EFFECT OF CASTRATION AND ORAL MELOXICAM ON INFLAMMATION, ANIMAL BEHAVIOR, AND GROWTH PERFORMANCE IN BEEF CATTLE

by

Shelby Leann Roberts

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Approved as to style and contents by:

Dr. John T. Richeson	Date
Chairman, Dissertation Committee	
Dr. Jeff Carroll	Date
Member, Dissertation Committee	
Dr. Jeremy Powell	Date
Member, Dissertation Committee	
Dr. Brock Blaser	Date
Member, Dissertation Committee	
Dr. Bridget Guerrero	Date
Member, Dissertation Committee	

Head, Major Department	Date
Dean, Graduate School	Date

ABSTRACT

Castration is a common, yet painful management practice that is utilized by beef producers throughout the United States. There has been an increase in public concern for welfare within livestock species that warrants further investigation into pain mitigation practices that could be implemented during castration. Three studies were conducted to investigate the use of meloxicam, a non-steroidal anti-inflammatory drug, and its effect on inflammation, growth performance and behavior in castrated beef cattle. Study one utilized thirty yearling crossbred bulls assigned to three treatment groups including surgical castration with or without meloxicam administration and intact males used as positive controls. This study indicated that oral meloxicam administration at 1 mg/kg BW reduced inflammation and the stress response, while delaying the febrile response after castration. Study two was conducted to evaluate meloxicam administration in younger-aged beef bulls. Eighty-three crossbred beef bulls averaging 68 days of age were utilized in this study and were randomized into 1 of 3 treatments: intact bulls serving as positive controls, surgical castration and surgical castration with 1 mg/kg BW oral meloxicam administration. This study suggested that meloxicam administration in younger bulls does not clearly alter the immune response or animal behavior. Meloxicam administration did improve ADG for the first two weeks post-castration; however, BW was not different following castration. Study three was conducted using yearling bulls entering the feedlot to evaluate the effect of castration method and meloxicam administration on animal performance, behavior, carcass traits and inflammation. This study was conducted over a

3 year period and consisted of a total 194 crossbred beef bulls assigned to 1 of 5 treatments: negative control animals that were castrated near birth, surgical castration with or without meloxicam administration, and band castration with or without meloxicam administration. Animal performance was affected by method of castration; surgical castration reduced performance compared to band castration in the first week post-castration, while band castration reduced performance in the second week post-castration. Average daily gain for the entire study was not different with castration method; however, meloxicam administration did improve overall ADG compared to castrated controls, regardless of method. Meloxicam administration also improved several carcass traits compared to castrated control animals. Animal behavior, the first week post-castration, was altered with castration. The method of castration had differing affects with surgical castration causing increased standing time, while band castration increased the number of steps and the number of lying bouts post-castration. Haptoglobin concentration was also reduced for band castration compared to surgical castration with meloxicam mitigating the magnitude of haptoglobin concentration in surgically castrated animals. These studies indicate that meloxicam may be an effective pharmacological method to reduce pain and inflammation in yearling-age castrated beef cattle; whereas, the method of castration had divergent behavior and performance responses but neither method is clearly advantageous.

Keywords: castration, meloxicam

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REVIEW OF LITERATURE

Introduction

Castration is known to be a stressful event that can modulate physiological responses along with behavior (Robertson et al., 1994). Physiological responses to stress results in activation of the hypothalamic-pituitary-adrenal (HPA) axis ultimately resulting in the release of cortisol (Cohen et al., 1990), production of acute phase proteins (APP; Brown et al., 2015) and altered blood leukocyte concentration (Chase et al., 1995). An increase in the risk of developing bovine respiratory disease (BRD) has also been reported when castration is delayed until arrival to the feedlot (Coetzee et al., 2012a; Richeson et al., 2013). Method (i.e, banding vs. surgical) and age of cattle during castration both influence the stress response and animal performance observed following castration of beef animals (Bretschneider, 2005).

Moral and ethical treatment of livestock throughout the various agricultural production systems is a growing concern within society (Rollin, 2004). The pain associated with some production practices has a negative impact on public perception, especially when they are performed without pain mitigation techniques (Benson and

Rollin, 2008). These growing societal concerns, coupled with the potential to improve animal production outcomes, are primary reasons to develop management practices that manage pain. There are numerous pharmaceutical products that may be useful in reducing or eliminating pain, such as local anesthetics, nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, α2-antagonists and N-methyl D-aspartate (NMDA) receptor antagonists (Coetzee, 2011). Management factors, namely the timing or age of the animal during application, should also be considered.

History of Beef Cattle Castration

Castration is a traditional management practice utilized by beef producers, with written accounts describing the procedure dating back to as early as the sixth century (Trow-Smith, 2013). Domestication of cattle has been a crucial milestone in the development of human societies. Oxen were relied upon to help cultivate the land. However, bulls were difficult to control, so castration was performed as a "physiological aid to taming" (Clutton-Brock, 1992). The beef production system has become more confined as the need for commercial food production has grown (Stafford and Mellor, 2005). In the modern U.S. beef production system, roughly 15 million bull calves are castrated each year (APHIS, 2011). Like ancient civilizations, cattle producers still need the ability to safely handle animals when raised in confinement. The aggressive behavioral traits associated with intact males are a result of testicular production of the androgen testosterone (Ford and D'Occhio, 1989). Testosterone production rapidly

increases around five to six months of age in bulls (Miyamoto et al., 1989; Rawlings et al., 1978). Beef bulls reach puberty around seven to ten months of age (Wolf et al., 1965). Puberty is defined as the entire period when the gonads secrete hormones in amounts sufficient to cause accelerated growth of genital organs and the appearance of sexual characteristics (Donovan and Ten Bosch, 1965). Aggressive and sexual behavior traits are usually displayed once a bull reaches puberty. Theses behavioral traits are comprised of sexual behavior, such as male-male mounting, and aggressive behavior including head pushing, butting and pawing/goring (Hinch et al., 1982). Hinch et al. (1982) also reported that in a grazing production setting bulls aged 15 to 18 months exhibited more fighting and mounting behavior. Management of bulls in more confined production settings could increase the occurrence of this behavior increasing the likelihood of animal injury. However, the size of the pen may influence the amount of activity with increased pen space allowing room for more fighting. Fighting will also be increased as hierarchy is established within the pen.

There are other benefits associated with castration in addition to modification of animal temperament and behavior; differences in carcass composition can also be observed between gender. As an animal reaches maturity muscle growth decreases and fat deposition increases; however, bulls have a higher proportion of muscle compared to fat than steers. (Berg and Butterfield, 1968). Carcass composition differences are a result of differences in physiological growth as steers appear to enter the fattening phase earlier reducing muscle growth, while bulls have a greater impetus for muscle growth before heavy fat deposition (Berg and Butterfield, 1968; Shahin et al., 1993). Increases observed in growth performance of bulls are primarily linked to testosterone's anabolic properties

(Steen, 1995). Bulls are more efficient and have faster rates of gain compared to castrated males (Champagne et al., 1969; Worrell et al., 1987). Conversely, steers have improvements in meat quality due to higher quality grades and increased meat tenderness when compared to intact males (Field, 1971; Klosterman et al., 1954; Seideman et al., 1982). Consumer studies have indicated that tenderness is an important aspect of quality that they would be willing to pay more for (Huffman et al., 1996; Miller et al., 1998). Due to decreased meat quality, bull carcasses command lower prices at market when compared to steer carcasses (Faulkner et al., 1992).

Modulators of Castration Response

Method of Castration

Castration of intact bulls can be performed using various techniques or tools (Stafford and Mellor, 2005). These techniques include physical methods of castration as well as chemical and hormonal methods. There is no current consensus on which method is best, so castration method varies throughout the beef industry. Surgical and rubber band castration are the two most commonly utilized methods among cow-calf producers that castrate males with 49.2% using surgical castration and 47.3% using rubber band (USDA, 2009). Castration of bulls upon feedlot entry show a similar trend with 52.3% surgically castrated, 41.1% band castrated and 6.6% not castrated at all (APHIS, 2011). The adoption of both hormonal and chemical castration methods have been slow within the beef industry.

Physical methods to castrate animals consist of the surgical removal of the testes or restricting blood flow to the testes using a rubber band or Burdizzo clamp. Rubber

banding and the use of the Burdizzo clamp cause damage to the blood vessels that supply the testes resulting in atrophy and necrosis of testicular tissue and eventual loss of the testes. Castration can also be performed using chemical and hormonal methods. Chemical methods cause loss of function in the testes through injection of sclerosing or toxic agents (Cohen et al., 1990; Fordyce et al., 1989). Intra-testicular injection of lactic acid has previously been used as a method of chemical castration. However, this product is no longer available due to necrosis of surrounding tissue (Capucille et al., 2002). Hormonal castration, also known as immunocastration, results in antibody production against gonadotrophin releasing hormone (GnRH) causing a decrease in hormone production (Adams et al., 1996; Molony et al., 1995). Immunization against GnRH must occur prior to the animal reaching puberty. Gonadotrophin releasing hormone is responsible for regulating testes function by controlling the secretion of luteinizing hormone (LH). Luteinizing hormone is necessary for androgen production including testosterone.

Different techniques produce different behavioral and physiological responses within the animal. Surgical castration has been observed to cause greater changes in behavior following castration, as well as a greater haptoglobin response compared to banded counterparts (Fisher et al., 2001). Plasma cortisol concentration, which can be used as an indicator of acute pain, has been observed to peak immediately following surgical castration when compared to banding methods (Chase et al., 1995; Molony et al., 1995). Conversely, Petherick et al. (2014) reported that band castration had greater cortisol concentration 2 hours post-castration compared to surgical castration. However, that study further reported that 24 hours post-castration the surgically castrated treatment had the greater cortisol concentration. The Burdizzo method of castration resulted in the

cortisol response being intermediate when compared with surgical (greatest peak) and banding (least peak); however, cortisol concentrations quickly returned to normal (within 72 minutes) with application of the Burdizzo (Robertson et al., 1994). Measures of systemic inflammation, such as serum haptoglobin, have been observed to increase on d 2 post-surgical castration, but banding resulted in a delayed inflammatory response until d 15 (Warnock et al., 2012). Petherick et al. (2014) observed that in surgically castrated bulls, haptoglobin concentrations were increased in the first week post-castration when compared to banded bulls. However, banded bulls had increased haptoglobin concentrations in weeks 2 and 3 post-castration (Petherick et al., 2014). Molony et al. (1995) reported that banding methods might produce a more chronic stress response due to prolonged inflammation and increases in abnormal standing that became evident 6 days post-castration. Chronic stress may be prolonged in banded calves due to continued afferent pain input following castration, whereas in surgical castration the nociceptors are removed (Robertson et al., 1994). The efficiency of banded cattle may also be influenced by age. Scrotum loss in 21 month old cattle occurred as early as 5 days and as late as 35 days post-castration (Chase et al., 1995), whereas; 14 month old cattle were reported to experience scrotal loss starting at day 28 and as late as 56 days post-castration (Fisher et al., 2001; Knight et al., 1999b). Performance loss commonly occurs within the first couple weeks after castration. Decreases in ADG of 50% and 70% were reported for rubber band and surgical castration, respectively (ZoBell et al., 1993). However, overall growth performance following castration is not typically affected by the method used (Bretschneider, 2005; King et al., 1991). No method of castration is pain-free, but Burdizzo appears to be the least painful method (Fisher et al., 2001; Molony et al., 1995).

Animal Age at Castration

Castration of calves occurs at various times within the beef production cycle, with only 59.2% of cow-calf producers castrating male calves prior to selling them (USDA, 2009). Studies have shown that castration at a younger age, less than 6 months old, reduces physiological stress experienced by the animal (Bretschneider, 2005; King et al., 1991). Cortisol concentrations, measured after castration, suggest that animals castrated at an older age experience greater levels of physiological stress compared to younger animals (Mellor et al., 1991).

The current dogma within the cow-calf industry is that bulls will have heavier body weight at weaning compared to steers. However, the difference in productivity of bulls and steers isn't realized until an animal reaches puberty, which occurs at an average age of seven to ten months in beef cattle (Wolf et al., 1965). This growth difference is due to the anabolic activity of androgens primarily testosterone. The anabolic properties of androgens cause's bulls to have 19% increase in average daily gain compared to steers (Steen, 1995). Knight et al. attempted to capture the superior growth and feed conversion of bulls and the superior quality associated with steers by castrating bulls post puberty followed by a finishing period (Knight et al., 1999a; Knight et al., 1999b). However, the weight advantage that animals gained as bulls was not retained following castration due to increased weight loss. A review article used data from 19 published articles to create a regression model that shows the effect of castration age on weight loss (Bretschneider, 2005). This regression model showed that weight loss in the first month following castration is increased as the age of the animal increases. Castration that occurs relatively close to birth shows that weaning weights of steers does not differ from bull calves

(Champagne et al., 1969; Worrell et al., 1987). Weaning of beef cattle normally occurs around 6 to 9 months of age before the animals reach puberty and can benefit from androgen production. No differences were observed in weaning weights or overall final body weights between calves castrated at 2 months of age or 10 months of age (Heaton et al., 2004; Micol et al., 2009). Similarly, Brown et al. (2015) reported no differences in carcass performance in animals castrated near birth or at weaning. Therefore, age of castration prior to puberty does not appear to affect overall growth performance, but does impact the level of stress an animal will experience.

Regardless of performance effects, castration prior to feedlot entry can have economic benefits for producers. Castration at an early age before calves enter the feedlot has been reported to reduce the odds of diagnosis with bovine respiratory disease (Richeson et al., 2013). This increased risk for treatment for respiratory disease results in bulls being sold in an auction market at a discounted price ranging from $0.04 to $0.17/ kg (Massey et al., 2011).

Pain Response

Pain is a complex experience that involves transduction of noxious stimuli from the environment followed by emotional and cognitive processing by the brain (Julius and Basbaum, 2001; Viñuela-Fernández et al., 2007). The International Association for the Study of Pain has defined pain as "an unpleasant sensory and emotion associated with actual or potential tissue damage, or described in terms of such damage" implying that pain can be experienced even when an injury does not occur (ISAP, 1979). Since animals lack the ability to communicate the degree of pain that they are experiencing a separate definition for animal pain must be used. In 1997, Molony and Kent defined animal pain

as “an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal’s physiology and behavior to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery.” Experiencing pain serves as a protective role as it actually warns the animals of tissue damage and coordinates reflexes and behavioral changes to keep damage to a minimum (Woolf and Mannion, 1999).

Pain experienced by an individual usually consists of various phases including acute pain, followed by sub-acute pain which may persist and cause chronic pain (Niv and Devor, 1998). Acute pain is the unpleasant sensory experience that occurs at the time of injury and causes activation of the peripheral nervous system (Chapman et al., 2008). Acute pain does not persist after the injury has healed (Molony and Kent, 1997). Acute pain is considered as a warning to damage or potential damage, while the sub-acute phase provokes protective behavior that aids in healing (Bateson, 1991; Millan, 1999). Chronic pain, however, is persistent pain that is often times not associated with any particular injury (Chapman et al., 2008). Chronic pain generally endures longer than the healing time of the injury (Bonica, 1990) and can be viewed as “non-functional pain” (Molony and Kent, 1997). The origin of chronic pain is from within the central nervous system due to damage to the mechanisms that control the pain process (Molony and Kent, 1997). Acute pain might be beneficial to the animal as it warns the body of actual or potential injury along with aiding in the healing process by enhancing inflammation. Chronic pain, however, has adverse effects such as decreased growth and immune function (Anil et al., 2005).

The early explanations of pain were simple connections that followed the pathway of the peripheral nerve (nociceptor) to the spinal cord to thalamus and finally ending at the cerebral cortex (Willis Jr, 1984). However, pain is now described in a plastic and integrative model that involves detection of a painful stimulus in the periphery that results in autonomic nervous responses, as well as endocrine and immune responses. This indicates, that the nervous, endocrine and immune systems must interact to produce a complex, coordinated response that allows the body to recover and return to homeostasis (Chapman et al., 2008).

Nociception

Julius and Basbaum (2001) describe nociception as the detection of painful stimuli in the sensory neurons through neurophysiological processes that involves detection, transduction and transmission of noxious stimuli to the central nervous system (CNS). Nociception and pain are not the same phenomenon; nociception is the physiological process of pain, while pain involves an emotional component (Iggo, 1984). Nociceptors are the sensory organs that detect tissue damage in the periphery. In 1910, the concept of peripheral nerve fibers that conduct pain signals was first described (Sherrington, 1910). Later, it was determined that there are several classes of these nerve fibers that are considered nociceptors (Raja et al., 1999). The free nerve endings that are classified as nociceptors are unmyelinated C fibers and thin myelinated Aδ fibers (Chapman et al., 2008; Westlund, 2005). Nociceptors are anatomically and physiologically specialized terminal structures that are innervated throughout the body in skin, muscle, blood vessels, viscera, connective tissue and bone (Byers and Bonica, 2001; Westlund, 2005). Noxious stimuli that activate nociceptors are overlapping with

mechanical, thermal and chemical stimuli activated both Aδ and C fibers (Bashams, 1993; Brooks and Tracey, 2005). The type of nociceptor will determine the speed at which the pain signal is conducted with Aδ fibers being rapid conductors while C fibers are slower conductors (Julius and Basbaum, 2001). The rapid conduction of Aδ fibers are associated with the "first" pain response which is the rapid, acute, sharp pain following injury. "Second" pain is mediated with the slower C fibers and produce a delayed, more diffuse, dull pain (Basbaum and Jessell, 2000).

Once an adequate number of nociceptors have been activated, an afferent volley is created and travels along the peripheral nociceptor to the dorsal horn (Basbaum and Jessell, 2000). The dorsal horn is a complex structure that forms synaptic connection with differentiated nociceptors into eight distinct laminae that process sensory information that serves multiple functions (Brooks and Tracey, 2005). The dorsal horn receives and integrates multiple sensory modalities and interfaces with both the internal and external environment. The primary functions of the dorsal horn includes production of spinal reflexes, relaying nociceptive messages to higher structures and either inhibiting or facilitating nociceptive transmission (Chapman et al., 2008; Le Bars, 2002). Glutamate is the primary neurotransmitter that is released by the peripheral neurotransmitters. Glutamate binds to ionotropic glutamate receptors, which mediate the majority of excitatory neurotransmission in the CNS, activating neurons within the dorsal horn (Dingledine et al., 1999). Central sensitization to nociceptive information is due the ionotropic glutamate receptor *N*-methyl-D-aspartate (NMDA) which are abundantly expressed throughout the dorsal horn of the spinal cord (Julius and Basbaum, 2001; Leem et al., 1996; Woolf and Thompson, 1991). These NMDA receptor channels are ordinarily

blocked by magnesium ions and depolarization is required to remove the magnesium block followed by binding with glutamate to open these channels (Woolf and Thompson, 1991).

Besides transmitting afferent signals to the spinal cord dorsal horn, activation of nociceptors also initiates neurogenic inflammation. Neurogenic inflammation is promoted by the release of neurotransmitters which promote the release of inflammatory substances from neighboring non-neuronal cells and the vascular tissue (Basbaum and Jessell, 2000; Julius and Basbaum, 2001). The substance released by nociceptors that help facilitate inflammation include substance P (SP), neurokinin A (NKA), as well as other peptides and nitric oxide (Chapman et al., 2008). The substrates cause increased chemotaxis and migration of macrophages, lymphocytes and mast cells to the wound area and interact with SP and NKA to create a chemical "soup" of substrates that further mediate inflammation (Kessler et al., 1992). The results of this chemical soup are vasodilation, extravasation of plasma proteins, and release of additional chemical mediators such as cytokines and prostaglandins (Rittner and Stein, 2005). Peripheral hyperalgesia is promoted through the changes associated with tissue damage and the ensuing inflammatory response. Hyperalgesia is the collective sensitization of normally high threshold nociceptors that expand the receptive fields of non-injured areas near the wound (Ji et al., 2003).

Central Pain Processing

Cells within the laminae of the dorsal horn send ascending axonal projections across the spinal cord and form white matter bundles that are connected to the thalamus and brainstem (Brooks and Tracey, 2005). The nociceptive information ascends to the

thalamus, which is located above the brainstem, via the contralateral spinothalamic tract. Nociceptive information is also directed to the medulla and brainstem via the spinoreticular and spinomesencephalic tracts, respectively (Dostrovsky and Craig, 2006). Nociceptive signals from the spinal cord to the brainstem are essential for integration with processes that maintain homeostasis, arousal and autonomic nervous system. The brainstem also has the ability to influence both the forebrain and spinal activity, thus playing a direct role in affecting the pain experience (Tracey and Mantyh, 2007). Following the brainstem and thalamus activity, the nociceptive information subsequently activates a large distributed brain network first described by Melzack as the pain "neuromatrix", but now it is commonly referred to as the "pain matrix" (Melzack, 1999; Tracey and Mantyh, 2007). The most common regions of the brain activated during acute pain are primary and secondary somatosensory, insular, anterior cingulate, and prefrontal cortices as well as the thalamus. Other regions of the brain, including basal ganglia, cerebellum, amygdala, hippocampus and areas within the parietal and temporal cortices, are active in the pain matrix depending of the circumstance of the pain stimulus (Apkarian et al., 2005). However, the literature is not consistent when it comes to activation of the pain matrix due to the fact that some regions of the brain have more or less activity that depends on the type of pain that is perceived (Tracey and Mantyh, 2007). In conclusion, the brain engages in a massive, distributed, parallel processing in response to noxious stimuli and the type of response is dependent on the type of stimuli.

Stress Response

In 1946, Hans Seyle documented the response of laboratory animals to noxious stimuli, which he termed the "general adaptation syndrome." Seyle further classified the

syndrome as having three distinct phases: 1) an alarm reaction, 2) a resistance phase, and 3) exhaustion of the biological system if the stress is not dissipated (Selye, 1946). Seyle concluded that the response to a noxious stimuli was 'non-specific' because different stimuli appeared to produce seemingly similar physiological responses. Contrary to Selye's hypothesis, it is now widely accepted that the stress response is specific with different stressors having different endocrine responses within and between different species of animals (Dantzer and Mormède, 1985; Mason, 1968).

A more recent definition of stress is a "state in which homeostasis is threatened or perceived to be so; homeostasis is re-established by a complex repertoire of behavioral and physiological adaptive response of the organism" (Chrousos and Kino, 2005). Moberg proposed a stress model that divides the stress response into three distinct stages: 1) recognition of a stressor, 2) biological defense against the stressor, and 3) consequences of the stress response (Moberg, 2000). The perception that a stressor potentially threatens homeostasis begins in the central nervous system. These coordinated physiological responses observed within the body in response to the wound formed during castration are activation of the HPA axis and the sympathetic nervous system; both mechanisms attempt to restore homeostasis within the body (Elenkov et al., 2000). Maintenance of homeostasis is achieved through hormonal signaling; therefore, the release of stress hormones can influence metabolism, reproduction, growth and the immune system (Matteri et al., 2000)

There are a variety of stressors that cattle may experience throughout their lifetime and can be grouped into one of the three following categories: 1) psychological stressor, 2) physical stressor, and 3) physiological stress (Carroll and Forsberg, 2007).

These are broad categories that can encompass many different environmental or management induced stressors. Psychological stress is typically any type of stress that is related to fear. Beef cattle specific stressors included in this category would be predation, commingling, novel environments, handling and restraint. Physical stressors can include injury, thermal stress, hunger or thirst, fatigue and disease. Lastly, physiological stress is the result of deviations in normal endocrine and neuroendocrine functions cause by nutritional deficiencies, glandular disorders and other endocrine disruptors (Carroll and Forsberg, 2007; Grandin, 1997) . The duration an animal is exposed to a stressor can be classified as either acute or chronic stress. The length of the stress response can influence the type of the physiological response the animal will experience. Acute stress is proposed to endure less than 12 hours and prime the immune system, while chronic stress that lasts for longer than 24 hours may be immunosuppressing (Dhabhar, 2009). Within the beef production system, calves arriving to the feedlot can be experiencing multiple stressors at the same time. These stressors could include weaning, marketing, transportation, castration, commingling and nutritional deficiencies. Understanding the type of stress condition the animal is likely experiencing is essential in order to develop management practices to minimize the negative effects of stress.

