THESIS

PAIN EVALUATION AND MITIGATION IN THE BOVINE

Submitted by

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ABSTRACT

PAIN EVALUATION AND MITIGATION IN THE BOVINE

The objectives of this research were to evaluate the effects of: 1) castration, 2) use of analgesia after castration on measures of behavior, feedlot performance, and physiological responses in cull bulls, and 3) efficacy of analgesia when applied to an induced pain model in cull dairy cows.

In the first study, our objectives were to evaluate the effects of ketamine-stun (**KET**) and oral meloxicam (**MEL**) at the time of band castration on performance and behavioral response of Angus bulls not selected as breeding stock immediately post-weaning. Angus bulls (n = 119, 291.3 ± 29.1 kg, 241.9 ± 21.6 d of age) were blocked by BW in a complete randomized design via a 2 × 2 factorial arrangement with 1 additional treatment group remaining intact. Bulls to be castrated on d 0 were randomly assigned to 1 of 4 treatments: 1) meloxicam with no ketamine-stun (**MEL**), 2) meloxicam and ketamine-stun (**MEL+KET**), 3) ketamine-stun with no meloxicam (**KET**), or 4) no meloxicam or ketamine-stun (**CON**). Meloxicam was administered on d 0, 7, and 14 (3 mg/kg) via oral bolus. Ketamine stun consisted of butorphanol (0.0125 mg/kg), xylazine (0.025 mg/kg), and ketamine (0.050 mg/kg) and was administered approximately 10 min before band application via a single subcutaneous injection. Animals not receiving KET or MEL received a subcutaneous injection of saline or an empty bolus, respectively. Castration was performed by banding using a Calicrate bander. Bulls that remained intact were subjected to sham manipulation of the scrotum associated with castration on d 0, but without band application. Subjective chute score (**CS**) was collected on d -7, 1, 7, 14, 21, and

28. Objective exit velocity (**EV**) was collected on d -7, 0, 1, 7, 14, 21, and 28. Blood samples taken on d 0, 1, 7, 14, 21 and 28 were analyzed for plasma cortisol concentration. Range of vertical head motion (**DIST**) during castration or sham was used as a behavioral pain indicator during castration. Video analysis was conducted blind to treatment group and procedure following castration. Animals were observed at 3 min intervals for 15 min immediately post castration or sham to evaluate behavior response by trained evaluators blind to treatments. Performance measurements analyzed over the 28-d period included ADG, DMI, and G:F. Chute scores were greater in castrated animals ($P \leq 0.02$) on d 14 and 28. There was a tendency ($P = 0.10$) for KET to reduce EV on d of castration. Vertical head movement tended to be greater ($P = 0.06$) in castrated animals than sham bulls. There was an interaction ($P = 0.001$) among main effects for DIST. The CON group had greater ($P < 0.01$) DIST than all other treatments. Mean percent lying down immediately post-castration was greater ($P < 0.001$) for castrates than bulls, and a there was a main effect of KET ($P < 0.001$) resulting in increased lying behavior. Bulls exhibited greater G:F ($P = 0.02$) and ADG ($P < 0.001$) than castrates. There was no effect of KET or MEL on G:F ($P \geq 0.12$) or ADG ($P \geq 0.13$). Plasma cortisol concentrations were greater ($P < 0.001$) in castrates than intact bulls throughout the observation period. In conclusion, castration resulted in more head motion, less favorable chute scores and increased plasma cortisol concentrations. Further, post-castration behavior was altered due to castration and use of KET. Data suggest that neither KET nor MEL alter feed performance among bulls castrated by banding.

The second study consisted of 2 experiments used to evaluate if pain (nociception) associated with an oxytetracycline (**OT**) i.m. injection site inflammatory response can be objectively measured using a pressure algometer. The second objective was to determine if

flunixin meglumine (**FM**) can mitigate pain (nociception) associated with OT injection site inflammation, as measured objectively by a pressure algometer. In Exp. 1, non-lactating cull Jersey cows (n = 5) and in Exp. 2 non-lactating cull Jersey and Holstein cows (n = 10) were randomly assigned to 1 of 2 treatments: 1) flunixin meglumine 2.2 mg/kg (**FM**), or 2) equivalent volume 0.9% saline (**SALINE**). Both treatments were administered i.v. at 24 h intervals. Researchers were blinded to all treatments. At 0 h, animals were administered an i.m. injection of **OT** (LA-200, 200 mg/ml) at 5 mg/kg on one site in the neck and one site in the hind leg (Exp.1) or just the hind leg (Exp. 2) to induce an inflammatory response and stimulate acute pain. In both experiments mechanical nociception threshold (**MNT**) was measured at the OT site and the same location on the opposite neck/leg (**non-OT**) using a pressure algometer. Pressure was applied to the site until the animal exhibited a conscious and visible reaction to pressure. Pressure readings were taken in random order in triplicate and an average of the readings was used. In Exp. 2, blood samples were obtained every 24 h via jugular venipuncture to measure fibrinogen content, as indicator of inflammatory response. In Exp. 1, there were no differences ($P > 0.05$) in MNT between the OT site and the non-OT site in the neck, and there were no differences ($P > 0.05$) in MNT between treatments (FM vs. SALINE) at the OT site in the neck.

In both experiments there were differences ($P \leq 0.05$) in MNT observed between OT and non-OT sites in the leg starting on d 0 throughout the rest of the observation period. In Exp. 1 there were differences ($P \leq 0.05$) in MNT between treatments (FM vs. SALINE) at the OT site starting on d 3 through the rest of the observation period. In Exp. 2 there were also differences ($P \leq 0.05$) in MNT between treatments (FM vs. SALINE) at the OT site starting on d 3 through 9, with tendencies ($P \leq 0.10$) on d 5 and 8. Mean fibrinogen content in animals treated with FM was lower ($P \leq 0.05$) than CON animals at multiple time points. Data suggest OT induced an

inflammatory reaction and FM was effective in reducing the pain sensitization associated with injection site inflammation, both measurable with an algometer.

