associated with a response score as pain (AVMA, 2009), though it is impossible to determine if these response scores are truly indicative of pain response. Though collected immediately post-castration, a study

examining the effects of butorphanol and xylazine on castration of 6 to 9 mo-old bulls included the evaluation of chute activity. Ranked in a similar fashion as response scores noted above, chute activity was determined by the degree of movement in the chute post-castration (Faulkner et al., 1992). The authors found that both castration and drug administration increased escape behavior from the chute relative to the intact or non-medicated counterparts. This did not evaluate the acute response to castration in the chute, nor did it evaluate the residual effect of castration or drug administration on subsequent processing dates.

*Subjective Chute Score.* Similar to a procedure response score, a subjective chute score evaluates an animal's degree of resistance upon head restraint in the chute. Gruber et al. (2010) used a 15-cm line divided into five, 3 cm classifications of response (0 = calm, 5 = aggressive). The authors found that feeder steers and heifers (n = 79, n =77, respectively) that exhibited an adverse response score to chute restraint also exhibited greater LM WBSF, plasma epinephrine at the chute, and plasma lactate. A study evaluating temperament effect on ADG used a similar 5-point system evaluating degree of movement of the chute as a measurement of docility (Voisinet et al., 1997). The authors revealed that *Bos taurus* cattle exhibiting lower temperament scores (less aggressive) exhibited greater ADG. Similarly, *Bos indicus* cattle exhibiting greater temperament score exhibited reduced ADG. Though this early study was on the forefront of developing a means of quantifying disposition, the study was divided into 2 experiments, evaluating *Bos taurus* and *Bos indicus* cattle separately. This was a problem in that different scales were used to evaluate temperament with Exp. 1 using a 5-point scale, and Exp. 2 using a 4-point scale (Voisinet et al., 1997). This discrepancy reveals the subjective nature of this behavioural measurement as there is no definite scale reported throughout the literature.

Though the subjective chute system as described by Gruber et al. (2010) has not been validated in response to a post-treatment pain, this scoring system may reveal differences post-castration as animals may respond more aggressively to chute restraint after being castrated. This has not been validated in the literature, but there is a possibility that cattle develop a habitual response to restraint on days post-castration.

*Exit Velocity.* Chute exit velocity has been shown to objectively measure flightiness in *Bos indicus* cattle (Curley et al., 2006). Exit velocity has also shown a negative correlation with ADG in *Bos taurus* cattle (Müeller and Von Keyserlingk., 2006). A study examining the effects of sodium salicylate in drinking water co-administered with a sub-anaesthetic ketamine stun, showed that Holstein bull calves that did not receive pain mitigation at surgical castration (n = 10) exhibited greater exit velocity than those that received a ketamine stun or co-administered ketamine stun and sodium salicylate (n = 10; Baldridge et al., 2011).

*Vertical Head Distance.* Head movement and velocity has been evaluated in response to methods of branding (Schwartzkopf-Genswein et al., 1998). The authors noted that animals that underwent hot-iron branding exhibited greater vertical head movement range and velocity when compared to freeze-branding and sham-branding. It can be assumed that this response variable would measure response to castration effectively as well.

*Video Documentation.* Post-castration video documentation has been used in a number of studies to assess castration-induced behavioural changes. Pen level documentation post-castration of cattle that underwent band castration with or without a xylazine epidural and flunixin meglumine revealed no difference in the percentage of cattle lying in the pen at various points, but did reveal decreased step-length in castrates not receiving pain mitigation (Gonzalez et al., 2010). The authors used 210 d-old Angus bull calves (n = 46) and steer calves (n = 43)

were assigned to 1 of 4 pens. Video documentation to study step-length was only collected on 2 animals·treatment$^{-1}$·pen$^{-1}$. This small sample size yielded differences across treatments, but further investigation with a greater sample size may allow for detection of differences in responses other than hind step-length. A similar study noted mitigation in step length for 8 h post castration in surgically castrated animals with flunixin meglumine administration compared to non-medicated castrates that exhibited decreased step-length (Currah, 2009).

## Physiological Responses to Castration

Response to a painful stimulus activates a cascade of autonomic nervous system and endocrine responses (Stewart et al., 2010). Pain activates the sympathetic nervous system which releases chemicals involved in both inflammatory and pain sensitization response. These chemicals (e.g. glucocorticoids and catecholamines) lead to arousal of the cardiac and respiratory systems among others (Anderson and Muir, 2005). In quantifying pain and distress, previous studies have implemented an array of physiological responses in regards to castration.

*Heart Rate.* Increased heart rate (**HR**) is not necessarily indicative of pain, but both pain and increased HR can be clinical signs of disease (Anderson and Muir, 2005). Increased HR was appreciated in calves 120 min after surgical castration of calves (Schwartzkopf-Genswein et al., 2005). One potential factor influencing HR may be due to an increase of inflammatory mediators (Cahn and Line, 2005). Catecholamines, such as epinephrine and norepinephrine, promote vasoconstriction, increased HR, and increased blood pressure and have been used as a measure of sympathetic nervous system response to stress (Stewart et al., 2010). The authors used heart rate variability (**HRV**) in 4 mo-old Fresian bull calves (n = 30) as an indicator of response to

castration. Castrated calves, regardless of pain mitigation, exhibited greater HR for 2 min after castration and then returned to baseline. Administration of local anaesthesia induced greater HR than calves that did not receive a local anaesthetic for 2 min after administration. Calves that were castrated without local anaesthesia exhibited greater HRV than sham calves and calves castrated with local anaesthesia (Stewart et al., 2010). As noted in the previous study, HRV is measured using a sophisticated HR monitor and analysis software. These methods of collection are often available in a clinical setting, but not readily applied to production-research settings.

Respiration rate is also influenced by catecholamines, thus stress and potentially pain influence respiration rate (Stewart et al., 2010). Though respiration has not been used as a physiological response to castration in the literature, there is potential for respiration to be indicative of stress, disease and pain.

Elevated rectal temperature can also be a clinical sign of disease, like pain. Increased rectal temperature may be attributed to increased inflammatory mediators associated with castration procedures (Cahn and Line, 2005). Pang et al. (2006) evaluated rectal temperature in 5.5 mo-old Fresian bulls in association with administration of carprofen (an NSAID) at band and Burdizzo castration. The authors found that 2 d post-castration, all castrates (band and Burdizzo) regardless of carprofen administration, exhibited greater rectal temperature than intact control bulls.

Cortisol is a glucocorticoid secreted in response to a stress-causing event; pain is considered such an event (Anderson and Muir, 2005). Though pain cannot be directly extrapolated from cortisol concentrations, it does serve as a measure of distress with varying peaks and duration (Coetzee, 2011).

Cortisol in the bovine has been measured in 2 ways: 1) plasma or serum cortisol derived from blood samples, or 2) salivary cortisol extracted from saliva from the animal's mouth. Typically analysis of each sample is completed via radio immunoassays.

Serum cortisol response to castration was studied using a 2 × 2 factorial arrangement of treatments with factors being butorphanol co-administered with xylazine or placebo, and surgical castration or sham castration (Faulkner et al., 1992). The authors found that both castration and medication increased serum cortisol on d 3 post-castration. The previous study, in our opinion, confounded stress on d 0 since tattooing, deworming and collecting a baseline blood sample were completed immediately before castration, and in fact serum cortisol on average was highest on d 0 vs. d 3 and 7 (Faulkner et al., 1992).

A more recent study evaluated serum cortisol in association with castration and dehorning, sodium salicylate, ketamine stun and co-administered treatments in Holstein calves (Baldridge et al., 2011). The authors found that mean serum cortisol was reduced in calves receiving a ketamine stun and in calves receiving sodium salicylate in the first hour hr post castration and dehorning and the first 6 hr post-castration (respectively). This study incorporated multiple painful procedures in a small set of calves (n = 40), which causes some difficulties in the interpretation of results given that a single procedure (castration or dehorning) could not be isolated temporally in regards to serum cortisol.

A study examining the effects of the method of castration on 9 and 14 mo-old bulls found that mean plasma cortisol did not differ in either age class of bull, though castrates exhibited greater plasma cortisol on d 14 than intact males (Fisher et al., 2001).

Plasma cortisol was not affected in 4 to 6 mo-old beef calves castrated in associated with iv sodium salicylate, oral acetylsalicylic acid, control castration or no castration (Coetzee et al.,

2007). This may be a function that calves of that age class do not respond to the same magnitude as older, more developed bulls, or it could speak to the efficacy of drugs used in that study.

Salivary cortisol has recently been incorporated into castration studies as it negates the need for venepuncture which may induce cortisol release in itself. Gonzalez et al. (2010) utilized 210 d-old intact bull calves and previously castrated steers to examine the use of a xylazine epidural and flunixin meglumine at the time of castration or handling (steers). Salivary cortisol analysis yielded no differences in any treatment of castration or medication, although it should be noted that samples were only collected on a subset (2 animals·treatment$^{-1}$·pen$^{-1}$; Gonzalez et al., 2010).

*Substance P.* Substance P (**SP**) is an 11-amino acid neuropeptide released from nerve endings to increase capillary permeability and contribute to inflammation in response to tissue damage (Boron and Boulpaep, 2009). Furthermore, SP regulates the sensitivity of nociceptive neurons on the dorsal horn of the spinal column (Coetzee et al., 2011). In the literature surrounding human medicine, SP was found to be greater in patients suffering from tissue damage than healthy patients (Onuoha and Alpar, 1999). Work done by Coetzee et al. (2008) was on the forefront of SP in bovine castration literature. Coetzee et al. (2008) used 10, 4 to 6 mo-old beef calves subjecting them to sham or surgical castration. Results indicated that at almost all time points, save 90 min post-castration, castrates exhibited greater SP than sham calves (Coetzee et al., 2008). Disadvantages associated with SP are intra-subject variability (Coetzee et al., 2008) and instability associated with the neuropeptide during collection, transport and analysis.

## Feeding Behaviors and Performance

*Average Daily Gain.* Likely the most common response variable examined for castration experiments is ADG. Gain in terms of BW is an obvious performance parameter of financial importance to producers; as well, ADG is assumed to be indicative of an animal's general well-being. Studies comparing recently castrated animals to intact males and cattle castrated much earlier in life revealed that castration, regardless of pain mitigation strategy, negatively impacts ADG for some amount of time (Faulkner et al., 1992; Fisher et al., 2001; Gonzalez et al., 2010). Some studies failed to find differences in ADG between control and castrated cattle (Fisher et al., 1996).

Examining the effect of castration method on ADG, there are mixed results associated with which method results in the least detrimental impact on ADG. One study did not find differences between band and surgical castration of 21 mo-old beef bulls, though castrates still had a tendency ($P = 0.13$) to exhibit reduced ADG compared to sham castrates (Chase et al., 1995). Likewise, a study examining the effects of band vs. surgical castration in 2 age classes of bulls found that band and surgical castrates gained at a similar rate, but exhibited reduced growth compared to intact males (Fisher et al., 2001). However, the authors reported that when ADG was evaluated via a regression analysis, band castrates grew at a reduced rate compared surgically castrated 14 mo-old bulls.

Within the context of pain mitigation, there is a myriad of products that have been tested in association with different methods of castration in different classes of bulls. Specifically examining local anaesthesia of lidocaine, a study investigating lidocaine at Burdizzo, surgical

and band castration found that lidocaine reduces the impact of surgical castration on ADG, but not with other tested methods (Fisher et al., 1996).

Studies examining the effects of NSAID's at the time of castration vary in regards to ADG. Gonzalez et al. (2010) noted no difference in ADG associated with flunixin meglumine administration, but this was completed in conjunction with a xylazine epidural.

Another NSAID, meloxicam, was studied in association with surgical castration of "high risk" weaned beef calves, and no differences were observed in ADG across treatment of oral meloxicam vs. placebo treated animals (Coetzee et al., 2011). However, the authors reported that meloxicam treated animals exhibited reduced respiratory disease morbidity when compared to placebo treated castrates.

*Dry Matter Intake*. Castration and administration of pain mitigation have been shown to affect DMI to varying degrees in cattle. Primitive means of estimating ADG could be the estimation of time spent grazing. Though, since no mass associated with intake can be accounted for, it is assumed that animals grazing consume more than those not. Fisher et al. (2001) found that banded cattle spent a greater portion of time grazing than surgically castrated cattle. At the same time, both groups of castrates exhibited reduced grazing time when compared to intact bulls.

Gonzalez et al. (2010) was one of the first studies to implement a radio frequency identification (**RFID**)-linked bunk system (Growsafe Systems Ltd., Airdrie, AB, CA), which allowed for individual animal intake measurements in a group-feeding environment. The authors reported that cattle receiving pain mitigation had reduced DMI compared to non-medicated cattle. Likewise, feed intake in band castrates, when compared to steers castrated much earlier, was less in only the fourth wk post-castration.

Coetzee et al. (2011) also examined DMI, though on a pen-level, and found no impact of meloxicam on average DMI. Unfortunately, pen level DMI measurements do no account for variation within each individual animal, but only the experimental unit: pen.

A previous study examining the effect of butorphanol and xylazine in association with castration of 9 mo-old beef calves found no differences across castration treatment or pain mitigation treatment (Faulkner et al., 1992). Although the authors did not note the means of collecting DMI data, it can be assumed that animals were housed in group feeding environments without the ability to account for individual intake, thus not accounting for individual animal intake variability.

*Feeding Behaviors*. Individual feed intake data acquired via RFID-linked feed bunks have emerged in the last decade (Growsafe Systems Ltd.). The capacity of this system has made evaluation of each individual animal's feeding behavior readily available without the need for video or manual documentation. A recent study validating an older edition (Growsafe 4000E) of this program reported that video data and system output were highly correlated within bunk visit duration and frequency (Mendes et al., 2011).

To the best of our knowledge, only 1 study has incorporated RFID-linked feed intake into a castration study (Gonzalez et al., 2010). The authors reported that bunk visit frequency was greater in control cattle not receiving pain mitigation than control cattle receiving pain mitigation and cattle that were band-castrated with medication. Control cattle did not differ from cattle banded without pain mitigation. Meal duration did not differ across treatments of castration or pain mitigation; however, meal size was greater in control cattle than all other treatments (Gonzalez et al., 2010).

These data presented by Gonzalez et al. (2010) could be interpreted several ways in regarding pain responses and feeding behaviours. Regardless of interpretation, building the body of literature surrounding these behaviours will be essential in growing the collective understanding of pain at castration of cattle.

.

## Justification for research

As societal concerns continue to grow (Rollin, 2004; HSUS, 2011) and consumers begin to demand more verification of humane practices throughout animal rearing (GAP, 2009), castration will likely enter an unknown realm of scrutiny. Many countries have already mandated the use of analgesia, or service of a veterinarian in certain classes of cattle castration (MAFF, 1992; NAWAC, 2005). Without a complete body of scientific literature addressing the concerns of pain mitigation and method of castration on pain response in bulls, there is a great potential for future legislation not to be guided by the tenets and findings of science, but rather public opinion.

Currently there is no drug approved in the US for the treatment of pain in food animals (Compendium of Veterinary Products, 2010). Given the nebulous understanding of how to identify pain in the bovine, achieving FDA approval of any drug for the mitigation of pain will prove difficult, if not impossible, as there is no clear means of identifying pain to be treated.

It would be unfeasible to say that producers would eliminate castration on a certain age class of bulls, not to mention the potential for eliminating castration in bulls as a whole. It is for these reasons there must be a concerted effort to address the issue of castration through veterinary, animal science and behavior research.

