IMPACTS OF BANAMINE INJECTION ON PAIN RESPONSES OF EITHER RUBBER RING CASTRATED AND TAIL DOCKED OR SURGICALLY CASTRATED AND DOCKED LAMBS

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Our hypothesis was that administering Flunixin Meglumine (**FM**) to lambs that were both rubber ring castrated and docked or surgically castrated and emasculator docked would decrease behavioral stress, serum cortisol, haptoglobin concentration, and wound and swelling score, but increase average daily gain. Rambouillet ram lambs (n = 181) were allocated with a completely randomized design in a 2 x 2 factorial arrangement of treatments. Treatment combinations included rubber ring castration and docking (on ram lambs with administration of saline or FM) or surgical castration and emasculator docking (on ram lambs with administration of saline or FM). Ram lambs were castrated at 12.5 ± 5.5 days of age. Overall, results indicate that the method of castration and docking in lambs affected short lived behavioral and physiological stress. The administration of FM surgical castration following castration decreased cortisol levels after 140 minutes. Economic analysis showed that rubber-ring castration was the cheaper method.

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LIST OF ABBREVIATIONS

ADG..Average daily gain

APP ....Acute phase proteins

BAN ....Castration by callicrate bander

DFI ....Daily feed intake

DWI..Daily water intake

EGF ....Epidermal growth factor

FM..Flunixin Meglumine

H..Handled

HD..Hot docking iron

HDMET ....HD + Muelsing (operation where skin is removed around the tail and rump in sections) + ear tagging

HDRRCMET ....HD + Mulesing +castration by RR + ear tagging

HPA..Hypothalamic pituitary adrenal

REQ..Restlessness + roll/jumping + foot stamp/kicking + easing quarters

RFI ....Residual feed intake

RR ....Rubber Ring

RRC..Rubber ring castration only

RRT..Rubber ring tail docking only

RRTRRC..Combination of docking and castration by both rubber ring

SC..Surgical Castration

TGF-β..Transforming growth factor beta

1. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Pain is defined as an aversive sensory or emotional event that causes the animal to experience stress (Molony and Kent, 1997). Stress is a biochemical response that can occur from a physical or psychological response on an individual (Eberhard and Veisser, 2007) in any type of situation. Stress is a stimulus that can disturb homeostasis (Smith and Dobson, 2002). In livestock production, animals experience stress during periods of transportation or handling. For example, lambs experience pain and stress while being castrated and docked.

Castration and docking are management tools used for livestock health management. Castration is the removal of testicles and there are several different recognized methods to perform the procedure. Castration reduces aggressive behavior and prevents unwanted pregnancies (SID, 2015). Docking is the partial or complete removal of the tail. Docking prevents flystrike occurring in lambs, which occurs when the soft feces collect at the head of the tail (SID, 2015). This collection of feces attracts flies that lay their eggs on the lamb resulting in eggs that mature and hatch into maggots. The lamb will become ill because of the open wound from the maggots (SID, 2015). There are both benefits and consequences for castration and docking. Castration and docking cause pain and stress to the lamb, which can last for days. However, castration and docking usually improve overall animal welfare and provide economic advantages to the producer. Producers should be aware of the pain their animals are experiencing and use humane techniques. Analgesics may be the solution to pain management in livestock production. Scientists are currently studying analgesic work for sheep and cattle to provide pain relief to livestock during stressful periods.

1.2. Stress in the brain and pain receptors

Stress can be broken into four different categories: behavioral, autonomic nervous system, neuroendocrine, or immune response. The behavioral stress response happens when an animal tries to escape from the stressor; for example, rams seeking shade because of high temperatures. The second stress response is the autonomic nervous system, also known as fight or flight response. During the fight or flight response, multiple biological systems work together to prepare the animal for the stressor. For example, a coyote is closing on a flock of sheep and a ewe notices the predator. This causes an increase in heart rate, breathing and blood pressure. This accelerated heart rate will cause a buildup of adrenaline and decrease action in the digestive systems, (Figure 1.1). These response to different body systems in the body prepared the ewe for life and death situations (Moberg and Mench, 2000). Neuroendocrine system responses can have an enduring effect on the body because all the biological systems are working together when the stressor occurs. The nervous system is affected because of the sudden release of hormones for the body to take into effect. Hormones can take a toll on different biological systems; it can also be tied with failed reproduction, such as abortion (Moberg and Mench, 2000). The immune stress response is affected by the immune response due to the hypothalamic pituitary adrenal (**HPA**) axis and other stress induced incidences. Stress can also have an effect with altering metabolizable energy and immunity causing lower immunity and poor performance traits (Moberg and Mench, 2000).

The animal senses a stressor that triggers a response to the sympathetic nervous system, releasing catecholamine (epinephrine and norepinephrine) from the sympathetic nerve terminals and adrenal medulla. Figure 1.1 shows the cascade of events when stress occurs. This release causes an increase in heart rate and breathing and a decrease in digestion. Simultaneously, an increase of glucose is released from muscle and liver and a decrease of insulin production in the pancreas. Blood vessels then constrict and send blood to areas that may be injured (Hill et al., 2004 and Smith, 2002). This causes the animal to release corticotropin -releasing hormone (**CRH**) into the hypothalamo-hypophyseal portal system. The anterior pituitary then releases adrenocorticotrophic hormone (**ATCH**). This response helps the animal learn the cause and effect of the stressor to prepare them for future occurrences. Glucocorticoids are released from the adrenal gland to the selected area where the stress originated. Glucocorticoids, such as cortisol, reinforce biological systems to release energy into the bloodstream. As a result, protein is catabolized from the muscle and bone, which stimulates the liver to release amino acids to start liver glycogenesis. Glucocorticoids stimulate fat catabolism to utilize fatty acids as an alternative energy source. This ensures that glucose is being produced for the requirement for physical energy and enforced fasting. The amino acids that are released from muscle catabolism are available for tissue repair (Hill et al., 2004 and Smith, 2002). The HPA axis responds to any type of stress to an animal whether it be emotional, environmental, or physiological (Hart, 2012).

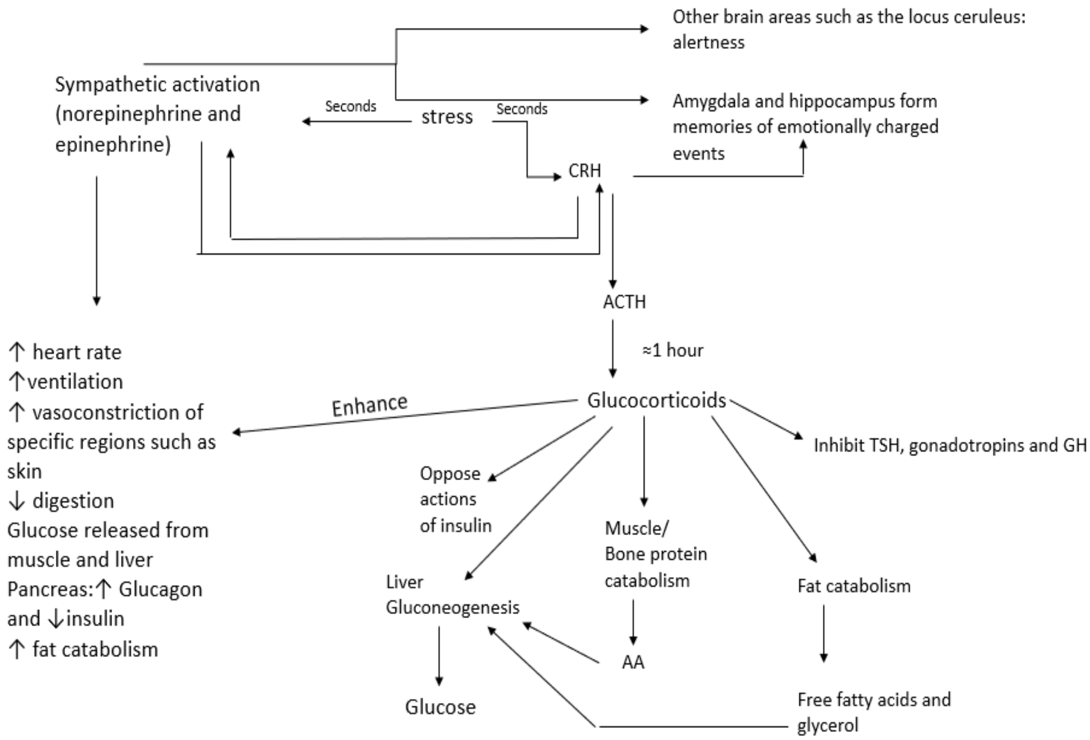

Figure 1.1: Diagram of the Hypothalamic pituitary adrenal axis when stress occurs (adapted from Hill et al., 2004 and Smith, 2002).

1.2.1. Measurements of stress

Cortisol concentration is one blood metabolite that is used to measure the amount of stress an animal is enduring. The corticosteroid role is to protect and defend the body against the stressor (Smith and Dobson, 2002). Cortisol is useful for measuring different types of response to different stressors. Cortisol can be found in blood, hair, and saliva. While most studies use blood serum collection, some cattle studies have used saliva or hair to detect cortisol levels. However, cortisol concentrations in saliva are considered a less-valued result for measuring stress and pain (Marti et al., 2017) compared with serum.

1.3. Wound healing

A wound is defined as the breaking of cellular tissue and the repair process that follows (Kumar, 2013). Wounds can originate from different sources, such as a paper cut or a surgical operation, but the healing process is similar regardless of the source of injury. Wound repair is a 4-step process, where treatment and recovery depend on the severity of the wound (Mendonça and Coutinho-Netto, 2009). Wounds go through the inflammatory phase on day zero through five. Trauma from the infliction of the wound can cause pain and blood distribution to the skin, causing hemorrhaging. Wound trauma starts by filling the wound with blood that contains plasma-enriched platelets. As platelets start to aggregate, a matrix barrier is generated and homeostasis is restored by forming a buffer, and blood starts to clot. This barrier helps protect the wound from any microorganisms that could potentially enter the body. The matrix releases cytokine and has growth factors to help the next stage of wound healing. Platelets within the blood are essential for forming the buffer because they secrete different growth factors and mediators to the damaged area (Mendonça and Coutinho-Netto, 2009). Thrombin is induced by these platelets for platelet degranulation and releases other growth factors. Vascular endothelial growth factor (**VEGF**), platelet derived growth factor (**PDGF**), epidermal growth factor (**EGF**), transforming growth factor beta (**TGF-β**), and transforming growth factor alpha (**TGF-α**) are the growth factors for this stage of wound healing (Ganapathy et al., 2012 and Mohd et al., 2012). Other important glycoproteins for the extracellular matrix include fibronectin and thrombospondin. Coagulation begins and ends with the growth factors and activation of the parenchymatous cells from the wound. Parenchymatous cells will produce different factors that help recruit the inflammatory cells to the wound. The inflammatory cells function for phagocytosis, produce growth factors and begin preparing the wound for the second phase of wound healing (Ganapathy et al., 2012 and Mohd et al., 2012).