Keywords: Beef bulls, Castration, Dairy cattle, Mechanical nociceptive threshold, Pain mitigation

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CHAPTER 1

REVIEW OF LITERATURE

Introduction

Global population continues to grow and food demand reaches levels higher than ever before, there is increased pressure for cost-and resource-efficient food production. Although people want abundant, cost-effective food there has also been an increased interest in the desire to know that food animals live decent lives (Rollins, 2004). As this demand by consumers intensifies, it is crucial that scientific data are available to aid producers in meeting new production demands in the most effective and efficient manner possible.

Recently there has been movement by niche markets to provide animal welfare conscious consumers with a meat product from animals that have been audited based on welfare standards. Whole Foods has developed a welfare auditing system to address consumer concerns via the Global Animal Partnership (**GAP**). The GAP consists of a 5-Step Animal Welfare Rating Standards that "recognizes and rewards producers for their welfare practices, promotes and facilitates continuous improvement, and better informs consumers about production systems they choose to support" (Global Animal Partnership, 2009). Starting at level 1, each level increases the requirements which producers must meet with level 5 requiring on-farm slaughter and no physical alteration of the animal. Programs like GAP offer consumers that are willing and able to pay a premium for their food to buy with confidence; however, this remains a niche market in the beef industry. Although this non-governmental organization welfare standard program doesn't

make up a large percentage of the industry, similar programs are increasing in popularity indicating that consumer-driven welfare verification is becoming a retail focus (PAACO, 2004).

As societal concern about ethical livestock treatment intensifies, common livestock management practices, such as castration, are key areas of concern regarding animal agriculture (Weary et al., 2006). In the U.S. there are currently no Food and Drug Administration (**FDA**)-approved drugs labeled for the treatment of pain in cattle (Compendium of Veterinary Products, 2010). According to the FDA, "validated methods of pain assessment must be used in order for a drug to be indicated for pain relief in target species" (FDA-CVM, 2009). In order to validate drug use for pain mitigation, there is need for identification and validation of biomarkers that objectively measure pain and the efficacy of analgesia during painful procedures (Baldridge et al., 2011; Coetzee et al., 2011). Pain in animals can only be estimated by examining reactions, both behavioral and physiological (Le Bars et al., 2001). Pain assessment in prey animals, such as cattle, is complex given the instinctual reaction to conceal pain (Underwood, 2002). The American Veterinary Medical Association (**AVMA**) states that although regulatory obstacles are present, "pain and physiological stress resulting from castration should be minimized to provide for the overall welfare of the animal" (AVMA, 2012).

Other countries have already adopted stronger stances on analgesia associated with common livestock practices. The European Union (**EU**) formed the Treaty on the Functioning of the European Union in 2009. Within Title II, Article 13 of this treaty states, "In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the Member States relating

in particular to religious rites, cultural traditions and regional heritage" (European Union, 2009). States within the EU have the right to implement animal welfare laws beyond those mandated across the entire EU.

The Canadian Veterinary Medical Association (**CVMA**) recommends castrating animals as young as possible, with use of appropriate analgesia and technique appropriate to the situation. The CVMA also encourages development and implementation of analgesic protocols that target both acute and long-term pain associated with castration. In addition, the CVMA states that "regardless of castration technique chosen and age of the patient, all ruminants benefit from the use of systematic analgesia and/or a local anesthetic" (CVMA, 2012).

Pain Pathway

Pain is defined as "an adverse feeling or sensation associated with actual or potential tissue damage resulting in physiological, neuroendocrine, and behavioral changes that indicate a stress response" (Molony and Kent, 1997). Nociception, or pain perception, occurs through the transduction of chemical signals at the infliction site into electrical energy. The electrical signal transmits via nerve fibers up the spinothalamic tracts where modulation occurs into the dorsal horn (Muir and Woolf, 2001). Pain is consciously perceived when the impulse from the dorsal horn is projected to the brain (Gottschalk and Smith, 2001). The pain initial response is short and localized and relatively proportional. Following this is a prolonged response that is more diffuse and causes hypersensitivity around the initial point of stimulus (Gottschalk and Smith, 2001; Coetzee 2011). Central sensitization, referred to as "wind-up," is the result of central nervous system changes causing hypersensitivity to pain (Kissin, 2000; Gottschalk and Smith, 2001).

Damage to tissue initiates an inflammatory response causing the release of chemicals such as histamine, cyclooxygenase, prostaglandins and cytokines. The presence of these chemicals, in combination with others, cause peripheral sensitization (Woolf and Slater, 2000). Chronic peripheral sensitization induces an influx of neurotransmitters, such as substance P and glutamate, which removes the barrier of Mg^{2+} to N-methyl-D-aspartate receptors which causes increased pain response (Woolf and Slater, 2000).

Based on this understanding of pain perception and response, there are 2 phases of pain associated with painful procedures: direct localized phase and prolonged inflammatory phase caused by tissue damage (Kissin, 2000). In order to be comprehensive, a pain mitigation strategy must target both phases of pain perception.

Pain Mitigation

Local Anesthesia. Local anesthetics are the most commonly used pre-emptive analgesic drug in food animal treatment (Muir et al., 1995). Local anesthetics cause reversible loss of sensitization in a localized area by blocking Na channels of nerve cells, preventing generation and propagation of nerve impulses (Webb and Pablo, 2009). Lidocaine and bupivacaine are a few local anesthetics that have been examined as candidates for use in livestock. Both drugs have advantages and disadvantages. Lidocaine has rapid onset of activity (2 to 5 min) but has a short duration of action (90 min) while bupivacaine offers a long duration of action (5 to 8 h) but has a slower onset of activity (20 to 30 min; Webb and Pablo, 2009). Researchers observed similar reduction in plasma cortisol concentrations using lidocaine and bupivacaine prior to castration of

dairy calves (Boesch et al., 2008). This suggests that despite the longer duration of activity bupivacaine may not offer clinical advantages (Coetzee, 2011).