The following chapters of this document explain research that has been conducted over the past 2 yr to address the issues of: 1) catration, 2) method of castration and 3) pain mitigation at castration, 4) performance in association with castration, and 5) behaviours associated with castration.

Pain mitigation can be delivered in a variety of ways. Though practical application of these means must be addressed, we first aimed to determine if the use of a multimodal analgesia would impact behaviors and feedlot performance at castration. As noted earlier, there are a multitude of pathways by which nociception occurs. An MMA allows for multiple pathways to be addressed in a synergistic manner.

Literature surrounding method of castration in older bulls has left room for conclusions to be drawn as to which method induces the least overall amount of pain. To this end, band and surgical castration were applied while both acute pain and chronic or residual pain were studied.

While addressing performance and behavioural response to castration, both objective and subjective measurements must be incorporated until a more objective means of measuring what could be perceived as a painful response is developed. Behaviors in the subsequent chapter include: response score, chute score (over time), exit velocity (over time) and various feeding behaviours collected by an RFID-linked feeding system. Performance parameters are always of importance in a production setting, and given most castrations occur within a production setting, ADG and DMI will be addressed.

Though a multimodal analgesia protocol as noted above may not be readily applied to production systems, practical recommendations must be made that can readily be adopted by the domestic beef industry. Given the time taken to perform a surgical castration in an older bull, band castration will need to be focused on. That said, previous literature has noted an impact of

band castration on behaviors and performance, and in some cases more so than surgical castration. For this, the effect of castration method must be addressed.

Meloxicam has proven to have a greater half-life (27.54 h) than the current analgesics available for food animals (flunixin meglumine, 3-8 h; Anderson et al. 1990). Additionally, promise in oral administration of meloxicam has allowed for the exploration of meloxicam's application to the beef industry (Coetzee et al., 2009).

In addition, the behavioral measurements noted above, including vertical head movement at the time of castration, allow for an objective measure of resistance to a procedure as opposed to a subjective response score.

In identifying pain, the neuropeptide SP, could help objectively identify pain in association with castration via a blood sample.

It is the aims of the following research to attempt to identify behaviours associated with response to castration, differences, or lack thereof, in feeding behaviors, feedlot performance and physiological response to castration and pain mitigation's effects on these responses.

## Literature Cited


Abrahamsen, E.J. 2008. Chemical restraint in ruminants. Vet. Clin. Food Anim. 24:227-243.

American Veterinary Medical Association. 2009. Welfare implications of castration of cattle. Accessed 15 May 2012. http://www.avma.org/reference/backgrounders/castration cattle bgnd.pdf

American Veterinary Medical Association. 2007. Extralabel Drug Use. Accessed 15 May 2012. http://www.avma.org/reference/amduca/extralabel_brochure.pdf

Anderson, D.E. and W.W. Muir. 2005. Pain management in ruminants. Vet. Clin. Food Anim. 21:19-31.

Anderson, K.L., C.A. Neff-Davi, L.E. Davis, and V.D. Bass, 1990. Pharmacokinetics of flunixin meglumine in lactating cattle after single and multiple intramuscular and intravenous administrations. Am J. Vet. Res. 51:1464–1467.

Baldridge, S.L., J.F. Coetzee, S.S. Dritz, J.B. Reinbold, R. Gehring, J. Havel, 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. 72:1305–1317.

Boron W.F., and E.L Boulepap. 2009. Medical Physiology. Saunders, St. Louis, MO>

Boynton, C.S., C.F. Dick, and G.H. Mayor. 1988. NSAID's: an overview. J. Clin. Pharmacol. 28:512-517.

Branson K.R., and M.E. Gross. 2001. Opioid agonists and antagonists. Pages 268-98 In: Veterinary Pharmacology and Therapeutics, Adams H.R., ed. Iowa State University press, Ames, IA.

Cahn C.M., and S. Line. 2005. The Merck Veterinary Manual. 9th ed., Whitehouse Station, NJ.

Chase, C.C. Jr., R.E. Larsen, R.D. Randel, A.C. Hammond, E.L. Adams. 1995. Plasma cortisol and white blood cell Responses in different breeds of bulls: a comparison of two methods of castration. J. Anim. Sci. 73:975–980.

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. 135:192-213.

Coetzee, J.F., L.N. Edwards, R.A. Mosher, N.M. Bello, A.M. O'Connor, B. Wang, B. KuKanich, and D.A. Blasi. 2011. Effet of oral meloxicam on health and performance of beef steers relative to bulls castrated on arrival at the feedlot. J. Anim. Sci. 90: 1026-1039.

Coetzee, J.F., R. Gehring, J. Tarus-Sang, and D.E. Anderson. 2010a. Effect of sub-anesthetic xylazine and ketamine ('ketamine stun') administered to calves immediately prior to castration. Vet. Anaes. and Analg. 37:566-578.

Coetzee, J.F., B. KuKanich, R. Mosher, and P.S. Allen. 2009. Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. Vet. Therap. 10: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 concentration of substance P and cortisol in beef calves after castration or simulated castration. AVJR. 69:751-762.

Coetzee J.F., A.L. Nutsch, L.A. Barbur, and R.M. Bradburn. 2010b. A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. BMC Vet. Res. 6:12.

Compendium of Veterinary Products. 2010. Compendium of Veterinary Products 13th edition. Bayley, A.J. ed., North American Compendiums INC, Port Huron, MI.

Curley, K.O., J.C. Paschal, T.H. Welsh, and R.D. Randel. 2006. Technical note: Exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. J. Anim. Sci. 84:3100-3103.

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. 50:375-382.

European Union. 2009. The EU and animal welfare: policy objectives. Accessed 10 May 2012. http://ec.europa.eu/food/animal/welfare/policy/index_en.htm.

European Commission, Scientific Commission on Animal Welfare. 2001. Welfare of cattle kept for beef production. Accessed 10 May 2012. http://ec.europa.eu/food/fs/sc/scah/out54_en.pdf

Faulkner, D.B., T. Eurell, W.J. Tranquilli, R.S. Ott, M.W. Ohl, G.F. Cmarik, and G. Zinn. 1992. Performance and health of weanling bulls after butorphanol and xylazine administration at castration. J. Anim. Sci. 70:2970-2974.

FDA. 1994. Food and Drug Administration. Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994. Accessed 25 May 2012. http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/AnimalMedicinalDrugUseClarificationActAMDUCAof1994/default.htm.

Fell, L.R., Wells, R., Shutt, D.A., 1986. Stress in calves castrated surgically or by the application of rubber rings. Aust. Vet J. 63:16–18.

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. 74:2336-2343.

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. Aus. Vet. J. 79:279-284.

Gilbert, R.O., and S.L. Fubini. 2004. Surgery of the male reproductive tract. Page 355 in Farm Animal Surgery. S.L. Gilbert and N.G. Ducharme, ed. Saunders, St. Louis, MO.

GAP. 2009. Global Animal Partnership. Global Animal Partnership 5-Step™ Animal Welfare Rating Standards for Beef Cattle. Accessed 10 May 2012. http://www.globalanimalpartnership.org/wp-content/uploads/2011/01/5-Step-Animal-Welfare-Rating-Standards-for-Beef-Cattle.pdf

GAP. 2012. Global Animal Partnership. For retailers. Accessed May 10 2012. http://www.globalanimalpartnership.org/for-retailers/

González, L.A., K.S. Schwartzkopf-Genswein, N.A. Caulkett, E. 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, Escherichia coli, and salivary cortisol. J. Anim. Sci. 88:802-810.

Gruber, S.L., J.D. Tatum, T.E. Engle, P.L. Chapman, K.E. Belk, and G.C. Smith. 2010. Relationships of behavioral and physiological symptoms of preslaughter stress to beef longissimus muscle tenderness. J. Anim. Sci. 88:1148-1159.

Hopkins, T.J. 1972. The clinical pharmacology of xylazine in cattle. Aus. Vet. J. 48:109-112.

HSUS. 2011. Humane Society of the United States. An HSUS Report: The welfare of calves in the beef industry. Accessed 10 May 2012. http://www.humanesociety.org/assets/pdfs/farm/welfare_calves.pdf.

International Association for the Study of Pain (IASP).1979. Pain terms: a list with definitions and notes on usage. Pain. 6**:**249-252.

Kent, J.E., M.V. Thrusfield, I.S. Robertson, V. Molony. 1996. Castration of calves: a study of methods used by farmers in the United Kingdom. Vet. Rec. 138:384-387.

Lamont, L.A.. 2008. Multimodial pain management in veterinary medicine: the physiologic basis of pharmacologic therapies. Vet. Clin. Small Anim. 38:1173-1186.

MAFF. Ministry of Agriculture, Fisheries and Food. 1992. Summary of law relating to farm animal welfare. Accessed 10 May 2012. http://discovery.nationalarchives.gov.uk/SearchUI/Collection/Display?uri=C10769-

Masferrer, J.L. and P.S. Kulkarni. 1997. Cyclooxygenase-2 inhibitors: a new approach to the therapy of ocular inflammation. Surv. Opthalmol. 41:S35-S40.

Mendes, E.D.M., G.E. Carstens, L.O. Tedeschi, W.E. Pinchak, and T.H. Friend. 2011. Validation of a system for monitoring feeding behavior in beef cattle. J. Anim. Sci. 89:2904-2910.

Millan, M.J. 1990. Trend Pharmacol. Sci. 11:70.

Molony, V. 1997. Comments on Anand and Craig (Letters to the Editor). Pain. 70:293

Müller, R., M.A.G. von Keyserlingk. 2006. Consistency of flight speed and its correlation to productivity in *Bos taurus* beef cattle. Appl. Anim. Behav. 99:193-204.

NAWAC. 2005. National Animal Welfare Advisory Committee. 2005. Animal welfare (painful husbandry procedures) code of welfare 2005. Accessed 10 May 2012. http://www.biosecurity.govt.nz/files/regs/animal-welfare/req/codes/painful-husbandry/painful-husbandry.pdf

Onuoha, G.N., Alpar, E.K., 1999. Calcitonin gene-related peptide and other neuropeptides in the plasma of patients with soft tissue injury. Life Sci. 65:1351–1358.

Pang, W.Y., B. Earley, T. Sweeney, and M.A. Crowe. 2006. Effects 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. 84:351-359.

Plumb, D.C. 2005. Plumb's Veterinary Drug Handbook. Blackwell Pub., Ames, IA.

Rollins, B.E., 2004. Annual meeting keynote address: animal agriculture and emerging social ethics for animals. J. Anim. Sci. 82:955–964.

Schwartzkopf-Genswein, K.S., J.M. Stookey, T.G. Crowe, and B.M Genswein. 1998. Comparison of image analysis, exertion force, and behavior measurements for use in the assessment of beef cattle responses to hot-iron and freeze branding. J. Anim. Sci. 76:972-979.

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. 85:131–138.

Seideman, S.C., H.R. Cross, R.R. Oltjen, and B.D. Schanbacher. 1982. Utilization of intact males for red meat production: A review. J. Anim. Sci. 55:826.

Smith, W. L. and R. Langenbach. 2001. Why there are two cyclooxygenase isozymes. J. Clin. Invest. 107:1491-1495.

Smith G.W., J.L. Davis, L.A. Tell, A.I. Webb, and J.E. Riviere. 2008. Extralabel use of nonsteroidal anti-inflammatory drugs in cattle. JAVMA. 232:697-701.

Stafford, K.J., and D.J. Mellor. 2005. The welfare significance of the castration of cattle: A review. NZ Vet. J. 53:271-278.

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 the model. J. Diary Sci. 93:3602-3609.

Stone Manufacturing. 2005. Henderson equine castrating tool. Accessed 15 May 2012. http://www.stonemfg.net/images/brochures/Henderson/HendersonEquine_trifold%20(High%20Res).pdf

Svensson C.I., and T.L. Yaksh. 2002. The spinal phospholipase-cyclooxygenaseprostanoid cascade in nociceptive processing. Annu Rev Pharmacol Toxicol 42:553–583.

Thurmon, J.C., and G.J. Benson. 1986. Anesthesia in ruminants and swine. Pages 51-71 In Current Veterinary Therapy 2: Food Animal Pracitce. J.L. Howard ed. Saunders, Philadelphia.

USDA. 2000. NAHMS Part I: Baseline reference of feedlot management practices, 1999. Accessed 25 May 2012. http://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot99/Feedlot99_dr_PartI.pdf

USDA. 2008. NAHMS Beef 2007-08, Part I: Reference of beef cow-calf management practices in the United States, 2007-08. Accessed 25 May 2012. http://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/downloads/beef0708/Beef0708_dr_PartI_rev.pdf

USDA. 2009. National Agricultural Statistics Service Agricultural Statistics 2009. Accessed July 5, 2011. http://usda.mannlib.cornell.edu/usda/nass/Catt//2000s/2009/Catt-07-24-2009.pdf.

Voisinet B.D., T. Grandin, J.D. Tatum, S.F O'Connor, and J.J. Struthers. 1997. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. J. Anim. Sci. 75:892-896.

Webb, A.I., and L.S. Pablo, 2009. Injectable anesthetic agents. In: Veterinary Pharmacology and Therapeutics, 9th ed. Riviere, J.E., Papich, M.G. ed., Wiley-Blackwell, Ames, IA, p. 383.

Wilcke, J.R., L.E. Davis, C.A. Neff-Davis, and G.D. Koritz. 1983. Pharmacokinetics of lidocaine and its active metabolites in dogs. J. Vet. Pharm. Ther. 6:49-57.

Woolf C.J., and M.W. Slater. 2000. Neuronal placity: increasing the gain in pain. Science. 288:1765-1769.

Worrell, M.A., D.C. Clanton, and C.R. Calkins. 1987. Effect of weight at castration on steer performance in the feedlot. J. Anim. Sci. 70:2970-2974.

CHAPTER II

## Effects of pain mitigation and method of castration on behavior and feedlot performance in cull beef bulls

**SUMMARY:** The objectives of this study were to evaluate the effects of castration method (band vs. surgical) and use of analgesia on behavior and feedlot performance in cull bulls. Angus, Hereford, and Angus crossbred bulls (n = 20; initial BW = 384 ± 59.3 kg; 336 ± 20.1 d old) were housed in feedlot pens equipped with the ability to measure individual daily feed intake. A balanced randomized block design using a 2 × 2 factorial arrangement of treatments was utilized. Factors included: 1) castration method, and 2) analgesia presence. A multimodal analgesia protocol (**MMA**) was used and consisted of subcutaneous ketamine-stun containing butorphanol (0.01 mg/kg), xylazine (0.02 mg/kg), ketamine (0.04 mg/kg), and a local 2% lidocaine hydrochloride anesthetic block of the spermatic cords (10 mL per cord) and scrotum (10 mL) on d 0. Flunixin meglumine (1.2 mg/kg) was administered intravenously (**iv**) on d 0, 1, 2 and 3 to MMA cattle. Cattle were stratified to treatments based on breed, BW, age and a temperament score. Treatments included: 1) band castration without analgesia (**BAND)**, 2) band castration with analgesia (**BAND-MMA)**, 3) surgical castration without analgesia (**SURG**), and 4) surgical castration with analgesia (**SURG-MMA**). All castrations were performed on d 0. Chute exit velocity (**EV**) and time in chute (**TIC**) were collected on d -9, 0, 1, 2 and 13. Willingness-to-enter-chute (**WTE**) score, rectal temperature (**TEMP**), heart rate (**HR**), and respiration (**RESP**) were collected on d 0, 1, 2, 3 and 13. Cattle were weighed on d -9 and 13 while feeding behaviors were collected continuously for 57 d pre-castration and 28 d post-castration. There was a tendency ($P < 0.09$) for ADG to be greater in cattle receiving analgesia.