During the second stage of wound healing, clotting factors produced by the inflammatory cells and plasma generation occurs around the wound edges to close the wound and inflammation takes place. During this process, a buildup of mitogens and chemoattractants at to the site of the wound occurs. Prostaglandins and kinins are produced for vasodilatation and smaller blood vessels increase permeability to the wound regions, resulting in edema (Mendonça and Coutinho-Netto, 2009). Edema is the accumulation of abnormal amounts of fluid in the affected tissue (SID, 2015). Six hours after trauma is inflicted, neutrophils begin to fill the wound for protection and defense against bacteria. Polymorphonuclear neutrophils are the first white blood cells to enter the wound and begin phagocytosis (Kumar et al., 2013 and Mendonça and Coutinho-Netto, 2009). Polymorphonuclear neutrophils have a short life span and numbers of these blood cells decrease after day three. The macrophages are the next white blood cell defense for the wound, since they have a longer life span and keep producing until the wound healing is complete. The macrophage process helps control the repair by degrading and removing any damaged tissue from the inflicted injury (i.e. damaged connective tissue, elastin, and collagen). The macrophages also produce chemotactic factors that attract other inflammatory cells to the wound and produce many growth factors to stimulate the formation of granulation tissue (Kumar et al., 2013 and Mendonça and Coutinho-Netto, 2009).

Once the wound has been cleared of any foreign bacteria or material the third phase of wound healing occurs, called the proliferative phase, including the closing of the wound. The three sub stages of this phase are epithelialization, fibroplasia, and angiogenesis. This phase occurs between three and fourteen days after the wound trauma occurred. Epithelialization is the first stage of the proliferative phase. The proliferative phase begins by stimulation of keratinocytes by mitogenic and chemotactic stimulation from TGF-β and EGF. This stage is essential for the formation of granulation tissue (Mendonça and Coutinho-Netto, 2009). Fibroblasts appear on the third day after trauma and peak around the seventh day. Fibroblasts are used for the repair and production of the structural proteins for tissue repair. Fibroblasts produce collagen in large quantities for the main component in the extracellular matrix for wound healing, which is responsible for strengthening the scar (Mendonça and Coutinho-Netto, 2009). The production of new blood vessels that extend from previous vessels helps increase vascular permeability to the skin. Water and macromolecules come to the wound from pathological angiogenesis to form the edema. Increased activity from the capillary and blood vessels reduces the edema during pathological angiogenesis. Angiogenesis is the process when new blood vessels are made from old vessels. Angiogenesis produces new temporary granulation tissue and supplies oxygen and nutrients to the new growing tissue. Vasculogenesis occurs through the first stage of vascular development. The precursor cells of the endothelium cells that undergo differentiation, coalescence, and expansion to form a network of primitive tubules in the host. The new network of blood vessels is responsible for 60% of the tissue repair. Angiogenesis occurs on the cellular matrix in the wound by migration and the mitogenic stimulation of endothelial cells (Mendonça and Coutinho-Netto, 2009). The restoration of the epithelial tissue takes place on the surface and takes up to 48 hours depending on the size of the wound.

Step four is the maturation phase, which can last from seven days to a year depending on the severity of the wound and trauma. The maturation phase objective is the remodeling of collagen. Growth factors stimulate collagen synthesis and connective tissue molecules and help modulate the synthesis of metalloproteinase. Metalloproteinase is the enzyme that serves to breakdown the epithelial cell migrations (**ECM**) component (Kumar et al., 2013). The net results from the ECM process vs. degradation for connective tissue remodeling is an important aspect in chronic inflammation and wound healing. Collagen is the primary constituent of the matrix and is randomly distributed in the collagen fibers (Kumar et al., 2013 and Mendonça and Coutinho-

Netto, 2009). The collagen fibers become cross-linked, and the fibers begin to form fibrillar bundles to give strength and stiffness. After a five-day lag time, from early granulation tissue development and matrix formation (composed of hyaluronioc acid and fibronectin), scar development begins. Collagen synthesis and collagenase enzyme are main contributors for scar formation. The scar formation can be from six to twelve months depending on the severity of the wound and the rate of collagen synthesis. Remodeling of the scar could continue for up to a year after the trauma (Kumar et al., 2013 and Mendonça and Coutinho-Netto, 2009).

1.3.1. Inflammatory response

Acute phase proteins (**APP**) are a group of proteins in the extracellular body fluid that change due to stress, injury, or inflammation. Acute phase proteins are markers for inflammation and have been used for cattle. Sheep studies have been scarce using APP because concentrations of APP in sheep serum are low. Acute phase proteins can detect acute/chronic challenges or inflammation via serum (Miglio et al., 2018). The positive APP in the blood are haptoglobin, serum amyloid A, and C-reactive protein that are released by hepatocytes after cytokine stimulation occurs (Gruys et al., 2005). Haptoglobin is an APP associated with inflammation and infection (Meléndez et al., 2017) that can be used for inflammatory nonspecific response and immunity status for animals and can be useful as an indicator of a bacterial infection in sheep (Bretschneider, 2005 and Skinner and Roberts, 1994). Average haptoglobin levels found in multiple sheep breeds were 0.29 mg/mL, which were measured in lactating dairy ewes (Miglio et al., 2018). Positive APP, such as haptoglobin, are formed in the APP that is associated with either a change in diet, metabolism, or production (Gruys et al., 2005). Positive APP indicates the source of amino acid required by APP synthesis are from protein absorption in the digestive tract instead of catabolism of muscle tissue. Amino acid composition from the digestive tract differs from the protein found in the muscle. Haptoglobin binds with hemoglobin and forms an anti-inflammatory response by binding to CD11b/CD18 intergines, which are the major receptors in the leukocyte cell membrane. The quantity of haptoglobin may decrease due to large erythrolysis in hemolytic blood, producing inaccurate quantification of haptoglobin (Gruys et al., 2005).

Acute phase reaction is a response due to the disturbance of homeostasis caused by several factors such as tissue damage or a microorganism. The site of the injury sets a cascade of signals to release pro-inflammatory cytokines and inflammatory cells from the vascular system (Manteuffel, 2002 and Gruys et al., 2005). Mediators from the pro-inflammatory cytokines and inflammatory receptors circulate through the blood and diffuse in the extracellular fluid component. Cytokine receptors signal different cells to lead into different reactions within the HPA axis to target the biological system that reduce hormone secretion and cause physical changes. Physiological changes detected in the blood stream because of a decrease in white blood cell numbers, vitamin A, zinc, calcium, iron, and α-tocopherol and low to high-density lipoprotein density. Other physiological changes in the blood are increases of Adrenocorticotropic hormone and glucocorticoids, an activation of blood coagulants, and a change of APPs due to hepatic metabolism (Manteuffel, 2002 and Gruys et al., 2005). If a receptor is triggered multiple times, the acute phase response can become long-lasting. Hours after an infection entered the body, the liver is altered resulting in an increase of positive APP’s (Gruys et al., 2005).

1.4. Behaviors

Behaviors of animals can be used to signal the pain and distress they are experiencing. As each animal reacts differently to pain, observations of these behavioral responses can be subtle.

Producers observe different signs of pain and distress such as changes in postures, standing, and lying down so they can evaluate each animal and determine if the animal needs assistance. Animals can experience a painful situation in different ways. Animals in pain can show aggression while in pain where others tend to avoid other animals and humans to decrease pain (Gleerup et al., 2015).

Posture change and behavioral response can be voluntary and involuntary (Molony and Kent, 1997). The fight or flight response can help the animal escape safely away from the danger. The fight or flight response can overcome the pain the animal is enduring by the release of adrenaline or other hormones. Animals can sense and experience pain in different ways. Active pain avoidance behavior, also known as postural indicators, can be viewed as different indicators. Some animals can show pain right away in the form of vocalization, tail wagging, kicking, licking, biting, and more (Fitzpatrick et al., 2005 and Grant, 2004).

Postural indicators can be used to differentiate slight posture differences. Posture differences could have slight changes from walking normally to crawling on front knees. Any of these small differences could be indicators that the animal is in pain (Grant, 2004). Molony et al. (2002) describes two different types of normal lying and standing for lambs (Table 1.1).

Table 1.1: Behavioral positions adapted by Molony et al., (2002).

Position	Body position
Normal ventral lying	V1- With head down V2 With head up
Abnormal lying ventral	V3- One partial leg extension V4- Full extension of one or more legs
Abnormal lateral lying with a shoulder down	L1-Head up L2 Head down
Standing postures	S1- Normal standing, walking S2- Abnormal standing walking, with swaying ataxia, abnormal stance S3- Grossly abnormal standing/walking, on knees; walking backwards S4-Immobile or statue standing with arched back

Standing is another indicator of pain after castration and docking. There is a difference between normal and abnormal standing. For example, normal standing is defined as doing normal activities without any abnormalities. Abnormal standing can be defined as walking unsteadily, walking in circles or leaning for support on walls or other objects. Figure 1.2. Shows images of a lamb experiencing abnormal standing. Molony and Kent. (1995) suggest to prevent future pain from occurring, lambs decrease their movement showing less abnormal positions.

Figure 1.2: Lamb 299 (left) in a stressed position versus lamb 129 (right) in a normal standing position.

There are other indicators of lambs being in pain. Vocalization, restlessness, and locomotion can be an indicator of pain or distress (Molony et al., 2002). Vocalization is defined as the vocalization calls related to pain documented by the observer. Restlessness is defined when the lamb stands up, lies down, or gets on their knees multiple times during a time period (Molony et al., 2002). Locomotion can be defined by either stride length, kicking, rolling, jumping, or tail wagging (Molony and Kent., 1997).

Specifically, research evaluated facial cues of 150 dairy calves that received analgesic or a placebo prior to castration. The calves were visually evaluated for their facial cues after castration. The relaxed features that calves presented were ears forward and no tensed muscles. Other expressions include tense ear movement, ears pointed back, or tension in the face such as showing furrowing of the face. Facial cues can tell us many different behaviors such as aggression, fear, and pain (Gleerup et al., 2015).

Pain can be measured with different behavioral cues, but it all comes down to the same reaction, if an animal is distressed, then it is likely shows signs of pain. However, some animals show no symptoms of pain; therefore, producers dismiss their lack of symptoms as no pain is being explained. Fitzpatrick et al. (2005) reviewed studies that evaluated subjective measures of sheep on a pain scale of 0 to10 (0 being no pain and 10 experiencing most pain) for castration method, foot rot, flystrike, cesarean section, and chronic mastitis. Castration method used either the surgical or rubber ring castration. Researchers perceived that the rubber ring method was more painful (average pain scale 6) than open surgery castration (average pain scale of 5). When castration was compared to cesarean section or chronic mastitis, castration was observed to have higher scores of pains (RR = 6, surgical = 5, and cesarean section and chronic mastitis = 4). The evaluators responded that sheep experienced more pain than cattle from castration; however cesarean section and mastitis were perceived more painful for cattle than sheep (Fitzpatrick et al., 2005).

Molony and Kent (1997) evaluated 49 Dorset crossbred lambs that were paired with their dams. The ram lambs were castrated at five to six days of age and assigned to seven different treatment groups. Groups include bilateral castration and docking with a rubber ring, bilateral castration with a rubber ring, unilateral castration with a rubber ring, short scrotum castration, docking with a rubber ring, short scrotum castration with an analgesic, and a control-handled group. Cortisol plasma was collected at 20-minute intervals and postures at 2-minute intervals continuously for 180 minutes. Lying down behavior total time was not significant between any treatments. Abnormal ventral lying was the highest behavior among the groups, and the restlessness, roll/jumping, foot stamp/kicking, and easing quarters (**REQ**) summation scores, which were the total sum of scores for the 180 minutes that included restlessness, rolling, foot stamping, and easing of quarters which were separated from each other. Peak cortisol levels did not differentiate with either the high or low ranked treatment, but cortisol levels did differentiate between treatments of intermediate severity from high to low ranked procedures.