Nonsteroidal Anti-inflammatory Drugs. Nonsteroidal anti-inflammatory drugs (**NSAIDs**) reduce prostaglandin synthesis by inhibiting cyclooxygenase (**COX**) in both peripheral tissues and the central nervous system, producing analgesic and anti-inflammatory effects (Ochroch et al., 2003). There are 2 isoforms of COX. Type I cyclooxygenase induces the production of prostaglandins necessary for normal physiological function. Because of this function non-specific COX-inhibitors may increase risk of gastrointestinal and renal damage (Coetzee, 2011). Type 2 cyclooxygenases are constitutively expressed in the central nervous system and when targeted by NSAIDs may be an important mechanism in preventing pain sensitization (Ochroch et al., 2003). Some common NSAIDs include: carprofen, flunixin meglumine, ketoprofen, meloxicam, phenylbutazone and salicylic acid derivatives (Coetzee, 2011).

The administration of NSAIDs prior to castration can reduce the peak plasma cortisol concentration by an average of 10.8% and the area under the plasma cortisol concentration effect curve by an average of 29%, suggesting NSAID use may be optimized in a multimodal mitigation strategy to better optimize reduction of acute pain (Coetzee, 2011). One drawback to NSAID use is the slow onset of effect requiring a delay between drug administration and start of procedure which may not be practical in production settings (Coetzee, 2011).

Sedative-analgesic Drugs. Opioids, alpha-2 adrenergic agonists and N-methyl D-aspartate (**NMDA**) receptor antagonists are sedative-analgesic compounds commonly used in veterinary medicine. Because of the synergistic action of these compounds, they are often co-administered to optimize effects (Coetzee, 2011).

Opioid analgesics decrease propagation of pain signals by inhibiting voltage-gated calcium channels. Butorphanol is primarily a κ-receptor agonist and partial μ-receptor agonist or antagonist (Coetzee, 2011). Because opioids are designated as Schedule 3 drugs in the U. S. they are regulated by the Drug Enforcement Agency (**DEA**) and can only to be administered by a DEA-licensed veterinarian (Coetzee, 2011). Currently there are no opioid drugs labeled for use in cattle. Thus, there is a zero tolerance level for residues and regulatory withdrawal times have not been established.

Alpha-2 adrenergic agonists produce sedation, chemical restraint and analgesia in cattle by inhibiting positive the feedback mechanism for the release of norepinephrine from nerve endings through reduction of the conductance of Ca (Postner and Burns, 2009). Xylazine is the most commonly used α-2 adrenergic agonist in cattle. Administration at low dose causes sedation without recumbency, while higher doses cause recumbency and some analgesia (Coetzee, 2011). Xylazine, and other α-2 adrengeric agonists, are not currently labeled for use in cattle in the U.S.

N-methyl D-aspartate-receptor antagonists block central sensitization of pain modulation by disrupting the central nervous system (Plumb, 2005). Ketamine is an NMDA-receptor antagonist and produces analgesic and anesthetic effects (Postner and Burns, 2009). Ketamine is also a Schedule 3 drug in the U.S. and can only to be administered by a DEA-licensed veterinarian (Coetzee, 2011).

Multimodal Analgesia. Effective analgesia requires a multimodal analgesic approach (**MMA**) using compounds that act on different receptor targets on the pain perception pathway (Muir and Woof, 2001). A commonly used method of MMA is the combination of an opioid (butorphanol), an α-2 adrenergic agonist (xylazine), and an NMDA-antagonist (ketamine)

(Coetzee, 2011). The goal of an MMA is to maximize pain mitigation while minimizing adverse effects associated with individual drugs.

Extra-label Drug Use. Currently there are no analgesic drugs approved for pain relief in livestock by the FDA (Compendium of Veterinary Products, 2010). The only NSAID labelled for beef and dairy cattle treatment is flunixin meglumine, and it is not labelled for analgesia (Davis et al., 2009).

Any use of drugs for pain relief in cattle constitutes extra-label drug use (**ELDU**; Smith et al., 2008). Under the American Medicinal Drug Use Clarification Act of 1994 (**AMDUCA**; AMDUCA, 1994), ELDU can be used to relieve suffering in cattle given specific conditions are met. Some of these conditions include: supervision by a veterinarian, must be FDA approved animal or human drug, the health of the animal must be threatened, the drug cannot be fed and the use cannot result in violative drug residue in food intended for human consumption (AMDUCA, 1994). There is a zero tolerance level for residues and regulatory withdrawal times have not been established by the FDA.

Pain Assessment and Models

There are 3 main approaches to pain assessment in animals: measures of general body function and productivity, measures of physiological responses and measures of animal behavior (Weary et al., 2006). Each of these methods has advantages and limitations in context to livestock species. For example, measurements of basic productivity, such as DMI and ADG, are often easy to measure but only reflect what is happening to the animal over the observation period and not what has caused changes to the animal. Physiological responses, such as plasma

cortisol concentrations, catecholamines, substance P, and acute phase proteins, such as fibrinogen and haptoglobin, are useful in prey animals that may conceal behavior responses. However, stress associated with handling livestock animals could alter physiological responses, even in the absence of pain (Schwartzkopf et al., 1998). In addition, physiological responses are less useful in a practical, on-farm assessment of pain that could be applied outside of a research setting.

Behavioral observations are the most practical and easily observed indicators of pain include chute behavior, exit velocity, foot stomping and vocalization. The challenge with behavior measurement is determining if the measurement is valid in providing useful information about the pain that the animal is experiencing (Weary et al., 2006). Once a behavior is determined to be a reliable indicator of pain, the remaining challenged is the subjective nature of scoring methods which often limits accuracy and consistency not only between but also within observers (Stookey et al., 1994). It is important to consider the reliability of a measure, or the potential for obtaining the same result when the scoring is repeated (Weary et al., 2006).