Both SURG treatments exhibited greater TEMP on d 1 ($P < 0.001$) and 2 ($P < 0.05$) compared to BAND treatments. Mean DMI post-castration was greater ($P = 0.02$) in MMA treatments compared with non-medicated treatments. Meal duration was greater ($P < 0.05$) in band castrates than in surgical castrates in wk 1 post-castration. Results suggest that pain mitigation reduces the impact of castration on ADG and DMI.

**Key words:** Beef bulls, Behavior, Castration, Ketamine-stun, Pain

## Introduction

Castration of male cattle is a common practice in the beef cattle industry. The benefits of castration include reduced aggression, reduced sexual activity, reduced incidence of dark-cutting carcasses, improved carcass quality grade, and fewer unwanted pregnancies (Worrell et al., 1987; Faulkner et al., 1992), and are considered to outweigh the disadvantages. Castrating bulls has been shown to reduce DMI and ADG, and increase serum cortisol and haptoglobin concentrations when compared to intact bulls (Faulkner et al., 1992), indicating that there is both a physiological stress and inflammatory response to castration. When surveyed in 2011, veterinarians associated some level of pain with castration of beef calves (Fajt et al., 2011). Despite the reduction in animal performance and apparent pain caused by castration, roughly 15 million livestock castration procedures occur yearly (USDA, 2009).

There are 2 commonly used methods of castration in the beef industry: banding and surgical castration (USDA, 2000; USDA, 2008). Reduced acute pain response occurs with banding, but was associated with decreased ADG in 14 mo-old beef bulls (Fisher et al., 2001). Surgical castration with a scalpel was noted as the most common castration method in bulls

among veterinarians surveyed in the U.S. (Coetzee et al., 2010b). Local anesthetics at castration have been shown to reduce cortisol response (Thüer et al., 2007) and improve ADG in 5.5-mo-old bull calves compared to castrates that did not receive local anesthetics (Fisher et al., 1996). Non-steroidal anti-inflammatory drugs (**NSAID**), specifically flunixin meglumine, have been shown to visibly reduce pain response up to 8 h post-castration (Currah et al., 2009). Therefore, the objectives of this study were to evaluate the effects of castration method (band vs. surgical) and use of a multimodal analgesia (**MMA**) approach on behavior and feedlot performance in yearling cull bulls.

## Materials and Methods

*Animals.* This project was approved by the Institutional Animal Care and Use Committee at Colorado State University. Angus (n = 5), Hereford (n = 4), and Angus, Hereford, and Simmental crossbred (n = 11) cull bulls weighing 384 ± 59.3 kg and 336 ± 20.1 d old were castrated via 4 treatments in a 2 × 2 factorial arrangement with factors being: castration method, and MMA presence.

*Housing and Feeding.* All animals were housed in one 30-head feedlot pen (30 m × 60 m). Ad libitum feed was provided to the pen, though only 4 animals could access feed at one time. The diet was composed of corn silage, ground alfalfa hay, and a vitamin and mineral supplement and delivered via 4 radio frequency identification linked individual feedbunks (Growsafe Systems Ltd., Airdrie, AB, Canada) that enabled collection of daily individual animal feed intake, meal duration, and meal size in a group feeding environment. Animals were acclimated to the feeding system for 14 d, which began 80 d prior to castration. Bulls were

vaccinated with clostridium perfringens, types C & D tetanus toxoid (Bar Vac CD/T, Boehringer Ingelheim, St. Joseph, MO) 14 d prior to castration. All castrations occurred on d 0.

*Feeding Behaviors*. Dry matter intake, meal duration, and meal size were averaged for 57 d before castration to establish baseline feeding behaviors for each animal. Post-castration feeding behavior data were collected for each animal from d 0 to 28. A meal event was defined as an animal feeding with no more than 300 s passing between visits to the bunk. A meal event could have been composed of multiple feed events which were defined as an animal feeding for more than 20 s. Meal duration was calculated on a daily basis, and was the average duration of meal events on a given d. Meal size was the average the amount of feed consumed during all meal events on a given d (Garossino, 2011).

*Behavioral Measurements.* As animals entered the chute on d 0, 1, 2, 3, and 13, a willingness-to-enter-chute (**WTE**) score was assigned using a 9-point scale: 1 = entered chute without pressure on the animal's flight zone, 2 = entered chute with only an approach to the alley by the handler, 3 = entered chute with only an anterior-to-posterior stroke of the back, 4 = entered chute with light contact on the back from the handler, 5 = required a tail twist to enter the chute, 6 = required physical contact via a driving aid, 7 = required one application of an electric prod (i.e. "hot shot"), 8 = required more than 1 application of an electric prod, 9 = the handler was unable to drive the animal into the squeeze chute. All handlers were trained to evaluate animals using the WTE scoring system.

On d -9, 0, 1, 2, and 13, objective time in chute (**TIC**) and exit velocity (**EV**) values were collected using an infrared sensor timing system (FarmTek Inc., North Wylie, TX). The EV (m/s) was collected beginning 1.892 m from the head catch and ending 1.892 m beyond that point.

At the time of castration (d 0), bulls received a procedure response score (**RS**) on a 4-point scale which was determined by a technician observing the procedure. The score system included: 0 = standing on all 4 feet during the castration procedure, 1 = treading of front or rear feet during the castration procedure, 2 = 1 to 3 kicks of a rear foot during the castration procedure, 3 = more than 3 instances of kicking or hopping on rear feet during the castration procedure.

Immediately upon restraint on d -9, 0, 1, 2, 3, and 13, a subjective chute score (**CS**) was determined for each animal when the scorer marked a 15-cm long line scale as described by Gruber et al. (2010). Marks were converted to values on a 0 to 5 line scale, 0 = calm, 5 = aggressive (Gruber et al., 2010).

*Treatments.* Bulls were stratified by breed, BW, age, and a combined temperament score (EV + CS + vocalization) collected on d -9 and assigned to 1 of 4 treatments: band castration without analgesia (**BAND**), band castration with analgesia (**BAND-MMA**), surgical knife castration without analgesia (**SURG**), and surgical knife castration with analgesia (**SURG-MMA**).

*Procedures.* Analgesia treatment groups (BAND-MMA and SURG-MMA) received an injection containing butorphanol (0.01 mg/kg), xylazine (0.02 mg/kg), and ketamine (0.04 mg/kg) administered subcutaneously upon restraint in the chute (Abrahamsen, 2008). In addition, 2% lidocaine was used as a local anesthetic injected at 10 ml per spermatic cord and a 10 ml subcutaneous infiltration of the scrotum. Upon restraint on d 0, all animals were haltered and flunixin meglumine (Flunixin Injectable, Norbrook Laboratories, Ltd., Newry, Ireland) was administered iv (1.2 mg/kg) to MMA treatments. All MMA cattle also received flunixin meglumine (1.2 mg/kg iv) on d 1, 2, and 3. Body weight was collected on d -9 and d 13.

Surgical castrations (SURG and SURG-MMA) were prepared by scrubbing the scrotum with pieces of role cotton soaked in dilute betadine solution. The distal aspect of the scrotum was excised using a sterile scalpel blade. The external spermatic fascia was stripped away from the testes using 10.16 cm × 10.16 cm gauze pads. Once exposed, the spermatic cord was twisted and ligated using a Henderson Castration Tool (Stone Manufacturing, Kansas City, MO) attached to a 14 volt cordless electric drill (Dewalt Industrial Tool Co., Baltimore, MD). The same experienced veterinarian completed all surgical castrations to ensure consistency. Oxytetracycline (LA-200, Pfizer Animal Health, New York, NY) was administered subcutaneously (4 mg/kg) to both SURG and SURG-MMA treatments.

Band castrations (BAND and BAND-MMA) were completed by securing a latex band around the neck of the scrotum using a Callicrate Bander (NO-BULL Enterprises, St. Francis, KS). The same experienced technician completed all band castrations throughout the study.

Rescue analgesia (flunixin meglumine, 1.2 mg/kg iv) was given to one animal in the SURG treatment at 24 h post-surgery due to extreme discomfort (i.e. did not readily stand when approached recumbent in the pen). We did not account for the rescue analgesia administered to this animal in our statistical analysis, thus this animal remained in the study.

Heart rate (**HR**), respiration rate (**RESP**), and rectal temperature (**TEMP**) were collected on d 0, 1, 2, 3, and 13. All collection occurred after CS designation, and on d 0 before castration procedures.

*Statistical Analyses.* Average daily gain, G:F, TIC, RS and d 0 EV were analyzed using two-way analysis of variance with factors being method and presence of MMA (PROC MIXED, SAS Institute, Cary, NC). Heart rate, TEMP, RESP, EV, CS and WTE were analyzed in a repeated measures model with subject being individual animal (PROC GLIMMIX, SAS

Institute). In this analysis, subjects were classified by factors including method and MMA, and the repeated measurement factor was d. Day 0 values were used as covariates for HR, TEMP, and RESP when analyzed in the repeated measures model, while d -9 was used a covariate for EV, CS and WTE in analyzing least square means for all other d. Dry matter intake, meal size, and meal duration were analyzed using the same repeated measures model with average values from d -57 to -1 used as covariates (PROC GLIMMIX, SAS Institute). Additionally, post-castration weekly averages were studied because of high daily variability of feeding variables. Autocorrelated (across time) error structures were considered for repeated measures of feeding variables, but were not used based on the Akaike's Information Criteria. If interactions between method and presence of MMA were not significant ($P > 0.10$), method and MMA values averaged over main effects were compared separately by d, week, and overall. Interactions between method and d, and MMA and d were studied by comparing main effect values separately. Comparisons were made regardless of significance of corresponding effect because certain d were of *a priori* interest.

## Results and Discussion

There was no method × MMA interaction ($P > 0.10$) for ADG, and ADG did not differ across the main effect of method ($P > 0.10$). There was a tendency ($P = 0.09$) for ADG to differ across MMA main effect (Table 2.1) with MMA treatments gaining 0.37 kg/d greater than those that did not receive analgesia. An experiment that evaluated the effects of a xylazine epidural and flunxin meglumine administration at band castration found no differences in ADG between medicated and non-medicated groups (Gonzalez et al., 2010). In another study, growth was

greater in surgical castrates than banded castrates, and both groups had reduced growth when compared to intact bulls (Fisher et al., 2001). In contrast, in the current study there was no effect of castration method on ADG.

Method × presence of MMA interaction was not significant ($P > 0.10$) for DMI. Mean DMI for 28 d post-castration was greater ($P = 0.02$) in MMA treatments (Table 2.1). Across the main effect of method, band castrates exhibited 2.62 ± 0.716 kg greater ($P = 0.003$) DMI on d 1 (data not reported). And though there was no d × MMA interaction ($P > 0.10$), MMA treatments exhibited 1.57 ± 0.713 kg greater ($P = 0.03$) DMI on d 1, 2.83 ± 0.713 kg greater ($P < 0.001$) DMI on d 2 and 1.44 ± 0.713 kg greater ($P = 0.04$) DMI on d 3 than surgical castrates (data not reported). Our results differed from others reported in the literature (Gonzalez et al., 2010), in which pain mitigation decreased DMI post band castration and sham castration. This difference could be attributed to sedation strategy (subcutaneous ketamine stun in the current study vs. xylazine epidural in the previous) or administration of flunixin meglumine (d 0, 1, 2 and 3 in the current study vs. d 0 in the previous). When wk was incorporated in the model, the main effect of MMA differed as evident with MMA treatments exhibiting greater DMI in wk 1 ($P = 0.007$) and wk 2 ($P < 0.05$) than non-medicated animals. There was a tendency ($P = 0.08$) for DMI to differ by method during wk 1, with band castrates exhibiting greater DMI than surgical castrates (Table 2.1).

As seen in Table 2.1, there was increased DMI over the 28 d post-castration period among MMA cattle compared to cattle that did not receive MMA. As seen in Table 2.1, there was also a tendency ($P = 0.09$) for increased ADG in MMA treatments. The current study only examined the 28-d period after castration and it is not known if these effects would be compensated for by the time of slaughter, though by wk 3 the effect of MMA equilibrated.

Ketamine and butorphanol are both controlled substances and must be administered by a Drug Enforcement Administration licensed veterinarian, which could inhibit widespread use in the beef industry. Hence, further investigation into pain mitigation strategies that are more accessible to the industry is needed.

There was not ($P > 0.10$) a method × MMA interaction, or method effect or MMA effect for G:F (Table 2.1). This is likely a function of the fact that there were no differences in ADG.

There was no ($P > 0.10$) method × MMA or method × MMA × d interaction for meal size. Neither were there differences across the main effect of method ($P > 0.10$) nor across the main effect of MMA ($P > 0.10$). However, there was a method × d interaction ($P = 0.001$) with band castrates exhibiting greater meal size on d 5 ($P < 0.05$), 7 ($P = 0.004$) and 12 ($P = 0.01$) than surgical castrates (data not reported). When wk was included in the model, mean meal size for band castrates had a tendency ($P = 0.05$) to be greater than surgical castrates during wk 1 (Table 2.1). These results may be indicative of the acute response (first 7 d post-treatment) surgical castration has on meal size.

Temporal effects associated with meal size indicate that surgical castration elicits an adverse response to consumption at a single meal event. This is seen in differences in DMI across the main effect of method. Furthermore, it appears that a delayed response potentially associated with band castration does not occur with the same magnitude as surgical castration.

Method × MMA interaction was not significant ($P > 0.10$) for meal duration. And, neither the main effect of method nor the main effect of MMA differed ($P > 0.10$) for meal duration. As well, MMA × d interactions within meal duration were not significant ($P > 0.10$). That said, there was a method × d interaction ($P < 0.0001$) with band castrates exhibiting greater meal duration on d 2 ($P = 0.02$), 3 ($P = 0.005$), 5 ($P = 0.003$), 7 ($P = 0.001$) and 8 ($P = 0.03$) vs.

surgical castrates (data not reported). However, surgical castrates exhibited greater meal duration on d 12 ($P = 0.01$), 14 ($P = 0.02$), and 19 ($P = 0.04$) compared to band castrates (data not reported). The fact that band castrates exhibited greater meal duration earlier and surgical castrates exhibited greater meal duration later in the feeding period may indicate that surgical castrates had an acute response and band castrates had a delayed response to castration. When wk was included in the model, band castrates exhibited greater ($P = 0.002$) meal duration in wk 1. Across the MMA main effect, MMA cattle had a tendency ($P = 0.06$) to exhibit greater meal duration in wk 1 (Table 2.1) vs. no-medicated cattle. Within the main effect of method, differences in the first wk indicate an acute response to surgical castration. Additionally, overall response to castration in meal duration may potentially be mitigated by MMA.

Though meal duration has not been examined previously in regards to castration or pain, there were clear differences which could suggest adverse response to castration method. Band castrates exhibited greater meal duration for the first wk and on certain d. That said, the inverse with surgical castrates exhibiting greater meal duration on d 12, 14 and 19 suggests that there was an adverse response to band castration as well, but later in the feeding period. However, the numbers of d that differ suggests that the response is not to the same magnitude at least within the feeding period examined. The main effect of MMA shows that analgesia may mitigate the response to castration during the first wk post-castration.