Hypothalamic- Pituitary- Adrenal Axis

The most consistent response to stress would be activation of the HPA axis that results in the release of glucocorticoid. In cattle, cortisol is the primary glucocorticoid produced. The HPA axis is initiated in the hypothalamus by production of corticotropin-releasing hormone (CRH) and vasopressin (VP). CRH is produced by the hypothalamic paraventricular nucleus when stimulated by serotine, acetylcholine or nor-epinephrine

(Black, 1994). Vasopressin is produced by magnocellular neurons within the hypothalamus which extend directly into the posterior pituitary were VP is stored (Matteri et al., 2000). CRH and VP are released into the hypophysial portal vasculature and travel to the anterior pituitary gland. Activation of the anterior pituitary gland cause corticotrophs, a specialized cell, to produce adrenocorticotrophic horomone (ACTH) (Guillemin and Rosenberg, 1955; Saffran et al., 1955). Studies in cattle have shown that, CRH is more potent in stimulating production of ACTH when compared to VP, yet together, CRH and VP can have synergistic effects on ACTH production (Carroll, 1993; Jacob and Minton, 1993). Furthermore, CRH and VP can work independently to mediate the release of ACTH or they can also work together to control the magnitude of glucocorticoid release (Carroll and Forsberg, 2007). While, CRH and VP are the primary stimulators of ACTH the neurohormones epinephrine and oxytocin have also been reported to induce ACTH production (Giguère and Labrie, 1983). Adrenocorticotrophic hormone then travels to the adrenal gland via systemic circulation. The synthesis of glucocorticoids from the adrenal medulla is stimulated by ACTH through promotion of cholesterol uptake which is then enzymatically converted to cortisol (Matteri et al., 2000). There are also paracrine actions of CRH and VP within the adrenal gland that can directly stimulate cortisol production (Carroll et al., 1996; Jones and Edwards, 1990). The complexity and integration of the HPA axis indicates that the stress response is not an "all or none" response (Matteri et al., 2000).

Biological Effects of Cortisol

Secretion of cortisol occurs in a circadian rhythm and is essential for the maintenance of homeostasis. In cattle, cortisol concentration is known to be greatest in

the early morning (Hays et al., 1975). Cortisol exhibits a variety of biological effects that includes modulation of the immune response, protein catabolism and hyperglycemia (Matteri et al., 2000). The effects of cortisol production on immune function are discussed later.

Maintenance of an appropriate cortisol concentration is essential for preservation of homeostasis and overall survival of the animal. Cortisol serves as a negative feedback loop at both the anterior pituitary gland and the hypothalamus to prevent production of ACTH and CRH, respectively (Fink et al., 1991; McEwen, 1978). This minimizes the duration of tissue exposure to cortisol and reduces its catabolic, lipogenic and immunosuppressive effects (Charmandari et al., 2005). During a stress response, energy and oxygen are redirected to the central nervous system and the site of injury, where these substrates are needed most (Charmandari et al., 2005). The repartioning of nutrients inhibits the activity of the growth and reproductive systems. The biological effects of circulating catecholamines that cause an increase in heart rate, blood vessel constriction, bronchiole dilation and increased metabolism aid in the repartitioning of nutrients during the stress response (Carroll and Forsberg, 2007).

Activation of the HPA axis is well documented among castration studies in beef cattle (Cohen et al., 1990; Macaulay and Friend, 1987; Robertson et al., 1994). Administration of NSAIDs during castration has resulted in reductions of peak cortisol concentrations (Earley and Crowe, 2002; Roberts et al., 2015; Webster et al., 2013). Administration of NSAIDs to modulate the stress response may be useful to help reduce the pro-inflammatory actions of cortisol.

Sympathetic Nervous System

Stimulation of the HPA axis is not the only physiological response to stressors; stress also activates the sympathetic nervous system. The sympathoadrenomedullary (SAM) axis was first coined the "fight or flight reaction" were the stress response was linked to production of the catecholamines (Chrousos and Gold, 1992). The SAM axis endocrine response involves the production and release of the catecholamines, epinephrine and nor-epinephrine, into systemic circulation from chromaffin cells of the adrenal medulla and noradrenergic neurons. Activation of the SAM axis is caused by acetylcholine production from preganglionic neurons (Chapman et al., 2008). Biological effects of catecholamines include increase heart rate, vasoconstriction to some body parts, and vasodilation to vital organs needed to escape. Vasoconstriction reduces hemorrhage, fosters platelet aggregation, and keeps healing factors within the wound (Chapman et al., 2008).

Immune System

The immune system is comprised of two major components: innate and adaptive immunity. The two components are characterized by pathogen specificity and speed of reaction. The innate component of immunity is relatively nonspecific and can provide more rapid host defense. Adaptive immunity is characterized by is antigen-specific response and can take several days to weeks to provide protection in response to antigen (Janeway et al., 2008). Relative to tissue injury that occurs with castration, innate immunity is the predominant type of immune response.

Innate Immunity

Innate immunity is the body's first defense in the internal environment protecting the host animal from viral and bacterial pathogens, trauma or stress. The innate immune system is composed of multiple and diverse components including physical barriers (i.e., skin), leukocytes, and complement. These individual components are part of an the early-defense system and are antigen-nonspecific that work together to prevent infection, eliminate potential pathogens and initiate inflammation (Janeway et al., 2008). The immune cells associated with innate immunity include the leukocytes (neutrophils, monocytes, eosinophils, basophils, and natural killer cells) and dendritic cells.

Leukocytes

Macrophages are the major phagocyte and resident macrophages are located in various tissue throughout the body. Monocytes migrate from the blood into tissues throughout the body and differentiate into a macrophage upon tissue entry. Neutrophils are the most abundant phagocytic cell; however, they are short-lived cells with approximately 6-hour half-life unless activated. Neutrophils are not present in healthy tissue and must be recruited to the site of infection. Following recruitment, neutrophils will quickly outnumber macrophages. Eosinophils and basophils are both granulocytes, along with neutrophils. Eosinophils and basophils are the chief defense against parasitic infection and are active in the allergic reaction (Janeway et al., 2008). However, eosinophils have recently been associated with the resolution of inflammation due to their production of anti-inflammatory and pro-resolving lipid mediators (Isobe et al., 2012).

Phagocytes are active at the site of infection where they engulf and kill pathogens before that can replicate within the body (Carroll and Forsberg, 2007). Phagocytes are activated when pattern-recognition receptors on the cell wall surface recognize highly

conserved structures, called pathogen-associated molecular patterns, which are present on a wide variety of microorganisms (Janeway et al., 2008). The pattern-recognition receptors on phagocyte cell walls can be divided into three functional groups: 1) circulating humoral proteins, 2) endocytic receptors that are expressed on the cell surface and mediate phagocytosis and 3) signaling receptors, such as toll-like receptors that are expressed on the surface of the cell (Mann, 2001).The cytokines interleukin (IL) -1β, IL-6 and tumor necrosis factor-alpha (TNF-α) are released from activated macrophages (Gruys et al., 1998; Murtaugh et al., 1996). These pro-inflammatory cytokines serve a wide range of biological effects such as production of acute phase proteins, neutrophil mobilization, fever due to activation of the hypothalamus, and the mobilization of protein and energy to facilitate the increased body temperature (Janeway et al., 2008; Klasing, 1988).

Inflammation is initiated and regulated by chemokines and cytokines. Inflammation is usually initiated within minutes following tissue injury or infection. Inflammation is caused by the infiltration of leukocytes, mainly neutrophils and monocytes which differentiate into macrophages, into the site of infection. In order for leukocytes to leave the blood, changes in the endothelial cells that comprise blood vessels surrounding the wound occur. The changes in local vasculature include vasodilation, expression of cell adhesion molecules, and permeability and clotting of microvessels at the site of infection to prevent the spread of pathogens (Simon and Green, 2005). Vasodilation increases the blood flow to the area of infection along with reducing the velocity of blood flow, thus allowing more leukocytes to make contact with the endothelium of the blood vessel. Pro-inflammatory cytokine production induces the upregulation of the adhesion molecule, E-selection, on the local vasculature endothelium.

Vasodilation allows for the leukocytes to slow down in speed allowing for the L-selectin present on their cell walls to form bonds with E-selectin. This bond however is at low affinity and results in a "rolling" of leukocytes along the vascular endothelium. Rolling allows for a more stable bond to form between integrin present on leukocytes and the intercellular adhesion molecule type 1 (ICAM1) on the vascular endothelium. This stable bond "stops" the leukocyte allowing it to migrate through the spaces in the endothelial cell wall, following the chemotactic gradient created by production of the chemokine IL-8 and chemoattractant C5a and other cytokines. These chemoattractants are produced by activated macrophages and complement, respectively, at the site of infection (Janeway et al., 2008; Ley, 1996; Parkin and Cohen, 2001).

Activation of inflammation can also be a result of interaction between the immune and nervous system. Afferent neurons induced by tissue injury or the cytokine IL-1β stimulate the release of the immunostimulatory peptides SP and NKA (Chapman et al., 2008).

Acute Phase Response

The acute phase response (APR) is a dynamic and complex series of events that is a part of innate immunity (Baumann and Gauldie, 1994; Cray et al., 2009). An APR can be triggered by both internal and external stimuli including stress, tissue damage, inflammation and infection (Cray et al., 2009; Murata et al., 2004; Petersen et al., 2004). The acute phase response is classically known to produce two biological responses: 1) fever and 2) alterations in liver metabolism resulting in the release of acute phases proteins (APP) from hepatocytes (Carroll and Forsberg, 2007; Suffredini et al., 1999). Naturally, variations in the APR occur between and among species that act as modulators

of innate immunity and the stress response. In cattle, variation in the APR can be attributed to breed, gender and temperament (Hughes et al., 2013). The acute phase response is detectable for several days after the stimuli depending on the extent of tissue damage (Kushner and Mackiewicz, 1987). Acute phase protein concentrations peak within 24 to 48 h after activation of the APR, with resolution on the APR occurring within 4 to 7 days after activation (Ballou and Kushner, 1992).

The febrile response is the result of activation of the hypothalamus by the cytokines IL-1β, IL-6 and TNF-α through induction of prostaglandin E_2 (Klasing, 1988). Fever is a phylogenetically very old and ubiquitous defense whose purpose is to destroy pathogens. The increase is temperature is sufficient to destroy many bacteria and microorganisms within the infected host. Proliferations of immune cells also is a product of fever. The febrile response is highly adaptive and a reduction in fever could reduce the survival rate of the host (Carroll and Forsberg, 2007).

Acute phase proteins are defined as proteins produced by hepatocytes whose concentrations change by at least 25% during systemic inflammatory conditions (Morley and Kushner, 1982). Acute phase proteins are primarily produced by hepatocytes within the liver (Murata et al., 2004); however, extrahepatic production can also occur (Uhlar and Whitehead, 1999; Vreugdenhil et al., 1999). Induction of APP production is specific based on the type of cytokine caused by different receptors located on the surface membrane of the hepatocyte (Mackiewicz, 1997; Suffredini et al., 1999). The primary regulator of most APP has been recognized to be IL-6 (Mackiewicz et al., 1993); IL-6 knockout mice have shown to have a significant depression of APP secretion when compared to normal mice (Kopf et al., 1994). There have also been studies that have

reported that physical stress can induce production of APP via the production of glucocorticoids. (Alsemgeest et al., 1995; Murata and Miyamoto, 1993).

Classification of Acute Phase Proteins

Activation of the APR can cause drastic concentration changes in more than 200 proteins. Changes in blood concentration varies depending on the protein and APPs can be classified as either negative or positive (Kaneko, 1997; Kushner and Mackiewicz, 1987). Positive APP are those proteins whose magnitude increases while negative APP are those proteins whose magnitude decrease following activation of the APR (Cray et al., 2009). Positive APP have several classes depending on the magnitude of change produced following activation of the APR. The response pattern of APP is specific according to species, except for albumin in which a 10-30% serum decrease has been shown in all species studied (Mackiewicz, 1997). In cattle, the primary positive APP are haptoglobin (Hp) and serum amyloid A (SAA), which are classified as major APP (> 10-fold change). The minor positive APP (1- to 10-fold increase) are fibrinogen (Fb), ceruloplasmin (Cp) and α1-acid glycoprotein (Cray et al., 2009; Petersen et al., 2004). Albumin is the primary negative APP that is reported in all animal species (Kushner and Mackiewicz, 1987). Acute phase proteins can also be divided according to which pro-inflammatory cytokine causes their induction. Serum amyloid A and Cp have drastic serum concentration increases early after inflammation signal and normalize quickly. These APP are considered rapid acting and are primarily induced by IL-1 and TNF-α (Gabay and Kushner, 1999; Kushner and Mackiewicz, 1987). Second line APP, such as Hp, are induced by IL-6 and are characterized by a later serum increase and remain

elevated for up to two weeks (Gabay and Kushner, 1999; Kushner and Mackiewicz, 1987).

Biological functions of Acute Phase Proteins

In cattle, the acute phase response is different from other species due to the fact that Hp is considered a major APP (Eckersall and Bell, 2010). The principal function of Hp is to form stable bonds with free hemoglobin which prevents iron loss (Allison, 1958; Keene et al., 1965). Free hemoglobin, when present in plasma, is toxic and pro-inflammatory as a result of oxidative damage that occurs during hemolysis (Wagener et al., 2001). Iron is essential for bacterial growth; therefore, a bacteriostatic effect occurs as Hp limits the availability of iron for bacterial use (Bullen, 1981; Eaton et al., 1982). Haptoglobin may also have a role in immunomodulation with a variety of effects. Suppression of lymphocyte proliferations with increase Hp concentration has been reported (Murata and Miyamoto, 1993; Oh et al., 1990). Haptoglobin also has inhibitory effects on granulocyte chemotaxis and phagocytosis (Rossbacher et al., 1999). Increased Hp concentrations are present during several inflammatory diseases including endometritis, pneumonia, enteritis, peritonitis, mastitis and endocarditis (Gómez-Laguna et al., 2011; Murata et al., 2004; Petersen et al., 2004). Circulating concentrations of Hp are non-negligible in normal cattle; however, during times of systemic inflammation Hp concentration can increase 100-fold or greater (Conner et al., 1988) making it an ideal clinical parameter to determine occurrence and severity of inflammation (Makimura and Suzuki, 1982). Quantification of Hp may be a useful field study parameter for respiratory disease detection due to the prolonged Hp response which results in a higher test sensitivity (Angen et al., 2009).

Serum amyloid-A has several roles that are associated with immunomodulation and down regulation of the inflammatory response (Ceciliani et al., 2002; Petersen et al., 2004). The immunomodulatory effects observed are chemotaxis of monocytes, polymorphonuclear leukocytes and T cells (Badolato et al., 1994; Xu et al., 1995), inhibition of oxidative burst (Linke et al., 1991) and platelet activation (Zimlichman et al., 1991). Serum amyloid A has been shown to transport cholesterol from dying cells to hepatocytes (Liang and Sipe, 1995). As a major APP in cattle, SAA is also a valuable tool that can be used to diagnose cattle experiencing inflammation (Alsemgeest et al., 1994; Boosman et al., 1989; Horadagoda et al., 1999). When used as a marker for inflammation, SAA appears to be more sensitive than Hp concentration. Serum amyloid A had 100% sensitivity and Hp had 76% specificity when distinguishing animal suffering from acute versus chronic inflammation (Horadagoda et al., 1999). However, application of SAA assays in veterinary diagnosis are not as common as Hp assays due to the difficulty that is associated with measuring SAA concentration (Eckersall, 2000).

The physiological functions of alpha 1-acid glycoprotein consist of drug binding and anti-inflammatory actions through immunomodulation. Albumin is the major drug-binding protein present in the serum of a healthy animal; however, it is a negative APP that will decrease in serum concentration following the APR. Alpha 1-acid glycoprotein (AGP) functions to bind and transport endogenous or exogenous substance and may counteract the decrease in drug carrying capacity experience with a decrease in albumin (Fournier et al., 2000). The anti-inflammatory actions performed by AGP through inhibition of neutrophil activations and increased secretion of IL-1 receptor antagonist from macrophages (Fournier et al., 2000). Alpha 1-acid glycoprotein also binds directly

to lipopolysaccharide (LPS) from the bacterial cell wall, thus neutralizing the antigenicity and toxicity of LPS (Moore et al., 1997). In cattle, serum AGP has been successfully used as an indicator of inflammation (Carter et al., 2002; Conner et al., 1986).

Fibrinogen functions to restore homeostasis by way of providing a substrate for fibrin formation which repairs damaged tissue (Thomas, 2000). Enhancement of degranulation, phagocytosis and antibody-dependent cellular cytotoxicity are all results of intracellular signaling that occurs after Fb binds with integrins present on the cell wall of phagocytes (Rubel et al., 2001; Sitrin et al., 1998). In cattle, serum Fb can be measured to indicate inflammation, bacterial infection or surgical trauma (Cheryk et al., 1998; Hirvonen and Pyörälä, 1998; Hirvonen et al., 1996). Unlike other APP, fibrinogen concentrations associated with tissue injury seem to be independent of cortisol concentrations (Fisher et al., 1997a).

Ceruloplasmin is a copper-containing ferroxidase that has the capability to oxidize toxic ferrous iron into a non-toxic form (Patel et al., 2002). The reduction of toxic ferrous iron present in tissue prevents tissue damage due to iron-mediated free radicals (Inoue et al., 1999). Anti-inflammatory actions are also observed with Cp through reduction in the number of neutrophils that can attach to endothelium (Broadley and Hoover, 1989). Serum Cp concentration have been measured previously as an indicator of infection in cattle; however, it is not commonly measured (Conner et al., 1986; Sheldon et al., 2001).

Stress and the Immune Response

There is bi-directional cross-talk between the stress and immune response that is mediated predominately by cytokines. The anti-inflammatory functions of cortisol may

also serve a beneficial function by limiting tissue damage that can result from heightened inflammation. Glucocorticoids can also have detrimental effects on the immune system that impact overall health with animals experiencing chronic stress having more occurrence of disease (Blecha, 2000). The length of a response plays an important role in the physiological response that an animal will experience. An acute stressor, cortisol appears to have an immuno-priming effect; conversely, cortisol in response to a chronic stressor has an immuno-suppressing effect (Hughes et al., 2013).

In vitro studies that incubated bovine immune cells with high concentration of cortisol reported decreases in lymphocyte proliferation, interleukin-2 production and neutrophil function (Blecha and Baker, 1986; Salak et al., 1993; Westly and Kelley, 1984). Neutrophilia, an increase in neutrophil concentration, is a common response reported in stressed animals and is primarily caused by changes in neutrophil trafficking after glucocorticoid exposure and changes in synthesis of neutrophils from the bone marrow (Blecha, 2000; Kelley et al., 1981; Murata et al., 1987; Roth and Kaeberle, 1982; Roth et al., 1982). One anti-inflammatory mechanism of cortisol is through down-regulation of L-selectin on the surface of neutrophils. L-selectin is an adhesions molecule that is critical in trafficking neutrophils to the site of infection (Weber et al., 2001). Flow cytometry analysis from blood collected from cattle given dexamethasone, a synthetic glucocorticoid, demonstrated that down-regulations of L-selectin on neutrophils was present 8 hours following injection and lasted 48 hours post-injection (Burton et al., 1995). *In vivo* studies have also demonstrated that cortisol affects the APR. Glucocorticoids are also able to stimulate an APR as demonstrated by incubation of bovine liver cells with the addition of glucocorticoid in the culture media (Higuchi et al.,

1994) and corticotropin-releasing hormone challenged steers that produced an acute phase reaction (Cooke and Bohnert, 2011). This is further supported by a report that indicated suppression of cortisol, through administration of metryapone, has been shown to reduce the production of APP following castration (Fisher et al., 1997b).

Pain Assessment in Animals

Unlike humans, animals are not able to verbally communicate the type or intensity of pain that they are experiencing, so changes in behavior patterns or biological responses have become the primary methods for pain perception in animals (Short, 1998). Pain identification and assessment are critical before pain alleviation methods can be administered. The problem with detection of pain in animals lies in the subjectivity and variation of the response between species and even individual animals within the same species (Anil et al., 2005). Direct measurements of subjective responses are not possible, so the assessment of pain in animals are based upon human judgements determine by indirect measurements and visual observations (Molony and Kent, 1997). When attempting to diagnose pain in animals the species and their evolutionary responses to pain should be considered (Short, 1998). Prey and predator species will have different response to pain. Prey species, such as cattle, are often considered stoic and often attempt to minimize the outward signs of pain that are displayed because sick and injured animals are often the target of predation (Livingston, 1994). It is important to note, not all painful stimuli will produce the same response, and so behavioral measures should be based on the species and the type of stimuli. Molony and Kent (1997) recognized four typical pain response in farm animals: 1) behavioral modifications that enable the animal to avoid recurrence of the painful experience 2) automatic response that protect parts or the whole

animal (ex. withdrawal reflexes) 3) response that minimize pain and assist healing, and 4) responses designed to elicit help or to stop another animal from inflicting more pain (ex. vocalization). For more accurate pain identification, use of multiple measurements may be beneficial instead of a single parameter. Pain is a complex response and a combination of both behavioral and physiological parameters could be beneficial and help evaluators make more informed diagnosis of whether an animal is experiencing pain (Anil et al., 2002).

Behavioral Responses

Behavioral responses are the most common parameter evaluated during assessment of pain in farm animals. Behavior variables can easily be collected in field settings and are non-invasive (Rutherford, 2002; Short, 1998; Weary et al., 2006). Quantitative or qualitative approaches can be used to measured abnormal behavior that may indicate pain. Behavior measurements are primarily based on visual appraisal of the animal; however, this method could be a confounding factor since some animals may be affected by a phenomenon called "predator-induced analgesia" (Kavaliers, 1988). Humans are often viewed as predators by prey animals and may result in the masking of painful behavior (Caine, 1992). Methods of animal behavior that minimize the interaction between evaluators and animals are ideal. These methods can evaluate behavior using video surveillance or pedometers/accelerometers (Currah et al., 2009; White et al., 2008).

Behavior observed after castration have been quantified by either measuring frequency of the event or the duration of a behavior (Viñuela-Fernández et al., 2007). The behavior response is dependent on the type of painful stimuli (i.e., different castration methods) and age of the animal (Lester et al., 1996; Stafford and Mellor, 2005). Behavior

observed in cattle following castration includes restlessness, foot stomping, kicking at the scrotum, statue standing, number of steps taken and abnormal lying positions (Currah et al., 2009; Molony et al., 1995). Molony et al. (1995) reported the differences in behavior as a result of castration method. Band castrated calves displayed increased restless behavior, such as kicking at their abdomen and a decrease in normal lying behavior. In contrast, surgically castrated animals displayed the behavior known as statue standing. Those authors suggested that the behavior observed in the banded animals might not be considered beneficial to the healing process, but rather are attempts to escape the pain. Pedometers and accelerometers are non-invasive monitoring devices that can be attached to an animals leg (White et al., 2008). Pedometers were used by Currah et al. (2009) to evaluate activity following castration and they reported that surgical castration caused animals to take fewer steps after the procedure compared to steps taken prior to castration. White et al. (2008) used accelerometers to evaluate standing and lying time following castration and found that surgical castration caused animals to spend more time standing. Band castration has been reported to increase restless behavior in animals when compared to surgically and burdizzo castrated animals (Robertson et al., 1994). Those authors also reported that banded animals had a decrease in normal lying behavior and an increase in abnormal lying compared to surgical and burdizzo castration. Petherick et al. (2014) collected behavior, using pedometers, and reported that band castration in weaned bulls and mature bulls spend more time lying compared to surgically castrated animals..