In addition to these 3 more traditional measurements, researchers are also looking at neuroendocrine changes through neuropeptide substance P, infrared thermography and electroencephalography to measure the efficacy of analgesia (Coetzee et al., 2011). Further evaluation will determine if these objectively indicate pain. However, like physiological responses, neuroendocrine changes are less practical outside of a research setting.

According to Weary and associates (2006), the gold standard for validation of pain response measurements is an experimental design that allows for examination of responses both with (*P*) and without (*p*) a condition that causes pain, and also both with (*A*) and without (*a*) analgesics thought to treat the pain. This design allows researchers to compare *Pa* and *pa* to

determine if a measured change is associated with a condition that is thought to cause pain or if it simply due to environmental or other unintended factors. If there are environmental factors that cause behavior changes in animals both with and without pain, then conclusions cannot be made based on pain alone.

The use of analgesic treatment, comparing *PA* and *Pa*, allows researchers to determine the effect of analgesia on pain itself. The challenge with some analgesic treatments is that the analgesia itself can have inhibitory, excitatory or sedative effects on the animal and consequently the animal's behavior. However, with the experimental design suggested by Weary et al. (2006) we can compare *pA* and *pa* to determine if the change in behavior can be attributed to the analgesia even in the absence of pain.

When pain studies are conducted on conscious animals they are designated "behavioral studies" since all of the responses (behavioral, physiological, performance) are used to measure the impact of the nociception on the animal. Nociceptive models consist of an "input-output" system where a stimulus is applied and then the output, or reaction, by the animal is measured (Le Bars et al., 2001). Experimental studies of acute pain necessitate appropriate input to provoke the intended "pain" stimuli whether caused through electrical, thermal, mechanical or chemical stimulation.

In order for a behavioral pain model of nociception to be effective it should be characterized by specificity, sensitivity, validity, reliability and reproducibility (Le Bars et al., 2001). Specificity requires the stimulus to be nociceptive. If the stimulus does not cause nociception the model is ineffective. In addition, it must be possible in the model to differentiate responses to the nociceptive stimuli from responses to extraneous stimuli. Sensitivity requires the ability to quantify the response and to correlate the variability to the intensity of the stimulus.

The model needs to be sensitive enough to detect changes, such as pain mitigation interventions, that might reduce the nociceptive response. In order to be valid the model has to allow for differentiation between nonspecific behavioral changes and behavioral changes caused by the stimuli. Reliability entails the consistency of scores when animals are retested with equivalent forms of the test. And lastly, reproducibility requires that the model, when repeated, should yield the same results (Le Bars et al., 2001). With a well-designed model, researchers can measure the effect of the nociceptive stimuli and also measure any decrease in pain by use of mitigation strategies, validating presence of pain as well as efficacy of analgesia.

Physiological Responses

Nociception following a painful procedure activates the sympathetic nervous system releasing chemicals involved in both the inflammatory response and pain sensitization. Presence of these chemicals excites both the heart and respiratory system (Anderson and Muir, 2005). In quantifying pain and distress, previous studies examined the physiological response to castration in order to quantify the effect of painful procedures.

Heart Rate. Although increased heart rate is not necessarily indicative of pain, both pain and increased heart rate can be clinical signs of disease (Anderson and Muir, 2005). Repenning at al. (2103) found that the heart rate of surgical castrates was greater than band castrates on d 1. Increased heart rate could be caused by release of catecholamines in response to the surgical procedure. Catecholamines cause vasodilation as well as alter cardiac output (Stewart et al., 2010). Heart rate variability (**HRV**), or the variation in the intervals between heart beats, has been hypothesized to be a more detailed measurement of stress than heart rate alone (Stewart et

al., 2009). Heart rate variability is used to investigate the autonomic nervous system, specifically the balance between the sympathetic and vagal activity (von Borell et al., 2007). Heart rate variability data are analyzed using frequency domain measures: high frequency (**HF**), low frequency (**LF**) and the ratio of LF:HF (Stewart et al., 2009). Stewart et al. (2010) observed increased HF power from baseline in calves surgically castrated without anesthesia. Calves castrated surgically with local anesthesia exhibited a decrease in LF compared to baseline. The authors concluded that an increase in HF power is indicative of increased parasympathetic activity associated with deep visceral pain (Stewart et al., 2010).

Substance P. Substance P (**SP**) is an 11-amino acid prototypic neuropeptide that regulates excitability of dorsal horn nociceptive neurons and is involved in integration of pain and stress (Coetzee, 2011). In the literature surrounding human medicine, SP was found to be greater in patients suffering from tissue damage than healthy patients (Onuoha and Alpar, 1999). Coetzee et al. (2008) observed mean plasma SP concentrations higher in castrated calves when compared to uncastrated controls. The author indicated that increases in SP concentrations post castration suggest an association with nociception. Repenning et al. (2013) observed no differences in SP between band castrates and control animals. The authors hypothesized that band castration does not elicit the same response in SP concentration as surgical castration.

C*ortisol*. Cortisol is a glucocorticoid secreted in response to a stressful event (Anderson and Muir, 2005). Increased serum cortisol concentration isn't necessarily indicative of pain (Baldridge et al., 2011). However, cortisol measurements have been used frequently throughout past literature since its response magnitude, as indicated by peak response, duration of response and integrated response generally correlates with the predicted pain level of procedures (Mellor

et al., 2000). There are 2 phases of pain associated with surgical procedures: a direct localized phase followed by a prolonged inflammatory phase caused by tissue damage (Kissin, 2000).

Earley and Crowe (2002) observed increased mean plasma cortisol concentration for at least 12 h post-castration in surgical castrates when compared to control animals. In the same study, ketoprofen reduced the negative impact of castration on cortisol response. The area under the cortisol response curve was greater in all castrates regardless of treatment than control animals. Local anesthetic did not reduce the area under the cortisol response curve, but ketoprofen significantly reduced the area under the curve compared with control castrates (Earley and Crowe, 2002).