1.5. Castration and docking management

1.5.1. Castration and docking

Castration is the removal of the testicles and docking is partial or complete removal of the tail. There are several different procedures to castrate and dock lambs. The producer may choose a procedure due to cost, availability, training, or even convenience.

1.5.2. Elastration

The elastration method, commonly called the rubber ring (**RR**) method, is perhaps the most common method of castration and docking in the U.S. This technique passes the scrotum and testicles through a rubber or elastic ring (SID, 2015). The same concept is used with docking; the tail goes through the band (SID, 2015). The length of time for the tissue to become atrophic is determined by the environment and size of the scrotum or tail. A sheep producer can use this method with relatively little training, and it is an inexpensive method to use. However, there are some disadvantages with using the elastrator for castration and docking. One disadvantage is that age can dictate this method is preferred for younger lambs versus older lambs. Using the rubber rings for older lambs can be an issue because of the size of the scrotum. This method has the possibility of the lamb contracting tetanus, due to the open wound after the scrotum or tail falls off. If the lamb encounters the tetanus spore and are unvaccinated, they can be infected and potentially perish (SID, 2015; Daly, 2015).

1.5.3. Surgical castration

Surgical castration is the removal of the testicles by a scalpel or a sharp knife, cutting the lower third of the scrotum, exposing the testes. Then the testes are removed by pulling them out without cutting the spermatic cord. Cow-calf producers use this method 49.2% of the time in the United States, versus the RR method which is used 47.3% of the time (Roberts et al., 2018).

Using this method gives the calf proper drainage of the wound compared to the RR method (Daly, 2015).

Surgical castration causes controversy from an animal welfare standpoint. The lambs experience the pain and distress right away, compared to a delayed reaction from the RR method from an inflammatory response (Warnock et al., 2012). Meléndez et al. (2017) found that the use of surgical castration caused higher levels of cortisol concentrations and more painful behavioral cues at 2 months and 4 months of age.

1.5.4. Burdizzo

The burdizzo is a device that has two blunt jaws that is applied to the neck of the spermatic cord and on the tail and crushes the blood vessels. This method for docking usually needs a knife or scalpel to remove the tail. For castration, the burdizzo crushes the spermatic cord at the neck of the scrotum (SID, 2015). The burdizzo docking method has been modified to have a crush and cut method known as an emasculator. The emasculator puts pressure on the tail and sharp edges on the device cut the tail. When it is cut, the producer should hold that position for ten seconds, so the bleeding stops (SID, 2015). The burdizzo method causes immediate pain response for castration but wound healing was faster and had fewer complications when compared to the RR method (Melches et al., 2007).

1.5.5. Hot iron docking

Hot iron docking is a device that docks by heating blunt jaws by propane, electricity, or fire. The heated jaws are applied with pressure to remove the tail. The hot iron will cauterize the tail's blood supply and control the bleeding and any potential infection. However, there are some issues with this type of docking due to a prolonged hold on the tail and causing long-term tissue damage. This method takes longer to heal when compared to other methods (SID, 2015). Grant

(2004) evaluated different castration and docking methods to evaluate the behavioral responses of each group. Hot iron docking had the highest incidences of vocalization and time spent in abnormal positions. There was demonstrated as an increase in restlessness, foot stamping, kicking, and rolling at 90 minutes in the study, but this did not differ when compared to the handled group.

1.5.6. Advantages and complications to castration and docking

Castration reduces aggressive behavior and prevents unwanted pregnancies. Castration could help prevent any unwanted genetics and maintaining behavioral control. Aggressive animals can hurt livestock and producers due to dominance or sexual behavior. Aggressive animals can also damage equipment and land due to their behavior. Additionally, carcass composition can improve from castrating lambs (Thornton and Waterman-Pearson, 1999). Docking is the partial or complete removal of the tail. Docking prevents flystrike occurring in lambs, which occurs when the soft feces collect at the head of the tail. This collection of feces attracts flies that lay their eggs on the lamb, where these will hatch into maggots. The lamb can become ill and die because of the wounds maggots create (SID, 2015). Disadvantages of castration or docking are mostly due to pain and complications after the procedure is completed. Surgical castration can cause issues with inguinal hernias, where the intestine is exposed out of the wound. Issues with hot iron docking is if the device is left on for too long, it can cause severe burns and possible tissue and nerve damage to the area (SID, 2015).

1.5.7. Age of castration and pain

Castration and docking should be done with younger animals due to a delayed response to pain compared to older animals (Meléndez et al., 2017). Some producers may need to castrate at a later age because of pre-pubescent males did not reach market weight before puberty or due to economic factors and geographic location (Melches et al., 2007). Lyson-Johnson (1998) evaluated two different age groups of beef calves to find out how age affects castration (33 weeks vs. 36 weeks). The treatments were control, surgical castration, and rubber band castration. They used haptoglobin to indicate stress due to injury. Lyson-Johnson (1998) found that the older group of calves had a higher level of haptoglobin, and therefore were more stressed during castration. Other studies have reported that haptoglobin does increase during castration. However, a Marti et al. (2017) studied different age groups of beef calves (1 week, 2 months, and 4 months) and showed there was a lack of differences due to age. Bretschneider (2005) reviewed studies conducted on cattle castration. It was reported that age does affect weight loss following castration. The older the animal, the higher chance the animal will experience weight loss. They also reported from previous studies that calves castrated before six months of age will endure less stress than those over six months. The larger testicle that results in greater discomfort could possibly explained by testicular development.

1.5.8. Studies comparing castration

A study was conducted by Meléndez et al. (2017) that used three different age groups of beef calves (1 week, 2 months, or 4 months old) to compare the effects of the RR method and surgical castration. At 1 week old, the lying down position was a common sign after castration and docking around day 2 and 3 post castration. These authors suggested this happened due to stretching of the affected tissue causing pain and distress to the calves from changing positions. At four months of age, when the scrotal size was larger, calves had higher levels of cortisol, laid down more often, and increased number of standing calves. These authors reported calves castrated at an older age with the rubber band experience had more discomfort than at a younger age. Kent et al. (1998) evaluated Suffolk-cross lambs to test the RR method for behavioral stress.

Lambs experienced abnormal positions for two hours after castration and docking. The lambs had a peak of abnormal lying at 30 to 60 minutes and full extension of lying occurred between 60 to 120 minutes post procedure. The rubber ring lambs produced more abnormal behavior compared to the burdizzo method (device that has two blunt jaws that is applied to the neck of the scrotum and on the tail and crushes the blood vessels; Kent et al., 1998).

Grant (2004) evaluated different castration and docking methods to evaluate the lamb's behavioral response. The groups consisted of handled (**H**), ear tag only (**ET**), docking done by hot docking iron (**HD**), rubber ring (**RRT**), castration by rubber ring alone (**RRC**) and combination of docking and castration by both rubber ring (**RRTRRC**), HD and Muelsing (operation where skin is removed around the tail and rump in sections), and ear tagging (**HDMET**) and HD, Mulesing, castration by RR, and ear tagging (**HDRRCMET**). It was reported that the RR tail-docking group had higher incidences of abnormal postures and active pain behaviors at 40 - 60 minutes after castration and docking, when compared to castration by RR and RR castration and docking. The higher incidences in active pain behaviors up to 60 minutes and significant abnormal postures did not return until the end of the experiment. When combining docking and castration via RR method, they experienced the largest incidences in restlessness, rolling, kicking, and foot stomping. They had significance in agitation and reduced amount of time of lambs standing immobile. Lambs showed abnormal lying postures until the end of the 90-minute study period and returned to normal.

1.6. Growth performance traits

1.6.1. Average daily gain and daily water intake

Decreased feed intake can be an indicator an animal is experimenting stress. Studies conducted in cattle examined the effects of castration on feed efficiency. Warnock et al (2012), evaluated seventy-five Angus and Brangus bull calves. The bull calves were evaluated for the effects of castration on daily feed intake (**DFI**), daily water intake (**DWI**), residual feed intake (**RFI**), growth performance and inflammation indicators. Calves were weaned for seven days and then were transported to a feedlot. After arrival, there was a 21-day acclimation period before the experiment. Calves from the control group were castrated before they were weaned at 52 ± 21 days. The treatment groups were the intact bulls, castration by callicrate bander (**BAN**), castration by Henderson castrating tool (**HEN**), and surgical castration (**SUR**). There were two data collection periods at 0-14 days and 0-84 days. The pens were equipped with two Grow Safe feed nodes to monitor feed intake.

Results from ADG showed that from day 0-14 there was a tendency for a treatment effect with the castrated calves having less ADG than the intact group. However, in the overall experiment calves from all the treatments had similar ADG, indicating that calves recovered from the stress of castration. Feeding behavior was not affected, as trips to the feed bunk was not affected by treatment. Daily water intake was similar across treatments from day 0 to 14. Daily water intake per week was not influenced by treatment. Gain to feed ratio (**G: F**) was similar for days 0-14 and for days 0-84.

Meta-analysis conducted by Sales (2014) examined rams versus wethers for carcass traits and meat quality. They reported ADG of wethers was lower when compared with rams when evaluating the random effects in the Bayesian model. Rams also had a higher growth rate when compared to wethers on different levels of nutrition (low level or high energy).

Melches et al. (2007) examined seventy white Swiss Mountain or 50% crossbred lambs. The treatment groups were control, RR, burdizzo, and surgical castration method and treated with either lidocaine or bupivacaine. The day before castration, there was no difference in body weight between treatments. In the first three weeks after treatment, weight gain was affected by treatment, with the surgical method having a loss of 2.5 kg of body weight, whereas other treatment groups maintained or gained weight. After day 21, weight gain was not different among treatments. However, Melendez et al. (2017) found no difference in feed intake in 1 week, 2 month and 4-month-old Angus calves when the surgical castration or RR method was compared to control. Each group was castrated by the rubber band or surgical method while control calves were handled but not castrated. Age at castration did not influence ADG. The studies concluded that pain caused from castration can influence ADG and feed intake. However, it has been observed that animals recover from the pain in the short-term after castration resulting in no differences in performance over the entire growing period.

1.6.2. Feed intake

Warnock et al. (2012) used seventy-five Angus and Brangus bull calves to examine the effect of castration on daily feed intake (DFI). Feed intake from day 0 to 14 was similar for all the treatments. There was an interaction between treatment and day for DFI. Henderson castrating tool calves had decreased DFI when compared to the control, bull calves, and callicrate bander (**BAN**) on day 0. The HEN group had similar DFI with SC and control group on day 2. Castration by callicrate bander calves tended to have a decrease in DFI when compared to the control and the SC calves on day 10 and on day 14 with control calves and intact calves. Decreased DFI can indicate different losses of appetite due to method of castration. The decrease in feed intake could be due to inflammation and pain associated with castration. The overall DFI was not affected by castration treatment for the overall experiment from day 0 to 84.

Melches et al. (2007) evaluated seventy White Swiss Mountain or 50% crossbred lambs. The groups were the control, RR, Burdizzo and surgical method and treated with either lidocaine or bupivacaine. Feed intake and eating frequency was significantly less in the surgical group than other treatments between 2.5 to 9 hours. The burdizzo group spent less time eating between hours 2.5 to 9, compared to the RR group. Poor feed intake could have been due to the anesthesia. Lambs would not be awake for several hours and did not eat as often as other treatment groups. Overall, the surgical group had the most concerns with feed intake. Feed intake was investigated in the meta-analysis by Sales (2014) and they observed no difference between rams and wethers.