Physiological Responses

Objective pain assessments that measure physiological changes often are the result of subjective conclusion that the changes in the measurements are related to pain

(Mellor et al., 2000). Physiological measures may be beneficial for use in prey animals that often try to hide behavioral responses to pain (Weary et al., 2006) Physiological response can measure products of the immune response and endocrine pathways.

Activation of the stress response occurs following a painful event; therefore, measuring serum concentration of the end products of the stress response is commonly used to quantify pain. Hormones produced by both the HPA axis and the sympathetic nervous system are often quantified from serum following a painful experience. The glucocorticoid, cortisol, is the primary hormone synthesized in response to the activity of the HPA stress axis in animals. Measuring the magnitude of the stress response is subjective due to the wide variety of situations and stressors that can affect the stress response (Conzemius et al., 1997; Mellor et al., 2000). However, measuring the magnitude and duration of the stress response could be a useful measurement of distress. Distress can be defined to include the effects of both pain and other adverse experiences an animal encounters (Fisher et al., 1997a; Mellor et al., 2000). Effects resulting from activation of the sympathetic nervous system can also be evaluated. Production of catecholamines, changes in blood pressure, increased heart rate, respiration and body temperature are all responses observed in the body following activation of the sympathetic nervous system (Sanford et al., 1986). Direct measurement of catecholamines can be used as an assessment of pain; however, it rarely used because several difficulties exist in quantification of catecholamines. This includes the extremely rapid response that is observed in catecholamines and is better suited as a measure of acute pain response (Ley et al., 1992).

Productivity measures such as feed and water intake along with changes in body weight and average daily gain can be measured with relative ease by producers (Stafford and Mellor, 2005). These measurements do not reflect what is happening in the animal currently, but instead what happened to the animal over a specific amount of time. Castration, regardless of method, does reduce animal weight gain compared to non-castrated animals (Berry et al., 2001; King et al., 1991). The decreases in ADG are likely a result of anorexia and sick behavior that occurs due to cytokine production (Johnson, 1998) However, production measurements are critical for animal-welfare research to influence animal producers to change the management procedures implemented.

Pain Management

Understanding the pathways associated with the pain response are essential when developing strategies to minimize or treat pain (Anderson and Edmondson, 2013). There are two methods to manage pain: 1) local anesthesia which causes loss of sensation within a localized area, and 2) systemic analgesia resulting in reduction of prolonged pain and inflammation. The goal of pain management should not be to eliminate all of the pain experienced by an animal because complete desensitization could result in further damage to the wound. Rather, the goal of pain management should be mitigate pain and allow the animal to resume normal self-care behavior like eating and drinking (Anderson and Edmondson, 2013; George, 2003). Pain management strategies can be utilized as either preemptive or reactive in response to painful procedures. Preemptive management attempts to control pain throughout the noxious stimulus which could be hours, days or even weeks. Reactive pain management is applied either following the painful procedure or when pain is recognized in the animal (Coetzee, 2011). Preemptive pain management

is thought to be more effective and optimize the animals well-being, while reactive management practices are less successful and may impede healing of the wound (Anderson and Edmondson, 2013). However, in large production systems preemptive pain management strategies may not be practical due to the increased need for labor and handling of the animals.

Local Anesthetics

Local anesthetic drugs produce reversible loss of sensation without causing loss of consciousness and are the most commonly used drugs during surgery of farm animals (Muir III and Hubbell, 2014). When administered in the tissue, the anesthetic activity is local and does not result in systemic anesthesia or behavioral effects. However, when administered intravenously or at extremely high doses in the tissue, systemic anesthesia can be reached (Anderson and Edmondson, 2013). The mode of action for anesthetic drugs is to block nerve fibers through inhibition of Na channels to impede the nerve conduction by preventing the depolarization of the nerve fiber (Coetzee, 2011; Muir III and Hubbell, 2014). Lidocaine hydrochloride (2%) and bupivacaine are two anesthetics that have been commonly used in beef cattle. The onset of activity for lidocaine is 2 to 5 minutes and it has duration of action of approximately 90 minutes. Bupivacaine is among the most potent local anesthetics in cattle with 20 to 30 minutes before onset of activity and anesthesia enduring for up to 58 hours; however, bupivacaine is toxic when administered intravenously (Coetzee, 2013). Lidocaine is the most utilized local anesthetic due to its rapid effect, lower toxicity, and low cost (Anderson and Edmondson, 2013). A survey of practicing veterinarians in the Unites States reported that only 22% administer a local anesthetic before castration (Coetzee et al., 2010). Anesthetics and

similar drugs that inhibit detection or perception of pain should not be considered as therapeutic beyond the benefit of lessening the distress experienced by the animal during a painful procedure. The anesthetic drugs do not treat disease or any of the processes that cause noxious stimulation like inflammation (Anderson and Edmondson, 2013; Otto and Short, 1998).

Coetzee (2013) reported the results of cortisol production from a meta-analysis of studies that utilized local anesthetics to achieve analgesia during castration. Coetzee reported that use of a local anesthetic caused a 25.8% reduction in peak cortisol concentration and a reduction of 16.3% in the area under the effect curve (AUEC) compared to castrated controls. These results suggest that overall animal distress can be reduced using local anesthetics, but only a modest change in the AUEC should be expected. Animal performance was observed in several castration studies that evaluated effects of anesthesia, and no effect on feed intake or weight gain were reported (Earley and Crowe, 2002; Fisher et al., 1996; Ting et al., 2003). The minimal change is AUEC and lack of effect on performance are probably due to the short term effect of local anesthetics whose effects do not extend the anesthetic period far enough into the post-castration period.

Nonsteroidal Anti-Inflammatory Drugs

Analgesic treatment may benefit the animal through pain mitigation, reduction in inflammation at the site of tissue damage and a more rapid patient recovery (Otto and Short, 1998). Compounds that are classified as a non-steroidial anti-inflammatory drug (NSAID) provide analgesic relief; with their primary role being mitigation of the pain associated with inflammation. The NSAIDs function through reduced synthesis of

inflammatory prostaglandin, primarily prostaglandin $E_{2\alpha}$, from arachidonic acid by inhibition of cyclooxygenase (COX) enzymes (Ochroch et al., 2003). There are two isoforms of the COX enzyme, each with unique biological functions. COX-1 is constitutively expressed in both peripheral tissues and the central nervous system with expression being enhanced by pain and inflammation mediators and COX-2 is ubiquitous in the central nervous system but only becomes the major enzyme for PG synthesis after induction by factors released during cell damage and death (Smith and Lagenbach, 2001). The COX-1 enzymes are recognized to be primarily involved with mechanisms that are associated with maintaining homeostasis. Whereas, COX-2 enzymes are preferentially induced in response to tissue injury. Specifically, COX-2 binding NSAIDs have been shown to be a less potent analgesic that COX-1, but they are thought to be safer for long-term use. Drugs that cause COX-1 inhibition may interfere with homeostasis of the abomasal mucosa; therefore, patient risk formation of abomasal ulcers with prolonged use (Anderson and Edmondson, 2013; Coetzee, 2011; Otto and Short, 1998). Drugs that cause nonspecific COX inhibition are aspirin and flunxin meglumine, while carprofen and meloxicam preferentially bind COX-2 enzymes(Coetzee, 2011)

Coetzee (2013) reported the cortisol production results from a meta-analysis of studies that utilized analgesics to achieve analgesia during castration. Analgesia following castration reduced the peak cortisol concentration by 10.8% and the AUEC was reduced by 29% when compared to castrated control animals. The provision of NSAIDs has also been shown to reduce Hp concentration following castration (Brown et al., 2015; Roberts et al., 2015).

Flunixin meglumine

Flunixin meglumine (Trade name: Banamine, Merck Animal Health, Madison, NJ) is the only NSAID that is approved by the Food and Drug Administration for the use in food animals within the United States; however, it is approved for anti-pyretic purposes only (Smith et al., 2008). Flunixin meglumine must be administered intravenously requiring administration by a skilled technician or veterinarian. The half-life of flunixin meglumine is 6.2 hours and requires that administration occurs every 12 to 24 hours to maintain analgesia (Anderson and Edmondson, 2013). The need for frequent handling and administration and the route of administration may result in added stress for the animal and reduce producer acceptance of its use.

Meloxicam

Meloxicam is another type of NSAID that may be an effective analgesic in cattle. Currently, meloxicam is not approved for use in food animals in the United States; however, it is approved for use in the European Union and Canada for use in beef cattle (Coetzee, 2011). Meloxicam causes systemic analgesia and reduces inflammation by reducing prostaglandin (PG) synthesis. Meloxicam preferentially, but not exclusively, binds to COX-2 receptors on various tissue types. Studies suggest, that the maximum serum concentration for oral administration of meloxicam (l mg/kg of BW) ranges from 12 (Coetzee et al., 2009) to 24 hours (Fraccaro et al., 2013). Fraccaro et al. (2013) suggested that this difference might a result of differences in the oral formulation. Coetzee (2009) used an oral drench suspension of crushed meloxicam tablets in water, whereas Fraccaro et al., (2013) combined meloxicam tablets with whey in a bolus. Meloxicam has a relatively long half-life estimation of 28 hours (Coetzee, 2009).

Meloxicam has been shown to modulate immune responses in cattle. Following castration, Roberts et al., (2015) reported that oral meloxicam administration at 1 mg/kg of BW reduced white blood cell count, and the specific concentration of neutrophils, eosinophils, monocytes and red blood cells compared to intact (bull) control animals. Oral meloxicam administration (1 mg/kg of BW) during long term transportation also resulted in a reduction in total leukocyte count and lymphocyte count (Van Engen et al., 2014). Contrary results were reported by Brown et al., (2015), who observed no difference in total leukocyte count with oral meloxicam administration during surgical castration of weaning age calves. After surgical castration of yearling beef bulls, oral meloxicam also resulted in Hp reduction (Roberts et al., 2015). Furthermore, oral meloxicam administration reduced Hp concentrations after transportation (Filho et al., 2014).

Meloxicam has also been observed to modulate the stress response in cattle. A reduction in cortisol concentration was also reported in yearling steers that were administered oral meloxicam during surgical castration (Roberts et al., 2015). A reduction in cortisol 6 hours was reported in cautery dehorning in dairy calves following an intramuscular injection of meloxicam at a dosage of 0.5 mg/kg of BW (Heinrich et al., 2009). A study that utilized transportation as a stress model reported that blood meloxicam concentration had an inverse relationship with circulating cortisol concentrations (Van Engen et al., 2014). Substance P, a neuropeptide associated with pain response, has also been evaluated following meloxicam administration. Substance P was reported to be reduced in scoop dehorned calves following intravenous administration of meloxicam at a dosage of 0.5 mg/kg of BW (Coetzee et al., 2012b).

Conversely, Repenning et al. (2013) observed an increase in SP concentration following band castration and repeated oral meloxicam administration. Similarly, there was no effect of oral meloxicam on SP concentration following long-distance transportation (Van Engen et al., 2014). These conflicting reports may be associated with a reduced magnitude of SP response to transported and band castration compared with dehorning.

There have also been contradictory reports of the impact of meloxicam administration on growth performance in cattle. Administration of oral meloxicam suspension in water (1.0 mg/kg of BW) administered 24 hours prior to surgical castration upon feedlot arrival did not alter ADG, G:F or pen-level DMI when compared to surgically castrated control animals (Coetzee et al., 2012a). Similar results were reported in cattle that were band castrated upon feedlot arrival with no differences observed in ADG and pen-level DMI following repeated oral administration of meloxicam (Repenning et al., 2013). However, meloxicam administration prior to long-term transportation and upon feedlot arrival did increase ADG, DMI and G:F in cattle used in another study (Filho et al., 2014). Average daily gain was also improved with meloxicam administration when bulls were castrated at weaning (Brown et al., 2015). A study evaluating behavior following dehorning showed that calves administered meloxicam spent more time at the feed bunk compared to control calves (Theurer et al., 2012). Repeated administration of oral meloxicam at 1.0 mg/kg BW, 0.5 mg/kg BW and 0.5 mg/kg on d -1, 0, and 1, respectively, showed no difference in pen-level behavior following band castration (Repenning et al., 2013).

Meloxicam has also been reported to have health benefits when administered as ancillary treatment of bovine respiratory disease (BRD). Coetzee et al., (2012a) reported that meloxicam administration decreased the occurrence of treatment of BRD in castrated

beef cattle. Meloxicam administration in conjunction with an antibiotic has also been shown to decrease the extent of lung lesion as well as increase ADG in animals compared to antibiotic treatment alone (Friton et al., 2005).

Current Recommendations and Oversight on Pain Management

Beef cattle production practices that induce pain are a growing animal welfare concern. The American Association of Bovine Practitioners recommends the use of long-acting NSAIDs to extend the period of analgesia after painful management procedures (AABP, 2014). Currently, there is no NSAID approved by the United States Food and Drug Administration for use of pain mitigation in livestock animals. There is one NSAID that is approved for use in food animals; however, flunixin meglumine is approved for use as an anti-pyretic not for use for pain relief. The American Veterinary Medical Association "supports the use of procedures that reduce or eliminate the pain of dehorning and castration of cattle" (AVMA, 2009); however, a recent survey showed that only 52.9% of veterinarians provide analgesia during castration of cattle older than 6 months (Fajt et al., 2011). Under the guidelines of the Animal Medicinal Drug Use Clarification Act, NSAIDs can be prescribed by a veterinarian to reduce pain in livestock animals (Coetzee, 2011). However, a valid veterinarian-client-patient relationship must exist prior to extra-label use of a drug.

Conclusion

Castration is a painful and stressful, yet necessary procedure commonly experienced by livestock. The timing and method of castration both impact the type of stress response that an animal will experience. Castrating at a young age is recommended

to help reduce the intensity of pain and stress experienced by the animal. As consumer awareness of animal welfare increases, the need for producers to address painful management practices also grows. Administration of NSAIDs may be another useful management practice that could be implemented by producers in an attempt to mitigate pain and inflammation in castrates. Since animals lack the ability to communicate the level of pain they are experiences there is a need to accurately identify parameters that can be used as indicators of pain. Quantification of pain in farm animals is difficult; however, physiological and behavioral measures can be evaluated and used as a proxy for pain. Combining quantitative (physiological) and qualitative (observational behavior) when trying to elucidate the pain response is essential. The ideal behavioral and physiological parameters may change depending on the type of pain stimulus. Further research is needed to elucidate the effects of meloxicam effects on carcass performance and animal behavior.

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CHAPTER II

EFFECT OF SURGICAL CASTRATION WITH OR WITHOUT ORAL MELOXICAM ON THE ACUTE INFLAMMATORY RESPONSE IN YEARLING BEEF BULLS

S. L. Roberts[1], H. D. Hughes[1], N. C. Burdick Sanchez[2], J. A. Carroll[2], J. G. Powell[3], D. S. Hubbell[3], and J. T. Richeson[1]

[1]Department of Agricultural Sciences, West Texas A&M University, Canyon, TX 79016

[2]Livestock Issues Research Unit, USDA-ARS, Lubbock, TX 79403

[3]Department of Animal Science, Division of Agriculture, University of Arkansas, Fayetteville, AR 72701

[4]Livestock and Forestry Research Station, Division of Agriculture, University of Arkansas, Batesville, AR 72501

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ABSTRACT

Pain management and welfare are increasingly prevalent concerns within animal agriculture. Analgesics may alleviate pain and inflammation associated with castration of beef cattle. This study was conducted to elucidate the effects of surgical castration on the acute inflammatory response and immunomodulation, and whether concurrent oral administration of meloxicam (1 mg/kg BW) would alter these responses. On d -1, crossbred bull calves (n=30; initial BW = 227.4 ± 10.3 kg) were fitted with indwelling jugular cathetors and rectal temperature (RT) recording devices, placed into individual stanchions, and assigned randomly to 1 of 3 treatments. Treatment application occurred at h 0 and consisted of: 1) intact bulls with sham castration (CON), 2) surgical castration (CAS), and 3) surgical castration with oral meloxicam (MEL). Blood samples were collected at 0.5-h intervals from h -2 to 4 h, 1.0-h intervals from h 4 to 8 h, and 12-h intervals from h 12 to 72 h. Serum was analyzed for cortisol and haptoglobin (Hp) concentrations using ELISA. Whole blood was analyzed for complete blood counts at -2, 0, 2, 4, 6, 8, 12, 24, 36, 48, 60, and 72 h, and RT was recorded in 5-minute intervals. Post-castration RT was greatest for MEL (39.04), intermediate for CAS (38.99), and least for CON (38.93°C; $P \leq 0.01$). Serum cortisol was increased ($P < 0.001$) for CAS (12.3) and MEL (11.3) compared to CON (6.7 ng/mL) during the post-castration period. At 0.5 and 1.5 h, cortisol concentration was greater in CAS and MEL than CON; whereas, at 2 and 2.5 h cortisol concentration was greatest for CAS, intermediate for MEL , and least for CON (trt x time; $P < 0.001$). Total white blood cell ($P \leq 0.04$), lymphocyte ($P \leq 0.02$) and monocyte ($P \leq 0.002$) counts were greatest for CAS, intermediate for MEL and least for CON. Administration of MEL reduced ($P \leq 0.002$) eosinophil counts during the post-

castration period when compared to CON and CAS. The change in serum Hp, relative to baseline values, was reduced for MEL at 36 ($P < 0.01$) and 60 h ($P \leq 0.03$), and the overall Hp concentration was least for MEL ($P < 0.001$). Oral administration of meloxicam at the time of castration reduced the acute inflammatory response in castrates, as evidenced by a reduction in Hp and certain leukocyte concentrations, it also caused a delayed increase in RT. Further research is needed to determine if this reduced acute inflammatory response would equate to improved health and/or performance post-castration.

Key words: beef cattle, castration, cortisol, inflammation, meloxicam

INTRODUCTION

Castration of beef bulls is a painful and stressful, yet common management practice within the United States. Physical castration methods are known to have adverse effects such as transiently reduced gain performance (Faulkner et al., 1992; Fisher et al., 1996) or increased bovine respiratory disease risk (Richeson et al., 2013), and result in both physiological and behavioral changes that suggest pain. Pain is difficult to assess in farm animals; however, some biomarkers that can be used as a proxy for pain include changes in cortisol, white blood cells, substance P and/or acute phase proteins (Molony et al., 1995).

The American Association of Bovine Practitioners recommends that long-lasting non-steroidal anti-inflammatory drugs (NSAIDs) be used to extend the postoperative period analgesia. Pain management strategies could be implemented to help mitigate the negative effects associated with castration, but extensive research is needed to establish

their efficacy and potential for adverse effects. Currently, there are no NSAIDs approved by the FDA for analgesic use in cattle. Meloxicam is an NSAID that is approved for pain management in companion animals, and under the guide of the Animal Medicinal Drug Use Clarification Act, meloxicam can be prescribed by a veterinarian to reduce pain and inflammation in recently castrated beef calves (Coetzee, 2011). Meloxicam is known to preferentially bind to Cyclooxygenase-2 (COX2) receptors within the peripheral tissue. Inhibition of COX2 receptors reduces the synthesis of inflammatory prostaglandins, primarily $PGE_2\alpha$, from arachidonic acid (Ochroch et al., 2003). Therefore, we hypothesized that oral administration of meloxicam would mitigate pain by reducing inflammation following surgical castration of yearling beef bulls. The objective of this study was to elucidate the effect of surgical castration on the acute inflammatory response and immunomodulation, and determine if concurrent oral administration of meloxicam would alter these responses.

MATERIALS AND METHODS

This study was conducted during June 2013 at the USDA-ARS Livestock Issues Research Unit near Lubbock, TX. All animals were cared for in accordance to the acceptable practices and experimental protocols approved by the West Texas A&M University (WTAMU; protocol # 04-05-13) and USDA-ARS Livestock Issues Research Unit Animal Care and Use Committees (protocol # 2013-04-JTR01).

Animals

Thirty crossbred beef bulls from the University of Arkansas Livestock and Forestry Research Station in Batesville, Arkansas were utilized for this study. Following

weaning, animals were preconditioned on the ranch-of-origin for 42 days before being transported approximately 1,233 km to the WTAMU Nance Ranch. After a 7-d rest period, cattle were then transported approximately 177 km to the USDA-ARS Bovine Immunology Research and Development facility where the study was conducted. Average BW at the start of the study was 227.4 ± 10.3 kg. On d -1, an indwelling jugular vein catheter was placed into all animals to facilitate intensive, serial blood collection. Animals were also fitted with an indwelling rectal temperature (RT) monitoring device described previous by Reuter et al. (2010) with some modification. Animals were housed in individual stanchions for the duration of the 3-d study. Once animals were placed in their assigned stanchion, a 24-hour acclimation period was allotted before castration was performed to mitigate stress experienced from acclimation to the stanchions and jugular catheterization procedure.

Treatments

On d -1, animals were ranked by BW and assigned randomly to 1 of 3 castration treatments: 1) intact bulls that were treated with sham castration to serve as a positive control (CON; n = 10), 2) surgical castration without meloxicam administration (CAS; n = 10), and 3) surgical castration with oral meloxicam (1 mg/kg BW) administration (MEL; n=10). Meloxicam tablets (Carlsbad Technology, Inc., Carlsbad, CA) were pulverized, placed into gelatin capsules and administered with a bolus device. Individual animal dosage was determined using the d -1 BW. The boluses containing meloxicam were administered directly into the rumen via esophageal intubation. Administration of meloxicam was concurrent with the castration procedure in order to mimic practical industry use. Oral meloxicam has been reported to reach mean peak plasma concentration

within 11.64 h, mean residence time of 44.9 h and has an elimination half-life of 27.54 h (Coetzee et., 2009). Van Engen et al., (2014) reported that oral administration of MEL at 1 mg/kg provided circulating MEL concentration for up to 5 d.

Castration was performed in the stanchions using a rope to restrain a hind leg, removing the ventral one-third of the scrotum was removed surgically, and pulling the testes ventrally exposing the spermatic cords, which were then severed with a scalpel using aseptic technique. Aseptic technique included rinsing hands in an anti-septic wash and changing scalpel blades between each animal. Wounds were also sprayed with anti-septic solution. To mimic common industry practices, testes were not prepared prior to castration and local anesthetic was not administered. To account for the influence of handling stress from castration, the CON calves were subjected to a sham castration procedure. Sham castration was performed by administering an empty gelatin bolus and these animals had their hind leg restrained for 2 min to impose the stress involved in handling the animals for the surgical castration procedure.

Blood Collection and Analysis

Blood was collected via jugular catheter, every 30 min from -2 to 4 h, hourly from 4 to 8 h and every 12 h from 12 to 72 h relative to castration (h 0). Blood was collected into 2 commercial blood collection tubes. A sampling tube without an additive (Sarstedt, Inc., Newton, NC) was used to collect blood used to harvest serum, which was stored in triplicate aliquots at -80°C after centrifugation at 1500 × *g* for 20 min. For complete blood count (CBC) analysis, whole blood was collected at hours -2, 0, 2, 4, 6, 8, 12, 24, 36, 48, 60 and 72 into 4 mL evacuated tubes with 10.8 mg K_2 EDTA (Vacutainer, BD Franklin Lakes, NJ) and CBC analysis was immediately performed using an automated

hemocytometer (ProCyte DX Hematology Analyzer, IDEXX Laboratories,Westbrook, ME). Rectal temperature was recorded by the indwelling device every 5 min from d -1 to 3, then averaged to hour intervals for statistical analysis.