Acute Phase Proteins. At the site of tissue injury there are numerous responses in both the tissue and its vasculature including platelet aggregation, clot formation and cytokine production. Inflammatory cytokines signal liver to produce and release of acute phase proteins, such as haptoglobin and fibrinogen (Faulkner et al., 1992). Earley and Crowe (2002) found that fibrinogen and haptoglobin were increased in castrated calves when compared to controls following castration.

Behavioral Measurements

Behavior measurements are commonly used in pain assessment for the bovine. Changes in animal behavior following a procedure are thought to indicate pain or discomfort. Although there are many commonly used methods, the challenge with behavior measurement is determining if the measurement provides useful information about the pain that the animal is experiencing (Weary et al., 2006). Once a behavior is determined to be a reliable indicator of

pain, the remaining challenge is the subjective nature of scoring methods. Behavior observations often lack accuracy and consistency (Stookey et al., 1994).

Subjective Chute Score. Chute scores are used to evaluate an animal's resistance behavior to head restraint in a chute. Gruber et al. (2010) used a 15-cm line divided into 5, 3 cm classifications of response (0 = calm, 5 = aggressive). Upon restraint of the animal, observer(s) mark a spot on the line to indicate the animal's temperament. The marked spot is then converted to a number between 0 and 5 giving the animal a measure of disposition. Other researchers have used a 4-point scale (1 = calm, 4 = struggling; Voisinet et al., 2011; Coetzee et al., 2012). Gruber et al. (2010) noted differences in feedlot performance and carcass quality associated with chute score. However, prior to use by Repenning et al. (2013) similar disposition measurements had not been applied to painful procedures, such as castration. The authors did not find differences associated with band castration, but concluded that because the animals were well acclimated to handling by the time of castration they did not exhibit differences during or in the days after castration. The thought process behind use of chute score in pain assessment is that animals subjected to painful procedures would be more resistant to restraint in the handling sessions following the procedure.

Exit Velocity. Chute exit velocity is a measurement of the speed at which an animal leaves the chute after restraint. Exit velocity can be measured using electronic sensors to observe the amount of time it takes for the animal to cover a preset distance. Chute exit velocity has been shown to objectively measure flightiness in *Bos indicus* cattle (Curley et al., 2006). In *Bos taurus* cattle, exit velocity had a negative correlation with ADG (Müller and Von Keyserlingk, 2006). Because exit velocity has been used in temperament and reactivity studies in cattle, it could also be useful in determining the effect of a painful procedure as well as determining the effect of

sedative treatment on animal behavior (Curley et al., 2006; Baldridge et al., 2011). A study examining the effects of sodium salicylate in drinking water co-administered with a sub-anesthetic ketamine-stun showed that Holstein bull calves that did not receive pain mitigation at surgical castration exhibited greater exit velocity than those that received a ketamine stun or co-administered ketamine stun and sodium salicylate. However, results were attributed to the sedative effect of ketamine stun (Baldridge et al., 2011). Repenning et al. (2013) looked at exit velocity in conjunction with both band and surgical castration and found no differences at the time of castration, even with use of ketamine stun. The authors suggested the difference between their results and previous literature was attributed to differences in age and breed of bulls.

Vertical Head Movement. Head movement and velocity has been measured in response to different methods of branding (Schwartzkopf-Genswein et al., 1998). The authors noted that animals that underwent hot-iron branding exhibited greater vertical head movement range and velocity when compared to freeze-branding and exhibited even less vertical head movement when subjected to sham-branding. Based on that study, head movement range increased with the likely severity of pain. Repenning et al. (2013) observed vertical range of head movement during band castration of weanling bulls (n = 19). The authors found that castration, regardless of meloxicam administration, caused greater head movement than sham castration. They concluded that head movement was indicative of pain response (Repenning et al., 2013).

Video Documentation and Observation. Post-procedure video documentation has been used in a number of studies to assess painful procedure behavioral changes. Pen level documentation post-castration of cattle that underwent band castration, with or without a xylazine epidural and flunixin meglumine, revealed no difference in lying behavior of cattle at various time points; however, castrates did display decreased step length compared to animals

who received pain mitigation (Gonzalez et al., 2010). A similar study noted increased step length post castration in surgically castrated animals with flunixin meglumine administration compared to non-medicated castrates that exhibited decreased step-length (Currah, 2009). In the same study observations using pedometers indicated that surgically castrated calves took significantly fewer steps compared to before the procedure. Fisher et al. (2001) found that bulls castrated surgically swished their tails, stomped their feet and grazed less following castration than both control bulls and band castrated bulls. Repenning et al. (2013) observed greater percentage lateral recumbence in castrates when compared to control bulls indicating a behavioral impact of castration.

Pressure Algometry. Algometers are used to quantitatively assess mechanical pain by application of pressure. The mechanical nociceptive threshold (**MNT**) is defined as the amount of pressure necessary to evoke a behavioral response indicative of pain (Haussler et al., 2007). Mechanical nociceptive thresholds can be used to measure pain as well as to assess the efficacy of analgesic interventions (Stubsjoen et al., 2010). Pressure algometry has been applied to cattle in association with dehorning, lesion classification, as well as claw pain and locomotion (Whay et al., 1998 and Dyer et al., 2007; Heinrich et al., 2010). Tapper et al. (2013) used pressure algometry in a study using induced transient lameness in sows (n = 12) to assess analgesic drugs for mitigation of lameness pain. Researchers found that the sows tolerated less pressure on the limb with induced lameness than their sound limb. However, there were no differences in mechanical nociceptive threshold found between control animals and those who received analgesics. Researchers noted that this study contributes to a growing body of literature across livestock species supporting the use of pressure algometry in pain assessment (Tapper et al., 2013).