There was no method × presence of MMA interaction ($P > 0.10$) for TIC. Across the main effect of castration method, TIC was greater ($P < 0.001$; Table 2.2) in surgical than band castration treatments by 318.0 ± 48.75 s. In cattle that received MMA, TIC was greater ($P = 0.03$) by 115.4 ± 48.75 s. These results indicate that both surgical castration and administration of analgesia are more time consuming than band castration and omission of analgesia. While

examining the application of these results, time has the potential to play a role in industry acceptability.

Exit velocity on d 0 collected immediately after castration was similar ($P > 0.10$) across the main effects of castration method or presence of analgesia. When mean EV (pooled across all d post-castration) was analyzed independent of d 0 with d -9 as a covariate, EV was 0.40 ± 0.145 m/s greater ($P = 0.04$) in MMA cattle than cattle that did not receive pain mitigation (Table 2.2). Though there was no MMA × d interaction, MMA treatments exhibited greater ($P = 0.03$) EV on d 13. There was a method × d interaction ($P = 0.003$) for EV, as shown by surgical castrates exhibiting greater ($P < 0.01$) EV on d 13 than band castrates (Table 2.2).

Chute score did not differ ($P > 0.10$) across main effects over time or on a given d (Table 2.2). To date, no studies have documented the effect of castration on CS beyond the time of castration. Previous studies revealed that more aggressive CS are associated with reduced ADG and carcass quality (Gruber et al., 2010), but did not evaluate CS in response to a painful stimulus.

There was no method × presence of MMA interaction ($P > 0.10$) for WTE. When measured in cattle after castration, WTE did not differ ($P > 0.10$) across main effects of castration method or presence of analgesia (Table 2.2).

There was no method × MMA interaction ($P = 0.46$) for RS. Response score was affected by the main effects of method, as seen by SURG animals exhibiting a greater ($P < 0.01$) RS than BAND, and by the main effect of MMA where animals not receiving analgesia exhibited a greater ($P < 0.01$) RS than MMA treatments (Figure 2.1). A study examining the effects of iv ketamine and xylazine administration at a dose of 0.1 mg/kg and 0.05 mg/kg, respectively, found that castration without any sedation resulted in a greater number of animals that exhibited violent

escape behavior at castration (Coetzee et al., 2010a). Findings from the current study support the contention that MMA decreases acute resistance responses when castrating yearling bulls at least based on behavior in the chute.

There was a method × MMA interaction ($P = 0.04$) for TEMP with SURG, SURG-MMA and BAND-MMA exhibiting greater ($P < 0.001$, $P = 0.001$, and $P = 0.01$, respectively) TEMP values than BAND cattle pooled across d 1, 2, 3 and 13 (Table 2.3). Since there was no method × MMA × d interaction, comparisons were made across main effects and d. There was a method × d interaction ($P = 0.04$) for TEMP (Table 2.4). Mean TEMP in the SURG treatments was greater ($P < 0.001$) than BAND treatments on d 1. On d 2, TEMP for SURG was greater ($P = 0.03$) than BAND treatments. Rectal temperature among surgical castrates in the present study may have been caused by an inflammatory response to soft tissue injury at the surgical site. Increased inflammatory mediators would be a consistent connection between the elevated TEMP observed in surgical castrates (Cahn and Line, 2005).

There was no method × MMA interaction ($P = 0.98$) for HR. There was a method × d interaction ($P = 0.005$) for HR, as shown with surgical castrates exhibiting 25.7 ± 7.38 beats/min greater ($P = 0.001$) than band castrates on d 1. This could be attributed to the soft tissue damage caused by surgical castration which induces the release of catecholamines (inflammatory mediators), subsequently causing vasodilation and altering cardiac output (Stewart et al., 2010). A study investigating the effects of a local anesthetic and castration on autonomic response found increased HR for 2 min post-local anesthetic administration and castration. However, the previous study did not examine HR beyond 12 min post-castration (Stewart et al., 2010). There were no other differences ($P > 0.10$) for HR across method or d (Table 2.4).

Within RESP, there was a neither method × presence of MMA interaction ($P > 0.10$) nor were there differences across main effects when pooled over time ($P > 0.10$). There was a tendency ($P = 0.06$) for an MMA × d interaction for RESP, as shown by treatments not receiving MMA exhibiting greater ($P = 0.06$) RESP on d 1 (Table 2.4) than MMA treatments. A potential reason for the RESP difference seen in MMA cattle may be attributed to the mitigating effect of analgesia on increased inflammatory mediators in response to castration.

As seen in the present study, MMA combines multiple analgesics to target an array of pain pathways. Previous studies have shown that the MMA principle allows for a synergistic effect of drugs by using a lower dose of each drug in combination to minimize the detrimental effect of each (Lamont, 2008).

The MMA protocol used in the current study incorporated an $\alpha_2$-adrenergic agonist (xylazine), an opioid (butorphanol), a N-methyl-D-asparate receptor antagonist (ketamine), an NSAID (flunixin meglumine) and a local anesthetic (lidocaine hydrochloride; Abrahamsen, 2010). Results of the current study suggest that MMA allowed for production and welfare benefits in regards to DMI and RS. It could be hypothesized that the absence of negative side-effects associated with the products used in the current study can be attributed to this MMA approach.

There is a relatively limited understanding of how to best measure pain response in the bovine. This makes it difficult to determine what behaviors to evaluate and how to score these behaviors during potentially painful procedures such as castration in order to evaluate methods of pain mitigation. The current study showed differences in DMI, HR, TEMP, RS and EV, and a tendency to differ in ADG, but not WTE or VOC. Further investigation into behaviors related to

pain associated with castration will be vital for the approval of more effective pain mitigation strategies for use with castration in food animals.

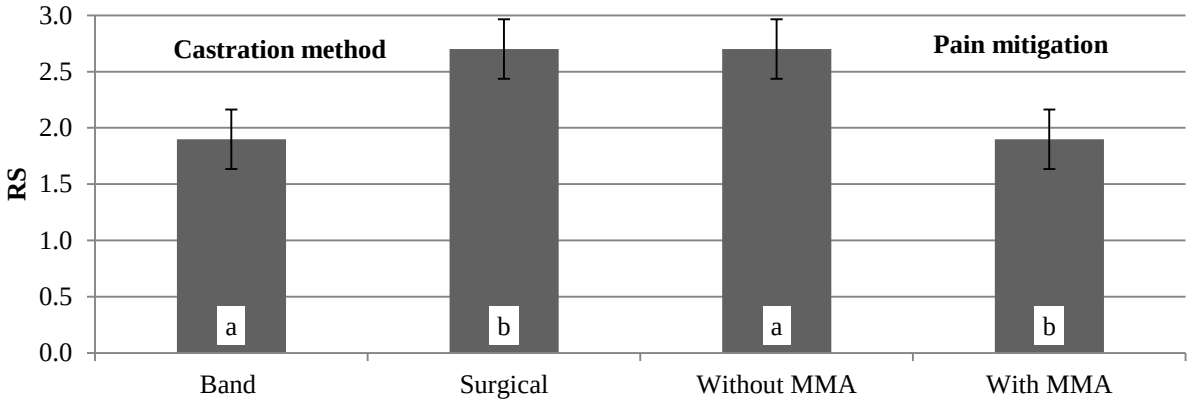


**Figure 2.1**. Mean response score (RS) ± SE at time of castration (d 0) by main effect of method of castration and pain mitigation presence[1,2,3,4]

[1]There was no method × pain mitigation interaction ($P > 0.10$), therefore the main effects of method of castration and presence of MMA were compared.

[2]Pain mitigation was provided via a multimodal analgesia approach (MMA).

[3]Band = band castration, Surgical = surgical castration, MMA= Subcutaneous ketamine stun and local lidocaine block at time of castration (d 0) and intravenous flunixin meglumine at d 0, 1, 2 and 3.

[4]Response score was determined as: 0 = standing on all 4 feet during the castration procedure, 1 = treading of front or rear feet during the castration procedure, 2 = 1 to 3 kicks with a rear foot during the castration procedure, 3 = more than 3 instances of kicking or hopping on rear feet during the castration procedure.

[a,b]Within each main effect, means without common superscripts differ ($P < 0.05$).

**Table 2.1.** Least square means ± SEM for ADG, DMI, G:F, meal duration, and meal size across the main effects of method of castration and presence of pain mitigation[1, 2]

| | Method | | | MMA | | |
|---|---|---|---|---|---|---|
| | Band | Surgical | SEM | Without | With | SEM |
| ADG (kg/d) | | | | | | |
| Overall | 0.91 | 1.13 | 0.150 | 0.84 | 1.21 | 0.150 |
| DMI (kg/d) | | | | | | |
| Week 1 | 10.16 | 9.39 | 0.437 | 9.16[b] | 10.39[a] | 0.304 |
| Week 2 | 10.12 | 10.37 | 0.437 | 9.81[b] | 10.68[a] | 0.304 |
| Week 3 | 9.48 | 10.01 | 0.437 | 9.51 | 9.99 | 0.304 |
| Week 4 | 10.10 | 10.57 | 0.437 | 10.10 | 10.56 | 0.304 |
| Overall | 9.96 | 10.07 | 0.275 | 9.63[b] | 10.40[a] | 0.273 |
| G:F | | | | | | |
| Overall | 0.09 | 0.13 | 0.020 | 0.09 | 0.12 | 0.020 |
| Meal Size (kg/meal)[3] | | | | | | |
| Week 1 | 0.81 | 0.68 | 0.045 | 0.69 | 0.79 | 0.044 |
| Week 2 | 0.82 | 0.90 | 0.045 | 0.85 | 0.87 | 0.044 |
| Week 3 | 0.81 | 0.85 | 0.045 | 0.85 | 0.80 | 0.044 |
| Week 4 | 0.84 | 0.90 | 0.045 | 0.88 | 0.85 | 0.044 |
| Overall | 0.82 | 0.83 | 0.036 | 0.82 | 0.83 | 0.036 |
| Meal Duration (s/meal)[4] | | | | | | |
| Week 1 | 847.9[a] | 677.1[b] | 36.26 | 712.0 | 812.0 | 36.07 |
| Week 2 | 767.4 | 853.9 | 36.26 | 791.5 | 829.9 | 36.07 |
| Week 3 | 755.2 | 776.9 | 36.26 | 795.1 | 736.9 | 36.07 |
| Week 4 | 829.9 | 868.8 | 36.26 | 848.3 | 850.4 | 36.07 |
| Overall | 798.9 | 791.3 | 27.50 | 784.4 | 805.7 | 27.27 |

[1]There was no ($P > 0.10$) method × MMA presence interaction for ADG, DMI, G:F, meal size, or meal duration. Therefore the main effects of method and MMA presence have been reported.

[2]A 2 × 2 factorial arrangement was used with factors being method of castration and pain mitigation. Method of castration was either: band castration or surgical castration. Pain mitigation was provided with a multimodial analgesia approach (**MMA**) which consisted of subcutaneous ketamine stun and local lidocaine block at time of castration (d 0) and intravenous flunixin meglumine at d 0, 1, 2 and 3.

[3]Meal size was defined as the average amount of feed consumed per meal event.

[4]Meal duration was defined as the average time elapsed per meal event, which was defined as an animal feeding for more than 300 s.

[a,b]Within main effect, means without common superscripts differ ($P < 0.05$)

**Table 2.2.** Least square means ± SEM for time in chute (TIC) exit velocity (EV), subjective chute score (CS) and willingness-to-enter-chute (WTE) across main effects of method and presence of pain mitigation over time.[1]

| | Method | | | MMA | | |
|---|---|---|---|---|---|---|
| | Band | Surgical | SEM | Without | With | SEM |
| TIC (s) | | | | | | |
| D 0 | 560.1[b] | 878.1[a] | 34.42 | 661.5[a] | 776.8[b] | 34.47 |
| EV[2] (m/s) | | | | | | |
| D 0 | 1.24 | 1.46 | 0.166 | 1.23 | 1.48 | 0.166 |
| D 1 | 1.30 | 1.15 | 0.157 | 1.04 | 1.41 | 0.106 |
| D 2 | 1.36 | 1.01 | 0.103 | 1.05 | 1.31 | 0.157 |
| D 13 | 1.30[b] | 2.18[a] | 0.178 | 1.45[b] | 2.02[a] | 0.176 |
| Overall | 1.32 | 1.44 | 0.095 | 1.18[b] | 1.58[a] | 0.099 |
| CS[3, 5] | | | | | | |
| D 0 | 1.91 | 1.76 | 0.351 | 1.71 | 1.96 | 0.351 |
| D 1 | 1.70 | 2.63 | 0.585 | 2.38 | 1.97 | 0.603 |
| D 2 | 2.38 | 2.37 | 0.387 | 1.96 | 2.79 | 0.397 |
| D 3 | 2.43 | 2.94 | 0.376 | 2.14 | 3.23 | 0.385 |
| D 13 | 2.55 | 2.10 | 0.387 | 2.23 | 2.42 | 0.395 |
| Overall | 2.64 | 2.51 | 0.256 | 2.17 | 2.61 | 0.271 |
| WTE[4, 6] (1-9) | | | | | | |
| D 0 | 2.50 | 1.90 | 0.403 | 2.10 | 2.30 | 0.403 |
| D 1 | 3.36 | 1.81 | 0.576 | 2.74 | 2.67 | 0.571 |
| D 2 | 1.94 | 1.07 | 0.669 | 1.83 | 1.18 | 0.667 |
| D 3 | 2.40 | 1.51 | 0.576 | 1.94 | 1.97 | 0.571 |
| D 13 | 3.30 | 2.21 | 0.576 | 2.14 | 3.37 | 0.571 |
| Overall | 2.81 | 1.65 | 0.471 | 2.16 | 2.30 | 0.464 |

[1]There was no ($P > 0.10$) method × MMA presence interaction for TIC, EV, CS, or WTE. Therefore the main effects of method and MMA presence have been reported.

[2]Exit velocity was collected upon animals leaving the chute using an infrared barrier system as the velocity exhibited 1.892 to 3.784 m beyond the head catch.

[3]Chute score was determined upon head restraint with 0 = calm and 5 = aggressive on a 15-cm line (Gruber et al., 2010).

[4]Willingness-to-enter-chute was determined 1 to 9 as: 1 = entered chute without pressure on the animal's flight zone, 2 = entered chute with only an approach to the alley by the handler, 3 = entered chute with only an anterior-to-posterior stroke of the back, 4 = entered chute with light contact on the back from the handler, 5 = required a tail twist to enter the chute, 6 = required physical contact via a driving aid, 7 = required one application of an electric prod (i.e. "hot shot"), 8 = required more than 1 application of an electric prod, 9 = the handler was unable to drive the animal into the squeeze chute.

[5]Least square means were studied independent of any other d for d 0, but d 1, 2, 3 (if collected) and 13, and overall means were studied using d -9 as a covariate.

[6]Least square means were studied independent of any other d for d 0, but d 1, 2, 3 and 13, and overall means were studied using d 0 as a covariate.

[a,b]Means within the same row and main effect without a common superscript were different ($P < 0.05$).