Haptoglobin (Hp) concentration was determined from sera at the WTAMU Ruminant Health and Immunology Laboratory using a commercial, bovine-specific sandwich ELISA kit (Immunology Consultant Laboratory, Inc., Portland, OR). Intra- and inter-assay CV's were calculated for each ELISA assay. Intra-assay CV are determined by averaging the CV across an entire plate. Inter-assay CV is determined by running one sample on every plate. CV's for that sample across all plates is then averaged for the inter-assay CV. Hp had intra- and inter-assay CV of 11.6% and 15.2%, respectively. Serum cortisol (Arbor Assays, Ann Arbor, MI) and pro-inflammatory cytokines (SearchLight, Aushon Biosystems Inc., Billerica, MA) were analyzed at the USDA-ARS Livestock Issues Research Unit laboratory using ELISA assays. Serum cortisol had intra- and inter-assay CV of 9.2% and 15.2%, respectively. The cytokine assays intra- and inter-assay CV were $\leq 6.7\%$ and $\leq 18.1\%$, respectively.

Statistical Analysis

For all dependent variables, the MIXED procedure with repeated measures (SAS Inst., Inc., Cary, NC) was used to analyze data. The model included the main effects of treatment, time, and treatment × time with individual animal serving as the subject. The repeated statement was time (h) and the covariance structure with the lowest Akaike information criterion for each dependent variable was used. Data were tested for normal distribution using PROC UNIVARIATE and non-parametric data was $\log_2$-transformed if normality was improved. Differences of least squares means were determined using the

PDIFF option in SAS. Variables from h -2 to 0 were averaged and used as the baseline covariate for data that depict change relative to baseline values. Effects of treatment, time and their interaction were determined to be statistically significant at $P \leq 0.05$ with $0.05 < P \leq 0.10$ considered a tendency.

RESULTS

A treatment × time interaction was detected ($P < 0.001$) for serum cortisol concentration (Figure 1). No treatment differences ($P = 0.15$) were detected before castration was performed. Immediately following castration (h 0.5) cortisol concentrations were elevated ($P \leq 0.05$) in both castrated groups (24.13 ± 2.2 and 26.27 ± ng/mL for CAS and MEL, respectively) compared to the CON group (13.88 ± 2.35 ng/mL). At h 2 and 2.5, all treatments differed ($P \leq 0.02$) with cortisol concentration being greatest for CAS, intermediate for MEL, and least for the CON group. At h 3, the CAS group had the greatest ($P \leq 0.02$) cortisol concentration compared to CON and MEL, whereas, MEL was greater than CON ($P = 0.05$). At h 4, cortisol concentration was reduced ($P \leq 0.05$) for CON compared to CAS and MEL with no differences ($P = 0.44$) between the two castrated groups. Likewise, cortisol concentration was increased ($P = 0.03$) in castrates compared to CON at h 6.

There was no treatment × time interaction ($P = 0.38$) for post-castration serum Hp concentration; however, there was a treatment effect ($P < 0.001$, data not shown) detected with CAS having the greatest Hp concentrations, CON having intermediate concentrations, and MEL having the least concentrations of Hp. Baseline values (h -2 to 0) were used as a covariate ($P = 0.14$) to determine the change in serum Hp concentrations, which resulted in a tendency ($P = 0.07$) for a treatment × time interaction

(Figure 2). The CAS treatment had a greater change ($P = 0.05$) in Hp at h 7, with MEL having the least and the change in the CON group being intermediate; this pattern was relatively maintained through h 24. At h 36, the CON group had the greatest ($P < 0.01$) change in Hp concentration with CAS being intermediate and MEL group having the least change compared to baseline values.

The change in RT (Figure 3) is reported due to unexplained treatment differences observed ($P = 0.01$) prior to castration. Baseline RT values were used as a covariate ($P < 0.001$) in this analysis. There was no treatment × time interaction ($P = 0.84$); however, there was an effect of both treatment ($P < 0.001$) and time ($P < 0.001$). The MEL group exhibited a greater RT compared to both the CON and CAS groups.

No treatment × time interaction ($P = 0.19$) was detected for total white blood cells (WBC); however, there was a post-castration treatment effect ($P < 0.001$) with CAS having the greatest number of WBC, and MEL and CON having the least WBC concentrations (Figure 4). There was a difference ($P < 0.001$) in post-castration neutrophil concentrations (Figure 5) with MEL having a decreased concentration as compared to the CON and CAS groups. A post-castration treatment × time interaction ($P = 0.02$) was detected for blood lymphocyte concentration (Figure 6). Control calves had a decreased ($P \leq 0.02$) number of circulating lymphocytes than the CAS and MEL groups from h 4 to 8. A treatment difference was detected ($P < 0.001$) in post-castration monocyte concentration with CAS having the greatest concentration, CON the least, and MEL being intermediate (Figure 8). A difference ($P < 0.001$) was also detected for blood eosinophil concentration. The CON treatment had the greatest eosinophil concentration, MEL the least, while the CAS group was intermediate (Figure 7). There was a post-

castration treatment effect on red blood cells ($P < 0.001$) that followed the eosinophil trend with CON having the greatest concentration and the CAS and MEL groups had the least concentrations (Figure 9). Platelets had a post-castration treatment effect (Figure 10; $P = 0.008$) with CON having the greatest values of this variable and there was no difference between the castrated groups. Hematocrit ($P < 0.001$) was affected similarly to platelets, CON (10.87 ± 0.8) was the greatest with no difference between CAS (10.22 ± 0.08) and MEL (10.11 ± 0.11) groups. No differences ($P \geq 0.16$) in neutrophil:lymphocyte or basophil concentrations were detected in this study (data not shown).

There was no treatment × time interaction ($P = 0.78$) observed for tumor necrosis factor-alpha (TNF-α); however, pre- ($P < 0.001$) and post-castration ($P < 0.001$) treatment effects were observed. The CAS group had the least TNF-α concentration both pre- ($P \leq 0.05$) and post-castration ($P < 0.001$). Pre-castration concentration indicated that the CON group had a greater TNF-α concentration compared to MEL; however, post-castration TNF-α was not different between CON and MEL groups (data not shown). Due to interferon- gamma (IFN-γ) treatment differences ($P = 0.04$) prior to castration, baseline IFN-γ concentrations were used as a covariate ($P < 0.001$) to show the change in IFN-γ. There was no treatment × time interaction for change in IFN-γ ($P = 0.77$), but there was a treatment effect ($P = 0.01$, data not shown). The CAS group had the greatest change in IFN-γ, and the MEL group had the least change while the CON group did not differ ($P \geq 0.06$) from either of the other two treatment groups. There was a post-castration treatment effect ($P = 0.04$, data not shown) observed for interleukin 6 (IL-

6). The CON group had the greatest concentration, the CAS group had the least, and the MEL group was intermediate.

The metabolic variables measured in this study were glucose and NEFA. There was no treatment × time interaction ($P = 0.94$) observed for glucose; however, there was a post-castration treatment difference ($P < 0.001$). No difference was observed between the CAS (186.92 ± 1.87 mg/dL) and MEL (182.76 ± 2.21 mg/dL) groups, yet glucose concentration was reduced for CON (173.05 ± 2.07 mg/dL). There were no differences ($P = 0.32$) observed in NEFA concentrations throughout this study (data not shown).

DISCUSSION

Castration of beef cattle is known to be a painful and stressful event that stimulates the hypothalamic-pituitary-adrenal (HPA) axis resulting in synthesis and secretion of the glucocorticoid, cortisol (Minton, 1994). Furthermore, immunomodulation such as alterations in total WBC numbers (Chase et al., 1995) and increases in acute phase proteins (APP; Chrousos et al., 1995) have been observed following castration of beef cattle. The negative perception associated with pain caused by castration and other management practices is a growing concern making the development of strategies to control pain an important area of research. Molony and Kent (1997) defined pain as "an aversive feeling or sensation associated with actual or potential tissue damage resulting in physiological, neuroendocrine and behavioral changes that indicate a stress response." Two distinct categories of pain associated with surgical castration are plausible: 1) the initial, acute pain associated with the incision, and 2) the prolonged pain due to the inflammatory response. Meloxicam is classified as an NSAID which can provide analgesic effects in the host. It is important to note that analgesia is a method to reduce

pain and inflammation in a systemic fashion, as opposed to local anesthesia, which causes loss of sensation within a localized area; therefore, the primary role of an NSAID is to mitigate the pain associated with inflammation.

Serum cortisol results from this study showed a rapid increase immediately following castration, particularly in the castrated treatments (CAS and MEL). Although the CON group experienced a lesser increase in serum cortisol following sham castration, this suggests a stress response associated with handling alone. A review by Dantzer and Mormede, 1983 summarizes that environmental stimulation causes hormonal changes, such as increased corticoid steroid release. There was an overall decrease in cortisol at h 1, however the CAS group had a secondary increase at h 2.5. A possible explanation for this could be the initial increase in cortisol was a result of handling and tissue damage experienced during castration, while the secondary increase is a response to pain. Since meloxicam was administered concurrent with castration, its effects would not be realized until blood concentrations reached the therapeutic threshold. Although the pharmacokinetics of meloxicam were not directly measured in the current study, at h 2, there was a sharp decrease in cortisol concentration of the MEL group but not for CAS, possibly indicating the time required for meloxicam to reach efficacious blood concentrations in the animals. This data is supported by Allen et al., 2013 who measured Prostaglanding E2 which is an inflammatory prostaglandin whose synthesis is known to be inhibited by meloxicam. Allen et al. 2013 reported that Prostaglandin E_2 concentrations were decreased at 6 hours post-dehorning in cattle administered oral meloxicam prior to dehorning. Similar to our serum cortisol observations, at approximately two hours following castration Early and Crowe (2002) reported a

decrease in cortisol for castrates administered ketoprofen, an NSAID similar to meloxicam. A study that utilized transportation as a stress model reported that blood meloxicam concentration had an inverse relationship with circulating cortisol concentrations (Van Engen et al., 2014). In the current study, the suppression of cortisol was provisional with concentrations being similar between castrated groups after h 3.5. Despite the seemingly short term effects, meloxicam was efficacious in modulating the acute cortisol response in surgically castrated animals.

The acute phase response (APR) is a non-specific component of innate immunity which may respond to infection, inflammation, tissue damage, or stress (Baumann and Gualdie, 1994; Hughes et al., 2014). Biological responses associated with the APR include fever, production of acute phase proteins (APP) from hepatocytes, and increases in circulating white blood cells (Carroll and Forsberg, 2007). The synthesis of APP is modulated by pro-inflammatory cytokines (i.e., TNF-α, IL-6) and perhaps cortisol (Carroll and Forsberg, 2007; Fisher et al., 1997b). Haptoglobin is an APP that possesses anti-inflammatory actions by binding free hemoglobin, a pro-inflammatory protein, within the plasma (Murata et al., 2004). Haptoglobin can be used as a clinical parameter to determine the occurrence and severity of the inflammatory response in cattle (Makimura and Suzuki, 1982). The change in Hp concentrations from this study demonstrates that Hp was reduced in castrates that received oral meloxicam. This would indicate that meloxicam was able to reduce the magnitude of inflammation following castration, possibly due to the reduction in cortisol production. Early and Crowe (2002) also reported a decrease in serum Hp concentrations following castration with the administration of ketoprofen. Oral meloxicam administration also reduced Hp

concentrations after transportation (Filho et al., 2014). The febrile response is also a product of the APR. The current study showed that there was an increased RT in the MEL group from h 40 to 48 compared to the CAS and CON groups, suggesting that meloxicam may result in a delayed febrile response in castrates. The increase in RT could be a result of decreased meloxicam serum concentration, which is supported by Coetzee et al., 2009 who reported that oral meloxicam had a mean residence time of 44.90 hours. Both MEL and CAS had a transient febrile response immediately following castration. Ting et al. (2003) reported a similar increase in RT of surgically castrated cattle administered ketoprofen compared to surgical castrates receiving no analgesia.

Total WBC, lymphocytes, monocytes and RBC were elevated for both castrated groups when compared to the CON group, which is similar to results reported by Chase et al. (1995). Oral meloxicam reduced concentrations of total WBC, neutrophils, eosinophils, monocytes, and red blood cells. When ketoprofen was administered prior to castration no differences were observed in leukocyte concentrations (Early and Crowe, 2002). Ting et al. (2004) reported that hydrocortisone infusion to mimic castration had no significant effects on leukocyte concentrations. The reduction in some hematological parameters, observed in this study, may suggest that oral meloxicam reduces the magnitude of inflammation following castration. Nevertheless, inflammation is an important process for tissue repair and resolution of infection and field studies are needed to determine if meloxicam administration may impede healing from castration. However, Mintline et al. (2014) reported that administration of the NSAID flunixin meglumine had no effect on the rate of wound healing following surgical castration.

The production of glucose from non-carbohydrate carbon substrates like pyruvate, lactate, glycerol, and amino acids, known as gluconeogenesis, can be stimulated by cortisol (Carroll and Forsberg, 2007) resulting in an increase in blood glucose concentration. The current study observed an increase in blood glucose concentrations in the castrated animals compared to CON. In contrast to our results, Ting et al. (2004) reported no difference observed in glucose concentration following Burdizzo castration.

In conclusion, oral administration of meloxicam was able to diminish the cortisol response, which suggests that the physiological stress associated with surgical castration was reduced by oral administration of meloxicam at 1 mg/kg BW. Meloxicam also reduced the magnitude of the inflammatory response in castrates, as evidence by a decrease in Hp, and certain peripheral blood leukocyte concentrations. However, there was a delayed febrile response observed with meloxicam administration following castration. Development of pain management strategies could be useful to address both health and animal welfare concerns in the beef industry. Further research is needed to determine whether administration of meloxicam may impede convalescence following surgical castration.

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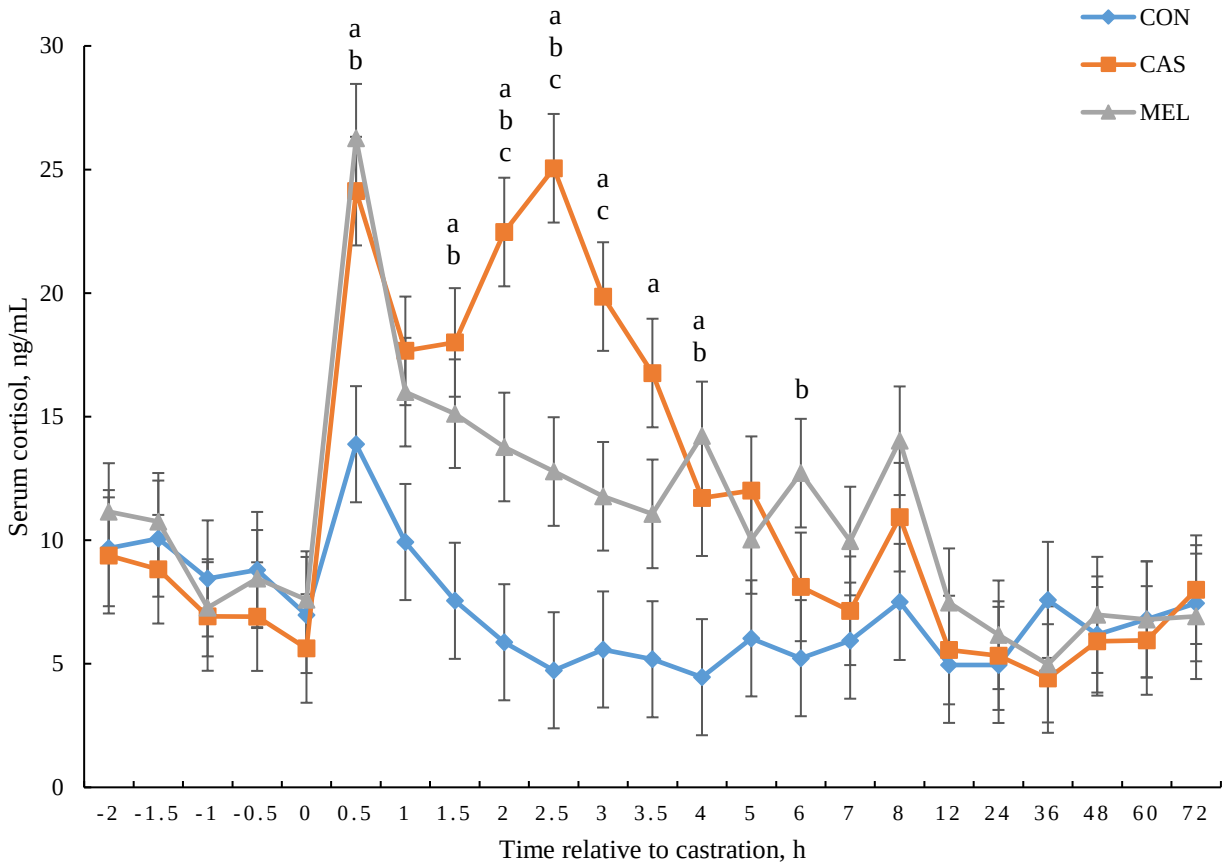

Figure 1. Serum cortisol concentration (± SEM) of intact bull calves treated with sham castration (CON; n=10), bulls surgically castrated without meloxicam administration (CAS; n= 10), and bulls surgically castrated with oral meloxicam (1 mg/kg BW) administration (MEL; n=10). A treatment × time interaction was detected ($P < 0.001$). Within hour, letters indicate the following mean comparisons ($P \leq 0.05$): a = CON vs. CAS, b= CON vs. MEL, and c= CAS vs. MEL.

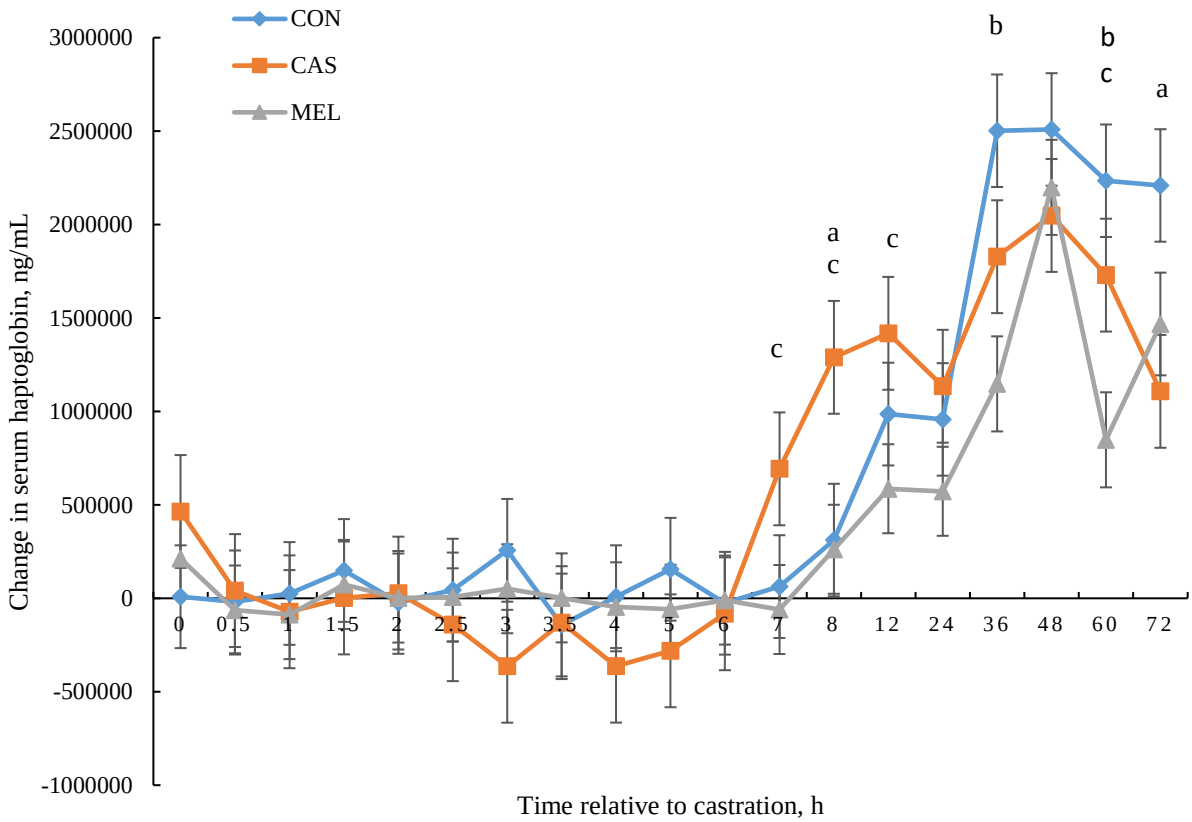

Figure 2. Change in serum haptoglobin concentration (± SEM) of intact bull calves treated with sham castration (CON; n=10), bulls surgically castrated without meloxicam administration (CAS; n= 10), and bulls surgically castrated with oral meloxicam (1 mg/kg BW) administration (MEL; n=10). Values collected before castration (h -2 to -0.5) were used as the covariate (P = 0.137). A tendency for a treatment × time interaction was detected (P = 0.074). Within hour, letters indicate the following mean comparisons ($P \leq$ 0.05): a = CON vs. CAS, b= CON vs. MEL, and c= CAS vs. MEL.

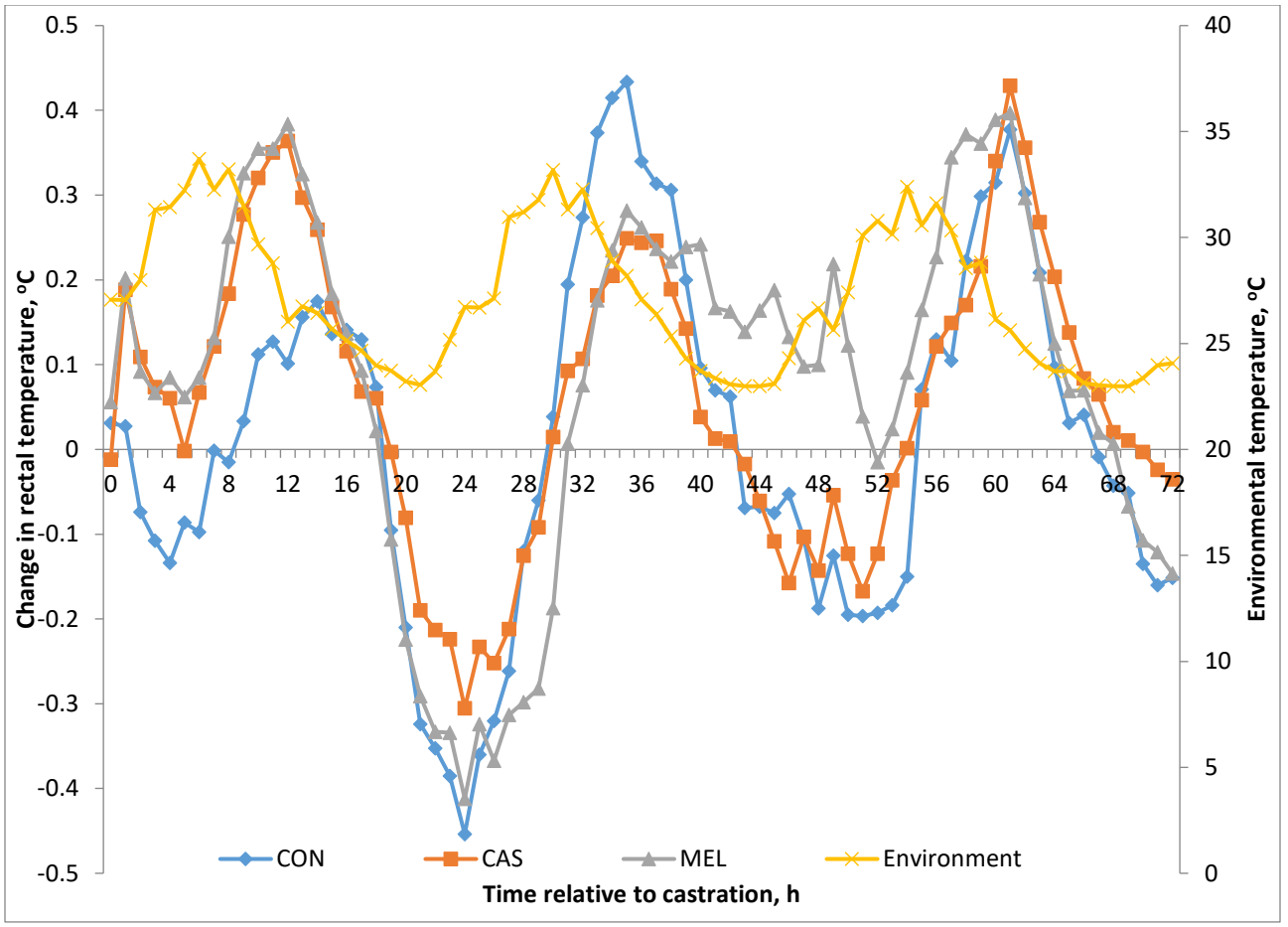

Figure 3. Change in rectal temperature of intact bull calves treated with sham castration (CON; n=10), bulls surgically castrated without meloxicam administration (CAS; n= 10), and bulls surgically castrated with oral meloxicam (1 mg/kg BW) administration (MEL; n=10). Values recorded before castration (h -2 to -0.5) were used as the covariate ($P < 0.001$). No treatment × time interaction was detected ($P = 0.84$); however, there was a significant treatment ($P < 0.001$) and time ($P < 0.001$) effect.