1.6.3. Residual feed intake

Warnock et al. (2012) computed RFI for days 0 - 14 and 0 - 84 and blood samples were taken for phase protein response. The treatment groups were the intact bulls, castration by BAN, castration by Henderson castrating tool (**HEN**), and surgical castration (**SC**). Residual feed intake had the tendency to be affected by treatments from day 0 to 14. The control bulls had similar for RFI but were less than surgical. The HEN and BAN were in the middle when compared to other groups. Castration did cause a change in RFI during the period for days 0 to 14. The overall RFI for 0 - 84 day was not affected by treatment. This shows that castration by different techniques does not affect the calves long-term; it was an initial effect for the calves.

1.7. Analgesics

Analgesics are drugs for pain suppression. There are many different examples of analgesic drugs (Clutton, 2018). Opioids are used to alleviate moderate to severe pain. Opioids include analgesics such as morphine and fentanyl. Non-steroidal anti-inflammatory drugs (**NSAIDS**) are for moderate pain and are useful for inflammatory responses. Flunixin, ketoprofen, and meloxicam are some examples of NSAIDS (Clutton, 2018). Non-steroidal anti-inflammatory drugs act by blocking and inhibiting. Prostaglandins synthesis is blocked by inhibiting cyclooxygenase that converts arachidonic acid to cyclic endoperoxides (Clutton, 2018). The inhibition of prostaglandin gives the effects of the analgesic, antipyretic, anti-inflammatory and platelet inhibiting action (National Center for Biotechnology Information, 2005). Local anesthetics such as lidocaine and bupivacaine can be used for laparoscopic, abdominal, or open surgery (Clutton, 2018). These anesthetics have an anti-inflammatory effect and prevents pain during surgery. N-Nitrosodimethylamine (**NDMA**) antagonist is used for animals undergoing surgery. An example of a NDMA antagonist is ketamine. Ketamine has a constant rate infusion to provide the anesthetic and prevent central sensation (Clutton, 2018).

Banamine (flunixin meglumine) is a non-narcotic, nonsteroidal, analgesic agent with anti-inflammatory and anti-pyretic activity (Banamine Intervet/Merck Animal Health, Madison, NJ). The molecular weight of flunixin meglumine is 491.46 g/mol and is organoammonium acquired by merging flunixin with one molar alike 1-deoxy-1-(methylamino)-D-glucitol (Figure 1.4.) Banamine can cause issues such as acute poison, corrosive and irritant to skin health hazards to the lungs, eyes, and other organs when inhaled and swallowed (National Center for Biotechnology Information, 2005).

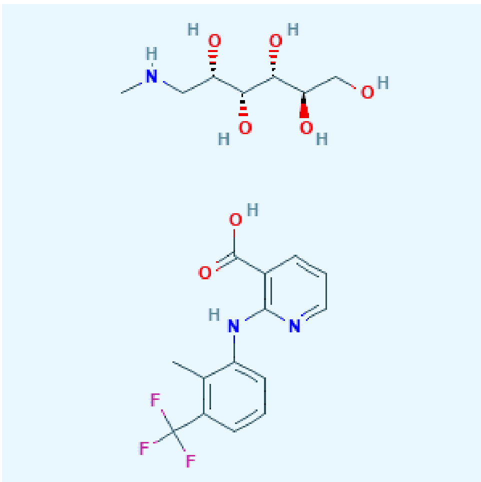

Figure 1.3: Chemical structure of Flunixin Meglumine from National Center for Biotechnology Information (2005).

Banamine injectable contains 50 mgs of flunixin, 0.1 mg edetate disodium, 2.5 mg of sodium formaldehyde sulfoxylate, 4.0 mg diethanolamine, 207.2 mg propylene glycol: 5.0 mg phenol for preservative, hydrochloric acid, and water (Banamine Intervet/Merck Animal Health, Madison, NJ). Castration relievers such as meloxicam or flunixin can be used for pain relief for cattle (Daly, 2015). Banamine can be administered either intramuscular, subcutaneous, or intravenous (Banamine Intervet/Merck Animal Health, Madison, NJ).

1.7.1. Analgesic studies

Paull et al. (2012) evaluated flunixin and meloxicam during castration on 48 Merino lambs. There were four groups: sham group; RR with saline (control); RR with 5 mls of flunixin; or RR with 5 mls of meloxicam. Blood was collected to evaluate the cortisol concentration. Cortisol peaked at 30 minutes after castration. At 90 minutes, there was a difference between each treatment group. The flunixin group was 48% lower and meloxicam group was 28% lower for their cortisol response when compared to the saline group. The flunixin group had reduced leg behavior, restlessness, foot stamping, kicking, and biting at the wound site. Meloxicam reduced pain behaviors such as elevated leg movement, foot stamping, and kicking. The medicated groups spent less time in abnormal positions and laying. Marini et al. (2017) evaluated different castration methods with the assistance of flunixin in the feed and injected for surgically castrated and hot iron docked lambs. Lambs experienced abnormal behavior in the hindquarters for the non-treated castrated and docked lambs when compared to meloxicam fed and control lambs. All treatments administered to the lambs had an significant effect at hour one. Lambs that did receive analgesics did experience less painful hindquarter postures when compared to the non-treated lambs. Postural behavior was similar for pen, time, and treatment but there was no interaction between time and treatment. Non-treated castrated lambs experience more summed up pain, when compared to the meloxicam-fed lambs, injected lambs, and the control. The difference between treatments was between 24 to 48 hours after castration and docking occurred.

1.7.2. Non-Flunixin studies

Graham et al. (1997) evaluated at 3-week-old Suffolk and Dorset lambs utilizing four different analgesics during tail docking. The control treatment was subcutaneous injection of 0.5 ml of bupivacaine hydrochloride. The other experimental group was epidural group,analgesic spray for three seconds and an injectable Diclofenac. The subcutaneous injection reduced abnormal behaviors and postures for all docking methods. The RR method was the most affected for total active behavior followed by burdizzo and hot iron docking. The RR and burdizzo method were observed in abnormal postures longer and had higher levels of cortisol concentrations compared to the control group. The control treatment reduced occurrences abnormal behavior. The epidural treatment had significant reduction of abnormal behavior and postures compared to the spray, and diclofenac had reduced abnormal postures and behavior. The spray was not as effective as the subcutaneous injection because it did not penetrate the skin. Diclofenac had more incidences with abnormal positions and behavior for the RR method. Overall, subcutaneous injection preformed the best for reducing pain for abnormal behavior.

Melches et al (2007) utilized seventy White Swiss Mountain or crossbred lambs to observe the analgesic response to RR, surgical, and burdizzo docking. Lambs were castrated at 10-24 weeks of age. Expression of pain during castration was significantly influenced by method of castration. Surgical and burdizzo treated lambs exhibited expression of pain when compared to the RR and handled group. The anesthetic did not have any effect on pain expression. Cortisol levels in the surgical group were significantly higher throughout treatment day when compared to the other groups. The burdizzo treatment with lidocaine was significantly higher at 1.5- and

2.5-hours post procedure and had the tendency to be higher at three hours. After 6 hours, they return to the normal basal levels. Abnormal lambs observed a significant change in the surgical group when compared to the other groups. The RR treatments had an increase in abnormal positions in the lidocaine treated lambs were significant from 2.5 to 9 hours when compared with the bupivacaine. There was no difference found between treatments for RR and burdizzo treated lambs. Total activity had less incidences observed in the burdizzo group. The burdizzo group had significantly less total activity when compared to the RR and handled groups. Day of castration and a three-day period following showed a difference in the burdizzo group, while the lidocaine group was less active when compared to bupivacaine. This was also observed in the RR group as there was no significance when compared to the control group. The surgical group had no significant difference between treatments.

In a study done by Thornton and Waterman-Pearson (1999), 4 different castration methods were evaluated with three different anesthetics provided. Local anesthesia was administered in the scrotum with 2% lidocaine. General anesthesia was administered with 1 - 2% halothane with 100% oxygen and recovery period was between three and four minutes when the lamb raised its head. Control lambs that were given no anesthesia observed no visual behaviors and lambs that were castrated had behaviors up to three hours. The RR non-anesthesia treatment had the highest peak of visual signs among the castration, with the combination group (RR and clamp) coming in second. The surgical treatment did have an increase of visual responses that remained until the 8 - hour period. Local anesthesia groups had a decrease in visual signs in activity in the control and surgically castrated lambs. Combined lambs in the anesthesia treatment observed similar levels of active and unresponsive behavior when comparing to the control. Active pain behavior was reduced in the surgical group, and the behavioral response was significantly different from the control group. In the surgical groups unresponsive behavior was not affected by the treatment. General anesthesia treatment had no significant effect for active pain behavior for the RR or surgical group. However, there was a declining trend for the RR and surgical groups. There was a reduction for the combination group that showed no significance. Unresponsive behaviors were greater in all castration groups when compared to no anesthesia. The interaction was unresponsive to the observer for the surgical group when comparing to the control. General anesthesia had no effect for scrotal pain among castration groups. Mechanical nociceptive threshold observed that castrated lambs administered with no anesthesia had a rise in their threshold response when comparing to the control group RR group produced a slight rise in their response but was not significantly different from the control group. Local anesthesia did not change any pain response in the surgical group. General anesthesia observed a rise in thresholds in the surgical group when compared to RR, combined and control group. The rise was significantly lower when general anesthesia was used for castration compared to the other groups. RR or combination was not significantly different when it came to thresholds.

Small et al. (2014) evaluated the use of oral buccal meloxicam to understand if using an analgesic can reduce painful behavior for castration and docking in Merino lambs. Sixty merino lambs were utilized at 6 -7 weeks of age and were randomly allotted to treatment. Treatments were administered into the buccal cavity immediately before any castration or docking occurred. Lambs were placed in either a placebo or meloxicam group then were surgically castrated and hot iron docked. Lambs were placed on their back in a cradle position for 60 seconds during the castration and docking procedure. After the castration and docking procedure was completed, lambs was released to join the ewes. Behaviors were recorded from 15-minute intervals for an 8-hour period. Two evaluators observed and recorded the lamb’s behavior and posture such as standing and lying down. Lambs were then evaluated for wound and swelling score at days 4 and 7. Meloxicam had a reduction in combined abnormal behavior, abnormal postures, and spent more time in normal lying position and grazing than the control. At 24 hours, the meloxicam treatment spent more time laying down. At eight hours after treatment was administered, a significant difference was found in behavioral postures such as standing hunched over, normal posture, and in stretched posture. Meloxicam treatment had a decrease in combined postures when compared to the placebo (5.2% to 0.7%). Meloxicam treated lambs had a slight decrease (41.4%) of flystrike when compared to the control (46.1%). Meloxicam lambs had higher scrotal wound appearance on day four and on day seven. Tail wound appearance was significantly higher in the meloxicam lambs. Some tail wounds had increased swelling due to maggots from fly strike.