**Table 2.3.** Least square means (± SEM) for rectal temperature (TEMP) ± SEM across castration method and analgesia treatments averaged over time.[1,2]

| | BAND | BAND- | SURG | SURG- | SEM |
|---|---|---|---|---|---|
| TEMP (ºC)[3] | 39.27[b] | 39.58[a] | 39.82[a] | 39.74[a] | 0.0868 |

[1]Method × MMA interaction was significant ($P < 0.05$), therefore least square means for each treatment were presented separately.
[2]BAND = band castration, BAND-MMA= band castration with subcutaneous ketamine stun and local lidocaine block at d 0 and intravenous flunixin meglumine at d 0, d 1, d 2 and d 3, SURG = surgical castration, SURG-MMA = surgical castration with subcutaneous ketamine stun and local lidocaine block at d 0 and intravenous flunixin meglumine at d 0, 1, 2 and 3.
[3]Rectal temperature was collected on d 0, 1, 2, 3 and 13.
[a,b]Means without common superscripts differ ($P < 0.05$).

**Table 2.4.** Least square means ± SEM for heart rate (HR), respiration rate (RESP), and rectal temperature (TEMP) across main effects of method of castration and MMA presence over time.[1]

| | Method | | | MMA | | |
|---|---|---|---|---|---|---|
| | Band | Surgical | SEM | Without | With | SEM |
| TEMP (ºC)[2] | | | | | | |
| D 1 | 39.2[b] | 39.9[a] | 0.09 | 39.4 | 39.7 | 0.03 |
| D 2 | 39.7[b] | 39.8[a] | 0.09 | 39.6 | 39.7 | 0.03 |
| D 3 | 39.4 | 39.7 | 0.09 | 39.5 | 39.6 | 0.03 |
| D 13 | 39.6 | 39.7 | 0.09 | 39.6 | 39.7 | 0.03 |
| HR (beats/min)[3] | | | | | | |
| D 1 | 95.0[b] | 121.0[a] | 5.15 | 107.2 | 108.8 | 5.20 |
| D 2 | 107.0 | 106.0 | 5.15 | 102.6 | 110.4 | 5.20 |
| D 3 | 116.0 | 117.0 | 5.15 | 113.4 | 119.6 | 5.20 |
| D 13 | 135.4 | 135.0 | 5.15 | 131.8 | 138.6 | 5.20 |
| Overall | 113.4 | 119.7 | 3.50 | 113.8 | 119.3 | 3.58 |
| RESP (breaths/min)[3] | | | | | | |
| D 1 | 61.9 | 60.5 | 2.96 | 65.9[a] | 56.5[b] | 2.98 |
| D 2 | 57.5 | 58.9 | 2.96 | 61.1 | 55.3 | 2.98 |
| D 3 | 58.7 | 62.5 | 2.96 | 59.5 | 61.7 | 2.98 |
| D 13 | 64.1 | 62.5 | 2.96 | 66.3 | 60.3 | 2.98 |
| Overall | 60.6 | 61.1 | 2.31 | 63.2 | 58.5 | 2.33 |

[1]A 2 × 2 factorial arrangement was used with factors being method of castration and pain mitigation. Method of castration was either: band castration or surgical castration. Pain mitigation was provided with a multimodial analgesia approach (**MMA**) which consisted of subcutaneous ketamine stun and local lidocaine block at time of castration (d 0) and intravenous flunixin meglumine at d 0, 1, 2 and 3.

[2]There was a method × MMA interaction ($P < 0.05$), but no method × MMA × d interaction ($P > 0.10$) for TEMP. Therefore, overall least square mean TEMP by main effect of castration method and presence of MMA were not reported.

[31]There was no ($P > 0.10$) method × MMA presence interaction for HR or RESP. Therefore the main effects of method and MMA presence have been reported.

[a,b]Within each main effect (method of castration or presence of analgesia), means without common superscripts differ ($P < 0.05$).

## Literature Cited


Abrahamsen, E.J. 2008. Chemical restraint in ruminants. Vet. Clin. Food Anim. 24:227-243.

Cahn C.M. and S. Line. 2005. The Merck Veterinary Manual. 9th ed., Whitehouse Station, NJ.

Coetzee, J.F., R. Gehring, J. Tarus-Sang, and D.E. Anderson. 2010a. Effect of sub-anesthetic xylazine and ketamine ('ketamine stun') administered to calves immediately prior to castration. Vet. Anaes. and Analg. 37:566-578.

Coetzee J.F., A.L. Nutsch, L.A. Barbur, and R.M. Bradburn. 2010b. A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. BMC Vet. Res. 6:12.

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. 50:375-382.

Faulkner, D.B., T. Eurell, W.J. Tranquilli, R.S. Ott, M.W. Ohl, G.F. Cmarik, and G. Zinn. 1992. Performance and health of weanling bulls after butorphanol and xylazine administration at castration. J. Anim. Sci. 70:2970-2974.

Fajt, V.R., S.A. Wagner, and B. Norby. 2011. Analgesic drug administration and attitudes about analgesia in cattle among bovine practitioners in the United States. J.A.V.M.A. 238:755-767.

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. 74:2336-2343.

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. Aus. Vet. J. 79:279-284.

González, L.A., K.S. Schwartzkopf-Genswein, N.A. Caulkett, E. 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, Escherichia coli, and salivary cortisol. J. Anim. Sci. 88:802-810.

Gorossino, K. 2011. Feed and meal events. Technical services. Growsafe Systems Ltd. Airdrie.

Gruber, S.L., J.D. Tatum, T.E. Engle, P.L. Chapman, K.E. Belk, and G.C. Smith. 2010. Relationships of behavioral and physiological symptoms of preslaughter stress to beef longissimus muscle tenderness. J. Anim. Sci. 88:1148-1159.

Lamont, L.A.. 2008. Multimodial pain management in veterinary medicine: the physiologic basis of pharmacologic therapies. Vet. Clin. Small Anim. 38:1173-1186.

Thüer, S., S. Mellema, M.G. Doherr, B. Wechsler, K. Nuss, and A. Steiner. 2007. Effect of local anaesthesia on short and long-term pain induced by two bloodless castration methods in calves. Vet. J. 173:333-342.

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 the model. J. Diary Sci. 93:3602-3609.

USDA. 2000. NAHMS Part I: Baseline reference of feedlot management practices, 1999. Accessed 25 May 2012. http://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot99/Feedlot99_dr_PartI.pdf

USDA. 2008. NAHMS Beef 2007-08, Part I: Reference of beef cow-calf management practices in the United States, 2007-08. Accessed 25 May 2012. http://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/downloads/beef0708/Beef0708_dr_PartI_rev.pdf

USDA. 2009. National Agricultural Statistics Service. Agricultural Statistics 2009. Accessed 5 July 2011. http://usda.mannlib.cornell.edu/usda/nass/Catt//2000s/2009/Catt-07-24-2009.pdf

Worrell, M.A., D.C. Clanton, and C.R. Calkins. 1987. Effect of weight at castration on steer performance in the feedlot. J. Anim. Sci. 70:2970-2974.

CHAPTER III

## Impact of castration and oral meloxicam administration on feedlot performance and behavioral and physiological responses in cull beef bulls

**SUMMARY:** Two experiments evaluated the effects of band castration, and oral administration of an analgesic in association with castration on performance and behavioral and physiological responses in yearling beef bulls. In Exp. 1 Angus and Charolais-crossbred bull calves (n = 127; 309.8 ± 59.04 kg) and in Exp. 2 Hereford, Angus, and Hereford × Angus crossbred bulls (n = 30; 300.8 ± 4.96 kg), were stratified by BW and randomly assigned to 1 of 3 treatments: 1) band castration (**BAND**), 2) band castration with oral administration of meloxicam (**BAND-MEL**), and 3) sham castration (**SHAM**). The BAND and SHAM procedures were completed on d 0. The SHAM treatment consisted of all animal manipulations associated with band castration without band application. Meloxicam was administered on d -1, 0, and 1 (1.0 mg/kg, 0.5 mg/kg and 0.5 mg/kg, respectively) via an oral bolus. Body weight and a subjective chute score (**CS**) were collected on d -1, 0, 1, 7, 14, 21 and 28 (Exp. 1 only). In Exp. 1, jugular blood samples were collected immediately before castration and 24 hr post-castration for Substance P (**SP**) analysis. In Exp. 2, video documentation on d 0 was used to determine range of vertical head motion (**DIST**) on a subset of animals during treatment administration. In both experiments, ADG was similar ($P > 0.10$) between BAND and BAND-MEL, but ADG in SHAM cattle was greater ($P < 0.001$) and tended ($P = 0.07$) to be greater than castrates in Exp. 1 and 2, respectively. In Exp. 1, CS did not differ ($P > 0.10$) between BAND and BAND-MEL on any d, but castrates exhibited less desirable CS on d 1 and 28 than SHAM cattle. In Exp. 2, CS was not affected ($P > 0.10$) by castration or the presence of meloxicam. In Exp. 1, DIST did not differ ($P > 0.10$) between

BAND and BAND-MEL, but when pooled, castrates exhibited greater ($P$ = 0.04) DIST than SHAM. In Exp. 1, plasma SP concentrations were similar ($P$ > 0.10) between BAND and BAND-MEL, and castrates vs. sham cattle. Results indicate no impact of meloxicam administration on performance or behavioral and physiological responses to band castration. However, there was an impact of castration on ADG and DIST.

**Key words**: Band castration, Beef cattle, Meloxicam, Welfare

## Introduction

Castration of beef bulls is a common practice in the United States with over 15 million castrations occurring each year (USDA, 2009). With growing concern for animal welfare amongst consumers (Rollins, 2004; Broom, 2010), there has become a need to address not only means of mitigating pain associated with potentially painful practices, but also development of reliable techniques to identify pain and stress in livestock. Substance P (**SP**) has proven to be effective to identify acute pain response. A previous study showed higher peak plasma SP in surgically castrated 4 to 6 mo-old beef calves when compared to calves that underwent simulated castration (Coetzee et al., 2008). Band castration is commonly implemented in beef herds, nonetheless, it is predicted be a painful procedure as a previous study appreciated prolonged wound formation and decreased ADG when compared to both surgical castrates and intact bulls (Fisher et al., 2001).

A previous study examining the effects of a caudal xylazine epidural and intravenous flunixin meglumine revealed decreased stride length and more frequent painful behaviors in non-

medicated band castrates (Currah et al., 2009). While flunixin meglumine is the only non-steroidal anti-inflammatory drug (**NSAID**) in the United States approved for food animals, legal extralabel use of certain NSAID's is gaining support in the veterinary community (Smith et al., 2008). Previous studies have shown welfare benefits of oral administration of the NSAID meloxicam. When studied at the time of surgical castration in high risk beef calves, oral meloxicam treatments exhibited reduced pen-level first-pull-rate versus those that did not (Coetzee et al., 2011). Given the chronic nature of band castration-induced wounds, the current study aimed to test the hypothesis of whether: 1) an NSAID with a longer half-life, such as meloxicam (Coetzee et al., 2009), could mitigate painful response to band castration in cull bulls, and 2) band castration diminishes performance and induces adverse behavioral and physiological response when compared to intact males.

## Materials and Methods

All animal handling and procedures were approved by the Colorado State University Institutional Animal Care and Use Committee.

### *Experiment 1.*

*Animals and Treatments.* Angus and Charolais crossbred bull calves (n = 127; 309.8 ± 59.04 kg) were weaned at 240 ± 23.4 d of age. All bulls were weighed, vaccinated, dewormed and assigned a radio frequency identification (**RFID**)-linked ear tag before being shipped 51.8 km to a feeding facility. Animals were received in large backgrounding pens for 48 hr and

received an ad libitum diet of long-stem-grass hay and top dress of the TMR cattle received on trial. On d -5, bulls were processed and assigned to 7-head pens. Pens were stratified by BW and randomly assigned to 1 of 3 treatments: 1) band castration (**BAND**), 2) band castration with oral administration of meloxicam (**BAND-MEL**), and 3) sham castration (**SHAM**). While on trial, cattle received a diet of corn silage, cracked corn, dried distillers grain, wheat straw, mineral supplement, calcium carbonate and monensin (Rumensin, Elanco Animal Health, Indianapolis, IN) in a TMR. Every morning orts were collected from each pen and weighed for pen-level DMI and bunk calls. Feed was delivered once daily with adjustments to each pen's delivery to achieve near ad libitum feed supply.

The BAND and SHAM procedures were completed on d 0. Band castrations were completed by securing a latex band around of the neck of the scrotum to cause subsequent necrosis of the scrotum and testis using a banding tool (Calicrate Bander, No Bull Enterprises, St. Francis, KS). Sham bulls were subjected to manipulation of the scrotum associated with castration, but without band application.

Castrates receiving meloxicam were administered 1.0 mg/kg, 0.5 mg/kg and 0.5 mg/kg on d -1, 0, and 1, respectively, by rounding to the nearest meloxicam tablet (Meloxicam 15 mg, Zydus Pharmaceuticals, Pennington, NJ). Tablets were then encapsulated in a porcine gelatin bolus (Torpac Inc., Fairfield, NJ) and administered *per os* via a stainless steel balling gun. Both SHAM and BAND cattle were given a placebo of an empty gelatin capsule.

*Blood Collection.* Coccygeal blood samples were collected in 6 mL lithium heparinzed vaccuatiner tubes from 3 bulls per pen on d 0, 1, and 8. Plasma was collected by centrifuging at 2,000 × g and upon which samples were stored at -20º C.

Plasma concentrations of meloxicam were determined using high-pressure liquid chromatography (Surveyor MS Pump and Autosampler, Thermo Scientific, San Jose, CA) with mass spectrometry detection (TSQ Quantum Discovery MAX, Thermo Scientific). Plasma samples, plasma spikes, and plasma (**QC**) samples, 0.200 mL, were treated with 30% perchloric acid (20 µL) after addition of 40 ng/mL of the internal standard piroxicam. The samples were vortexed for 5 s and centrifuged for 20 min at 2,500 × *g* to sediment the precipitate. A portion of supernatant, 80 µL, was transferred to an injection vial fitted with a glass insert containing 120 µL of 1.9% ammonium hydroxide in 25% aqueous acetonitrile. The injection volume was set to 12.5 µL. The mobile phases consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile at a flow rate of 0.225 mL/min. The mobile phase began at 25% B with a linear gradient to 95% B at 7 min, which was maintained for 1.5 min, followed by re-equilibration to 25% B. Separation was achieved with a solid-core C18 column (KinetexXB -C18, 100 mm × 2.1 mm, 2.6 µm particles, Phenomenex, Torrance, CA) maintained at 40°C. Piroxicam eluted at 4.85 min and meloxicam at 5.95 min. Four selected reaction monitoring (**SRM**) transitions were monitored for meloxicam and 3 SRM transitions were used with the internal standard, piroxicam. The quantifying ions for meloxicam were 72.99, 88.01, 114.99, and 140.98 m/z and 77.97, 94.98, and 120.98 m/z for piroxicam. Sequences consisting of plasma blanks, calibration spikes, QC samples, and bovine plasma samples were batch processed with a processing method developed in the Xcalibur software (Thermo Scientific). The processing method automatically identified and integrated each peak in each sample and calculated the calibration curve based on a weighted (1/X) linear fit. Plasma concentrations of meloxicam in unknown samples were calculated by the Xcalibur software based on the calibration curve. Results were then viewed in the Quan Browser portion of the Xcalibur software. The standard curve in bovine plasma was

linear from 0.005 to 10.0 µg/mL. The correlation coefficient exceeded 0.995 and all measured values were within 15% of the actual values with most of the values less than 5% difference from the actual values. The accuracy of the assay for meloxicam in bovine plasma was 99 ± 3% of the actual concentration while the coefficient of variation was 5% determined on 4 sets of replicates for each of the following concentrations: 0.015, 0.15, and 1.5 µg/mL.