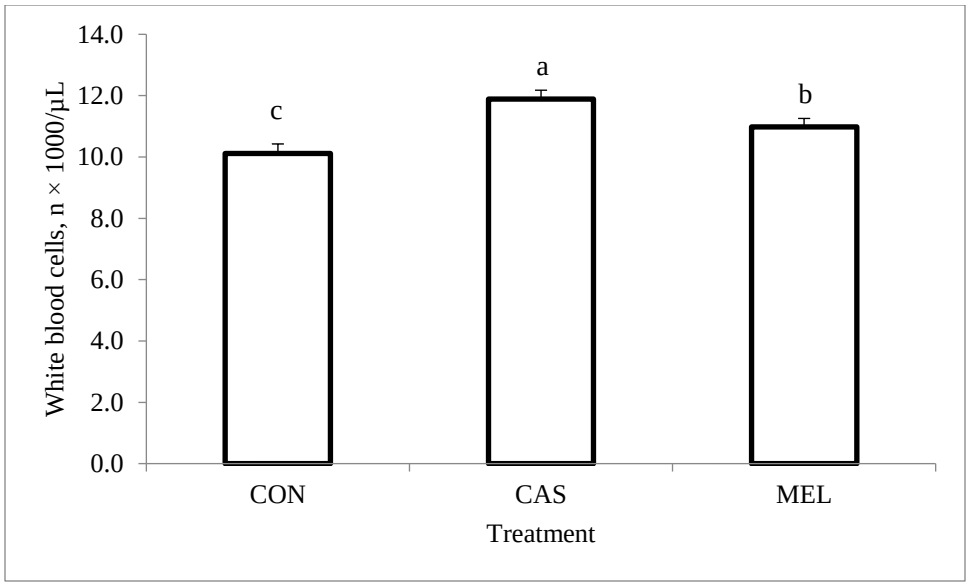

Figure 4. Post-castration (h 0.5-72) total white blood cell concentration (± SEM) of intact bull calves treated with sham castration (CON; n=10), bulls surgically castrated without meloxicam administration (CAS; n= 10), and bulls surgically castrated with oral meloxicam (1 mg/kg BW) administration (MEL; n=10). Treatment effect ($P < 0.001$); means with different letters differ ($P \leq 0.04$).

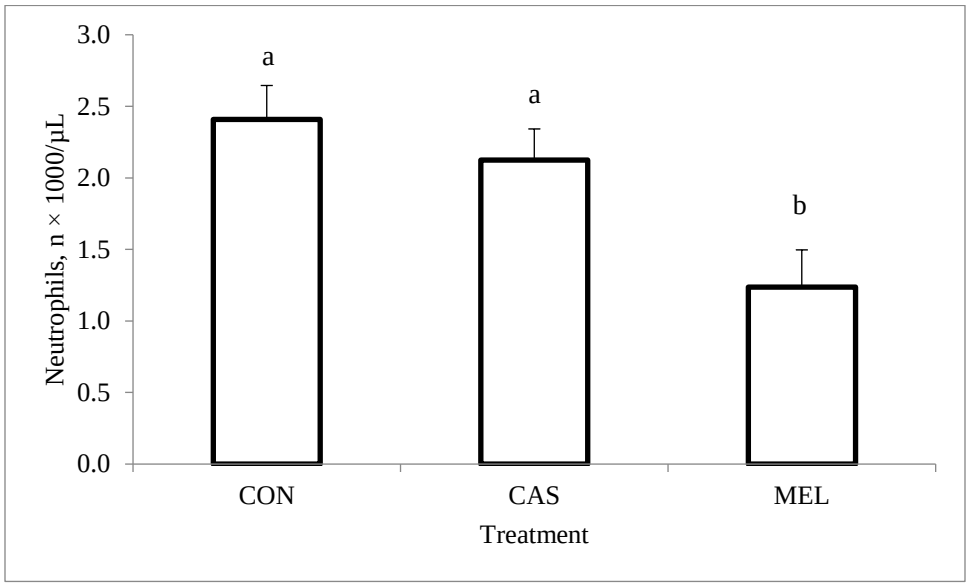

Figure 5. Post-castration (h 0.5-72) blood neutrophil concentration (± SEM) of intact bull calves treated with sham castration (CON; n=10), bulls surgically castrated without meloxicam administration (CAS; n= 10), and bulls surgically castrated with oral meloxicam (1 mg/kg BW) administration (MEL; n=10). Treatment effect ($P < 0.001$); means with different letters differ ($P \leq 0.01$).

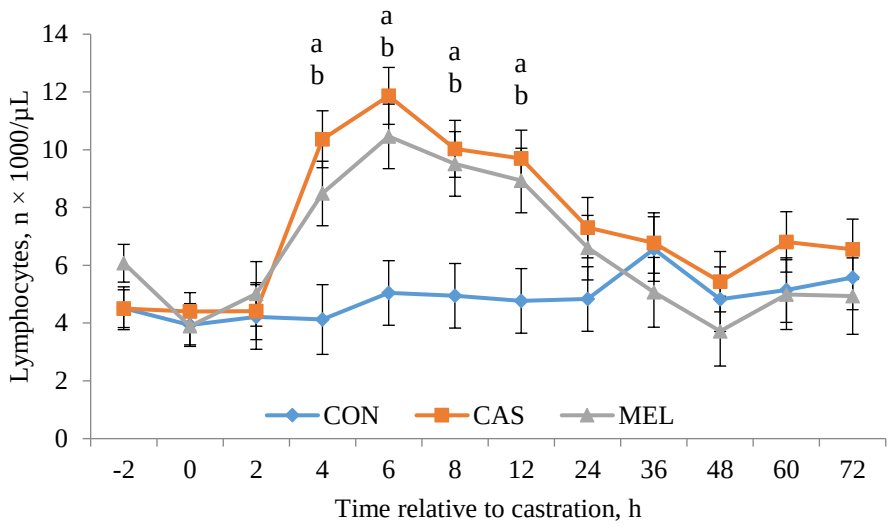

Figure 6. Blood lymphocyte concentration (± SEM) of intact bull calves treated with sham castration (CON; n=10), bulls surgically castrated without meloxicam administration (CAS; n= 10), and bulls surgically castrated with oral meloxicam (1 mg/kg BW) administration (MEL; n=10). A treatment × time interaction was detected ($P = 0.016$). Within hour, letters indicate the following comparison ($P \leq 0.02$): a = CON vs. CAS, b= CON vs. MEL, and c= CAS vs. MEL.

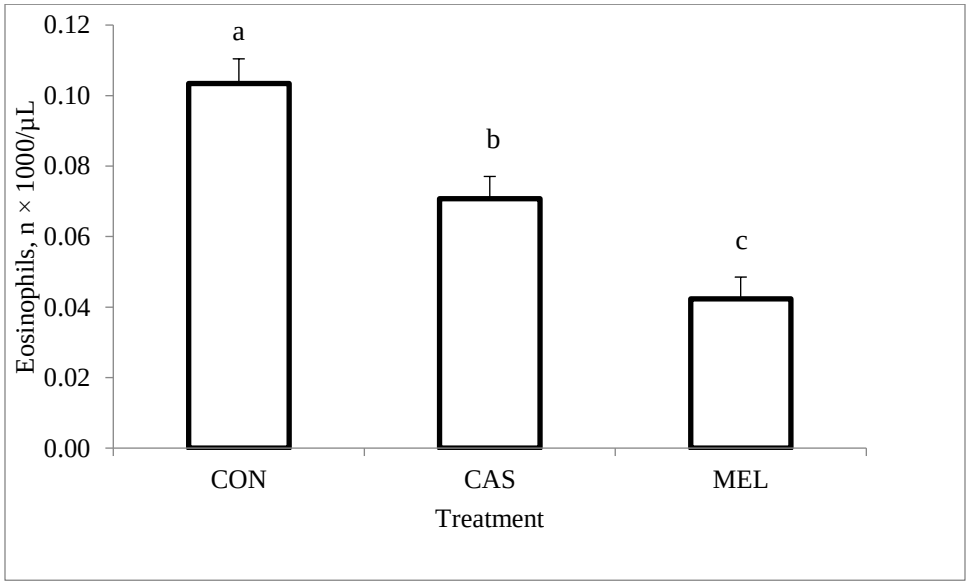

Figure 7. Post-castration (h 0.5-72) blood eosinophil concentration (± SEM) of intact bull calves treated with sham castration (CON; n=10), bulls surgically castrated without meloxicam administration (CAS; n= 10), and bulls surgically castrated with oral meloxicam (1 mg/kg BW) administration (MEL; n=10). Treatment effect ($P < 0.001$); means with different letters differ ($P \leq 0.002$).

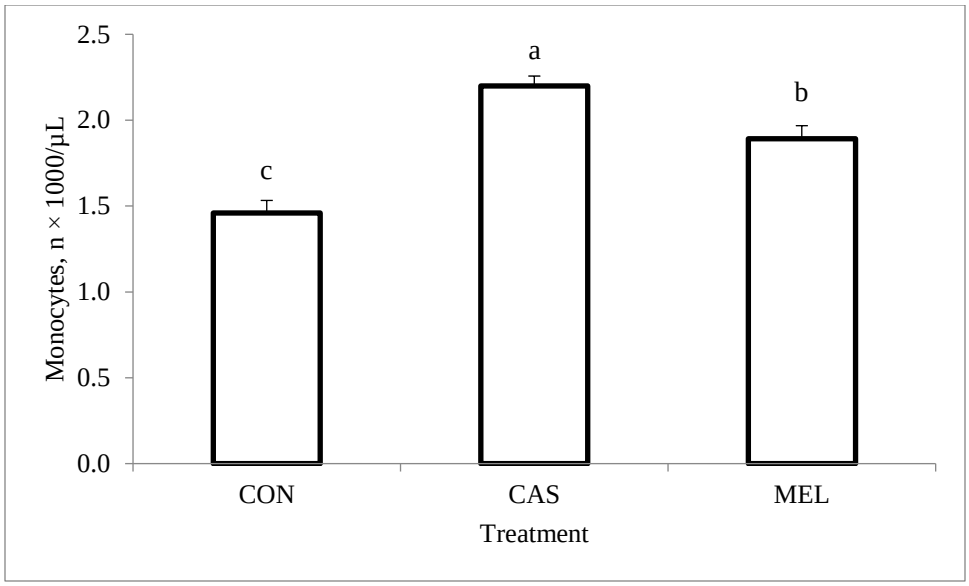

Figure 8. Post-castration (h 0.5-72) blood monocyte concentration (± SEM) of intact bull calves treated with sham castration (CON; n=10), bulls surgically castrated without meloxicam administration (CAS; n= 10), and bulls surgically castrated with oral meloxicam (1 mg/kg BW) administration (MEL; n=10). Treatment effect ($P < 0.001$); means with different letters differ ($P \leq 0.002$).

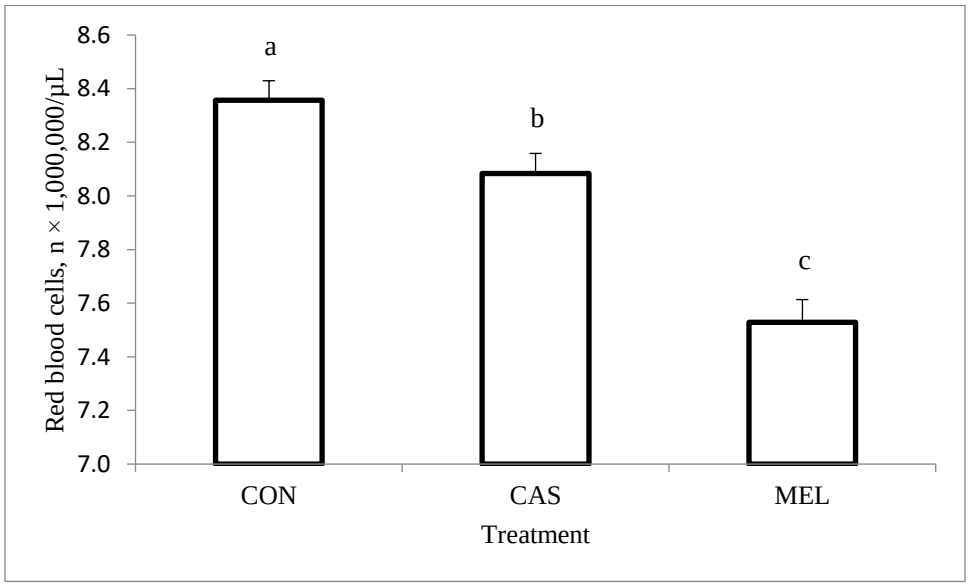

Figure 9. Post-castration (h 0.5-72) red blood cell concentration (± SEM) of intact bull calves treated with sham castration (CON; n=10), bulls surgically castrated without meloxicam administration (CAS; n= 10), and bulls surgically castrated with oral meloxicam (1 mg/kg BW) administration (MEL; n=10). Treatment effect ($P < 0.001$); means with different letters differ ($P \leq 0.01$).

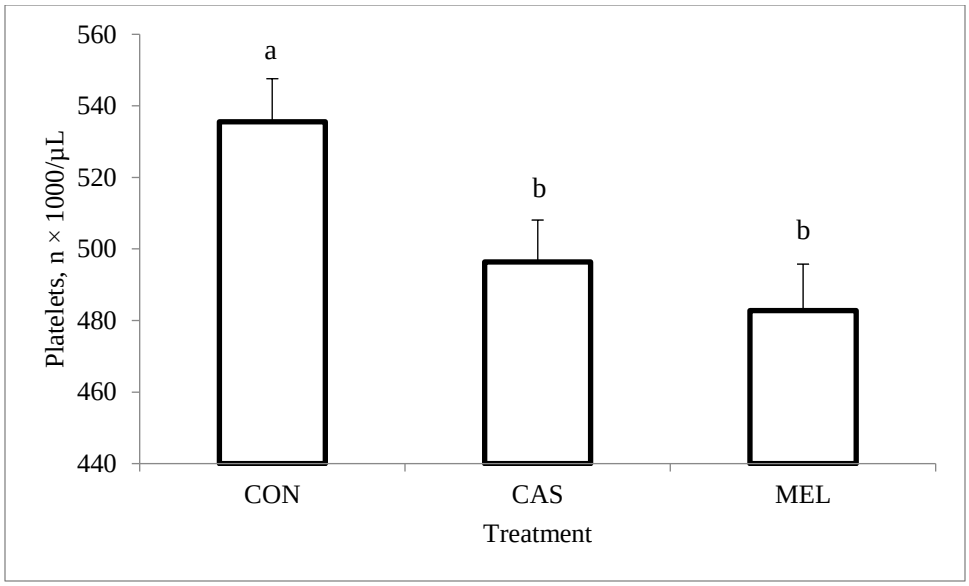

Figure 10. Post-castration (h 0.5-72) platelet concentration (± SEM) of intact bull calves treated with sham castration (CON; n=10), bulls surgically castrated without meloxicam administration (CAS; n= 10), and bulls surgically castrated with oral meloxicam (1 mg/kg BW) administration (MEL; n=10). Treatment effect ($P = 0.008$); means with different letters differ ($P \leq 0.02$).

CHAPTER III

EFFECT OF SURGICAL CASTRATION WITH OR WITHOUT ORAL MELOXICAM ON GROWTH, BEHAVIOR, AND INFLAMMATION IN PRE-WEANED BEEF CALVES

S. L. Roberts[1], J. G. Powell[2], D. S. Hubbell[3], M. S. Gadberry[4], P. A. Beck[5], E. A. Backes[2], J. L. Reynolds[2] and J. T. Richeson[1]

[1]Department of Agricultural Sciences, West Texas A&M University, Canyon

[2]Department of Animal Science, Division of Agriculture, University of Arkansas, Fayetteville

[3]Livestock and Forestry Research Station, Division of Agriculture, University of Arkansas, Batesville

[4]Cooperative Extension Service, Division of Agriculture, University of Arkansas, Little Rock

[5]Southwest Research and Extension Center, Division of Agriculture, University of Arkansas, Hope

ABSTRACT

Use of analgesics during painful management procedures such as castration warrants investigation, yet the efficacy of pain management strategies may be impacted by the age of animals in which the strategy is applied. The objective of this study was to evaluate the effects of oral administration of the analgesic meloxicam (1 mg/kg BW) on growth performance, behavior and inflammation in calves castrated at the time typically known as branding (~90 d of age). Crossbred beef bulls (n=83; 105.8 ± 7.1 kg) from a single herd were assigned randomly to 1 of 3 treatments: 1) positive control consisting of intact males through weaning (CON), 2) surgical castration at branding (CAS), and 3) surgical castration at branding with oral meloxicam (MEL). Calves were housed with their dams in 6 different cohort pastures with treatments similarly represented in each pasture. Body weights were recorded on d 0 (branding), 7, 14, 28, 56, 84 and 112 (weaning) to determine gain performance. Blood samples from a subset of calves (n=27) were collected on d 0, 0.25, 1, 3, and 7 and used to quantify serum haptoglobin (Hp) concentration as a proxy for systemic inflammation and complete blood count (CBC) was determined via automated hemocytometer using whole blood. Calves were fitted with accelerometers on d 0 to measure behavior variables. No differences in BW were detected at weaning ($P = 0.95$); however, performance was reduced ($P = 0.05$) in the CAS group for 2 wk following castration. The ADG from d 0 to 14 was 0.75, 0.44, 0.68 kg/d for CON, CAS and MEL, respectively. Meloxicam administration altered lying behavior following castration; time spent lying on sternum as a proportion of total lying time was decreased for MEL (48.0%) compared to CON (52.1%) and CAS (53.5%) groups ($P < 0.10$). Serum Hp concentration was less (trt x time; $P \leq 0.05$) for CON

compared to the CAS and MEL groups on d 0, 0.25, 1 and 3, but no differences were observed between castrated groups for those time periods. Total white blood cell count was increased (trt x time; $P < 0.001$) on d 0.25 for CAS with no differences observed between CON and MEL. Both CAS and MEL resulted in increased Hp concentration relative to CON; whereas, ADG was transiently reduced for CAS but not CON or MEL.

Key words: beef cattle, castration, meloxicam

INTRODUCTION

Castration in beef cattle is a painful management practice that can modulate the physiological responses and behavior of the animal (Robertson, 1994). Physiological responses include stimulation of the immune system, acute phase protein production and activation of the hypothalamic-pituitary-adrenal stress axis (Chase et al., 1995; Cohen et al.,1990; Brown et al., 2015; Roberts et al., 2015). The age of the animal during castration can modulate the magnitude of the stress response with younger animals, those less than 6 months old, experiencing less physiological stress than older animals (Bretschneider et al., 2005). This is supported by greater cortisol concentration in older animals that experienced greater levels of physiological stress when compared to younger animals at the time of castration (Mellor et al., 1991).

Timing of castration varies throughout the beef production system depending on the producer's management philosophy and resources. Only 59.2% of cow-producers castrate their bull calves prior to marketing (USDA, 2009), despite numerous studies that indicate the benefits of castration at earlier ages (Bretschneider et al., 2005; Champagne et al., 1969). Producers may choose to delay castration due to lack of facilities, increased

labor and the belief that at weaning bulls will have heavier BW compared to steers. The pain incurred as a result of routine production practices has a negative public perception, especially when they are performed without pain mitigation techniques (Benson and Rollin, 2008). Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID) that mitigates pain through reduction in inflammation at the site of tissue damage (Otto and Short, 1998). Meloxicam functions to reduce the synthesis of inflammatory prostaglandins, primarily prostaglandin $E_{2\alpha}$, from arachidonic acid by inhibition of cyclooxygenase (COX) enzymes (Ochroch et al., 2003).

Our hypothesis was that administration of oral meloxicam will mitigate the performance loss and inflammation in pre-weaned (branding) bulls (~ 60 days of age) following surgical castration. The objective of this study was to evaluate the effect of oral administration of the analgesic meloxicam on growth, performance, behavior and inflammation in calves surgically castrated at branding.

MATERIALS AND METHODS

This study was conducted during the summer and fall of 2015. All animals were cared for in accordance to acceptable practices and experimental protocol approved by the University of Arkansas Animal Care and Use Committee.

Animals

Eighty-three crossbred beef bulls (105.8 ± 7.1 kg) from the University of Arkansas Livestock and Forestry Research Station near Batesville, AR were utilized for this study. Calves were housed with their dams in 6 different pastures. Calves were separated from their dams and BW was recorded on d 0, 7, 14, 28, 56, 84, 112 and 123.

On d 112, animals were removed from their dams and weaned as a single group in a drylot facility.

Treatments

Animals were randomly assigned to 1 of 3 treatments including: 1) intact bulls serving as positive controls (CON), 2) bull calves surgically castrated (CAS), and 3) bulls calves surgically castrated with 1 mg/kg BW oral meloxicam administration (MEL). Castration occurred on d 0 at an average calf age of 68 ±15 days. Surgical castration was performed while calves were restrained in a hydraulic chute by removing the ventral one-third of the scrotum; the testes were pulled ventral exposing the spermatic cords which were then severed with a scalpel using aseptic technique. Meloxicam tablets (Carlsbad Technology, Inc., Carlsbad, CA) were placed in gelatin capsules (32 mL) and administered orally using a bolus device. The appropriate number of meloxicam tablets, according to BW, was counted and placed in the bolus to achieve 1 mg/kg of d 0 BW concurrent with castration. Maximum serum concentration of meloxicam occurs between 12-24 h after administration with a relatively long half-life of 28 h (Coetzee, 2009; Fraccaro et al., 2013). Following castration, calves and dams were returned to pasture. All treatments were equivalently represented among pastures.

Sample Collection and Analysis

Blood samples were collected on a subset of 27 calves (9 calves/treatment) on d 0, 0.25, 1, 3 and 7. Jugular venipuncture was performed to collect blood into 2 commercial blood tubes. Evacuated sampling tubes without additive (BD Vacutainer, Franklin Lakes, NJ) were used to harvest serum. Centrifugation was performed at 1,500 × *g* for 20

minutes at 4°C to separate serum from cellular components of whole blood. Serum was decanted and stored in duplicate at -40°C. Sera was used to determine haptoglobin (Hp) concentration using a commercially available, bovine-specific sandwich ELISA kit (Immunology Consultant Laboratory, Inc., Portland, OR). Whole blood was collected into an evacuated blood tube containing 7.2 mg K_2 EDTA (BD Vacutainer, Franklin Lakes, NJ) for complete blood count (CBC) analysis using an automated hemocytometer (Cell Dyn, Abbott Laboratories, Abbott Park, IL).