Kent et al. (1998) evaluated crossbreed lambs on their pain from castration and docking. Groups of eight lambs were used for control, with or without any anesthetic. The castration methods used for this experiment were burdizzo and RR methods. Lidocaine hydrochloride with 2% adrenaline was utilized as the local anesthetic. Injections were placed either by high pressure needle less injection of 0.2 ml to the middle of the testes, and right dorso-lateral tissues of the tail. The burdizzo method combined with RR was injected into the left and right dorso-lateral tissues of the tail or right and left dorso-laterally to the tail or into the spermatic cord of the testes. Time between the anesthetic and castration was 10 to 15 seconds for the anesthetic to take effect for RR castration. Time between injection and castrator was two minutes for burdizzo method. Behavioral responses were recorded every two and six minutes up to 96 minutes. Postures and active behaviors were observed and recorded with the use of summation of incidences. Different positions that were accounted for were abnormal lying and standing, walking, rolling, jumping, etc. Blood samples evaluated for cortisol concentrations of the lambs every 20 minutes up to 180 minutes, with the exclusion of 160 minutes. The control treatment had no significant effect due to anesthetic of both non-docked or castration for active behavior cues and scored 9 to 12 on the REQ score during the first 96 minutes after the treatment was administered. Castration done by the RR method without anesthetic averaged 216 REQ score while docking had an average score of 115. There was a significant difference found in head turning when compared to other treatments. Two hours after castration, two-thirds of the lambs were found to be in abnormal positions at 86 minutes. It was observed at 65 minutes that lambs were lying down in the abnormal position. Between 30- and 60-minutes lambs peaked in abnormal lying down positions either in lying down with full extension of legs or lying laterally with one shoulder on the ground. Partial lying with extension of one leg was observed between minute 60 and 120. Abnormal lying down was rarely observed between the RR treatments and partial lying occurred less. There was no significant difference between anesthetic use between castration method or standing and walking behaviors. But, the non-anesthetic and anesthetic treatment has a significant difference in the RR treatment as they observed more abnormal standing when compared to the control treatment or when compared to combined burdizzo treatment with or without the use of anesthetic. RR treatment averaged 10.9 minutes in abnormal positions when compared to the combined burdizzo at 3.5 minutes and the control treatment at 0 minutes. Mean cortisol levels were found not to exceed 45 $nmol•l^{-1}$ with or without anesthetic, the control treatment had the highest level at 34 $nmol•l^{-1}$. Mean cortisol concentrations was around 40 to 60 minutes at 180 $nmol•l^{-1}$ for castration and 60 $nmol•l^{-1}$ for docking. These concentrations are higher when compared to the control treatment. Anesthetic use with either a syringe or with a needle did reduce the REQ scores and abnormal postures for all castration treatments. Behaviors that were reduced by anesthetic was easing quarters and restlessness when compared to the control treatment. Time spent in abnormal lying positions were reduced and abnormal postures were highest in the first hour after castration was completed and labeled as abnormal standing and lying down with one leg partially extended. Tail docking REQ score was reduced due to the anesthetic, but the values were not found to be significantly different when compared to the control treatment. The abnormal waling and standing postures were reduced by anesthetic from either needle less or needle injection. Cortisol levels were decreased for the RR treatment by the anesthetic, and the site of injection had little effect. There was no significant effect for the cortisol level from administion method.

1.8. Conclusions

The objective of this section is to review overall stress and use of castration and docking, as well showing the mechanics of how stress occurs, wound healing, behavioral and performance traits affected by castration and docking, and the use of analgesics. Stress naturally occurs in the body when confronting a situation. The body has developed ways to prepare the individual for a fight or flight situation in a matter of minutes. After the stressor, if the body has an injury, the process of wound healing is extensive. Castration and docking techniques are an example of a stressor that can affect lambs at different ages and the body reacts differently with each method. Castration, docking and stress has potential research to evaluate how a lamb behaves and how stress impacts the lamb's body. Researchers have looked into the different aspects of lambs' performance such as behavior, blood serum, wound and swelling score and weight traits. The possibilities on the topic of stress, pain and analgesic could impact on the welfare of livestock.

Stress has always been a topic for research for livestock as the public's view of operations of livestock may seem harmful to the animal. Using analgesic to relieve animals' pain may benefit the animal, the producer, and the public perception. Researchers have perused analgesics to provide some aid into their pain and stress. Different analgesics have created different results, but research needs to be conducted at a larger scale. Stress and behavior have been studied on different size scales of species. However, current sheep research does not apply to how lambs react to castration and docking in the range environments of the western United States. Lambs need to be evaluated with the producers' techniques and how they react in their own environment, including the use of analgesics.

When castrating and docking lambs in a western range operation, we hypothesized that administering Flunixin Meglumine (**FM**) to lambs that were either rubber ring castrated and docked or surgically castrated and emasculator docked would decrease behavioral stress, serum cortisol, haptoglobin concentration, and wound and swelling, but increase average daily gain.

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2. IMPACTS OF FLUNIXIN MEGLUMINE ON PAIN RESPONSES OF EITHER RUBBER RING CASTRATED AND TAIL DOCKED OR SURGICALLY CASTRATED AND DOCKED LAMBS

2.1. Abstract

Our hypothesis was that administering Flunixin Meglumine (**FM**) to lambs that were either rubber ring castrated and docked or surgically castrated and emasculator docked would decrease behavioral stress, serum cortisol and haptoglobin concentration, but increase average daily gain. Rambouillet ram lambs (n = 181) were allocated with a completely randomized design in a 2 x 2 factorial arrangement of treatments to determine their effects on behavioral and physiological stress responses. Treatment combinations included rubber ring castration and tail docking (**RR**; placement of rubber band at distal end of caudal fold and around the base of the testicles) or surgical castration (**SC**; surgical tissue scissors removing the lower third of the scrotum and testicles removed manually) and emasculator docking (crushing the tail at the distal end of the caudal fold, followed by cutting of the tail) on ram lambs with administration of saline (**S**) or **FM** (2.2 mg/kg of BW; RR-S, RR-FM, SC-S, SC-FM). Ram lambs were castrated at 12.5 ± 5.5 days of age and observed for behavioral changes such as lying down, leg extension and walking for 180 min in 30-min increments. Blood samples were taken at -30, 30, 90- and 140-minutes post-treatment to observe cortisol and haptoglobin levels. Behavioral traits, physiological traits, and average daily gain were analyzed in PROC GENMOD (SAS Inst. Inc., Cary, NC). A treatment x time interaction ($P < 0.001$) occurred for lamb pain behavioral scale, where scores at 30, 60, and 180 minutes after castration and docking exhibited differences among treatments. Average daily gain was not affected by a treatment x time interaction ($P = 0.22$) or treatment ($P = 0.50$) main effect. Serum cortisol concentration exhibited a treatment x

time interaction ($P = 0.002$). The SC-FM lambs had lower cortisol concentrations ($P = 0.004$) than all other treatments at 90 min post-treatment, with the SC-S lambs having higher concentrations ($P < 0.001$) at 140 min post-treatment. Haptoglobin concentration did not exhibit a treatment x time interaction ($P = 0.99$), however treatment and age were significant ($P < 0.004$). The RR-S lambs had an increase concentration of haptoglobin with SC-FM lambs being intermediate. Wounds were assessed on days 4 and 7 post-treatment to determine physiological responses to the type of castration and tail docking technique with or without the administration of FM. The Wound Assessment Scale ranged from 1-5 (Small et al., 2014) and was determined from levels of discharge, scabbing, and wound healing. The swelling score was also a 1-5 scale, varying from no swelling, large amounts, or healed (Small et al., 2014). Lambs were manually restrained by trained personnel (castrating, docking, and bleeding). Wound and swelling score was analyzed with PROC GENMOD (SAS Inst. Inc., Cary, NC). Scrotal wound exhibited a treatment x day interaction ($P < 0.001$), however tail wound score ($P = 0.84$) and scrotal swelling ($P = 0.36$) were not affected. The SC treated lambs had a higher wound score on day 4 and 7 when compared to the RR treated lambs for both the scrotum and tail score ($P < 0.001$). Flunixin Meglumine had no effect on wound score of the scrotum ($P > 0.50$) and tail ($P > 0.43$) for both RR and SC groups. However, RR treated lambs had higher swelling scores on day 4 and 7 ($P < 0.001$), largely due to the elastic ring causing pressure to the tissue surrounding it. Flunixin Meglumine administration did not influence swelling score in treated lambs ($P > 0.43$) of the scrotum and tail ($P > 0.24$). Overall, results indicate that the method of castration and docking in lambs affected short-lived behavioral and physiological stress, with the administration of FM decreasing stress regardless of castration and docking method.

2.2. Introduction

Pain is defined as an aversive sensory or emotional event that causes the animal to experience stress (Molony and Kent, 1997). Stress is a biochemical response that can occur from a physical or psychological response on an individual (Eberhard and Veisser, 2007) in any type of situation. Stress is a stimulus that can disturb homeostasis (Smith and Dobson, 2002). For example, lambs experience pain and stress while being handled during castration and tail docking. In livestock production, animals experience stress during periods of transportation or handling.

Castration and docking are management tools used for livestock health management. Castration is the removal of testicles and there are several different recognized methods to perform the procedure. Castration reduces aggressive behavior and prevents unwanted pregnancies (SID, 2015). Docking is the partial or complete removal of the tail. Docking prevents flystrike, which occurs when the soft feces collect at the head of the lambs' tail (SID, 2015). The collection of feces attracts flies and the flies lay their eggs on the lamb. The eggs mature and hatch into maggots. The lamb will become ill because of the open wound caused by the maggots (SID, 2015). There are both benefits and consequences for castration and docking. Castration and docking may cause days of pain and stress to lambs (Marni et al., 2017). However, castration and docking can improve animal welfare and provide economic advantages to the producer. Producers should be aware of the pain their animals are experiencing and use humane techniques.

Analgesics are drugs for pain suppression. There are many different examples of analgesic drugs (Clutton, 2018). Analgesics may be a solution to pain management in livestock production. In cattle, Flunixin Meglumine is used for acute mastitis, pneumonia, or lung inflammation caused from bovine respiratory disease, and it can be used for castration (Banamine Intervet/Merck Animal Health, Madison, NJ). Scientists are currently studying analgesic work for sheep and cattle to provide pain relief. Research could assist livestock to endure less pain during this stressful period. The largest trial previous to the current study was Thornton and Waterman-Pearson (1999) using 216 lambs with three different analgesics. Our hypothesis was that administering Flunixin Meglumine (**FM**) to lambs that were either rubber ring castrated and docked or surgically castrated and emasculator docked would decrease behavioral stress, serum cortisol and haptoglobin concentration, but increase average daily gain.

2.3. Materials and methods

All procedures were approved by the Animal Care and Use Committee of North Dakota State University (**NDSU**; #A19006). This study was conducted at the NDSU Hettinger Research Extension Center (**HREC**) in Hettinger, ND.

2.3.1. Behavioral and performance responses

Animals and procedure: One hundred and eighty-one Rambouillet ram lambs (12.5 ± 5.5 days of age) were allocated in a randomized complete design with a 2 x 2 factorial arrangement of treatments to determine the effects on behavioral and physiological stress responses. Lambs were attained from the HREC flock and were housed in the lambing barn at the HREC.

Lambs were allotted to treatment by stratifying over time to ensure technicians could collect data accurately. Lambs were manually restrained by trained personnel for weight and blood collection. Lambs were randomly allotted to one of four treatments at the time of docking and castration (n ≈ 45 lambs/treatment): 1) rubber ring castrated and tail docked (**RR**) receiving an injection of saline 15 to 30 min prior to banding (2.2 mg/kg of BW SQ; **RR-S**), or 2) Flunixin Meglumine 15 to 30 min prior to banding (Flunixin Meglumine; 2.2 mg/kg of BW SQ; **RR-FM**), and 3) lambs surgically castrated (**SC**) and emasculator docked receiving an injection of saline 15 to 30 min prior to procedure (2.2 mg/kg of BW SQ; **SC-S**) or 4) Flunixin Meglumine 15 to 30 min prior to procedure (Flunixin Meglumine; 2.2 mg/kg of BW SQ; **SC-FM**). Lambs were injected with 29 gauge, ½ inch insulin syringe with FM or saline. Rubber band castration and tail docking included placement of rubber band at the base of the scrotum and at distal end of caudal fold. Surgical castration used surgical tissue scissors to remove the bottom third of the scrotum and removed the testicles. Surgical scissors were switched every 10 lambs. Emasculator docking crushed the tail at the distal end of the caudal fold, followed by cutting of the tail. Lambs were vaccinated at the time of castration and docking and at weaning (CD-T; Bar Vac CD/T; Boehringer Ingelheim, Ridgefield, CT).