*Behavioral Measurements.* Upon restraint, an evaluator assigned a subjective chute score (**CS**) by marking a 15-cm long line scale as described by Gruber et al. (2010). Marks were converted to values on a 0 to 5 line scale (0 = calm, 5 = aggressive) which were aimed to be indicative of the animals response to restraint (Gruber et al., 2010). Rectal temperature (**TEMP**) was collected after CS was determined on each d. All animals were weighed in a hydraulic crush chute (Silencer Inc., St. Francis, KS) on d -5, -1, 0, 1, 8, 14, 21 and 28 and assigned a CS.

Objective exit velocity (**EV**) was collected on d -5, -1, 1, 8, 14, 21 and 28 using an infrared laser timing system (Farmtek Inc., Wylie, TX) in Exp. 1. One set of sensors was placed 1.892 m beyond the head catch, while the second set was placed 1.892 m beyond the first set. Exit velocity was calculated from the time elapsed between the 2 sets of sensors and reported in m/s.

In Exp. 1, TEMP was collected on d -1, 0, 1, 8, 14, 21 and 28. Rectal temperature was collected after CS was determined.

Video documentation also occurred at a pen-level for 3-d before castration, on d 0 (immediately post-castration), and for 3-d post castration, for 2 hrs in the morning and 2 hrs in the evening. Video footage was taken every 15 min of individual 15 s clips for each pen. These clips were retrospectively analyzed by a third party blinded to all treatments and study design.

Data were reported as proportion of the pen (animals clearly distinguishable in the frame) standing, walking, lying lateral, lying sternal, at the feedbunk and at the water tank.

*Statistical Analyses.* Experimental unit for Exp. 1 was pen. Weekly averages for DMI were analyzed in a mixed repeated measures model (PROC GLIMMIX, SAS Institute, Cary, NC). Pre-castration DMI did not differ ($P > 0.10$) across treatments, therefore a pre-castration DMI value was not included as a covariate. Rectal temperature and ADG were studied in a repeated measures mixed model using mean values from d -1 and 0 as covariates. In a similar fashion, CS and EV were studied, though mean d -5 and -1 values were used as covariates. Within repeated measures models, time was considered a factor and examined for interactions. Random effect was pen within treatment.

Average G:F was analyzed using mean ADG divided by mean trial DMI·hd$^{-1}$·d$^{-1}$ with average d -1 and 0 BW used as a covariate in a mixed model (PROC GLIMMIX, SAS Institute).

Proportion of animals within a pen was determined by comparing the number of animals participating in a given activity divided by the total number of animals that could be distinguished in each frame of the footage. These proportions were studied in a generalized linear mixed repeated measures model, which applied a logit transformation. Numeric mean proportions were reported, though transformed values were used in simple comparisons (PROC GLIMMIX, SAS Institute). Orthogonal contrasts were completed by contrasting BAND and BAND-MEL, and then pooled castrate values were compared to SHAM for all response variables (LSMESTIMATE, SAS Institute).

***Experiment 2.***

Purebred Hereford and Angus, and Hereford × Angus crossbred bulls (n = 30, 300.8 ± 4.96 kg) were stratified by BW and randomly assigned to 1 of 3 treatments: 1) band castration (**BAND**), 2) band castration with oral administration of meloxicam (**BAND-MEL**), and 3) sham castration (**SHAM**). Bulls were housed in 3 adjacent 10-head feedlot pens and were fed an ad libitum diet of alfalfa hay, corn silage, wheat straw, whole corn and mineral supplement in a TMR.

The BAND and SHAM procedures were completed on d 0 as described in Exp. 1. Castrates receiving meloxicam were administered 1.0 mg/kg, 0.5 mg/kg and 0.5 mg/kg on d -1, 0, and 1, respectively as described in Exp. 1.

*Blood Collection.* Jugular blood samples were collected using 18 gauge 3.81 cm sterile needles and a syringe on d 0, 1 and 7. Blood was then transferred immediately into a sterile 6 mL lithium heparinized vacutainer tube and immediately place on ice for transport for processing. Plasma meloxicam was analyzed using the same method as in Exp. 1.

Blood samples for SP were injected in 10 mL EDTA vaccutainer tubes which were prepared with a benzamidine so that, with blood, the concentration was 1mM benzamidine. After benzamidine was added, tubes remained on ice until blood injection. Blood samples were immediately centrifuged at 1,700 × *g* chute-side and plasma was immediately placed on ice until transport within 3 hrs to a -80º C freezer until analysis. Analysis was conducted as noted previously by Coetzee et al. (2008).

*Behavioral Measurements.* Upon restraint in a hydraulic crush chute (Silencer Inc.), a subjective CS was assigned as described in Exp. 1. Rectal temperature was collected after CS was determined on each d and bulls were weighed. These data were collected on d -1, 0, 1, 7, 14 and 21.

Video documentation at the time of castration or sham treatment was collected on a subset of bulls. Video data were studied using video analysis software (Dartfish INC., Alpharetta, GA). This was a modified version of collection as noted by Schartzkopf-Genswein et al. (1998), in which they examined the effect of hot-iron branding, freeze branding and sham branding on head movement and velocity. The current experiment examined the maximum vertical range of head movement during the procedure as noted by differences in highest and lowest points of the nose in the frame of the video (Figure 3.1).

*Statistical Analyses.* Individual animal was the experimental unit. A repeated measures analysis of covariance was used with mean d -1 and d 0 values used as covariates to study TEMP, SP, and BW (PROC MIXED, SAS Institute). Mean ADG was calculated using average BW for d -1 and 0 as the initial value. Plasma meloxicam values were presented as least square means per treatment group. Rectal temperature and CS were examined over time by studying least squares mean value differences across treatments on a given d by including numeric d in the model statement. When there were no differences ($P > 0.10$) across treatments of castrations, orthogonal contrasts were completed for all response variables, contrasting BAND and BAND-MEL, and pooled castrate values and SHAM values. Analysis of DIST data was conducted using a Tukey's adjustment for unbalanced sampling (PROC MIXED, SAS Institute), as a greater proportion of animals documented were castrated. If values associated with BAND and BAND-MEL were similar ($P > 0.10$), values were averaged and compared to SHAM using orthogonal contrast (LSMESTIMATE, SAS Institute).

## Results

### *Experiment 1.*

Average daily gain did not differ between BAND and BAND-MEL ($P > 0.10$). However, when pooled and compared to SHAM, SHAM bulls exhibited greater ADG ($P = 0.0003$; Table 3.1) than banded bulls.

There were no differences ($P > 0.10$) for DMI between BAND and BAND-MEL, or between castrates and sham cattle. When wk was included in the model, mean DMI was greater ($P = 0.01$) for SHAM cattle than BAND and BAND-MEL treatments. At no point did DMI differ ($P > 0.10$) between BAND and BAND-MEL (Table 3.1).

There were no differences ($P > 0.10$) between BAND and BAND-MEL for G:F. However, SHAM cattle exhibited greater G:F than castrates throughout the trial (Table 3.1).

Plasma meloxicam concentrations for BAND-MEL were 1,402.1± 22.14 ng/mL, 913.3 ± 18.59 ng/mL, and 1.0 ± 1.77 ng/mL for d 0, 1 and 8 respectively (Table 3.1).

Overall CS did not differ ($P > 0.10$; Table 3.1) between BAND and BAND-MEL, nor did it differ when castrates were pooled and compared to SHAM. When d was included in the model, castrates exhibited less desirable CS than SHAM on d 1 ($P = 0.01$), and 28 ($P = 0.03$). Differences between castrates and SHAM on d 1 may be attributed to the temporal proximity of castration (d 0), but this would likely not explain the differences seen 28 d post castration.

Overall EV tended ($P = 0.06$; Table 3.1) to be greater for castrates than SHAM. By d, there was a tendency ($P = 0.06$) for castrates to exhibit greater EV than SHAM on d 8 and 14. On

d 28, castrates exhibited greater ($P = 0.02$) EV than SHAM. Meloxicam did not impact EV at any point ($P > 0.10$) during the trial.

Mean TEMP did not differ ($P > 0.10$) between BAND and BAND-MEL, nor did castrates differ ($P > 0.10$) from SHAM. When d was included in the model, there were no differences ($P > 0.10$) on any given d measured (Table 3.1).

Pen level video footage on d 0 revealed a greater proportion ($P = 0.03$) of animals at the bunk in a pen for SHAM treatments vs. castrates at 15 min post castration. Percentage of cattle at the bunk did not differ ($P > 0.10$) between castrates and SHAM at any other time. Meloxicam treatment had no effect ($P > 0.10$; Table 3.2) at any time point within 120 min post-castration on pen bunk behavior. Average percentage of cattle at the bunk on d 1 was greater ($P < 0.001$; Table 3.3) for BAND than BAND-MEL, but percentage of cattle at the bunk did not differ ($P > 0.10$) between pooled castrate values and SHAM. That said, on d 1, a greater ($P = 0.01$) percentage of SHAM cattle were at the bunk than BAND-MEL, while a greater ($P = 0.001$) percentage of BAND cattle were at the bunk than SHAM cattle (data not reported).

Percentage of animals at the water tank immediately post castration did not differ between BAND and BAND-MEL, or between castrates and SHAM cattle at any time points on d 0 or on d 1, 2, or 3 ($P > 0.10$; Tables 3.2 and 3.3).

When percentage of pen standing in the frame of the video was examined, a greater ($P = 0.02$) proportion of BAND-MEL cattle were standing than BAND cattle immediately post-castration (Table 3.2). There were no other differences between BAND and BAND-MEL on d 0. Castrates and SHAM were similar ($P > 0.10$) in percentage of cattle standing in the pen at every time point throughout the 120 min observation period on d 0. On d 1, BAND-MEL exhibited a greater percentage of cattle standing than BAND. There was no difference ($P > 0.10$) between

pooled castrate values vs. percentage of SHAM standing. Similarly on d 2, a greater ($P < 0.001$; Table 3.3) percentage of BAND-MEL cattle were standing than BAND cattle. On d 1, 2, and 3 BAND-MEL exhibited a greater percentage of cattle standing than SHAM. On d 3, SHAM castrates exhibited a greater ($P < 0.001$) percentage of cattle standing than pooled castrates.

There were no differences ($P > 0.10$; Table 3.2 and 3.3) in percentage of cattle in a pen walking between BAND and BAND-MEL, or castrates and SHAM at any time point on d 0, or on d 1, 2, or 3.

Cattle lying in the pen were divided into sternal and lateral classification. Percentage of cattle in the sternal position was greater ($P = 0.03$; Table 3.2) in castrates than SHAM at 60 min post-castration. There was also a tendency ($P = 0.08$) for castrates to exhibit sternal lying at a greater rate at 30 min post-castration than SHAM. Sternal response was similar ($P > 0.10$) for BAND and BAND-MEL throughout the 120 min period post castration on d 0. On d 1, a greater ($P = 0.03$; Table 3.3) percent of SHAM cattle were sternal recumbent than castrates. There was a tendency ($P = 0.08$) for SHAM cattle to have a greater percentage of cattle sternal recumbent than castrates on d 2.

Percentage of cattle exhibiting lateral recumbence was greater in castrates than SHAM treatments at 60 min post castration. There was a tendency for a greater ($P = 0.08$, $P = 0.06$, respectively; Table 3.2) percentage of castrates at 30 min and 120 min post castration to be lateral recumbent. There were no differences ($P > 0.10$) between BAND and BAND-MEL for lateral recumbence during the 120 min period post-castration. On d 1, castrates exhibited a greater ($P = 0.04$; Table 3.3) percentage of cattle that were lateral recumbent. Percentage of cattle lateral recumbent did not differ ($P > 0.10$) on d 2 or 3.

***Experiment 2.***

Average daily gain did not differ across treatments ($P = 0.17$, Table 3.4). However, when contrasted with pooled values of castrates, SHAM bulls had a tendency ($P = 0.07$) to exhibit greater ADG. Both groups of castrates experienced negative ADG over the 21 d trial, while SHAM bulls experienced positive gain.

Plasma SP values did not differ across treatments ($P = 0.88$, Table 3.5). Plasma meloxicam concentrations in BAND-MEL were 1,518.0 ± 86.85 ng/mL, 1,443.6 ± 91.77 ng/mL, and 1.4 ± 0.55 ng/mL for d 0, 1, and 7 respectively (Table 3.5). No meloxicam was detected in blood samples from either BAND or SHAM on d 0 or d 7, Plasma meloxicam was greater in BAND-MEL than BAND ($P < 0.001$) and SHAM ($P < 0.001$) on d 0. Plasma meloxicam samples for d 1 were not collected for BAND and SHAM treatments, thus comparisons could not be made on d 1. These concentrations are consistent with accepted therapeutic levels in calves (Coetzee et al., 2009; Coetzee et al., 2011).

Chute score did not differ ($P > 0.10$, Table 3.5) across BAND and BAND-MEL, or between castrates and SHAM. Furthermore, when treatment × d interactions were examined, neither BAND and BAND-MEL nor castrates and SHAM on a given d differed ($P > 0.10$), though there was a d effect ($P < 0.05$) independent of treatment (Table 3.5). Subjective CS, as used in the current study, has only been studied in the performance of beef cattle as a measure of disposition. Gruber et al. (2010) noted differences in carcass and feedlot performance associated with CS, but there is no evidence in the literature that this measurement has been used before in association with castration. These similarities could likely be a function of band castration's minimal impact, or it may also be attributed to the fact that these animals had been well

acclimated to handling by the time of castration and subsequently did not exhibit differences in CS response.

Video analysis revealed that when compared to SHAM cattle, castrates exhibited greater DIST ($P < 0.05$), while DIST did not differ ($P > 0.10$) between BAND and BAND-MEL (Table 3.4).

Mean TEMP did not differ across treatments ($P > 0.10$, Table 3.5), but there was a treatment × d interaction ($P = 0.001$, Table 3.5). On d 14, banded cattle exhibited greater ($P = 0.004$) TEMP than SHAM. On d 14, BAND and BAND-MEL did not differ ($P > 0.10$). On d 21, TEMP was greater ($P = 0.03$) for BAND-MEL than BAND. As well, SHAM exhibited greater TEMP than BAND and BAND-MEL ($P = 0.001$, $P = 0.07$, respectively) on d 21. On any other day, TEMP did not differ across treatments ($P > 0.10$). Differences on d 14 may indicate that by d 14 band castration increased inflammatory mediators to cause a rise in TEMP. Day 21 results are likely unrelated to the castration procedure.

## Discussion

Results from both Exp. 1 and 2 indicate no impact of oral meloxicam on ADG. Exp. 1 revealed that castration does have an adverse impact on ADG in beef bulls, with SHAM bulls exhibiting greater ADG than castrates. These results concur with findings of a previous study examining oral meloxicam at surgical castration (Coetzee et al., 2011) where meloxicam had no impact on ADG. Though our results were unique with castrates exhibiting a loss of BW in Exp. 2, in previous studies castrates exhibited reduced ADG when compared to intact bulls (Fisher et al., 2001). Though significant differences were not found for ADG within Exp. 2, the results of

Exp. 1 suggest this was a function of sample size being insufficient for a 21-d feeding period utilized in Exp. 2.