A subset of 36 calves had accelerometers (Onset Company, Bourne, MA) placed on their left rear leg on d 0 and the devices were removed on d 7. These animals were not used for blood collection to minimize handling effects on behavior data. Data ware excluded for animals that had loose accelerometers or lost data. Raw data from accelerometers were processed using the same methods as described in Brown et al. (2015).

Statistical Analyses

Animal performance data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Individual animal was identified as the experimental unit. Treatment was the fixed effect with sire and dam included in the model as random effects. Least square means were used to determine treatment differences with significance established for $P \leq 0.05$. Behavior data were analyzed as repeated measures using the GLIMMIX procedure of SAS. The covariate structure used was compound symmetry. A significance level of $P \leq 0.10$ was used to determine treatment effect on behavior data. Hematological variables and serum Hp concentration were evaluated using the MIXED procedure with repeated measures. For this repeated measures analysis, day

was identified as the repeated statement and fixed effects included treatment, day, and their interaction. Sire and dam were included in the model as random effects. The covariate structure for each repeated variable evaluated with the lowest Akaike information criterion (AIC) estimate was used. Before analysis of repeated data, PROC UNIVARIATE was used to test for normality. Dependent variables determined to be non-normal in their distribution were $\log_2$- transformed prior to statistical analysis and the back-transformed means are reported.

RESULTS AND DISCUSSION

Growth Performance

Body weight results are presented in Table 1. There was no treatment difference in BW throughout the entire study ($P \geq 0.72$). Interestingly, there was no difference ($P = 0.95$) in BW between bulls and steers at weaning (d 112). This lack of difference in BW is contrary to industry dogma that bulls have heavier BW at weaning compared to steers. Similar results have been published previously, where animals castrated near birth had no difference in BW at weaning compared to intact males (Champagne et al., 1969). Meloxicam had no effect ($P \geq 0.72$) on BW in the current study. Similarly, Brown et al. (2015) found when administered during castration at birth, meloxicam had no effect on BW. The lack of meloxicam effect noted in younger calves may be explained by the reduced stress and inflammatory responses from castration performed in young calves.

Average daily gain results are presented in Table 2. There was no difference ($P = 0.32$) in ADG at d 7 following castration. However, there was a difference ($P = 0.05$) in ADG between treatments for the initial two week period (d 0 to 14) following surgical

castration. Calves castrated without meloxicam administration had reduced ($P < 0.05$) ADG compared to CON and MEL, with no difference ($P = 0.61$) between CON and MEL. There was no difference ($P = 0.78$) in ADG through weaning (d 0 to 112).

Activity

There was no difference ($P > 0.10$) in percentage of time standing for the week following surgical castration. There was a difference ($P < 0.10$) in the time spent lying sternum as a percentage of total lying time. The animals castrated with meloxicam spent a lesser percentage ($P \leq 0.05$) of time lying in a sternum position compared to CON and CAS, with no difference ($P = 0.14$) between the CON and CAS treatments. Lying positon recorded by the accelerometer position is difficult to delineate if time spent in this position is an indicator or pain or animal contentment, especially since CAS treatment was not different from non-castrated controls. Mean duration of standing and lying on side events did not differ ($P = 0.03$) between treatments. However, there was a difference ($P < 0.10$) in mean duration of lying sternum events. Meloxicam administration during castration had the lowest mean duration (12 min), while the CAS calves had the largest mean duration (15.7 min) with CON calves being intermediate (14.3 min). The number of daily lying and standing events did not differ ($P > 0.10$) following castration. Because calves in this study were returned to pasture following castration, activity may be influenced by the activity of the calves' dams or other factors that may complicate these measurements.

Activity variables analyzed from this study suggests that castration at branding does not clearly alter behavior of calves. However, castration near birth has been previously reported to decrease the amount of time lying when compared to intact bulls

(Brown et al., 2015). Similar to our results, Brown et al. (2015) found that meloxicam administration reduced the proportion of time spend lying sternum following castration at birth. Similarly, Ting et al. (2003) reported that bulls surgically castrated at 11 month of age had a decreased incidence of lying posture compared to males left intact during a 6 h post-castration observation period. Furthermore, the authors reported that repeated administration of the NSAID, ketoprofen, did not modify behavior compared to controls. It was concluded that age at castration does affect behavioral modification following castration.

Hematology

Effects of meloxicam administration during surgical castration on hematological variables are presented in Table 4. Total white blood cell (WBC) count had a treatment × day interaction ($P < 0.001$). On d 0.25, WBC was decreased ($P < 0.001$) for the CON animals compared to castrates with no difference ($P = 0.76$) between the CAS and MEL treatments (Figure 1). Neutrophil concentration (Figure 2) and neutrophil percentage of total WBC (Figure 3) both had treatment × day interactions ($P < 0.001$). Neutrophil concentration was increased on d 0.25 for castrates compared to CON animals ($P < 0.001$) with no difference ($P = 0.89$) observed between the CAS and MEL treatments. On d 1, neutrophil concentration tended to increase ($P = 0.07$) for CAS compared to CON with no difference ($P > 0.13$) between MEL and CON or CAS. Neutrophil percentage was increased on d 0.25 ($P < 0.001$) for CAS and MEL treatments compared to CON. Lymphocyte percentage also had a treatment × day interaction ($P < 0.001$). On d 0.25, CAS and MEL had decreased ($P < 0.001$) lymphocyte percentage compared to the CON animals. There was an overall treatment effect ($P = 0.04$) for percent eosinophils;

meloxicam administration resulted in eosinopenia ($P < 0.03$). There was also a treatment effect ($P = 0.03$) on basophil concentration; CON had decreased ($P = 0.01$) number of basophils compared to CAS, with MEL not differing ($P > 0.05$) from CON or CAS.

In the current study, surgical castration resulted in increased total WBC, neutrophil, and lymphocyte concentrations; however, meloxicam administration did mitigate these increases. Similarly, Chase et al. (1995) reported an increase in WBC for castrates compared to intact males. Meloxicam administration has been reported to decrease total WBC and neutrophil concentration following surgical castration in yearling bulls (Roberts et al., 2015). Similar decreases with meloxicam administration have been observed following long-distance transportation (Van Engen et al., 2014). Eosinophil percentage was altered for meloxicam, which is in agreement with Roberts et al., (2015) who also observed eosinopenia following castration and meloxicam administration.

Haptoglobin

There was a treatment × day interaction ($P < 0.001$) for serum Hp concentration (Figure 5). Castrates had increased ($P < 0.02$) Hp concentration on d 0.25, 1, and 3 compared to CON; however, there was no difference ($P > 0.87$) between CAS and MEL. On d 7, MEL animals had increased ($P < 0.02$) Hp concentration compared to both CON and CAS animals.

Haptoglobin is an acute phase protein that is often used as a proxy for systemic inflammation. Haptoglobin is produced, primarily from hepatocytes, during the acute phase response in response to infection, inflammation, injury or stress (Warnock et al., 2012) In the current study, meloxicam administration was not observed to reduce peak

Hp concentration following surgical castration at branding. Conversely, Ballou et al. 2013 reported that in 3 month old Holstein bull calves, administration of a local anesthetic (lidocaine) and a general analgesic (flunixin meglumine) resulted in Hp reduction 24 hours post-castration. When meloxicam was administered during castration in yearling animals, a reduction in peak serum Hp concentration was observed (Roberts et al., 2015). The difference between studies is likely a result of different ages at castration; an NSAID such as meloxicam is probably more efficacious in older castrates with greater inflammation.

In conclusion, castration and administration of meloxicam, when performed at an average age of 68 ± 15 days, did not alter BW compared to bulls. Meloxicam briefly improved ADG in calves castrated at branding; however, inflammation was not clearly affected and weaning weights were not different. Castration that occurs early in life may have long-term animal performance benefits as weaning weight was not different between castrates and intact males, yet delay of the castration procedure until post-weaning is known to decrease performance and increase disease risk.

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Table 1. Effect of surgical castration and meloxicam administration on body weight of pre-weaned beef calves.

	Treatment[1]				
Item	CON	CAS	MEL	SEM	*P*-value
Body weight, kg					
D 0	105.9	106.5	106.3	5.1	0.99
D 7	112.5	112.8	117.8	6.8	0.72
D 14	117.2	113.0	117.0	4.5	0.75
D 28	126.7	126.5	127.6	4.8	0.98
D 56	153.0	152.0	152.0	5.9	0.99
D 84	176.9	175.5	176.7	6.7	0.98
D 112	203.2	200.4	200.8	7.8	0.95
D 123	205.1	208.0	204.0	8.4	0.89

[1] CON= positive control intact males; CAS= surgical castration without meloxicam administration; MEL= surgical castration with 1 mg/kg oral meloxicam administration.

Table 2. Effect of surgical castration and meloxicam administration of average daily gain of pre-weaned beef calves.

	Treatment[1]				
Item	CON	CAS	MEL	SEM	*P*-value
ADG, kg					
D 0 to 7	0.96	1.00	1.53	0.57	0.32
D 0 to 14	0.75[a]	0.44[b]	0.68[a]	0.15	0.05
D 14 to 28	0.63	0.89	0.74	0.14	0.17
D 28 to 56	0.92	0.89	0.83	0.06	0.40
D 56 to 84	0.80	0.80	0.83	0.07	0.92
D 84 to 112	0.91	0.85	0.83	0.07	0.46
D 0 to 112	0.85	0.83	0.82	0.04	0.78
Post-weaning					
D 112 to 123	-0.01	0.07	0.02	0.05	0.53

[1] CON= positive control intact males; CAS= surgical castration without meloxicam administration; MEL= surgical castration with 1 mg/kg oral meloxicam administration.

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

Table 3. Effect of surgical castration and meloxicam administration on activity variables of pre-weaned beef calves.

	Treatments[1]			
Item[2]	CON	CAS	MEL	Pooled SEM
Calves, n	11	10	12	1.31
Time Standing, %	46.9	45.5	45.3	1.31
Lying Sternum, % of total lying	52.1[a]	53.5[a]	48.0[b]	1.44
Standing Activity				
Average daily events, n	39.7	38.0	40.7	2.03
Mean duration per event, min	17.5	17.5	16.0	1.07
Lying on sternum activity				
Average daily events, n	28.2	26.7	31.2	2.04
Mean duration per event, min	14.3[a]	15.7[a]	12.0[b]	1.21
Lying on side activity				
Average daily events, n	20.2	19.2	23.9	1.89
Mean duration per event, min	18.2	19.5	17.6	1.52

[1] CON= positive control intact males; CAS= surgical castration without meloxicam administration; MEL= surgical castration with 1 mg/kg oral meloxicam administration.

2 Data collected using accelerometer and downloaded using HoBoware Lite Graphing and Analysis Software. Calf position was established by x and y axis position of the accelerometer.

[a,b] Least square means within a row with uncommon letter superscripts differ ($P \leq 0.10$).

Table 4. Effects of surgical castration and meloxicam administration on hematology of pre-weaned beef calves.

	Treatment[1]				*P*-value		
Item	CON	CAS	MEL	SEM	Treatment	Day	Treatment x day
Total white blood cells, $x10^3/\mu L$	9.06	10.68	10.35	0.72	0.24	<0.001	<0.001
Neutrophils,[2] $x10^3/\mu L$	3.30	4.77	4.20	-	0.05	<0.001	<0.001
Neutrophils,[2] %	35.45	42.51	37.03	-	0.15	<0.001	<0.001
Lymphocytes, $x10^3/\mu L$	4.67	4.34	4.90	0.58	0.71	<0.001	0.11
Lymphocytes, %	49.10	42.33	48.82	3.54	0.31	<0.001	<0.001
Monocytes,[2] $x10^3/\mu L$	1.20	1.35	1.28	-	0.32	<0.001	0.08
Monocytes,[2] %	13.85	13.28	12.61	-	0.56	<0.001	0.26
Eosinophils,[2] $x10^3/\mu L$	0.03	0.06	0.02	-	0.06	<0.001	0.29
Eosinophils,[2] %	0.33	0.56	0.15	-	0.04	<0.001	0.34
Basophils,[2] $x10^3/\mu L$	0.10	0.16	0.15	-	0.03	<0.001	0.07
Basophils,[2] %	1.19	1.42	1.35	-	0.15	<0.001	0.45
Red blood cells, $x10^6$ µL	9.55	9.47	9.36	0.40	0.93	<0.001	0.01
Hemoglobin, g/dL	11.82	11.56	12.01	0.65	0.77	<0.001	0.03
Hematocrit, %	31.73	30.41	31.63	1.29	0.55	<0.001	<0.001
Platelets, $x10^3/\mu L$	912	1128	1076	144	0.54	<0.001	0.16

[1] CON= positive control intact males; CAS= surgical castration without meloxicam administration; MEL= surgical castration with 1 mg/kg oral meloxicam administration.

[2] Data were log transformed before statistical analysis. Reported least square means are the geometric treatment means and *P*-values shown are for the log transformed data.

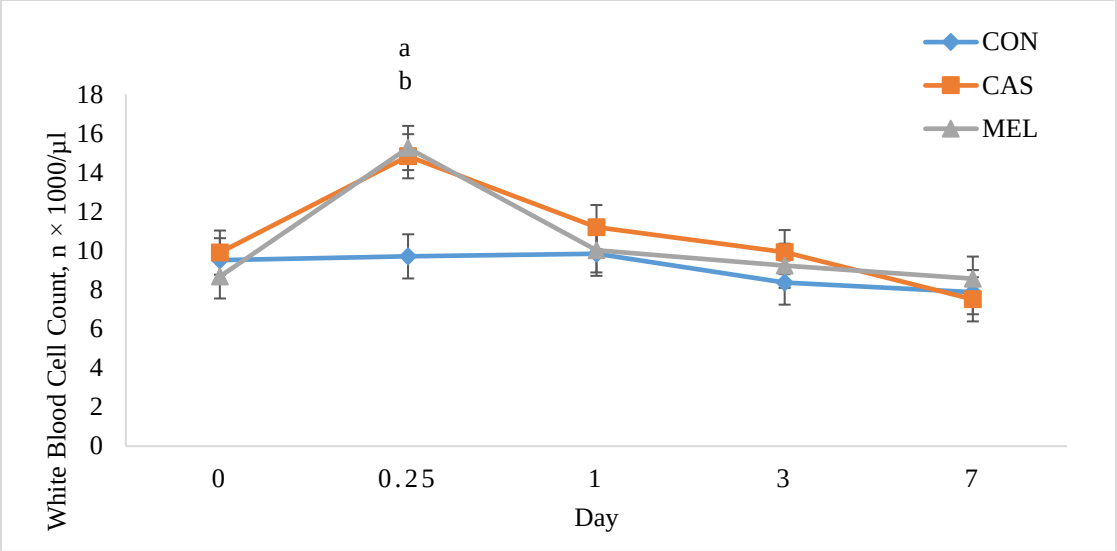

Figure 1. Effect of surgical castration and meloxicam administration on white blood cell count in pre-weaned beef calves. CON= positive control intact males; CAS= surgical castration without meloxicam administration; MEL= surgical castration with 1 mg/kg oral meloxicam administration. Data were log transformed before statistical analysis. Reported least square means are the geometric treatment means and *P*-values shown are for the $\log_2$-transformed data. Effect of treatment, $P = 0.24$; day, $P < 0.001$; treatment × day, $P < 0.001$. Within day, letters indicate the following comparison ($P < 0.001$): a = CON vs. CAS, b = CON vs. MEL, and c = CAS vs. MEL.

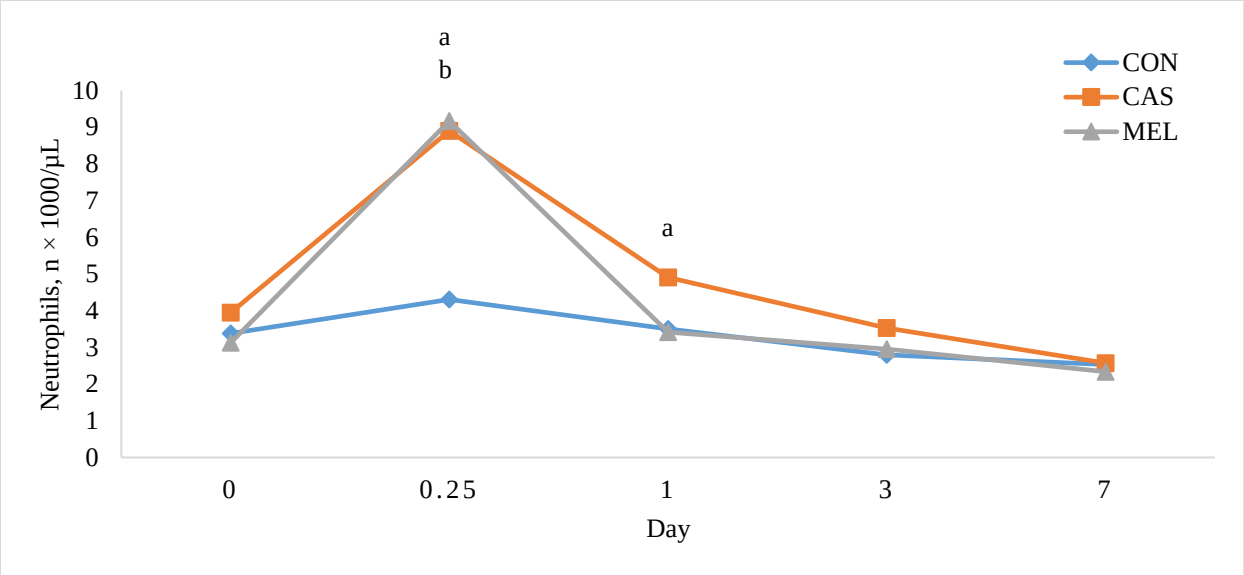

Figure 2. Effect of surgical castration and meloxicam administration on blood neutrophil concentrations in pre-weaned beef calves. CON= positive control intact males; CAS= surgical castration without meloxicam administration; MEL= surgical castration with 1 mg/kg oral meloxicam administration. Data were $\log_2$- transformed before statistical analysis. Reported least square means are the geometric treatment means and *P*-values shown are for the log transformed data. Effect of treatment, $P = 0.05$; day, $P < 0.001$; treatment × day, $P < 0.001$. Within day, letters indicate the following comparison ($P < 0.001$): a = CON vs. CAS, b = CON vs. MEL, and c = CAS vs. MEL.

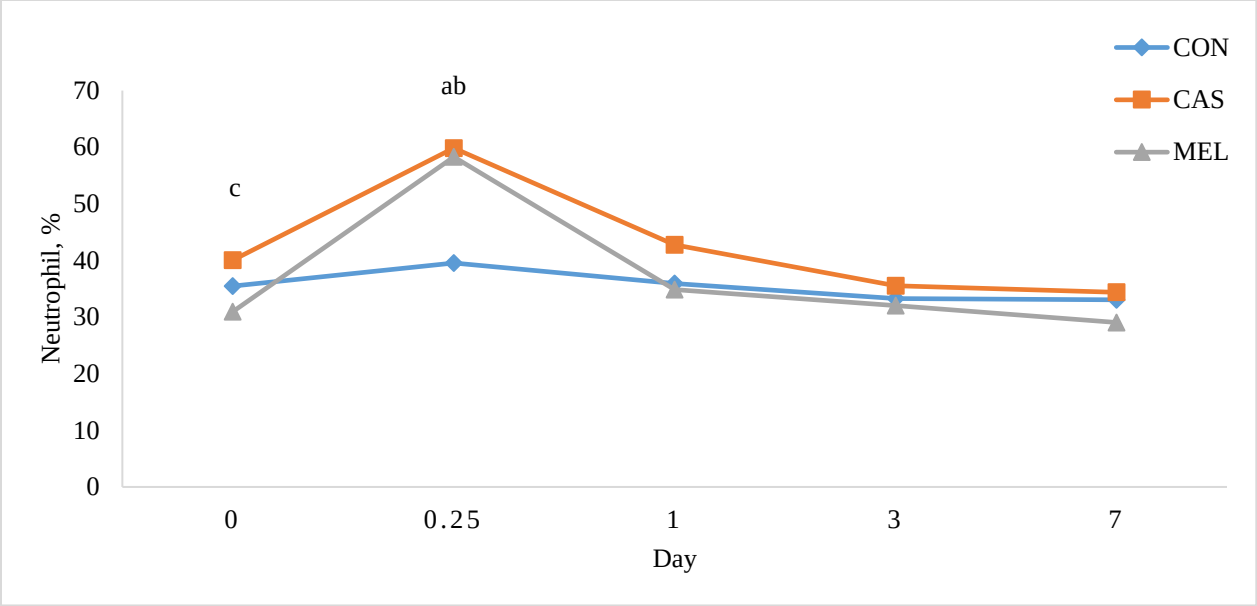

Figure 3. Effect of surgical castration and meloxicam administration on blood neutrophil percentage in pre-weaned beef calves. CON= positive control intact males; CAS= surgical castration without meloxicam administration; MEL= surgical castration with 1 mg/kg oral meloxicam administration. Data were $\log_2$- transformed before statistical analysis. Reported least square means are the geometric treatment means and *P*-values shown are for the log transformed data. Effect of treatment, $P = 0.15$; day, $P < 0.001$; treatment × day, $P < 0.001$. Within day, letters indicate the following comparison ($P < 0.001$): a = CON vs. CAS, b = CON vs. MEL, and c = CAS vs. MEL.

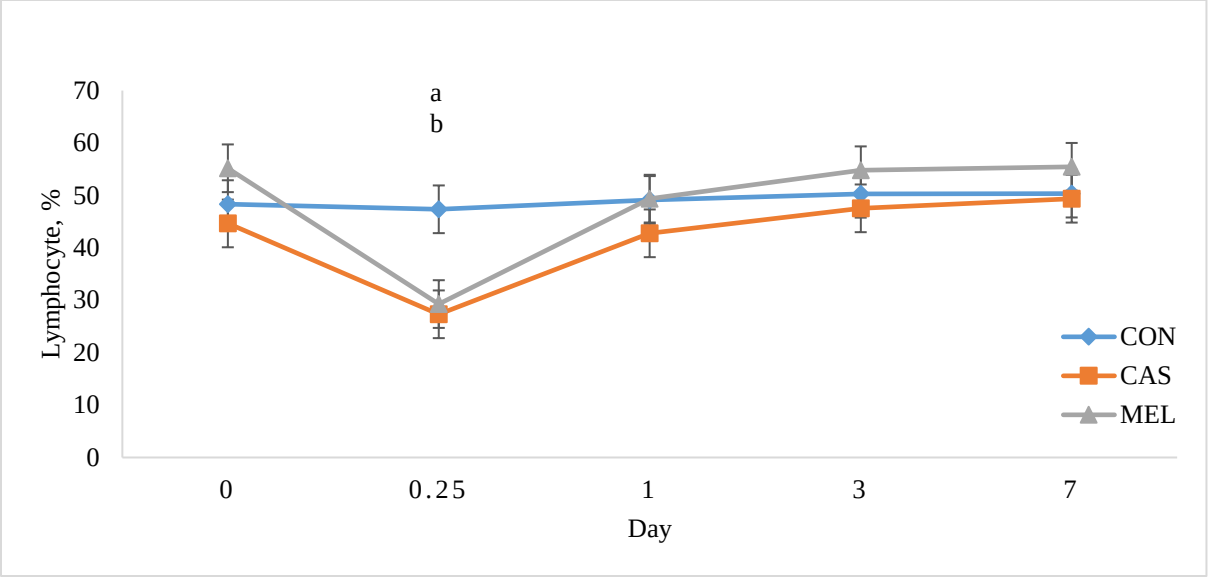

Figure 4. Effect of surgical castration and meloxicam administration on blood lymphocyte percentage (± SEM) in pre-weaned beef calves. CON= positive control intact males; CAS= surgical castration without meloxicam administration; MEL= surgical castration with 1 mg/kg oral meloxicam administration. Effect of treatment, $P = 0.31$; day, $P < 0.001$; treatment × day, $P < 0.001$. Within day, letters indicate the following comparison ($P < 0.001$): a = CON vs. CAS, b = CON vs. MEL, and c = CAS vs. MEL.