Lambs were grouped by age (between 7 to 18 days of age) in pens and were randomly allotted to treatment. Lambs were weighed, assigned to treatment, and given the respective dosage of the treatment followed by their treatment procedure. Lambs were injected using an insulin syringe (29 gauge, VWR International). To determine behavioral responses, the scale described by Molony et al. (1993, 1995) was utilized. An observer blindly went on the outside of the pen and if a lamb was not found, the observer went in the pen, waited for 2 minutes for the lambs to return to normal behavior. Postural indices were used for measurements seen in Table 1.1. These measurements were taken immediately post-castration and tail docking. Observations were also taken at 30, 60, 90, 120, 150- and 180-min post treatment

2.3.2. Physiological response

The same group of 181 male lambs were used as in behavioral and performance responses to determine the effects of rubber band castration and tail docking or surgical castration and emasculator docking on wound healing, acute-phase proteins, and serum cortisol concentration. The treatments were the same as performance collection (CONR, FMR, CONS, and FMS). Wounds were assessed by two scales utilized by Small et al. (2014) on days 4 and 7 to determine physiological responses to the type of castration and tail docking with or without FM. Lambs were restrained in the same manner as previously described. Scales include a swelling scale and wound appearance to assess the castration and docking method (Small et al., 2014; Table 2.1).

Table 2.1: Swelling and Wound appearance adapted by Small et al (2014).

Swelling Score	Wound appearance score
(1) No swelling	(1) Edges close together, dry scab
(2) Slight swelling along wound edges	(2) Small area < 1 cm or wet and oozing but no visible pus
(3) Large area of swellings, but soft	(3) Medium area 1 to 5 cm of wet and oozing with small amount of pus
(4) Large area of hard swelling; pitting edema	(4) Large area > 5 cm necrotic with copious pus drainage
(3) Reducing hard swelling with loose cover, healing phase	(3) Granulation tissue forming, but still oozing, healing
(2) Scarring or nodule or healed	(2) New skin evident, shiny but not oozing

2.3.3. Data collection

Blood was drawn -30, 30, 90, and 140 min after castration and docking. A trained handler extended the neck of the lamb and another trained personnel drew 3 mL of blood via jugular venipuncture using vacutainers (SST, VWR International) with a 21-gauge, 1" needle for the evaluation of serum cortisol. Additionally, a subset of lambs ($n \approx 25 - 27$) from each treatment group were randomly selected for the determination of haptoglobin concentration. An additional 3 mL of blood was collected at the same time to determine serum haptoglobin concentrations (EDTA, VWR International). Blood was cooled at 4°C for 2 h and centrifuged (3,640 × g, 15 degrees C, 20 min), and serum was harvested and stored (-20°C). Serum cortisol was analyzed at North Dakota State University using the Immulite 1000 (Siemens Healthcare Diagnostics).

Haptoglobin samples were analyzed at University of Guelph using the Roche Cobas 6000 c501 biochemistry analyzer (J. G. Skinner laboratory).

2.3.4. Statistical analysis

For analysis purposes, lamb behavioral responses were placed on a zero to four-pain scale to distinguish no pain, little, moderate, moderately high, and high amounts of pain. Group 0 was classified as V2 and S1. Group 1 is V1 due to the lamb having the head being down. Group 2 includes V3 and S2 because it indicates moderate pain. Group 3 includes L1 and S3 as it is the next level of pain and more stressful than the previous level. Group 4 indicates severe levels of pain and stress the lamb is enduring including SS, V4, and L2. Repeated measures were present for behavioral responses and ADG. Treatment, time, and treatment x time interaction were included as fixed effects. Age was included as a fixed covariate. Behavioral traits, physiological traits, and average daily gain were analyzed in PROC GENMOD (SAS Inst. Inc., Cary, NC) due to the lack of normality in the residuals when analyzed in PROC MIXED. Wound and swelling scores was separated into different categories for tail and scrotum analysis by the scale from Small et al. (2014). Swelling and wound score were analyzed with contrast and estimate statement.

2.4. Results and discussion

2.4.1. Pain behavioral scale

A treatment x time interaction ($P < 0.001$) occurred for the lamb pain behavioral scale, where scores at 30, 60, and 180-min after castration and docking exhibited differences among treatments (Figure 2.1). The RR treated lambs (both with and without FM) experienced the highest pain level rating at 30 min post-castration with a 40% increase in lambs standing in abnormal positions. At 30- and 60-min post-treatment, there was a castration and docking method difference; the first 60-min post castration and docking the RR group experienced higher levels of stress. At 90-min all treatment groups beside SC-S group experienced over 47% chance at pain level zero (i.e., no pain). Pain behavioral scores continued to decrease over time with 55 to 70% chance all groups' average scores being 1 or less (little pain exhibited) at 120-min after procedures were applied. Only the SC-S group exhibited 36% of pain level 2 when compared to less than 25% with other treatments at 180-min post treatment ($P < 0.05$). Lambs treated with FM had no significant difference ($P > 0.05$) for behavioral stress. Our results disagree with those of Marini et al. (2017), Paull et al. (2012), and Small et al. (2014), as lambs in their trials that were administered analgesics exhibited a reduced painful posture. Furthermore, our results disagree with results of Melches et al. (2007) who reported that surgical and burdizzo castration and docking methods resulted in more expression of pain in lambs when compared to the RR and the control group. Our trial found at different time points there was a difference between method of castration and docking and our results conclude that FM did influence painful behavior to reach negligent or no pain level prior to but not before 180 minutes.

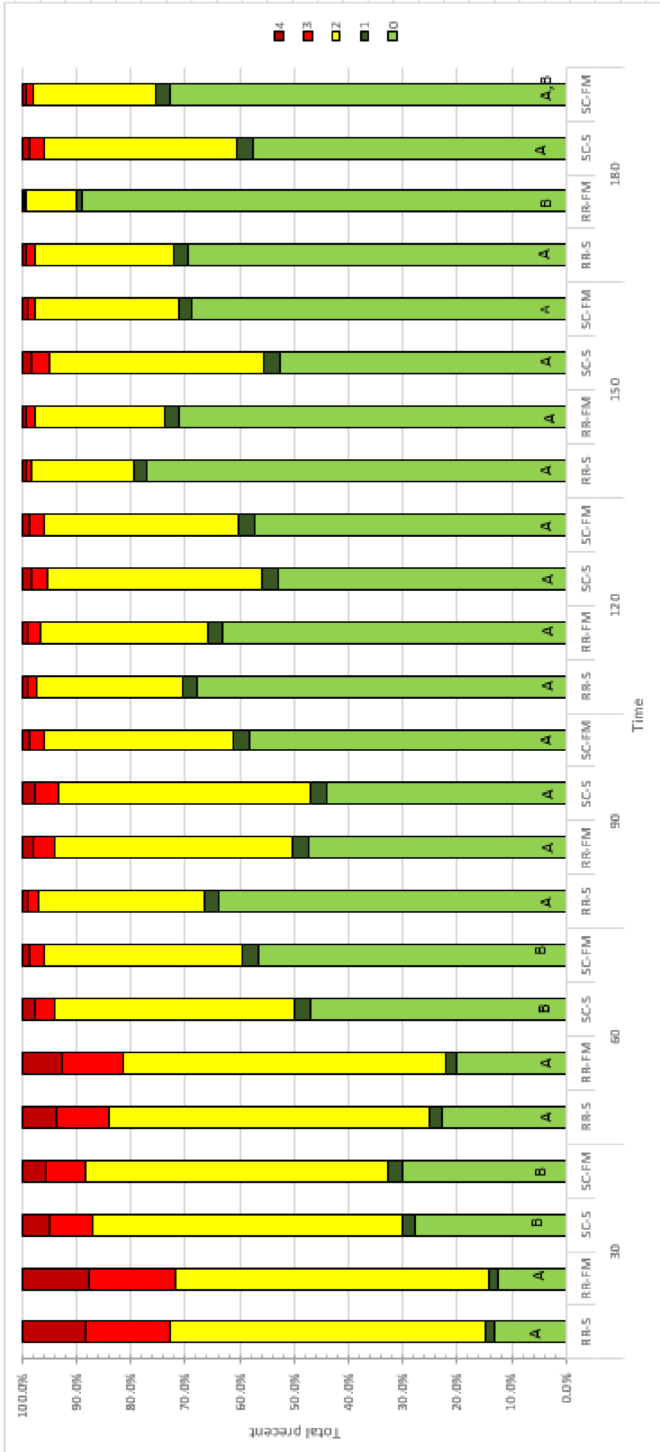

Figure 2.1: The effect of castration and docking technique and administration of Flunixin Meglumine on lamb behavior (Molony et al., 1993 and 1995).
Treatments include RR-FM (Rubber ring - Flunixin Meglumine), RR-S (Rubber ring - saline), SC-FM (Surgical castration - Flunixin Meglumine), and SC-S (Surgical castration - saline).
Means with different superscripts (A – B) differ ($P \leq 0.05$) within each time point.

2.4.2. Cortisol

Serum cortisol concentration exhibited a treatment x time interaction ($P = 0.002$; Figure 2.2.). Base-line cortisol concentration was taken at -30-min prior to castration and docking. Serum cortisol concentration peaked at 30-min post-castration and docking, with SC-S exhibiting the highest concentration and RR-S the lowest. At 90-min post-castration, there was a difference in lower cortisol concentrations ($P = 0.004$) with the SC-FM group having when comparing to SC-S. As time progressed to 140-min post-treatment, the SC-S remained significantly higher ($P < 0.001$) than all other treatments. The increase of cortisol for SC-S indicates the FM decreased cortisol concentration compared to saline. Results found for the SC treated lambs were similar to Molony et al. (1995) in cattle, who reported SC castration was more stressful than RR castration. Results found for the SC treated lambs were similar to Melches et al. (2007), who reported lambs that were surgically castrated had higher levels of serum cortisol when compared to RR and burdizzo method. The surgically castrated with FM administered lambs had decreased cortisol levels by 48% compared to SC-S lambs at 140 minutes. Our results disagree with Paull et al. (2012) and Kent et al. (1998) for RR castration, as there was no significant difference in administering FM to RR castration. Our study is more reliable as the amount of lambs were used at each time point and using only four time points to decrease environmental variability. Paull et al. (2012) used 28 lambs for their study and Kent et al. (1998) observed 64 lambs.