Similarities in DMI suggest that neither castration nor oral administration of meloxicam impacted overall DMI. That said, castrates exhibited reduced DMI in the fourth week of the trial, but at no point did BAND and BAND-MEL differ, suggesting no impact of oral meloxicam in association with castration on DMI. Exp. 1 of the current study utilized pen level DMI for 28 d post-castration. Future studies utilizing RFID-linked feedbunks would allow for individual bull to be the experimental unit, while also providing feeding behaviors beyond DMI. These feeding behaviors could help determine the impact of pain mitigation in association with castration on parameters beyond commonly accepted performance traits.

In Exp. 2, SP, a neuropeptide that has shown promise in objectively identifying painful response (Coetzee et al., 2008), did not reveal differences between band castration and sham. Nor was there an impact of oral meloxicam. The similar SP values may be accredited to collection interval used in the current study, as previous studies examined SP associated with surgical castration collected 10 times over 4 hrs post-castration (Coetzee et al., 2008). The current study used a greater collection interval (immediately before castration and 24 hr after) with the hypothesis that band castration would induce a delayed SP response relative to surgical castration as used in the previous study (Coetzee et al., 2008). Though the hypothesis, to the best of our knowledge, has not been tested, another explanation for the similarity may be that band castration does not elicit as marked a response in SP concentration as surgical castration in bulls. Additionally, given the instability of this neuropeptide, there is potential that SP was degraded during processing of the samples, though all samples would have undergone the same degradation. Beyond these explanations, there is a potential that the sample size utilized did not

have adequate statistical power to control Type II error given that, to our knowledge, no other studies have examined SP in response to band castration.

Future studies associated with SP and band castration are needed to determine the most advantageous time interval to isolate peaks in substance P response. Onuoha and Alpar (1999) studied healthy human controls and humans experiencing some variety of acute soft tissue damage and found that injured patients exhibited 27-fold greater SP than healthy controls. The current study did not reveal similar results in regards to band castrates vs. SHAM. However, this may be because band castration does not elicit tissue damage analogous to the human subjects in the previous study, thus not inducing significantly higher SP concentrations.

Bioavailability of oral meloxicam, as evident with plasma concentrations, appeared to adequately achieve therapeutic levels in cull beef bulls. This was verified by comparing plasma meloxicam levels to median plasma meloxicam levels in a previous study that administered 1.0 mg/kg oral meloxicam (Coetzee et al., 2009). The authors used a small sample size (n = 6) of Holstein calves, but found similar median plasma meloxicam concentration as the current study. Though in the current study meloxicam had a negligible impact on bulls at castration, there may be further application outside the realm of band castration.

Head movement associated with castration in the current study revealed that castration, regardless of meloxicam administration, induced a greater resistance behavior than sham castration. A previous study noted differences in DIST across method of branding (hot-iron, freeze, and sham) with hot-iron branding eliciting the greatest DIST response (Schwartzkopf-Genswein et al., 1998). Though the current study examined castration, we can assume that DIST is indicative of the magnitude of pain response. Results of the current study suggest that meloxicam does not impact DIST. Perhaps even if peak plasma meloxicam concentration was

achieved, though we can only extrapolate in the current study, meloxicam's anti-inflammatory effect was limited in the mitigation of acute pain associated with application of a band. Further investigation into painful behaviors and means of alleviating said behaviors will be critical in approving new drugs for pain mitigation.

Video documentation for 120 min post-castration revealed an impact of castration on percentage of animals at the bunk 15 min post-castration, and on percentage of bulls sternal recumbent 60 min post-castration. Oral meloxicam administration had no impact on pen-level behaviors documented at any point during the 120 min post-castration observation period. Perhaps a greater number of animals within each pen or evaluating on an individual-animal level in future studies would allow for differences to be observed. A previous study using Angus bulls (n = 46) and steers (n = 43) as controls, examining the effect of xylazine epidural and flunixin meglumine in association with band castration, found no difference between main effect of castration or main effect of medication on percent of cattle lying (Gonzalez et al., 2009). Similarly, the current study found no effect of medication on percentage of cattle lying in the pen, though certain time points revealed differences in the percentage of castrated cattle lateral recumbent vs. SHAM. Gonzalez et al. (2009) found rear leg step length post-castration was greater in both previously castrated steers and across the main effect of medication, but this analysis was conducted on a total of 8 animals/treatment. The current study was not able to document step-length with sufficient accuracy; therefore no step-length was measured.

Pen level video data revealed BAND-MEL cattle exhibited a smaller percentage of cattle at the feedbunk on d 1, 2, and 3 than both BAND and SHAM. These data were not reaffirmed by DMI differences in wk 1, assuming that there was a compensatory effect that equilibrated by d 7.

Percentage of cattle at the water tank and walking did not differ across meloxicam treatment or castration. These similarities suggest these behaviors are not valuable in defining differing behavioral patterns in association with band castration and oral meloxicam administration at the time-frame observed in the current study.

The greater percentage of lateral recumbence in castrates on d 1 vs. SHAM indicates that at 24 hr post-castration there is a behavioral impact of castration. Lateral recumbence may be a response to discomfort or pain, but the current study has no means of making this conclusion.

Previous studies investigating mulesing of Merino lambs performed pen-level observations after mulesing or sham in association with meloxicam or tolfenamic acid (Paull et al., 2008). Like castration, mulesing is a husbandry practice scrutinized for its impact on animal pain. The authors found that mulesed lambs, regardless of NSAID treatment, displayed similar behaviors in the pen (i.e. lying sternal, lying lateral, standing or walking). Mulesed lambs, regardless of NSAID treatment, also spent a greater portion of time standing hunched, as compared to sham lambs that spent a greater portion of time lying. The previous study administered NSAID's at the time of castration or 45 min prior to mulesing. This time interval may not have been adequate to prevent initial inflammatory response to mulesing-induced tissue damage. As well, both tolfenamic acid and meloxicam are cyclooxygenase (**COX**) 2 specific NSAID's (embody a favorable COX 2:1 ratio) which are advantageous in avoiding adverse gastrointestinal problems (Griswold and Adams, 1996). However, one would assume there would be adequate analgesia later in the observation period, though hyperalgesia (pain proliferation) may have already taken place by the time of peak drug levels.

Both Exp. 1 and 2 in the current sized utilized a sample size of weaned cattle with greater BW than most previously published research. Although feeder calves being castrated post-

weaning is typically a function of managerial restraints, there is a large population of seedstock producers that cannot castrate before a bull's mature phenotype (e.g. weaning weight, post-weaning gain, and conformation) can be fully evaluated. Further studies examining the effect of age at castration is important for bettering our understanding of pain response to castration, but there is still a need to examine the effects of post-weaning castration to address this sector of the beef industry.

Similar to results of a previous study investigating oral meloxicam in association with surgical castration (Coetzee et al., 2011), the current study revealed no impact of oral meloxicam on ADG. The authors of the previous study found that meloxicam impacted some aspects of morbidity, but no behavioral or feedlot performance parameters when compared to other castrates.

Continuing to investigate the impact of pain mitigation in association with castration on behavior is imperative in addressing societal concerns over castration. Furthermore, the literature must grow in the field of pain mitigation in order to approve effective products for pain management at castration.


**Figure 3.1.** Video image sequence of vertical head positions in response to band castration with initial and lowest point of the nose (A), highest point of the nose (B), and a reference line and measurement of distance between the 2 points, indicating vertical head movement range (C). Video was examined using video analyzing software (Dartfish Inc., Alpharetta, GA).

**Table 3.1.** Least square means (± SEM) for ADG, DMI, G:F, subjective chute score (CS), exit velocity (EV), rectal temperature (TEMP), and plasma meloxicam concentration across time and treatment of castration and oral meloxicam (Exp. 1)

| | | Treatments[1] | | | | Contrasts *P* = | |
|---|---|---|---|---|---|---|---|
| | | BAND | BAND-MEL | SHAM | SEM | CAST[2] | MELOX[3] |
| ADG (kg/d) | | | | | | | |
| | Overall | 1.67 | 1.62 | 2.07 | 0.071 | 0.0003 | 0.62 |
| DMI (kg/d) | | | | | | | |
| | Wk 1 | 5.2 | 5.2 | 5.4 | 0.36 | 0.60 | 0.99 |
| | Wk 2 | 7.1 | 6.8 | 7.1 | 0.36 | 0.75 | 0.66 |
| | Wk 3 | 7.9 | 7.7 | 8.1 | 0.36 | 0.50 | 0.73 |
| | Wk 4 | 8.6 | 8.6 | 9.8 | 0.36 | 0.01 | 0.93 |
| | Overall | 7.2 | 7.1 | 7.6 | 0.33 | 0.25 | 0.84 |
| G:F | | | | | | | |
| | Overall | 0.21 | 0.20 | 0.28 | 0.010 | 0.0001 | 0.40 |
| CS[4] | | | | | | | |
| | d 1 | 3.62 | 3.91 | 2.77 | 0.321 | 0.01 | 0.54 |
| | d 8 | 4.05 | 3.77 | 3.37 | 0.321 | 0.18 | 0.55 |
| | d 14 | 3.62 | 3.10 | 3.90 | 0.321 | 0.18 | 0.26 |
| | d 21 | 3.12 | 3.28 | 2.93 | 0.321 | 0.49 | 0.72 |
| | d 28 | 5.18 | 4.70 | 4.00 | 0.321 | 0.03 | 0.30 |
| | Overall | 3.92 | 3.75 | 3.39 | 0.230 | 0.14 | 0.62 |
| EV[5](m/s) | | | | | | | |
| | d 1 | 2.84 | 2.68 | 2.51 | 0.138 | 0.18 | 0.40 |
| | d 8 | 2.79 | 2.79 | 2.44 | 0.138 | 0.06 | 0.99 |
| | d 14 | 2.80 | 2.66 | 2.38 | 0.138 | 0.06 | 0.48 |
| | d 21 | 2.60 | 2.67 | 2.49 | 0.138 | 0.42 | 0.66 |
| | d 28 | 3.56 | 3.47 | 3.06 | 0.138 | 0.02 | 0.62 |
| | Overall | 2.93 | 2.84 | 2.58 | 0.088 | 0.06 | 0.69 |
| TEMP[6](ºC) | | | | | | | |
| | d 1 | 39.49 | 39.46 | 39.29 | 0.108 | 0.18 | 0.86 |
| | d 8 | 37.98 | 37.99 | 38.06 | 0.108 | 0.58 | 0.90 |
| | d 14 | 38.85 | 38.81 | 38.94 | 0.108 | 0.41 | 0.77 |
| | d 21 | 39.02 | 38.97 | 38.81 | 0.108 | 0.19 | 0.74 |
| | d 28 | 39.35 | 39.27 | 39.26 | 0.108 | 0.73 | 0.56 |
| | Overall | 38.94 | 38.90 | 38.87 | 0.053 | 0.53 | 0.61 |
| Meloxicam[7](ng/mL) | | | | | | | |
| | d 0 | NC | 1402.1 | NC | 22.14 | - | - |
| | d 1 | NC | 913.3 | NC | 18.59 | - | - |
| | d 8 | NC | 1.0 | NC | 1.71 | - | - |

[1]BAND = band castration on d 0, BAND-MEL = band castration on d 0 with oral administration of meloxicam on d -1, 0, and 0, SHAM = sham castration.
[2]CAST = Contrast between pooled BAND and BAND-MEL values and SHAM values.
[3]MELOX = Contrast between BAND and BAND-MEL values.

[4]Upon restraint, cattle were assigned a subjective CS with 1 being calm and 5 being aggressive as noted in Gruber et al. (2010).
[5]Chute exit velocity was collected as the velocity exhibited from 1.892 m to 3.784 m beyond the head catch using an electronic infrared barrier system.
[6]Rectal temperature (**TEMP**) was collected on d -1, 0, 1, 8, 14, 21 and 28. LS means for d 1, 8, 14, 21 and 28 were calculated using d -1 and d 0 means as a covariate.
[7]Plasma meloxicam was collected on d 0, 1, and 8 from a subset of BAND-MEL bulls only.

**Table 3.2.** Least square means (%) ± SEM for activity in the pen using video footage collected at 15 min intervals for 2 hr immediately post-castration across treatment of castration and administration of oral meloxicam(Exp. 1)

| | Treatments[1] | | | | Contrasts[2] *P* = | |
|---|---|---|---|---|---|---|
| (min) | BAND | BAND-MEL | SHAM | SEM | CAST[4] | MELOX[4] |
| At Bunk | | | | | | |
| 0 | 13.5 | 26.1 | 8.3 | 11.59 | 0.49 | 0.50 |
| 15 | 7.7 | 26.8 | 58.5 | 12.87 | 0.03 | 0.30 |
| 30 | 23.7 | 19.4 | 52.5 | 14.92 | 0.12 | 0.83 |
| 45 | 10.0 | 25.6 | 52.5 | 13.88 | 0.08 | 0.44 |
| 60 | 3.3 | 11.1 | 33.3 | 10.95 | 0.14 | 0.58 |
| 75 | 0.0 | 21.6 | 39.5 | 10.02 | 0.99 | 0.99 |
| 90 | 22.2 | 23.1 | 55.3 | 14.82 | 0.10 | 0.97 |
| 105 | 27.0 | 41.9 | 47.4 | 16.54 | 0.52 | 0.53 |
| 120 | 9.6 | 23.8 | 40.6 | 15.87 | 0.23 | 0.51 |
| At Water | | | | | | |
| 0 | 0.0 | 0.0 | 0.0 | 0.00 | 1.0 | 1.0 |
| 15 | 0.0 | 0.0 | 0.0 | 0.00 | 1.0 | 1.0 |
| 30 | 0.0 | 0.0 | 2.5 | 0.45 | 0.99 | 1.0 |
| 45 | 3.3 | 0.0 | 0.0 | 0.59 | 0.99 | 0.99 |
| 60 | 0.0 | 2.8 | 0.0 | 0.50 | 0.99 | 0.99 |
| 75 | 0.0 | 0.0 | 0.0 | 0.00 | 1.0 | 1.0 |
| 90 | 0.0 | 0.0 | 2.6 | 0.47 | 0.99 | 1.0 |
| 105 | 0.0 | 3.2 | 2.6 | 1.05 | 0.99 | 0.99 |
| 120 | 0.0 | 0.0 | 3.1 | 0.56 | 0.99 | 1.0 |
| Standing | | | | | | |
| 0 | 24.3 | 11.9 | 55.6 | 11.20 | 0.39 | 0.02 |
| 15 | 41.0 | 41.5 | 39.0 | 12.78 | 0.89 | 0.98 |
| 30 | 42.1 | 41.9 | 40.0 | 13.56 | 0.90 | 0.99 |
| 45 | 40.0 | 38.5 | 25.0 | 12.99 | 0.37 | 0.94 |
| 60 | 40.0 | 33.3 | 59.0 | 13.60 | 0.74 | 0.19 |
| 75 | 44.4 | 18.9 | 18.4 | 11.57 | 0.43 | 0.17 |
| 90 | 41.7 | 23.1 | 18.4 | 11.71 | 0.38 | 0.30 |
| 105 | 32.4 | 35.5 | 26.3 | 12.91 | 0.87 | 0.63 |
| 120 | 38.7 | 9.5 | 40.6 | 13.13 | 0.28 | 0.19 |
| Walking | | | | | | |
| 0 | 59.5 | 52.4 | 36.1 | 10.49 | 0.14 | 0.64 |
| 15 | 28.2 | 12.2 | 0.0 | 5.43 | 0.99 | 0.19 |
| 30 | 5.3 | 6.5 | 0.0 | 3.55 | 0.99 | 0.87 |
| 45 | 10.0 | 7.7 | 0.0 | 4.30 | 0.99 | 0.80 |
| 60 | 3.3 | 8.3 | 2.6 | 4.60 | 0.63 | 0.54 |
| 75 | 5.6 | 0.0 | 18.4 | 4.46 | 0.99 | 0.99 |
| 90 | 0.0 | 5.1 | 0.0 | 1.56 | 0.99 | 0.99 |
| 105 | 0.0 | 0.0 | 0.0 | 0.00 | 1.0 | 1.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 120 | 9.7 | 0.0 | 3.1 | 3.70 | 0.99 | 0.99 |
| Lateral Recumbent | | | | | | |
| 0 | 0.0 | 2.4 | 0.0 | 0.70 | 0.99 | 0.99 |
| 15 | 5.1 | 2.4 | 0.0 | 1.77 | 0.99 | 0.49 |
| 30 | 2.6 | 9.7 | 0.0 | 2.35 | 0.99 | 0.20 |
| 45 | 6.7 | 2.6 | 0.0 | 2.11 | 0.99 | 0.39 |
| 60 | 16.7 | 16.7 | 0.0 | 3.87 | 0.99 | 1.0 |
| 75 | 11.1 | 24.3 | 0.0 | 3.65 | 0.99 | 0.11 |
| 90 | 5.6 | 5.1 | 2.6 | 2.96 | 0.47 | 0.99 |
| 105 | 10.8 | 9.7 | 0.0 | 3.10 | 0.99 | 0.87 |
| 120 | 6.5 | 23.8 | 0.0 | 4.07 | 0.99 | 0.06 |
| Sternal Recumbent | | | | | | |
| 0 | 2.7 | 7.1 | 0.0 | 2.89 | 0.99 | 0.51 |
| 15 | 20.5 | 17.1 | 2.4 | 6.42 | 0.11 | 0.76 |
| 30 | 26.3 | 22.6 | 5.0 | 7.87 | 0.08 | 0.78 |
| 45 | 30.0 | 25.6 | 22.5 | 9.55 | 0.64 | 0.76 |
| 60 | 36.7 | 27.8 | 5.1 | 8.61 | 0.03 | 0.56 |
| 75 | 38.9 | 35.1 | 23.7 | 9.95 | 0.28 | 0.80 |
| 90 | 30.6 | 43.6 | 21.1 | 9.67 | 0.20 | 0.38 |
| 105 | 29.7 | 9.7 | 23.7 | 8.58 | 0.58 | 0.14 |
| 120 | 35.5 | 42.9 | 12.5 | 10.98 | 0.06 | 0.68 |