Feeding Behavior and Performance

Average Daily Gain. Average daily gain is often measured in animals undergoing painful procedures to indicate pain induced changes in normal body function and general well-being. Average daily gain plays an important role in the economic impact of painful procedures as well as the potential incentive for implementing pain mitigation. However, production parameters, such as gain, are often too variable to reflect the pain of animals following painful procedures (Stafford and Mellor, 2005). In addition, decreased gains following castration may be influenced by the decrease in testosterone caused by removal of the testes (King et al., 1991). Most studies comparing recently castrated animals to intact bulls and cattle castrated much earlier in life revealed that castration, regardless of pain mitigation strategy, negatively impacts ADG following castration (Faulkner et al., 1992; Fisher et al., 2001; Gonzalez et al., 2010). Despite the imprecise nature of ADG measurements, assessment of production parameters is critical in keeping research relevant to livestock producers (Coetzee, 2011).

Because there are many potential analgesics and anesthetic drugs, as well as combinations of these that could potentially be used for pain mitigation following painful procedures, there is a large body of literature looking at the effects on ADG with varying results. Baldridge et al. (2011) found that although castration and dehorning had significant impact on ADG among all treatment groups, calves treated with sodium salicylate and sodium salicylate in combination with ketamine-stun had significantly higher ADG for the 13 d following castration than those who received only ketamine-stun or no pain mitigation. Coetzee et al. (2012) looked at the differences in health and performance of steers relative to bulls surgically castrated upon arrival at a feedlot. Bulls castrated upon arrival exhibited lower ADG than steers for the initial 14

d; however, by 28 d there was no differences detected. Treatment with meloxicam had no effect on ADG. Similar to this study, Repenning et al. (2013) found no impact of oral meloxicam on ADG in band castrated bulls, despite an adverse impact on ADG by castration when compared to intact bulls.

Dry Matter Intake. Along with estimates of ADG, dry matter intake (**DMI**) can be used to look at how much time the animal is spending eating/grazing. Painful procedures and administration of pain mitigation have both been shown to have an impact of DMI, although the level of impact varies. Gonzalez et al. (2010) used a radio frequency identification (**RFID**)-linked bunk system, allowing for individual animal intake measurements to be observed despite a group setting. The authors reported that feed intake in band castrates, when compared to steers castrated much earlier, was less in the fourth wk post-castration. However, the authors reported cattle receiving pain mitigation had reduced DMI compared to non-medicated cattle.

Using a similar RFID-linked bunk system, Repenning et al. (2013) reported that banded castrates exhibited greater DMI than surgical castrates 1 d post-castration; however, DMI did not differ for the following 26 d of observation. Castrates (regardless of method) receiving multimodal analgesia (**MMA**), consisting of ketamine-stun and lidocaine block of spermatic cords, had greater DMI on d 1, 2, and 3 post-castration. Multimodal analgesia treatments resulted in greater DMI during the 28-d post-castration feeding period. These results differ from the previous literature by Gonzalez et al. (2010), which may be attributed to the sedation strategy as well as reduction in butorphanol dosage in the ket-stun.

Feed Behavior. Individual feed intake data acquired via RFID-linked feed bunks has made evaluation of each individual animal's feeding behavior readily available without the need for video or manual documentation. Gonzalez et al. (2010) reported that bunk visit frequency

was greater in control cattle not receiving pain mitigation than control cattle receiving pain mitigation and cattle that were band-castrated with medication. Control cattle did not differ from cattle banded without pain mitigation. Meal duration did not differ across treatments of castration or pain mitigation; however, meal size was greater in control cattle than all other treatments (Gonzalez et al., 2010). Repenning et al. (2013) reported that meal duration was greater in band castrates than surgical castrates during the first wk post-castration. Because RFID-linked feed bunks have emerged relatively recently there is a need for more research on the changes in eating behavior associated with painful procedures.

Justification for Research

Currently there are no FDA-approved drugs labeled for the treatment of pain in cattle (Compendium of Veterinary Products, 2010) and yet there is growing interest by consumers about the humane raising of food animals (Rollins, 2004). Because some indispensable production practices are thought to cause pain to livestock, it is only a matter of time before consumers demand pain intervention. However, according to the FDA "validated methods of pain assessment must be used in order for a drug to be indicated for pain relief in target species" (FDA-CVM, 2009). Despite abundant literature on behavioral, performance and physiological effects of painful procedures there are no validated methods of pain assessment or mitigation for livestock species. Continued research on the effects of analgesia during common animal husbandry practices, such as castration and dehorning, are integral in finding an effective pain mitigation strategy to ease consumer concern. In addition, development of pain models that can

be applied to livestock to validate the efficacy of analgesia are crucial to providing data to get proper analgesia approved by the FDA.

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CHAPTER II

Effect of ketamine-stun and meloxicam on behavior and performance associated with band castration in cull beef bulls

Our objectives were to evaluate the effects of ketamine-stun (**KET**) and oral meloxicam (**MEL**) at the time of band castration on performance and behavioral response of Angus bulls immediately post-weaning. Angus bulls (n = 119, BW 291.3 ± 29.1 kg) were blocked by BW in a complete randomized design via a 2 × 2 factorial arrangement with 1 additional treatment group remaining intact. Bulls to be castrated on d 0 were randomly assigned to one of 4 treatments: 1) meloxicam with no ketamine-stun (**MEL**), 2) meloxicam and ketamine-stun (**MEL+KET**), 3) ketamine-stun with no meloxicam (**KET**), or 4) no meloxicam or ketamine-stun (**CON**). Meloxicam was administered on d 0, 7, and 14 (3 mg/kg) via oral bolus. Ketamine stun consisted of butorphanol (0.0125 mg/kg), xylazine (0.025 mg/kg), and ketamine (0.050 mg/kg) and was administered 10 min before band application via a single subcutaneous injection. Animals not receiving KET or MEL received a subcutaneous injection of saline or an empty bolus, respectively. Castration was performed by banding using a Calicrate bander. Bulls that remained intact were subjected to sham manipulation of the scrotum associated with castration on d 0, but without band application. Subjective chute score (**CS**) was collected on d -7, 1, 7, 14, 21, and 28. Objective exit velocity (**EV**) was collected on d -7, 0, 1, 7, 14, 21, and 28. Blood samples taken on d 0, 1, 7, 14, 21 and 28 were analyzed for plasma cortisol concentration. Range of vertical head motion (**DIST**) during castration or sham was used as a behavioral pain indicator during castration. Video analysis was conducted blind to treatment group and procedure