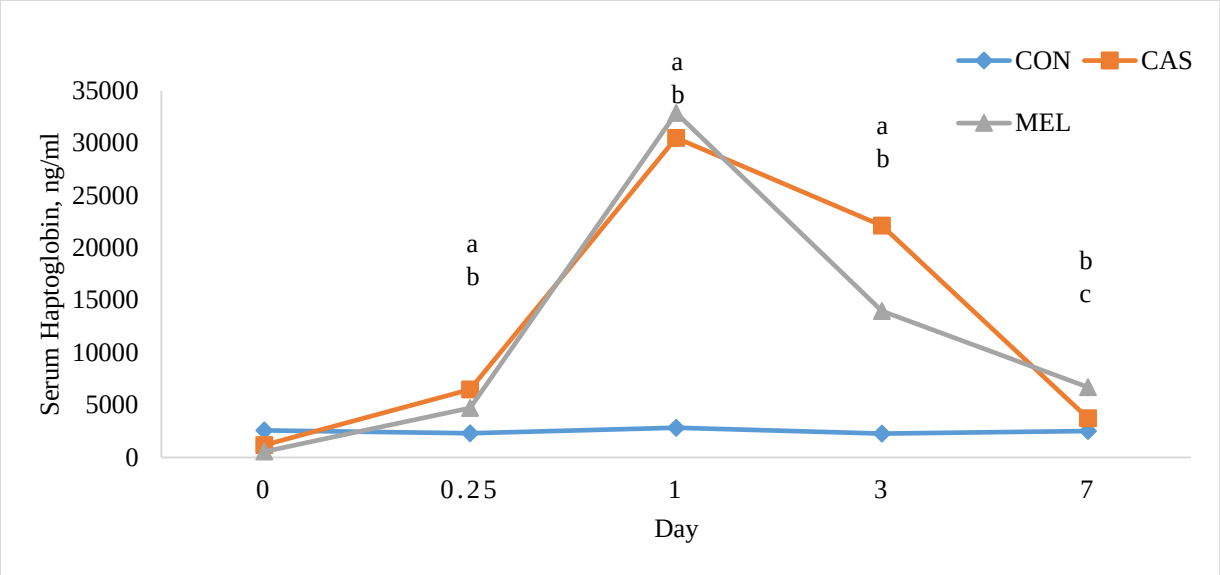

Figure 5. Effect of surgical castration and meloxicam administration on serum haptoglobin concentration in pre-weaned beef calves. CON= positive control intact males; CAS= surgical castration without meloxicam administration; MEL= surgical castration with 1 mg/kg oral meloxicam administration. Data were $\log_2$- transformed before statistical analysis. Reported least square means are the geometric treatment means and *P*-values shown are for the log transformed data. Effect of treatment, $P = 0.24$; day, $P < 0.001$; treatment × day, $P < 0.001$. Within day, letters indicate the following comparison ($P < 0.001$): a = CON vs. CAS, b = CON vs. MEL, and c = CAS vs. MEL.

CHAPTER IV

EFFECT OF CASTRATION METHOD AND MELOXICAM ON BEHAVIOR, INFLAMMATION, GROWTH PERFORMANCE, AND CARCASS TRAITS IN FEEDLOT CATTLE

S. L. Roberts[1], H. D. Hughes[1], J. G. Powell[2] and J. T. Richeson[1]

[1]Deparment of Agricultural Sciences, West Texas A&M University, Canyon, TX, 79016

[2]Department of Animal Science, Division of Agriculture, University of Arkansas, Fayetteville, AR, 72701

ABSTRACT

Castration is a painful, yet routine management practice that is known to transiently decrease performance, and the method of castration, provision of analgesia, or both may impact inflammation and growth in feedlot cattle. Our objective was to determine the effect of castration timing (birth vs. feedlot entry), method (surgical vs. banding) and use of the analgesic meloxicam (**MEL**) on behavior, inflammation, performance, and carcass traits in feedlot cattle. This study was a randomized complete block design conducted over a 3-year period. Single-source Angus × Hereford steer (n = 42) and bull (n = 152) calves were randomized at birth to 1 of 5 treatments arranged as a 2 × 2 + 1 factorial: 1) steers castrated near birth (**CON**), 2) bulls surgically castrated without MEL (**SUR**), 3) bulls surgically castrated with MEL (**SUR+MEL**), 4) bulls band castrated without MEL (**BAN**), and 5) bulls band castrated with MEL (**BAN+MEL**). Upon feedlot arrival (d -10), animals were blocked by initial BW (224 ± 4.5 kg) and assigned randomly to treatment pens (n = 6 pens/treatment). Oral MEL was administered at 1 mg/kg BW concurrent with castration on d 0. Body weight was not affected by castration method or MEL. From d 0 to 7, ADG was reduced for surgical (-0.42 kg/d) compared to band (0.43 kg/d) castration. Conversely, ADG was increased for surgical (1.74 kg/d) vs. band (1.46 kg/d) castration from d 14 to 32. There was also an overall improvement in ADG for CON ($P = 0.04$) and MEL ($P < 0.01$), but this was not influenced by method ($P = 0.80$). The CON had increased marbling score ($P = 0.03$) compared to castrated; whereas, backfat thickness was increased ($P < 0.05$) in SUR+M, but did not differ from CON ($P = 0.15$). There was a treatment × d interaction ($P = 0.04$) with SUR animals having the greatest ($P < 0.01$) concentration of Hp on d 1 and 4.

Meloxicam administered during surgical castration reduced ($P = 0.01$) Hp concentration relative to SUR on d 1. Method of castration had contrasting effects on specific behavior variables. Relative to baseline, standing duration for surgical castration increased 113 min ($P < 0.01$), while banding caused 6.7 more lying bouts ($P < 0.01$) immediately following castration on d 0. Steps were increased on d 0 for banded (2,723), intermediate for CON (2,216), and least (1,801 steps) for surgical ($P < 0.01$). Results suggest that MEL mitigated the more pronounced inflammation observed for surgical castration; whereas, behavior was differentially altered for castration method indicative of a divergent pain response. Castration near birth had long-term performance benefits compared to castration upon feedlot arrival. Castration, regardless of method, transiently reduced ADG, but MEL administration improved overall ADG for both methods.

Key Words: analgesia, beef cattle, castration, performance

INTRODUCTION

Castration is a traditional management practice utilized by beef producers within the United States, with nearly 15 million bull calves castrated each year (APHIS, 2011). Castration benefits include modifications of animal temperament, behavior and changes in carcass compositions. Negative behavioral traits associated with intact males are a result of testicular production of the androgen, testosterone (Ford and D'Occhio, 1989). These negative behavior traits are usually displayed once an animal reaches puberty, which occur around 7 to 10 months of age in beef cattle (Wolf et al., 1965). Steers have improvements in meat quality due to higher quality grades and increased tenderness when compared to intact males (Field, 1971; Klosterman et al., 1954; Seideman et al., 1982). Carcass composition differences are a result of difference in physiological growth, steers

appear to enter the fattening phase earlier reducing muscle growth, while bulls have a greater impetus for muscle growth before heavy fat deposition (Berg and Butterfield, 1968).

Surgical and rubber band castration are the two most common methods utilized by cow-calf producers and feedlot managers. Of cow-calf producers that castrate males, 49.2% use surgical castration and 47.3% use band castration (USDA, 2009). Castration of bulls upon feedlot entry show a similar trend with 52.3% surgically castrated, 41.1% band castrated and 6.6% not castrated at all (APHIS, 2011). These different methods of castration produce different behavioral and physiological responses within the animal. Surgical castration has been observed to cause greater changes in behavior following castration, as well as greater haptoglobin response compared to banded counterparts (Fisher et al., 2001). The use of analgesic drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs), may benefit the animal through pain mitigation, reduction in inflammation at the site of tissue damage and a more rapid patient recovery (Otto and Short, 1998). We hypothesized that delaying castration until feedlot entry will alter behavior, growth performance and carcass traits, as well as elicit an increased inflammatory response compared to calves castrated near birth. However, oral meloxicam administration will ameliorate these responses in castrated animals. The objective of this study was to determine if castration, method of castration (surgical vs. banding) and meloxicam administration upon feedlot entry affects growth, inflammation and carcass traits in finishing cattle.

MATERIALS AND METHODS

Animal methods and procedures were approved by the University of Arkansas (UA) and West Texas A&M University (WTAMU; protocol #03-05-13) Animal Care and Use Committees.

Animals

This study was conducted over 3 consecutive years (2013 to 2015). One hundred and ninety-four Angus × Hereford crossbred male calves (steers= 42 and bulls= 152 head) from the University of Arkansas Savoy cow herd were utilized for this study. At birth, calves were randomly assigned to 1 of 5 treatment groups: 1) calves castrated near birth serving (CON), 2) feeder cattle castrated surgically without oral meloxicam administration (SUR), 3) feeder cattle castrated surgically with oral meloxicam administration (SUR+MEL), 4) feeder cattle castrated via banding without oral meloxicam administration (BAN), and 5) feeder cattle castrated via banding with oral meloxicam administration (BAN+MEL). Treatments were designed in a 2 × 2 factorial to investigate the main effects of castration method (surgical vs. banding) and analgesia and their interaction with the addition of a control. Castration at birth was performed using the banding technique without meloxicam administration. Surgical castration was performed by removing the ventral 1/3 of the scrotum with a scalpel, followed by emasculation of the spermatic cords. The BAN treatments were castrated using a California bander (Inosol Co., El Centro, CA). Meloxicam was administered at 1 mg/kg and given concurrent with castration. Whole meloxicam tablets (Carlsbad Technology Inc., Carlsbad, CA) were placed in gelatin capsules. The bolus containing meloxicam were administered directly into the rumen via a bolus device.

Identical processing with health and performance products were similar within year. Calves were vaccinated for clostridial diseases and respiratory viruses (pentavalent MLV vaccine) at approximately 60 days of age. Calves were removed from their dams and moved to small weaning lots. Calves were administered a daily supplement at approximately 1% of their BW. Calves were then allowed to graze a mixed-grass pasture for a 56-d weaning period. At the beginning of the weaning period, an anthelmintic (Dectomax, Zoetis) was administered according to BW. Calves were transported to the WTAMU Research Feedlot at the end of the 56-d weaning period. Within year, calves were blocked within each treatment group into a light and heavy block using the weight collected at the end of the weaning period. Calves were held overnight in a receiving pen. The following morning (d -10), calves were processed and received subcutaneous injections of anthelmintic (Dectomax, Zoetis) and tulathromycin (Draxxin, Zoetis). Calves were assigned randomly to pens within treatment and BW block resulting in 2 pen replications/treatment/year (total of 6 pen replicates/treatment). A 10-d post metaphylaxis interval and acclimation period was implemented before castration was performed (d 0). On d 0 cattle were revaccinated with a pentavalent MLV respiratory vaccine (Bovi-Shield Gold 5, Zoetis), vaccinated with clostridial + tetanus vaccine (Covexin 8, Merck Animal Health). The light block was administered a growth implant (Ralgro, Merck Animal Health) containing 36 mg of zeranol for all 3 years. While in years 1 and 2, the heavy block was given a growth implant containing 40 mg estradiol and 200 mg trenbolone acetate (Revalor- XS, Merck Animal Health) and in year 3 Revalor- IS (8 mg estradiol and 80 mg trenbolone acetate) was administered. An additional reimplant was administered to cattle each year on average around d 80. In years 1 and 2, an additional

growth implant (Revalor- XS) was administered to the light block, while in year 3 the light block was administered Revalor 200 (20 mg estradiol and 200 mg trenbolone acetate) and the heavy block was administered Revalor- IS.

Sample Collection and Assay Procedures

Body weights were collected at birth, branding (60 days of age), weaning, feedlot arrival (d-10), 0, 1, 4, 7, 14, 32, 70 and a final BW determined from the average of 2 consecutive BW on the day prior to and day of harvest.

Blood was collected from a subset of 50 animals per year (total n= 150) at the time of castration (d 0), 6 hours post-castration (0.25), 1, 4, 7, 14, and 32. Blood was collected into an evacuated sampling tube without additives (BD vacutainer, Franklin Lakes, NJ). To harvest serum, blood was centrifuged at 1,500 × *g* for 20 mins at 4°C. Serum was decanted into triplicate aliquots and stored at -20°C. Serum was then analyzed for haptoglobin (Hp) concentration. A commercially available sandwich ELISA kit (Immunology Consultants Laboratory, Inc., Portland, OR) was used to determine serum Hp concentration.

The same subset of animals (total n= 150) used for blood collection were also used to collect behavior data. Behavior variables were collected via pedometers (IceQube, IceRobotics, Midlothian, Scotland, UK) fitted on the animals on d -10 and removed 32 d post-castration. The IceQube pedometers logged data every 15 mins and the parameters collected include: time spent standing, time spent lying, total steps taken, number of lying bouts, and a motion index which is determined by a proprietary method. A baseline value for each group was determined by average of days -9 to -1.

Cattle were fed a common feedlot finishing ration throughout the finishing period of all three years. Each year, cattle were harvested at a commercial facility according to BW and visual appraisal. Treatments within each weight block were harvested on the same day. Carcass data was collected by WTAMU Beef Carcass Research Center personnel.

Statistical Analyses

For all statistical analyses SAS version 9.4 (SAS Inst. Inc., Cary, NJ) was used with pen, within year, used as experimental unit. Fixed effects included in the model were method, meloxicam and their interactions with significance established at $P \leq 0.05$. Random effects in the model included block and year. An additional contrast of CON vs. castrated treatments was analyzed. Performance and non-categorical carcass variable were evaluated using the MIXED procedure. The MIXED procedure with repeated measures was used to evaluate haptoglobin data. Day was used at the repeated statement with fixed effects of treatment, day and treatment × day interactions. The GLIMMIX procedure was used to evaluate quality grade and activity variables with compound symmetry used as the covariance structure. Least square mean differences were determined for method, meloxicam, their interaction and the additional contrast of CON vs. castrates with significance set at $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth Performance

The effect of treatment on growth performance is presented in Table 1. There was no difference ($P = 0.55$) in BW at weaning between steers castrated near birth and intact

bulls. These results are contrary to current industry dogma that bulls will have heavier BW at weaning compared to steers. There was a tendency ($P \leq 0.10$) for CON to have heavier BW than their castrated cohorts on d 14, 32, and time of reimplant. Castration method had no effect ($P = 0.90$) on final BW, but there was a tendency ($P = 0.07$) for meloxicam administration to have heavier final BW, regardless of castration method. Despite weight loss following castration, castrates compensated for the transient decrease and final BW did not differ ($P = 0.17$) between castrates and CON steers. However, the numerical increase in final BW that existed for CON, although not statistically different, would be financially beneficial to the producer.

Average daily gain results are presented in Table 2. Castration method had an effect on ADG for the first two weeks following castration. The first week post-castration (d 0 to 7) surgical castration had decreased ($P = 0.01$) ADG compared to band castrated animals. Conversely, the second week post-castration (d 7 to 14) surgical castration had increased ($P = 0.05$) ADG compared to the banded animals. There was also a difference ($P < 0.001$) for d 14 to 32 with surgical castration having greater ADG compared to band castration during this time. Nevertheless, these effects were transient; there was no effect ($P = 0.81$) of castration method on overall ADG for the entire finishing period. Meloxicam administration had no effect ($P = 0.50$) on ADG from d 0 to re-implant. There was an effect ($P =0.02$) of meloxicam administration on overall ADG with the meloxicam treatments having greater ADG compared to castrated animals that did not receive meloxicam, regardless of castration method. We expected to observe performance effects for meloxicam-treated castrates in the early interim periods, near the time of meloxicam administration, yet differences did not occur until after d 30. Control animals

that were castrated at birth had greater ($P < 0.001$) ADG compared to castrated animals from d 0 to reimplant; however, the increase in performance for CON was not maintained ($P > 0.17$) after reimplant.

The similar final BW observed between treatments are consistent with reports from Jago et al. (1996) and Fisher et al. (2001) where cattle castrated post-puberty finished with harvest weights similar to cattle castrated at younger ages. Castration method was not shown to have an overall effect on ADG, which supports results from a meta-analysis of castration studies that reported castration method had no effect on overall growth performance (Bretschneider, 2005). Performance results from the current study agree with Warnock et al. (2012), who reported that calves castrated prior to weaning had greater initial growth rate at feedlot entry than animals castrated after weaning. There are contradictory reports on the impact of meloxicam administration on ADG. Brown et al. (2015) reported that meloxicam administration improved ADG the first week post-castration in animals surgically castrated at weaning. However, Coetzee et al. (2012) reported that meloxicam administration 24- h prior to surgical castration did not alter growth performance, although it did reduce BRD morbidity in the study. Meloxicam administration pre- and post-band castration also had no effect on animal performance in cattle band castration at 240 d of age (Repenning et al., 2013). Filho et al. (2014) reported improved ADG, G:F, and DMI during the feedlot receiving period in feeder steers that received repeated administration of meloxicam pre- and post- 24-h road transportation.

Carcass Traits

Overall effects of castration and meloxicam administration on carcass traits are presented in Table 3. There was no effect ($P > 0.36$) of castration method or method × meloxicam interaction ($P > 0.15$) on any carcass traits. There was a tendency for meloxicam to increase ($P = 0.10$) hot carcass weight with no difference ($P = 0.46$) between CON and castrates. Marbling score was increased ($P = 0.05$) in CON treatments compared to castrates, with no difference with meloxicam administration ($P= 0.51$). Meloxicam administration ($P = 0.05$) did increase backfat thickness compared to castrated controls. Ribeye area was increased ($P = 0.05$) with meloxicam administration. There was no meloxicam ($P > 0.19$) or CON vs castrates ($P > 0.31$) effect on percentage of animals that graded USDA choice or better, KPH percentage or USDA yield grade.

This study showed that castration method has no effect on carcass traits. Meloxicam administration during castration did increase ribeye area and backfat thickness. Champagne et al. (1969) reported similar carcass results; calf age at castration (birth vs. 2, 7, and 9 mon) had no impact on marbling score, backfat thickness, and *Longissimus* area. Brown et al. (2015) reported that castration timing (birth vs. weaning) and meloxicam administration had no effect on any carcass characteristics. The current study evaluated castration in older bulls upon feedlot arrival. The CON treatment had similar carcass traits to castrates, with exception of an increase in backfat thickness. Castration at a younger age did not impact the overall carcass performance, except for an improvement in marbling score.

Haptoglobin

Serum Hp results are present in Figure 1. There was a treatment × day interaction ($P = 0.04$). There was no difference ($P > 0.92$) in Hp concentration on d 0 or 0.25.

Haptoglobin synthesized by the liver is not an immediate response with acute phase protein concentration typically peaking 24 to 48 h following activation of the acute phase response (Ballou and Kushner, 1992). On d 1, there was a difference ($P < 0.02$) with both surgical castration treatments having increased Hp concentration compared to the band castration treatments and the CON group. Meloxicam administration had no effect ($P > 0.10$) on d 1 Hp concentrations. On d 4, SUR had the greatest ($P < 0.001$) Hp concentration compared to all other treatments. There was no difference in Hp concentration observed on d 7 or 14 ($P \geq 0.71$).

Haptoglobin is an acute phase protein synthesized by hepatocytes in response to stress, inflammation, tissue damage and/or infection (Cray et al., 2009; Petersen et al., 2004) and is often used a physiological indicator of inflammation. Results suggest that castration method does modulate inflammation post-castration with surgically castrated animals experiencing greater inflammation compared to band castrated animals. Similar Hp results due to castration method are reported by Warnock et al., 2012; surgical castration had increased Hp concentration 2 d post-castration compared to band castration. Warnock et al. (2012) also reported that on d 15 post-castration band castration had greater Hp compared to surgical castration. However, in the current study there was no difference in Hp concentration between treatments after d 4. Meloxicam administration reduced Hp concentration in surgically castrated animals but not band castration, possibly due to the reduced magnitude of inflammation observed for band castration. Similar decreases in Hp magnitude with meloxicam administration during surgical castration were reported by Roberts et al. (2015).

Activity

There was a treatment × day interaction ($P < 0.001$) observed for change in standing time (Figure 2). Surgical castration, regardless of meloxicam administration, spent more time standing ($P < 0.02$) compared to the band castration treatments and CON group immediately following castration (d 0) and was sustained for 2 d post-castration. There was no difference in standing time ($P > 0.28$) between CON, BAN, and BAN+MEL treatments on d 0, 1, and 2. On d 3 and 4, SUR+MEL spent more time standing ($P < 0.001$) compared to CON, BAN and BAN+MEL; however, standing time was not different ($P = 0.24$) from SUR. Standing time for the SUR treatment was similar ($P \geq 0.08$) to the CON and BAN+MEL treatments, but did differ ($P = 0.02$) from the BAN treatment. On d 5 and 6, SUR+MEL had increased ($P \leq 0.02$) standing time compared to BAN and BAN+MEL castration increasing standing time, while meloxicam administration had no effect ($P \geq 0.20$) on activity within castration method.

Number of lying bouts (Figure 3) also had a treatment × day effect ($P < 0.001$). Band castration, regardless of meloxicam administration, had an increase ($P < 0.02$) in the number of lying bouts on d 0 and 1 post-castration compared to SUR, SUR+MEL and CON. On d 2, the number of lying bouts were decreased ($P = 0.05$) for SUR compared to SUR+MEL with no difference ($P \geq 0.16$) between the other treatments. On d 3, SUR+MEL and BAN+MEL differed ($P = 0.02$) with BAN+MEL having fewer lying bouts. The BAN group had an increased ($P = 0.03$) number in lying bouts on days 5 and 7 compared to the BAN+MEL treatment.

There was no treatment × day effect ($P= 0.99$) for motion index, but there was a main effect of treatment ($P = 0.01$) and meloxicam ($P = 0.02$; Figure 4). Band castration treatment had an increased ($P < 0.001$) motion index compared to the surgically castrated

treatment. Meloxicam administration also decreased ($P < 0.001$) motion index compared to castrated controls, regardless of castration method used. Motion index is a proprietary variable that is calculated by an index of individual activity variables combined. Correspondingly, Petherick et al. (2014) reported that band castration in weaned bulls and mature bulls caused the animals to spend more time lying compared to surgically castrated animals.

Step count (Figure 5) did not result in a treatment × day interaction ($P= 0.99$), but there was a method ($P \leq 0.001$) and meloxicam effect ($P = 0.05$). Similar to motion index, step count was increased ($P < 0.001$) for the band treatment compared to the surgical treatment. Meloxicam administration decreased ($P = 0.04$) the number of steps taken regardless of castration method. Petherick et al. (2014) also reported a tendency for mature bulls surgically castrated to take less steps following castration when compared to banded mature bulls.

These activity variables altered in this study indicate that both methods of castration modulate animal behavior following castration. However, castration method had differential effects on individual activity variables. Surgical castration resulted in animals spending more time standing post-castration, while band castration caused animals to have an increase in lying bouts along with an increase in daily steps. These different behavioral patterns may be due to different types of pain being experience between castration methods. Standing and lying are appropriate responses that attempt to minimize stimulation of the sensitized nociceptors due to tissue damage (Handwerker and Reeh, 1991). Surgically castrated animals took less steps and spent more time standing, plausibly in attempt to avoid putting any direct pressure on the open wound. The increase

in motion index, steps, and lying bouts may indicate that banded animals have pain-induced hyper reaction to the pressure experienced by application of the band. Correspondingly, band castration has been reported to increase restless behavior in animals when compared to surgically and burdizzo castrated animals (Robertson et al., 1994). Those authors also reported that banded animals had a decrease in normal lying behavior and an increase in abnormal lying compared to surgical and burdizzo castration. The behavioral changes indicate that the surgical groups are experiencing a more acute pain, while the banded group may be experiencing discomfort rather than acute pain.