[1]BAND = band castration on d 0, BAND-MEL = band castration on d 0 with oral administration of meloxicam on d -1, 0, and 0, SHAM = sham castration.
[2]Contrast *P*-values were calculated using log transformed values for percentage of cattle.
[3]CAST = Contrast between pooled BAND and BAND-MEL values and SHAM values.
[4]MELOX = Contrast between BAND and BAND-MEL values.

**Table 3.3.** Least square means (%) ± SEM for activity in the pen using video footage collected at 15 min intervals for 2 hr periods during the morning and evening on d 1, 2, and 3 post castration (Exp. 1)

| | | Treatments[1] | | | | Contrasts *P* = | |
|---|---|---|---|---|---|---|---|
| | | BAND | BAND-MEL | SHAM | SEM | CAST[2] | MELOX[3] |
| At Bunk | | | | | | | |
| | D 1 | 36.9 | 17.2 | 24.2 | 2.48 | 0.35 | 0.0001 |
| | D 2 | 34.2 | 24.3 | 19.8 | 2.45 | 0.002 | 0.0039 |
| | D 3 | 25.6 | 14.0 | 20.3 | 2.46 | 0.86 | 0.0007 |
| At Water | | | | | | | |
| | D 1 | 1.8 | 1.9 | 2.3 | 0.53 | 0.47 | 0.87 |
| | D 2 | 1.9 | 1.3 | 1.4 | 0.51 | 0.71 | 0.44 |
| | D 3 | 2.1 | 1.5 | 0.8 | 0.51 | 0.13 | 0.71 |
| Standing | | | | | | | |
| | D 1 | 32.9 | 49.0 | 38.4 | 2.74 | 0.44 | 0.001 |
| | D 2 | 28.2 | 37.8 | 36.9 | 2.70 | 0.25 | 0.01 |
| | D 3 | 30.1 | 42.3 | 40.8 | 2.73 | 0.001 | 0.17 |
| Walking | | | | | | | |
| | D 1 | 1.4 | 1.6 | 1.5 | 0.54 | 0.99 | 0.74 |
| | D 2 | 0.5 | 0.89 | 1.6 | 0.54 | 0.19 | 0.62 |
| | D 3 | 0.3 | 1.4 | 0.4 | 0.54 | 0.50 | 0.16 |
| Lateral Recumbent | | | | | | | |
| | D 1 | 1.9 | 1.5 | 0.3 | 0.55 | 0.04 | 0.57 |
| | D 2 | 1.5 | 2.7 | 1.3 | 0.55 | 0.28 | 0.12 |
| | D 3 | 2.1 | 1.4 | 0.8 | 0.55 | 0.20 | 0.38 |
| Sternal Recumbent | | | | | | | |
| | D 1 | 25.4 | 28.8 | 33.6 | 2.47 | 0.03 | 0.31 |
| | D 2 | 33.6 | 33.0 | 38.6 | 2.46 | 0.08 | 0.86 |
| | D 3 | 39.6 | 39.3 | 36.6 | 2.47 | 0.36 | 0.92 |

[1]BAND = band castration on d 0, BAND-MEL = band castration on d 0 with oral administration of meloxicam on d -1, 0, and 0, SHAM = sham castration.
[2]CAST = Contrast between pooled BAND and BAND-MEL values and SHAM values.
[3]MELOX = Contrast between BAND and BAND-MEL values.

**Table 3.4.** Least square means ± SEM of ADG, and vertical head movement (DIST) across treatment of castration and oral meloxicam (Exp. 2).[1]

| | Treatments | | | | Contrasts[3,4] *P* = | |
|---|---|---|---|---|---|---|
| | BAND | BAND-MEL | SHAM | SEM | CAST | Melox |
| ADG (kg/d) | -0.54 | -0.06 | 0.81 | 0.497 | 0.07 | 0.50 |
| DIST[4](m) | 1.25 | 1.39 | 0.65 | 0.213 | 0.04 | 0.57 |

[1]BAND = band castration on d 0, BAND-MEL = band castration on d 0 with oral administration of meloxicam on d -1, 0, and 0, SHAM = sham castration.
[2]CAST = Contrast between pooled BAND and BAND-MEL values and SHAM values.
[3]MELOX = Contrast between BAND and BAND-MEL values.
[4]Head movement at the time of castration (DIST) was determined using video analyzing software in which the greatest difference in nose position longitudinally was measure during band castration or sham castration.

**Table 3.5.** Least square means ± SEM for subjective chute score (CS), rectal temperature (TEMP), plasma substance P (SP) concentration and plasma meloxicam across time and treatment of castration and oral meloxicam (Exp. 2).[1]

| | Treatments | | | | Contrasts[6,7] *P* = | |
|---|---|---|---|---|---|---|
| | BAND | BAND-MEL | SHAM | SEM | CAST | MELOX |
| CS[2] | | | | | | |
| D -1 | 2.87 | 2.61 | 1.74 | 0.603 | - | - |
| D 0 | 4.15 | 4.27 | 3.76 | 0.537 | - | - |
| D 1 | 3.17 | 2.83 | 3.29 | 0.445 | 0.41 | 0.65 |
| D 7 | 2.32 | 2.52 | 2.71 | 0.413 | 0.61 | 0.88 |
| D 14 | 2.38 | 1.95 | 2.04 | 0.413 | 0.76 | 0.42 |
| D 21 | 1.61 | 1.92 | 1.74 | 0.413 | 0.97 | 0.48 |
| Overall | 2.40 | 2.36 | 2.46 | 0.213 | 0.60 | 0.84 |
| TEMP[3](ºC) | | | | | | |
| D -1 | 39.74 | 39.54 | 39.71 | 0.114 | - | - |
| D 0 | 39.59 | 39.66 | 39.59 | 0.116 | - | - |
| D 1 | 39.49 | 39.66 | 39.66 | 0.108 | 0.55 | 0.29 |
| D 7 | 39.72 | 39.98 | 39.78 | 0.108 | 0.59 | 0.09 |
| D 14 | 39.77 | 39.81 | 39.39 | 0.108 | 0.004 | 0.77 |
| D 21 | 39.57 | 39.91 | 40.19 | 0.108 | 0.0001 | 0.03 |
| Overall | 39.64 | 39.84 | 39.75 | 0.06 | 0.66 | 0.05 |
| SP[4](pg/mL) | | | | | | |
| D 0 | 143.05 | 158.69 | 166.23 | 17.7 | - | - |
| D 1 | 167.24 | 159.66 | 151.45 | 22.46 | 0.67 | 0.81 |
| Meloxicam[5] (ng/mL) | | | | | | |
| D 0 | 0 | 1,518.0 | 0 | 86.85 | - | 0.001 |
| D 1 | NC[8] | 1,443.6 | NC[8] | 91.77 | - | - |
| D 7 | 0 | 1.4 | 0 | 0.55 | - | - |

[1]BAND = band castration on d 0, BAND-MEL = band castration on d 0 with oral administration of meloxicam on d -1, 0, and 0, SHAM = sham castration.
[2]Subjective chute score (CS) was determined upon head restraint as previously noted by Gruber et al. (2010).

[3]Rectal temperature (TEMP) was collected on d -1, 0, 1, 7, 14 and 21. LS means for d 1, 7, 14 and 21 were calculated using d -1 and d 0 means as a covariate.
[4]Plasma substance P (SP) was collected on d 0 immediately before castration or sham castration and on d 1 upon restraint in the head catch. Samples were analyzed as completed previously by Coetzee et al. (2008). Means for d 1 were analyzed using d 0 values as a covariate.
[5]Plasma meloxicam samples were collected on d 0, d 1 and d 7, and analyzed using HPLC.
[6]CAST = Contrast between pooled BAND and BAND-MEL values and SHAM values.
[7]MELOX = Contrast between BAND and BAND-MEL values..
[8]Samples for plasma meloxicam concentration on d 1 for BAND and SHAM were not collected (NC).

## Literature Cited


Broom, D.M. 2010. Animal welfare: an aspect of care, sustainability, and food quality required by the public. J. Vet. Med. Ed. 37:83-88.

Coetzee, J.F., L.N. Edwards, R.A. Mosher, N.M. Bello, A.M. O'Connor, B. Wang, B. KuKanich, and D.A. Blasi. 2011. Effet of oral meloxicam on health and performance of beef steers relative to bulls castrated on arrival at the feedlot. J. Anim. Sci. 90: 1026-1039.

Coetzee, J.F., B. KuKanich, R. Mosher, and P.S. Allen. 2009. Pharmacokinetics of intravenous and oral meloxicam in ruminant calves. Vet. Therap. 10: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 concentration of substance P and cortisol in beef calves after castration or simulated castration. AVJR. 69:751-762.

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*:*375–382.

Fisher A.D., T.W. Knight, and G.P Cosgrove. 2001. Effects of surgical or banding castration on stress responses and behavior of bulls. Aust. Vet. J. 79:279–284.

González, L.A., K.S. Schwartzkopf-Genswein, N.A. Caulkett, E. 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, Escherichia coli, and salivary cortisol. J. Anim. Sci. 88:802-810.

Griswold, D.E., and J.L. Adams. 1996. Constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2): rationale for selective inhibition and progress to date. Med. Res. Rev. 16:181-206.

Gruber, S.L., J.D. Tatum, T.E. Engle, P.L. Chapman, K.E. Belk, and G.C. Smith. 2010. Relationships of behavioral and physiological symptoms of preslaughter stress to beef longissimus muscle tenderness. J. Anim. Sci. 88:1148-1159.

Onuoha, G.N., and E.K. Alpar. 1999. Calcitonin gene-related peptide and other neuropeptides in the plasma of patients with soft tissue injury. Life Sci. 63:1351-1358.

Paull, D.R., C. Lee, and A.D. Fisher. 2008. Effects of meloxicam or tolfenamic acid administration on the pain and stress responses of Merino lambs to mulesing. Aust. Vet. J. 86:303-311.

Rollins, B.E., 2004. Annual meeting keynote address: animal agriculture and emerging social ethics for animals. J. Anim. Sci. 82:955–964.

Schwartzkopf-Genswein, K.S., J.M. Stookey, T.G. Crowe, and B.M Genswein. 1998. Comparison of image analysis, exertion force, and behavior measurements for use in the assessment of beef cattle responses to hot-iron and freeze branding. J. Anim. Sci. 76:972-979.

USDA. 2009. National Agricultural Statistics Service. Agricultural Statistics. 2009. Accessed 10 May 2012. http://usda.mannlib.cornell.edu/usda/nass/att//2000s/2009/Catt-07-24-2009.pdf.

# APPENDIX I

**SAS Code for Chapter II**

Code for analyzing weekly and daily feeding behaviors

```
data cast5;
set cast4;
week=1;
if d gt 7 and d lt 15 then week=2;
if d ge 15 and d lt 22 then week=3;
if d ge 22 then week=4;
nweek=week;
proc means data=cast5 noprint nway;
var msize;
class ID week;
id method ket msize_1;
output out=cast6 mean;
proc glimmix data=cast6;
class ID method ket week msize_1;
model msize=method|ket|week msize_1;
random intercept /subject=id;
lsmeans method*week/slicediff=week;
lsmeans ket*week/slicediff=week;
run;
proc glimmix data=cast4;
class ID method ket trt d;
model msize=method|ket|d msize_1;
random intercept /subject=id;
lsmeans method*d ket*d/slicediff=d cl;
run;
proc gplot data=castintake;
plot estimate*d=method;
run;
proc sgplot data=graph; loess y=mu x=nd/group=method;run;
```

Code for analyzing ADG

```
proc glimmix data=cast;
class ID method ket;
model ADG=ket|method;
output out=residuals pred=p resid=r;
random ID (ket*method);
lsmeans method ket method*ket/pdiff;
run;
```

**SAS code for Chapter III**

Code for analyzing ADG by pen with orthogonal contrasts

```
proc sort data=melox; by pen;
proc means data=melox noprint; by pen;
var cs vel;
where day in(-5,-1);
output out=baseline mean= vel0 cs0;
proc means data=melox noprint; by pen;
var bw temp;
where day in(-1,0);
output out=baseline mean=bw0 temp0;
data melox2;
merge baseline melox;
by pen;
ADG=(bw-bw0)/day;
run;
proc print data=melox2 (obs=5);
run;
proc glimmix data=melox2; where day gt 1;
class day trt pen ;
model ADG=trt|day bw0/ddfm=kr;
random pen(trt);
lsmeans trt|day/slicediff=day;
lsmestimate trt*day '1-2  Day8'        1 -1 0  0  0 0  0 0 0  0 0 0;
lsmestimate trt*day '3-(1+2)/2  Day8' -1 -1 2  0  0 0  0 0 0  0 0 0/divisor=2;
lsmestimate trt*day '1-2  Day14'       0  0 0  1 -1 0  0 0 0  0 0 0;
lsmestimate trt*day '3-(1+2)/2  Day14' 0  0 0 -1 -1 2  0 0 0  0 0 0/divisor=2;
run;
```