following castration. Animals were observed at 3 min intervals for 15 min immediately post castration or sham to evaluate behavior response by trained evaluators blind to treatments. Performance measurements analyzed over the 28-d period included ADG, DMI, and G:F. Chute scores were greater in castrated animals ($P \leq 0.02$) on d 14 and 28. There was a tendency ($P = 0.10$) towards the effect of KET to reduce EV on d of castration. Vertical head movement tended to be greater ($P = 0.06$) in castrated animals than sham bulls. There was an interaction ($P = 0.001$) among main effects for DIST. The CON group had greater ($P < 0.01$) DIST than all other treatments. Mean percent lying down immediately post-castration was greater ($P < 0.001$) for castrates than bulls, and a there was a main effect of KET ($P < 0.001$) resulting in increased lying behavior. Bulls exhibited greater G:F ($P = 0.02$) and ADG ($P < 0.001$) than castrates. There was no effect of KET or MEL on G:F ($P \geq 0.12$) or ADG ($P \geq 0.13$). Plasma cortisol concentrations were greater ($P < 0.001$) in castrates than intact bulls throughout the observation period. In conclusion, castration resulted in more head motion, less favorable chute scores and increased plasma cortisol concentrations. Further, post-castration behavior and exit velocity were altered due to castration and use of KET. Data also suggest that neither KET nor MEL alter feed performance among bulls castrated by banding.

Key Words: Animal welfare, Beef bulls, Castration, Ketamine, Meloxicam

Introduction

Castration of bull calves is a common livestock management practice in the U.S. with approximately 17 million calves castrated annually (USDA, 2009). The American Veterinary Medical Association (**AVMA**) states that although regulatory obstacles are present, “pain and

physiological stress resulting from castration should be minimized to provide for the overall welfare of the animal" (AVMA, 2012). In addition there has been increased consumer concern about the well-being and ethical treatment of animals (Rollins, 2004). The author also reported that consequently, public perception of pain associated with castration has been increasingly negative. There has been amplified pressure to develop practices to mitigate pain and suffering.

One of the confounding issues associated with pain mitigation is that there are currently no analgesic drugs approved for pain relief in livestock by the U.S. Food and Drug Administration (**FDA**; Smith, 2013). According to the FDA, "validated methods of pain assessment must be used in order for a drug to be indicated for pain relief in target species" (FDA-CVM, 2009). However, validated methods of pain assessment are extremely limited in the scientific literature. Pain assessment in prey animals, such as cattle, is complex given the instinctual reaction to conceal pain (Underwood, 2002).

The hypothesis of the current study was that treatment with sub-anesthetic ketamine-stun (**KET**) and nonsteroidal anti-inflammatory drug (**NSAID)** meloxicam (**MEL**) would decrease the negative impact of castration on behavior and resulting performance of beef cull bulls. The objective of this study was to evaluate the effects of KET and oral MEL at the time of band castration on performance and behavioral response of Angus bulls immediately post-weaning.

Materials and Methods

Animals. This project was approved by the Institutional Animal Care and Use Committee at Colorado State University. One hundred and nineteen recently-weaned Angus bulls (BW 291.3 ± 29.1 kg) from a seedstock operation were used for the study. Thirty five bulls remained intact and were randomly assigned to 1 of 4 pens (**BULL**). The remaining 84 bulls to be castrated were

blocked by BW (heavy and light) and randomly assigned in a 2 × 2 factorial design to 1 of 4 treatments: 1) MEL, 2) MEL+KET, 3) KET, or 4) no analgesia/anesthesia (**CON**). Each treatment consisted of 4 pens with 5 or 6 animals per pen.

Data Collection. All animals were fed a total mixed ration. Feed was delivered once daily at 0800. Refusals were recorded daily by a trained bunk reader, to supply ad libitum feed. Orts were collected weekly and weighed in order to calculate DMI. Orts were then composited and a representative sample was dried for 48 h in a forced air oven at 60ºC to determine percent DM. Nutrient analysis of orts was conducted by a commercial laboratory (SDK Laboratories, Hutchinson, KS). Performance measurements analyzed over the 28-d period included ADG, DMI and G:F. Body weight was collected on d -7, 0, 1, 7, 14, 21 and 28.

Procedures. Bulls were band castrated d 0 (Calicrate Bander, No Bull Enterprises, St. Francis, KS). Bulls that remained intact were subjected to sham manipulation of the scrotum associated with castration, but without band application. Band castrations were completed by securing a latex band around of the neck of the scrotum to cause necrosis of the scrotum and testis using a Calicrate Bander (No Bull Enterprises, St. Francis, KS).

Castrates receiving MEL were administered 3.0 mg/kg on d 0, 7, and 14, respectively, by rounding to the nearest tablet (Meloxicam 15 mg, Zydus Pharmaceuticals, Pennington, NJ). Tablets were then encapsulated in a porcine gelatin bolus (Torpac Inc., Fairfield, NJ) and administered via a stainless steel balling gun. All cattle not receiving MEL were given a placebo of an empty gelatin capsule. All researchers were blind to treatment group except for the person administering the bolus.

Castrates receiving KET were administered a single subcutaneous injection consisting of butorphanol (0.0125 mg/kg), xylazine (0.025 mg/kg), and ketamine (0.050 mg/kg)

approximately 10 min prior to band application. Animals not receiving KET received a subcutaneous injection of saline. Researchers were blinded to the injection being administered.