In conclusion, castration at the cow-calf origin had long-term production and welfare benefits through the finishing period. Both castration methods reduced performance; however, the reductions in performance occurred at different times post-castration. Surgical castration decreased performance during the first week post-castration, while band castration decreased performance during the second week post-castration. Meloxicam administration improved overall ADG, regardless of castration method. Band castration decreased the peak serum haptoglobin concentration compared to surgically castrated animals, yet meloxicam was able to reduce peak inflammation following surgical castration. Both castration methods altered animal behavior indicative of acute pain, but with opposing adaptations. Further research, in large pen studies, is needed to determine if the performance and production benefits observed with meloxicam administration are repeatable in the commercial setting.

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Table 1. Effect of castration method and meloxicam administration on body weight of feedlot cattle.

	Treatment[1]						Contrasts, *P*-value			
Body weight, kg	CON	SUR	SUR+MEL	BAN	BAN+MEL	SEM	Method	Meloxicam	Interaction	CON vs Castrates
Weaning	204	201	200	200	202	22.02	0.96	1.00	0.77	0.55
D 0	245	242	244	246	247	24.21	0.55	0.79	0.94	0.96
D 7	249	237	240	248	249	22.44	0.03	0.65	0.86	0.24
D 14	272	260	259	266	266	25.32	0.17	0.92	0.92	0.07
D 32	310	293	274	296	296	22.47	0.25	0.40	0.69	0.10
Re- Implant	407	390	398	395	396	34.42	0.77	0.50	0.60	0.10
Final	618	593	619	601	614	13.55	0.90	0.07	0.52	0.34

[1]CON = calves band castrated near birth, SUR = bulls castrated surgically at feedlot arrival without oral meloxicam administration, SUR+ MEL = bulls castrated surgically at feedlot arrival with 1 mg/kg oral meloxicam administration, BAN = bulls castrated via banding at feedlot arrival without oral meloxicam administration, BAN+MEL = bulls castrated via banding at feedlot arrival with 1 mg/kg oral meloxicam administration (BAN+MEL).

Table 2. Effect of castration method and meloxicam administration on average daily gain of feedlot cattle.

	Treatment[1]						Contrasts, *P*-value			
ADG, kg	CON	SUR	SUR+MEL	BAN	BAN+MEL	SEM	Method	Meloxicam	Interaction	CON vs. Castrates
D 0 to 7	0.92	-0.45	-0.39	0.42	0.44	0.81	0.01	0.90	0.94	0.01
D 7 to 14	2.69	2.78	2.36	2.23	2.06	0.52	0.05	0.16	0.55	0.15
D 0 to 14	1.81	1.17	0.98	1.32	1.25	0.32	0.09	0.28	0.61	<0.001
D 14 to 32	1.81	1.63	1.85	1.46	1.46	0.28	<0.001	0.18	0.19	0.02
D 0 to Reimplant	2.14	1.91	1.98	1.93	1.93	0.15	0.80	0.50	0.46	<0.001
Reimplant to Final	1.59	1.50	1.66	1.52	1.62	0.11	0.78	0.01	0.56	0.81
Overall	1.78	1.66	1.78	1.68	1.75	0.13	0.81	0.02	0.51	0.17

[1]CON = calves band castrated near birth, SUR = bulls castrated surgically at feedlot arrival without oral meloxicam administration, SUR+ MEL = bulls castrated surgically at feedlot arrival with 1 mg/kg oral meloxicam administration, BAN = bulls castrated via banding at feedlot arrival without oral meloxicam administration, BAN+MEL = bulls castrated via banding at feedlot arrival with 1 mg/kg oral meloxicam administration (BAN+MEL).

Table 3. Effect of castration method and meloxicam administration on carcass traits of feedlot cattle.

	Treatment[1]						Contrasts, *P*-value			
Item	CON	SUR	SUR+MEL	BAN	BAN+MEL	SEM	Method	Meloxicam	Interaction	CON vs. Castrates
HCW, kg	375	359	377	366	373	11.28	0.82	0.10	0.48	0.46
Marbling Score	50.11	45.85	48.17	46.08	45.87	2.97	0.52	0.51	0.43	0.05
USDA Choice[2], %	87.62	77.25	84.02	78.40	77.50	12.10	0.71	0.68	0.59	0.31
Backfat, in.	0.69	0.64	0.73	0.65	0.68	0.07	0.36	0.01	0.12	0.50
Ribeye, in^2	13.63	12.96	13.71	13.31	13.53	0.40	0.73	0.05	0.30	0.36
KPH, %	3.50	3.41	3.81	3.67	3.66	3.42	0.71	0.19	0.15	0.41
Yield Grade	3.28	3.21	3.37	3.22	3.28	0.92	0.76	0.42	0.70	0.92

[1]CON = calves band castrated near birth, SUR = bulls castrated surgically at feedlot arrival without oral meloxicam administration, SUR+ MEL = bulls castrated surgically at feedlot arrival with 1 mg/kg oral meloxicam administration, BAN = bulls castrated via banding at feedlot arrival without oral meloxicam administration, BAN+MEL = bulls castrated via banding at feedlot arrival with 1 mg/kg oral meloxicam administration (BAN+MEL).

[2]Percent of treatment group grading USDA Choice or better

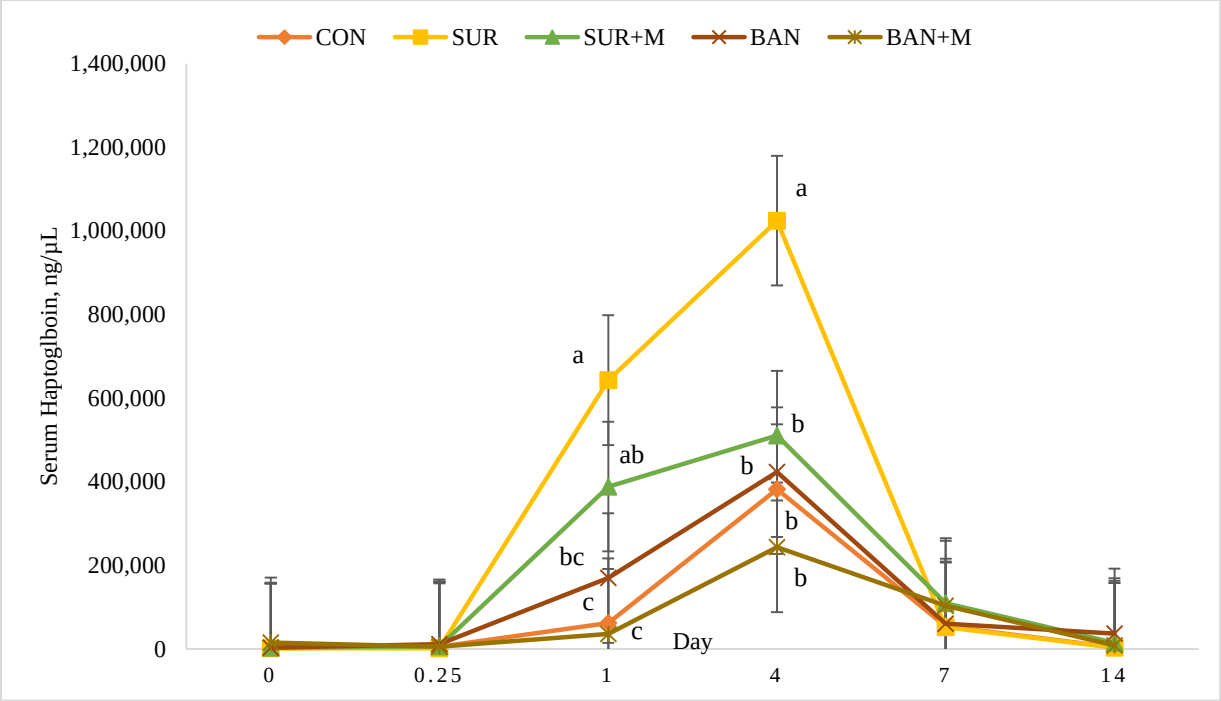

Figure 1. Effect of castration method and meloxicam administration on serum haptoglobin concentration of feedlot cattle. CON = calves band castrated near birth, SUR = bulls castrated surgically at feedlot arrival without oral meloxicam administration, SUR+ MEL = bulls castrated surgically at feedlot arrival with 1 mg/kg oral meloxicam administration, BAN = bulls castrated via banding at feedlot arrival without oral meloxicam administration, BAN+MEL = bulls castrated via banding at feedlot arrival with 1 mg/kg oral meloxicam administration (BAN+MEL). Effect of treatment, $P = 0.05$; day, $P < 0.001$; and treatment × day; $P = 0.04$. Contrasts = Method, $P = 0.01$; Meloxicam, $P = 0.13$; Interaction, $P = 0.52$; and CON vs. Castrates, $P = 0.21$.

^{abc} least square means with uncommon superscript letters differ within day, $P < 0.05$

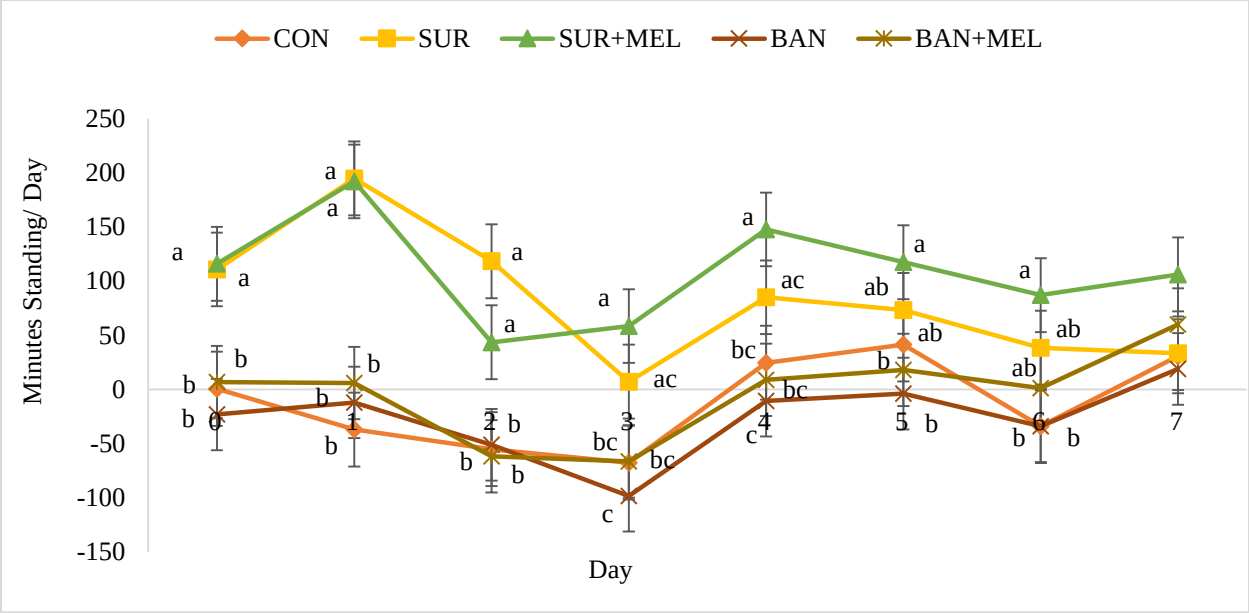

Figure 2. Effect of castration method and meloxicam administration on change in time spent standing from baseline values in feedlot cattle from d 0 to 7. CON = calves band castrated near birth, SUR = bulls castrated surgically at feedlot arrival without oral meloxicam administration, SUR+ MEL = bulls castrated surgically at feedlot arrival with 1 mg/kg oral meloxicam administration, BAN = bulls castrated via banding at feedlot arrival without oral meloxicam administration, BAN+MEL = bulls castrated via banding at feedlot arrival with 1 mg/kg oral meloxicam administration (BAN+MEL). Effect of treatment, $P < 0.001$; day, $P < 0.001$; and treatment × day, $P < 0.001$, Contrasts = Method, $P < 0.01$; Meloxicam, $P = 0.06$; Interaction, $P = 0.60$; and CON v. Castrates $P < 0.001$.

[1]Change from baseline (average of d -9 to -1).

[abc] least square means with uncommon superscript letters differ within day, $P < 0.05$

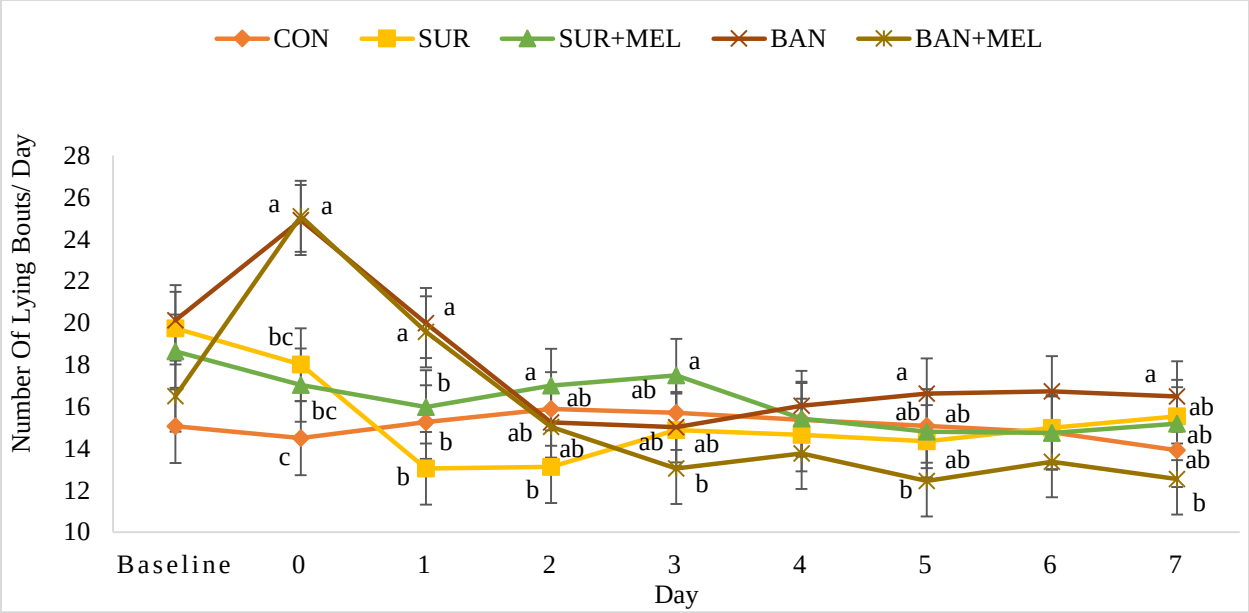

Figure 3. Effect of castration method and meloxicam administration on number of lying bouts per day of feedlot cattle from d 0 to 7. Baseline value is the average of d -9 to -1. CON = calves band castrated near birth, SUR = bulls castrated surgically at feedlot arrival without oral meloxicam administration, SUR+ MEL = bulls castrated surgically at feedlot arrival with 1 mg/kg oral meloxicam administration, BAN = bulls castrated via banding at feedlot arrival without oral meloxicam administration, BAN+MEL = bulls castrated via banding at feedlot arrival with 1 mg/kg oral meloxicam administration (BAN+MEL).Effect of treatment, $P < 0.001$; day, $P < 0.001$; and treatment × day, $P < 0.001$. Contrasts = Method, $P < 0.001$; Meloxicam, $P < 0.001$; Interaction, $P = 0.01$; andCON vs Castrates, $P = 0.02$.

[abc] least square means with uncommon superscript letters differ within day, $P < 0.05$

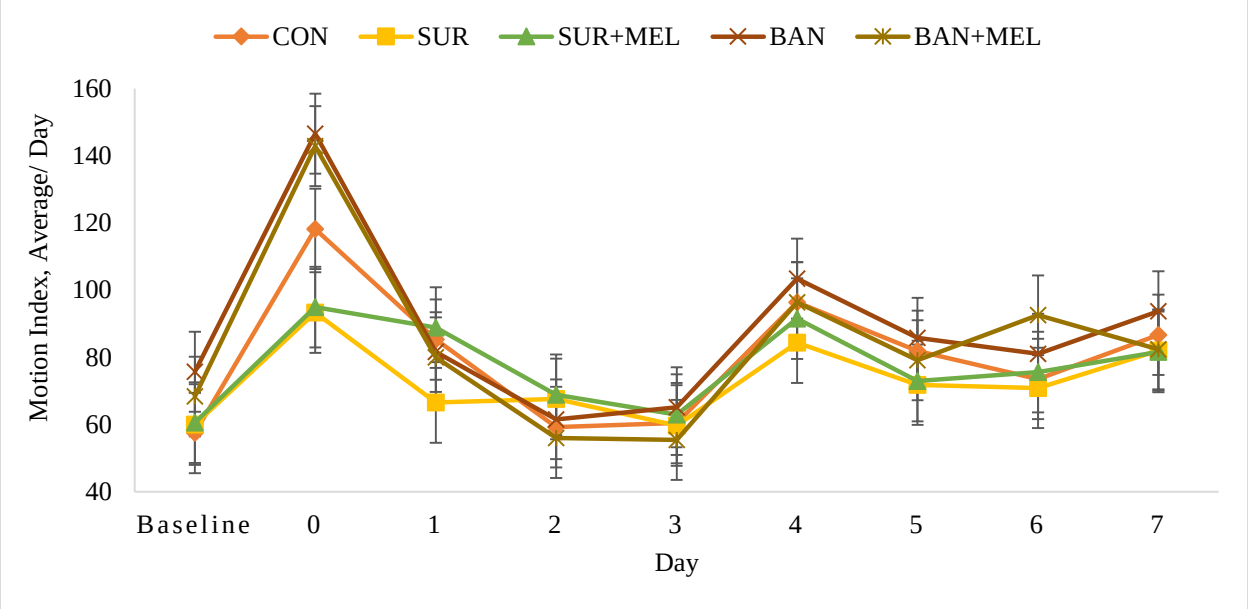

Figure 4. Effect of castration method and meloxicam administration on daily motion index of feedlot cattle from d 0 to 7. Baseline value is the average of d -9 to -1. CON = calves band castrated near birth, SUR = bulls castrated surgically at feedlot arrival without oral meloxicam administration, SUR+ MEL = bulls castrated surgically at feedlot arrival with 1 mg/kg oral meloxicam administration, BAN = bulls castrated via banding at feedlot arrival without oral meloxicam administration, BAN+MEL = bulls castrated via banding at feedlot arrival with 1 mg/kg oral meloxicam administration (BAN+MEL). Effect of treatment, $P < 0.001$; day, $P < 0.001$; and treatment × day, $P = 0.99$. Contrasts- = Method, $P = 0.01$; Meloxicam, $P = 0.02$; Interaction, $P = 0.11$; and CON vs. Castrates, $P = 0.20$.

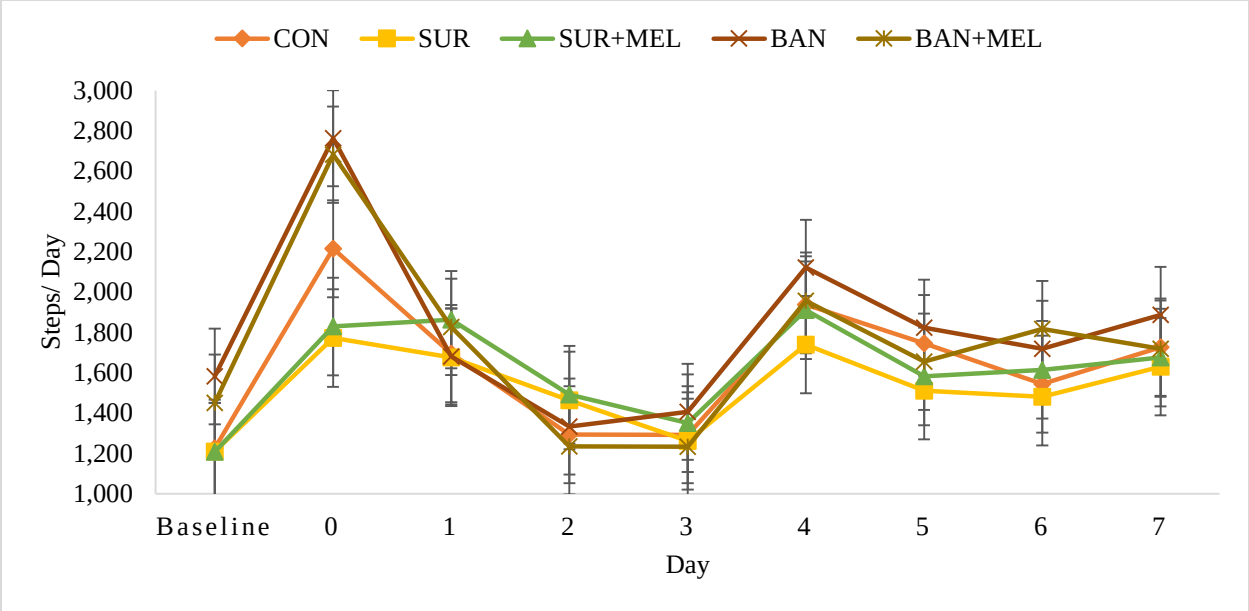

Figure 5. Effect of castration method and meloxicam administration on number of steps per day by feedlot cattle from d 0 to 7. Baseline value is the average of d -9 to -1. CON = calves band castrated near birth, SUR = bulls castrated surgically at feedlot arrival without oral meloxicam administration, SUR+ MEL = bulls castrated surgically at feedlot arrival with 1 mg/kg oral meloxicam administration, BAN = bulls castrated via banding at feedlot arrival without oral meloxicam administration, BAN+MEL = bulls castrated via banding at feedlot arrival with 1 mg/kg oral meloxicam administration (BAN+MEL).Effect of treatment, $P < 0.001$; day, $P < 0.001$; and treatment × day, $P = 0.99$. Contrasts = Method, $P < 0.001$; Meloxicam, $P = 0.05$; Interaction, $P = 0.19$; and CON vs. Castrates $P = 0.26$.

CHAPTER IV

CONCLUSIONS

These three studies show that meloxicam does mitigate the immune and inflammation response when given concurrent with castration. However, this modulation of the immune system was only seen in older animals (> 9 months of age). No difference in the immune response or inflammation was detected when meloxicam was administered to animals castrated at approximately 3 months of age. Meloxicam did reduce eosinophil concentration compared to control animals; however, the reason for this drug effect is unknown.

These three studies also demonstrate that older animals will experience greater magnitude of inflammation following castration as compared younger animals. It appears that castration performed in younger animals could improve animal welfare. Method of castration also affects the magnitude of inflammation with surgical castration causing greater inflammation compared to band castration. Castration at younger age has no effect on overall growth performance and decreases the degree of inflammation than the animal will experience. However, not all producer have the equipment and the workforce necessary to perform castration prior to marketing; resulting in castration at feedlot arrival. Meloxicam administration, at this time, can reduce the magnitude of inflammation that an animal will experience when castrated at an older age. The total cost for administering meloxicam in older animals is minimal at $0.59/ head in the heavier

animals (~ 225 kg). Carcass results from our feedlot study indicate that meloxicam administration may increase the final body weight, hot carcass weight and ribeye area. This improvement in carcass performance, even if slight, would have economic benefits considering the minimal input cost of meloxicam administration.

Further studies are needed to determine if meloxicam administration impedes convalescence of the wound following castration. Also, larger pen studies are needed to determine if the improvement in growth and carcass performance reported in the feedlot study are repeatable in large pen settings.