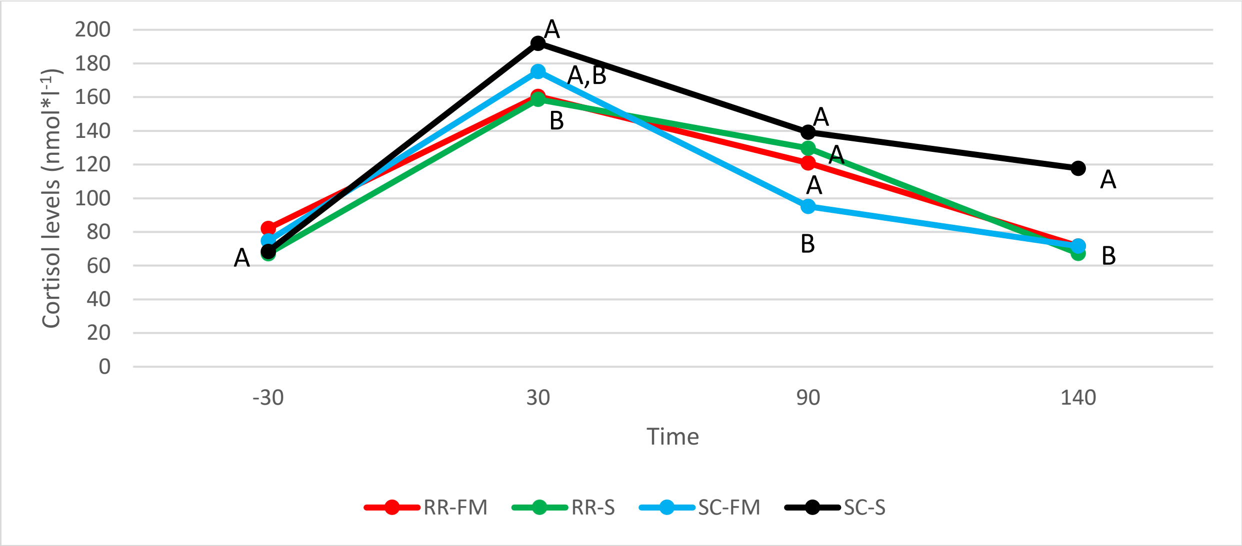

Figure 2.2: The effect of castration and docking technique and administration of Flunixin Meglumine on serum cortisol concentration from least square means.
Treatments include RR-FM (Rubber ring - Flunixin Meglumine), RR-S (Rubber ring - saline), SC-FM (Surgical castration - Flunixin Meglumine), and SC-S (Surgical castration - saline).
Means with different superscripts (A – B) differ ($P \leq 0.05$) within each time point.

2.4.3. Haptoglobin

Serum haptoglobin did not exhibit a treatment x time interaction (*P* = 0.99) or time main effect (*P* = 0.85); however, treatment (*P* = 0.001) and age (*P* = 0.004) did influence serum haptoglobin concentration. There was a difference found between age 7, 12 and 15 days of age (P < 0.0006). The RR-S lambs had an increase in haptoglobin concentration (*P* = 0.001; Table 2.2.) compared to other treatments, with SC treated lambs receiving FM being intermediate. Our results are similar to results reported by Marini et al. (2017) who observed that flunixin-fed lambs had lower levels in haptoglobin concentration than the control. Haptoglobin was numerically but not significant different (*P* = 0.07) when comparing the RR-saline treatment group. However, further research needs to be conducted to evaluate levels of haptoglobin because research from Marini et al. (2017) reported significant linear increase after 12 hours.

Table 2.2: The effect of castration and docking technique and administration of Flunixin Meglumine on serum haptoglobin concentration.

Treatment[1]	n	Haptoglobin (g/l)	SEM	*P*-value[2]
RR-FM	25	0.123	0.21	0.20
RR-S	27	0.29	0.39	<0.01
SC-FM	27	0.15	0.23	0.01
SC-S	25	0.13	0.24	0.16

[1]Treatments include RR-FM (rubber ring - Flunixin Meglumine), RR-S (rubber ring saline), SC-FM (surgical castration - Flunixin Meglumine), and SC-S (surgical castration-saline).

2.4.4. Wound and swelling score analysis

Wound score analysis of the scrotum exhibited a treatment effect ($P < 0.001$) and a tendency for a time by treatment interaction ($P = 0.07$). There was no difference between Flunixin Meglumine and saline ($P > 0.83$) for swelling or wound score, disagreeing with the results of Small et al. (2014) for surgical castration and docking. The wound score analysis of the tail found a treatment effect ($P < 0.0001$) in the surgical treatments, where no significance was found in the RR group ($P = 0.12$). There was no difference between Flunixin Meglumine and saline treatment for wound analysis ($P = 0.92$ for tail, $P = 0.96$ for scrotum), disagreeing with Small et al. (2014) for surgical castration and docking. Swelling score analysis had similar results to tail and scrotum swelling finding a treatment effect ($P < 0.0001$) for both scrotum and tail. A day effect was found in the scrotum ($P = 0.04$) and a day by treatment interaction in the tail ($P < 0.0001$). Swelling of the tail observed in the RR group had higher levels on day 7 while the surgical group decreased in swelling (Table 2.3). There was no difference found for the FM ($P = 0.99$) between RR and SC treatments. Wound swelling of the scrotum had similar results as the RR group had higher scores for swelling when compared to the surgical group. Banamine did not have any significance for both castration and docking groups ($P > 0.05$). Our results for wound and swelling disagree with Small et al. (2014) and is likely due to no incidences of flystrike. No incidences of fly strike occurred as the experiment was conducted in the fall and flies were not present.

Table 2.3: The effect of castration and docking technique and administration of Flunixin Meglumine on swelling and wound score.

Treatments[1]	SST[2]	SEM	*P*-value	SSS[2]	SEM	*P*-value	WST[2]	SEM	*P*-value	WSS[2]	SEM	*P*-value
RR-FM	2.95	0.11	<0.001	2.94	0.12	<0.001	1.12	0.06	0.71	1.14	0.07	0.002
RR-S	2.99	0.12	<0.001	3.03	0.13	<0.001	1.22	0.06	0.90	1.22	0.07	0.0002
SC-FM	2.00	0.08	<0.001	1.85	0.08	<0.001	2.37	0.11	<0.0001	1.83	0.09	<0.0001
SC-S	1.99	0.08	<0.001	1.78	0.08	<0.001	2.28	0.11	<0.0001	1.71	0.09	<0.0001

[1] Treatment include RR-FM (Rubber ring- Flunixin Meglumine), RR-S (Rubber ring saline), SC-FM (Surgical castration-Flunixin Meglumine), and SC-S (Surgical castration-saline).
[2] Variables include SST (swelling score tail), SSS (swelling score scrotum), WST (wound score tail), and WSS (wound score scrotum).

2.4.5. Average daily gain

Average daily gain did not exhibit a treatment x time interaction (*P* = 0.22) with the use of FM. Initial and final weights were similar across treatments ($P \geq 0.09$), resulting in an ADG of 0.21 kg/d (SEM = 0.02; Table 2.4.). Similar results have been reported for the effects of castration technique for performance traits in cattle (Bretschneider, 2005 and Warnock et al., 2012). Warnock et al. (2012) reported a decrease in daily feed intake (DFI) at days 10-14 in Angus and Brangus calves, however overall DFI was not affected by castration treatment for the overall experiment from day 0 to 84. Bretschneider (2005) reviewed studies that analyzed performance traits in beef cattle. Average daily gain was not affected by castration method or use of FM.

Table 2.4: The effect of castration and docking technique and administration of Flunixin Meglumine on growth performance.

	Treatment [1]					
Item	RR-FM	RR-S	SC-FM	SC-S	SEM	*P*-value[2]
Initial weight, kg	7.40	7.50	7.40	7.10	0.18	0.34
Final weight, kg	15.80	16.30	16.30	17.00	0.35	0.09
Final ADG, kg/d	0.21	0.23	0.19	0.22	0.02	0.72

[1] Treatments include RR-FM (Rubber ring-Flunixin Meglumine), RR-S (Rubber ring-saline), SC-FM (Surgical castration-Flunixin Meglumine), and SC-S (Surgical castration-saline).
[2]*P*-value across treatments (n ≈ 42).

2.4.6. Conclusion

Castration and docking method did have an effect on several traits. The use of Flunixin Meglumine did have an effect on lambs for both castration and docking methods. Painful behaviors were increased by method of castration and Flunixin Meglumine decreased for the RR method. Serum cortisol concentration was increased by use of FM for surgical castration and emasculator docking. Haptoglobin did not increase by treatments, however observations needs to be extended to longer periods of time to have a significance. Type of castration and docking did increased wound appearance in SC lambs and swelling appearance in RR lambs. Flunixin Meglumine did not increase or decrease wound and swelling appearance in both RR and SC lambs. Average daily gain did not increase or decrease by method of castration/docking and use of Flunixin Meglumine. Overall, the surgical castration and emasculator docking did cause more stress when compared to the RR method.

2.4.7. Implication

The use of Flunixin Meglumine lowered serum cortisol concentration in the surgical group but not the RR group. However, Flunixin Meglumine did not increase or decrease on haptoglobin concentration, behavior, wound and swelling score and average daily gain. Comparing the use of Flunixin Meglumine with other castrating and docking methods may be worth investigating further while evaluating other industry standards. Variables to be investigated are age and day of castration, looking at blood serum days later, and observation weight at 24 hours, 1 week and biweekly. Longer time intervals such as 12 hrs past castration and docking could show results of performance and physiological stress of lambs and give a better understanding of stress.

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3. ECONOMIC ANALYSIS OF THE IMPACT OF FLUNIXIN MEGLUMINE ON PAIN RESPONSES OF EITHER RUBBER RING CASTRATED AND TAIL DOCKED OR SURGICALLY CASTRATED AND DOCKED LAMBS

3.1. Abstract

Our hypothesis is the use of Flunixin Meglumine (**FM**) will decrease cost in the research flock. Castration is the removal of the testicles and docking is partial or complete removal of the tail. There are different procedures to castrate and dock lambs. The choice of method made by the producer may be due to cost, availability, training or even convenience. Rubber ring castration and docking, surgical castration and emasculator docking methods will be acceptable for any producer to choose. One hundred and eighty one male Rambouillet lambs (approximately 7 to 18 days of age) were used in a complete randomized design with a 2 x 2 factorial arrangement of treatments to determine the effects of rubber ring (**RR**) castration and tail docking (placement of rubber band at distal end of caudal fold) or surgical castration (surgical scissors removing the bottom third of the scrotum and testicles removed manually) and emasculator docking (crushing the tail at the distal end of the caudal fold to decrease bleeding, followed by cutting of the tail) on male lambs with or without the administration of FM on behavioral and physiological stress responses. A partial budget was used to evaluate castration and docking methods and the use of Flunixin Meglumine within the flock. Budget was comprised of castration and docking products, vaccines used during the time of castration/docking and weaning, labor and products used during the project (Flunixin Meglumine and insulin syringes). Total cost was calculated for the flock (RR, n = 90 and SC, n = 91) and cost per lamb was calculated. Castration and docking technique had a difference between the rubber ring method and surgical castration and emasculator docking. There was a $4.94

difference between methods (Table 3.1 and 3.2), with surgical castration and emasculator docking being the most expensive, due to the price of the emasculator tool. On a per lamb basis, it was a $0.10 increase with the use of surgical castration and emasculator docking when compared to RR method. Flunixin Meglumine did have a difference in cost for both methods of castration and docking. There was a $20.91($0.23 cents per lamb) difference for the RR method and $29.76 ($0.33 cents per lamb) for the surgical method. In similar to the methods cost, the RR method cost less than the SC method with the administration of FM.

3.2. Introduction

Castration is the removal of the testicles and docking is partial or complete removal of the tail. There are different procedures to castrate and dock lambs. The choice of method made by the producer may be due to cost, availability, training or even convenience. The elastration method, commonly called the rubber ring (RR) method, is the most common method of castration and docking. This technique uses a rubber or elastic ring that is placed dorsally over the scrotum and testicles. Docking is the same concept; the tail goes through the band and is positioned at the length the producer so chooses (SID, 2015). Surgical castration is another practice but not as common as the RR method. Surgical castration is the removal of the testicles by a scalpel or a sharp knife, cutting on the lower third of the scrotum, exposing the testes. Then the testes are removed by pulling them out without cutting the spermatic cord (Daly, 2015).

Flunixin Meglumine (**FM**) is a non-narcotic, nonsteroidal, analgesic agent with anti-inflammatory and anti-pyretic activity (Banamine Intervet/Merck Animal Health, Madison, NJ). In cattle, Flunixin Meglumine is used for acute mastitis, pneumonia, or lung inflammation caused from bovine respiratory disease, and it can be used for castration.

Cost analysis has not been conducted to see if the use of FM is cost effective for sheep producers. Additional costs discourage producers because if it costs too much, they will find an alternative drug or commodity to help their flock, or not use the drug at all. Our hypothesis is that there will be an added cost with the use of Flunixin Meglumine and that surgical castration and emasculator docking will cost more than using the RR method.

3.3. Materials and methods

All procedures were approved by the Animal Care and Use Committee of North Dakota State University (NDSU; #A19006). This study was conducted at the NDSU Hettinger Research Extension Center in Hettinger, ND.

3.3.1. Behavioral, physical, and performance responses

Animals and procedures: One hundred and eighty one male Rambouillet lambs (7 to 18 days of age) were used in a complete randomized design with a 2 x 2 factorial arrangement of treatments to determine the effects of rubber band castration and tail docking (placement of rubber band at distal end of caudal fold) or surgical castration (surgical scissors removing the bottom third of the scrotum and testicles removed manually) and emasculator docking (crushing the tail at the distal end of the caudal fold to decrease bleeding, followed by cutting of the tail) on male lambs with or without the administration of FM on behavioral and physiological stress responses. Lambs were attained from the NDSU Hettinger Research Extension Center (**HREC**) flock and were housed in the lambing barn at the HREC. Lambs were born during the two fall lambing cycles starting on September 15th and finishing on December 12th.

Lambs were allotted to treatment by stratifying them over time to ensure technicians could collect all of the intensive data accurately. Lambs were processed throughout the lambing period, where trained personnel were evaluating the behavior of the lambs. Lambs were manually restrained by trained personnel (castrating, docking, and bleeding - manually holding the lambs to limit movement). Lambs were allotted to one of four treatments at the time of docking/castration (n ≈ 45 lambs/treatment):1) rubber ring castrated and tail docked (**RR**) receiving an injection of saline 15 to 30 min prior to banding (2.2 mg/kg of BW SQ; **RR-S**), 2) Flunixin Meglumine 15 to 30 min prior to banding (Flunixin Meglumine; 2.2 mg/kg of BW SQ; **RR-FM**), 3) lambs surgically castrated (**SC**) and emasculator docked receiving an injection of saline 15 to 30 min prior to procedure (2.2 mg/kg of BW SQ; **SC-S**) or 4) Flunixin Meglumine 15 to 30 min prior to procedure (Flunixin Meglumine; 2.2 mg/kg of BW SQ; **SC-FM**). Lambs were selected by pen age and lambs were randomly selected for treatment. Lambs were weighed, assigned to treatment, and then given the respective dosage of the treatment followed by either banding the tail and scrotum or surgical castration and emasculator docked. Lambs were injected using an insulin syringe for the proper dosing (VWR International). Lambs were weighed every two weeks until weaning (approximately 74 d of age) to determine behavioral responses to the stress event. Data was recorded using a scale by Molony et al. (1993, 1995) to assess stress. Postural indices were used for measurements, including various forms of lying and standing categorized further by the positions of the limbs and head. The same group of 181 male lambs were used to determine the effects of rubber band castration and tail docking or surgical castration and emasculator docking on wound healing, acute-phase proteins, and serum cortisol concentration.

3.3.2. Economic analysis and objective

A partial budget was used to evaluate castration and docking methods and the use of FM within the flock. The budget was comprised of castration and docking products, vaccines used during the time of castration/docking and weaning, labor, and products used during the project

(FM and insulin syringes). Total cost was calculated for the flock (RR, n = 90 and SC, n = 91) and cost per lamb was calculated.

3.4. Results and discussion

Castration and docking technique had a difference between the RR ring method and surgical castration and emasculator docking numerically. There was a $4.94 difference between methods (Table 3.1 and 3.2), with surgical castration and emasculator docking being the most expensive, due to the price of the emasculator tool. On a per lamb basis, it was a $0.10 increase with the use of surgical castration and emasculator docking when compared to RR method. Sheep producers commonly use the RR method as a cheaper, bloodless, and easier to use method when compared to the surgical, castration and emasculator docking.

Flunixin Meglumine did have a difference in cost for both methods of castration and docking. There was a $20.91 ($0.21 cents per lamb); (Table 3.3) difference for the RR method and $23.77 ($0.09 cents per lamb); (Table 3.4) for the surgical method. The RR method cost less than the surgical castration and emasculator docking with the administering of FM. While there is an added cost to the use of FM in the flock, today's welfare standards may start enforcing use of analgesics to have some type of pain relief for lambs and to help the urban population understand and show that producers do care about the welfare of their livestock. The trial conducted did not indicate differences between FM and saline for ADG and haptoglobin. The use of FM did reduce pain postures at 150 and 180 minutes. The use of FM should be considered for reduction of painful behavior and help with the perception of pain in the agricultural world. However, when using the surgical castration and emasculator docking, the use of FM would help with pain relief and the swelling following the days after castration and docking. As the agricultural world connect with consumers, farmers and ranchers need to evaluate their animals for pain. Potential economic incentive may arise for producers that ensure animal well-being through the use of analgesics to help reduce pain. While pain management is not regulated or enforced, there may be some added bonus for the farmers and ranchers for years to come.

3.5. Conclusion

The use of analgesic for pain management could be implemented during times of stress. The use of FM showed benefit with lower cortisol levels and improved behavior in the surgical castration and emasculator docking treatment. Per lamb for SC group, it cost $0.22 more to use FM when compare using saline. Per lamb for RR group, it cost $0.21 more to use FM when compare using saline. It is the produces preference if using the FM to prevent stress and painful behavior for an added cost. Possible things to look into would be a 5-year budget with added cost such as feed and veterinary to see if the adding FM into the budget is a feasible cost for the producer.

Table 3.1: Cost analysis for RR method without Flunixin Meglumine.

Item	Cost	Total	Cost per lamb
RR[1]	$1.60 per 100 CT	$4.80	$0.11
CDT vaccine[2]	$38.33/250 mls	$76.66	$1.70
Needles	$15.00	$15.00	$0.33
Labor	$15.00/hr. (1.25 hrs.)	$18.75	$0.42
Total		$115.21	$2.56

1Treatment RR (rubber ring).
[2] CDT (Clostridium perfringens type C + D and tetanus).

Table 3.2: Cost analysis for SC without Flunixin Meglumine.

Item	Cost	Total	Cost per lamb
CDT vaccine[1]	$38.33/250 mls	$76.66	$1.70
Needles	$15.00	$15.00	$0.33
Labor	$15.00/hr. (1.5 hrs.)	$22.50	$0.50
Scissors	$5.99	$5.99	$0.13
Total		$120.15	$2.66

[1] CDT (Clostridium perfringens type C + D and tetanus).

Table 3.3: Cost analysis for RR with Flunixin Meglumine.

Item	Cost	Total	Cost per lamb
RR[1]	$1.60 per 100 CT	$4.80	$0.10
CDT vaccine[2]	$38.33/250 mls	$76.66	$1.67
Needles	$15.00	$15.00	$0.33
Labor	$15.00/hr. (1.25 total)	$18.75	$0.41
Flunixin Meglumine	$12.22 for 58 doses	$12.22	$0.07
Insulin syringes	$8.69	$8.69	$0.19
Total		$136.12	$2.77

[1]Treatment RR (rubber ring).
[2] CDT (Clostridium perfringens type C + D and tetanus).

Table 3.4: Cost analysis for SC method with Flunixin Meglumine.

Item	Cost	Total	Cost per lamb
CDT vaccine[1]	$38.33/250 mls	$76.66	$1.67
Needles	$15.00	$15.00	$0.33
Labor	$15.00/hr. (1.5 hrs.)	$22.50	$0.49
Flunixin Meglumine	$12.22 for 58 doses	$12.22	$0.07
Scissors	$5.99	$5.99	$0.13
Insulin syringes	$8.69	$8.69	$0.19
Total		$149.91	$2.88

[1] CDT (Clostridium perfringens type C + D and tetanus).

3.6. References

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4. IMPLICATIONS AND FUTURE RESEARCH

Welfare and stress have shown to affect livestock performance. Performance traits are looked at to see the value of the livestock. Stress and pain can have a widespread definition to different spectators. Different tools used in the livestock industry have been criticized among non-agriculturists. Castration and docking have been tools used in the sheep industry. For producers, the stress and pain that is associated with the risk of non-castration and docking is found to be inhumane. While castration and docking does cause pain for the lamb, the benefits outweigh the consequences such as flystrike and aggressive behavior. One possible way to remove the stress and pain is through analgesics. It may be an added cost, but it could bring added value to the producer’s stock, with consumers acknowledging that the lamb had pain reliver during castration and docking. With research that looks into pain and stress during castration and docking, the addition of pain relievers could benefit livestock production. Our results contribute a small sum of information about the use of analgesic (Flunixin Meglumine) and castration method for behavioral, performance, and physiological traits. To our knowledge, this is the largest trial conducted utilizing Flunixin Meglumine to alleviate pain during castration and docking. The largest trials previous to the current study utilized 48 lambs (Paull et al., 2012), and Thornton and Waterman-Pearson (1999) used 216 lambs with three different analgesics. There have been various trials conducted with different types of analgesics on different behavioral, performance, and physiological traits. The current trial is unique by looking at physiological traits that are not included in previous studies, such as haptoglobin. Cattle research has previously studied haptoglobin concentrations, but little information is available on sheep.

The use of Flunixin Meglumine did have a reduction in serum cortisol and behavior for the surgical castration and emasculator docking. After 90 minutes, the SC-FM group had decreased levels of cortisol and behavior scores at 150 minutes, therefore having lower levels of stress when compared to saline. Comparison of castration and docking methods showed that in the first 60 minutes, the RR method had higher levels of stressful behavior, whereas the SC treatment exhibited stressful behavior after 150 minutes. Swelling was prominent in the RR group whereas wound score was prominent in the SC group.

4.1. Future directions

Improvements for research should be done for each variable. Researchers should investigate behavior prior to the experiment and past 180 minutes, where it is suggested that researchers investigate days after castration and docking. Producers across the United States have their own procedure for castrating and docking. Scientist should look into common practices used by producers to see if there is any stress at different ages. Differences have been seen in cattle but not seen in sheep. Evaluating behavior prior to castration and docking could help set a baseline for normal behavior. Cortisol and haptoglobin should be investigated longer, as haptoglobin levels seen in cattle have showed results up to two days after treatment occurred. Wound score was evaluated at four and seven days after castration and docking, results should look into possibly a day after castration to weeks after to evaluate the overall swelling and wound healing process of the lambs. Weight traits should be improved by evaluating 24 hours, 48 hours, 1 week, and biweekly after to evaluate the weight change of lambs. Weight traits have been evaluated at different time points, but 48 hours has not been looked at.

4.2. References

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