Behavioral Measurements. Upon restraint in the chute, a single evaluator assigned each animal a subjective chute score (**CS**) by marking a 15-cm long line scale as described by Gruber et al. (2010). Chute scores were recorded on d -7, 1, 7, 14, 21 and 28 and the observer was blinded to treatments. Chute scoring did not occur on the d of band application. Marks were converted to values on a 0 to 5 scale (0 = calm and 5 = aggressive) where values were indicative of the animal's response to restraint (Gruber et al., 2010).

Objective exit velocity (**EV**) was collected on d -7, 0, 1, 7, 14, 21 and 28 using an infrared laser timing system (Farmtek Inc., Wylie, TX). Sensors were set up at 1.892 m beyond the head catch and 1.892 m beyond the first set of sensors. Exit velocity was calculated from the time elapsed between the 2 sets of sensors and reported in m/s.

Video documentation at the time of castration or sham was collected for each animal. Video data were analyzed using video analysis software (Dartfish INC., Alpharetta, GA). This was a modified version of collection as noted by Schwarzkopf-Genswein et al. (1998), in which they examined the effect of hot-iron, freeze, and sham branding on head movement and velocity. The current experiment examined the maximum vertical range of head movement (**DIST**) during the procedure as noted by differences in highest and lowest points of the nose in the video frame. In addition, animals were observed by 2 trained observers blinded to the study at 3 min intervals for 15 min immediately post band application or sham to evaluate behavior response (**OBS**). At each 3 min time point, observers classified each animal's current behavior as either standing or lying.

Blood Collection. Jugular blood samples were collected using 18 gauge 3.81 cm sterile needles and a syringe on d 0, 1, 7, 14, 21 and 28 for serum cortisol concentration. Blood was transferred immediately into a sterile 10 mL vacutainer tube and placed on ice for transport and processing. Cortisol concentrations were averaged across pen based on the cortisol concentration present in the serum sample. Samples were analyzed by competitive chemiluminescent immunoassay for cortisol (Coetzee et al., 2007).

Jugular blood samples were collected using 18 gauge 3.81 cm sterile needles and a syringe on d 0, 1, 7, and 14 for substance P (**SP**) concentration. Blood samples were injected into 10 mL EDTA vacutainer tubes, which were prepared with a benzamidine. Blood samples were immediately centrifuged at 1,700 x *g* at 4ºC chute-side and plasma was immediately placed on ice until transported to -80ºC freezer.

SP assay was performed as described in Liu et al., (2008) with slight modifications using non-extracted plasma. Validation of utilizing non-extracted plasma was based on results of excellent recovery percentage following addition of a known standard concentration (Van Engen et al., 2013). Samples were analyzed in duplicate with a double antibody radioimmune assay using a primary antibody (polyclonal rabbit anti-Substance P) (1:20,000; Phoenix Pharmaceutical, Inc, Burlingame, CA). EDTA (13mM) and benzamidine (1 mM) were added as protease inhibitors. SP was assayed using ^{125}I-[Tyr^{8}]-Substance P tracer (approximately 18000 cpm; PerkinElmer, Inc, Waltham, MA) and the least detectable concentration was 2.5 pg/ml. The intra- and inter-assay coefficients of variation were 15% and 29%, respectively.

Statistical Analyses. Pen was the experimental unit for all data analyses. The alpha level was set at $P = 0.05$. Average daily gain, G:F, DMI, DIST, CS, EV and cortisol were analyzed via SAS (PROC MIXED, SAS Institute, Cary, NC) using two-way analysis of variance with factors

being ketamine and meloxicam). Consistent with our objectives, data were analyzed in a 2 × 2 + 1 factorial design to determine the effect of castration and the main effects of MEL and KET using single degree of freedom contrasts. For CS and EV, d -7 was used as a covariate and for cortisol and SP concentration d 0 was used as a covariate. If an interaction among the main effects (MEL and KET) was present ($P < 0.10$), the 4 castrate treatment means were compared.

Results and Discussion

Video analysis at the time of castration or sham indicated that castrates tended ($P = 0.06$, Table 2.1) to have greater DIST than bulls. Among castrates, there was an interaction ($P = 0.001$) between main effects for DIST. As a result, means for the 4 castrate treatments were compared individually, and the CON group had greater ($P < 0.01$) DIST than all other treatments, other treatments means did not differ ($P > 0.10$). Head movement associated with castration revealed that animals being castrated had greater resistance behavior to constraint than sham castration.

Although the effect of KET on head movement was expected due to the sub-anesthetic effects of ketamine-stun, it is inexplicable why MEL administered immediately before band application affected head movement. A previous study noted differences in DIST across method of branding (hot-iron, freeze, and sham) with hot-iron branding eliciting the greatest DIST response (Schwartzkopf-Genswein et al., 1998). Though the current study examined castration, we can assume based on the previous study that DIST provides an indication of pain response.

In the current study, overall CS did not differ ($P = 0.21$, Table 2.1) when castrates were pooled and compared to BULL, and there were no main effects ($P > 0.14$) of KET or MEL. When d was included in the model, castrates exhibited higher CS ($P = 0.01$) on d 14 than BULL; however, on d 28 castrates exhibited lower CS ($P = 0.02$) than BULL.

Subjective CS, as used in the current study, has been most widely applied to studies of performance in beef cattle as a measure of disposition. Gruber et al. (2010) noted differences in feedlot performance and carcass characteristics associated with CS. Repenning et al. (2013) looked at subjective CS in association with castration but found no differences between castrates and controls. However, the authors concluded that this could be attributed to the minimal impact of band castration or that animals were well acclimated to handling by the time of castration.

In the current study, differences between castrates and bulls could indicate increased resistance to restraint by animals following painful procedures following castration. As defined by Molony and Kent (1997) pain is "an adverse feeling or sensation associated with the actual or potential tissue damage resulting in physiological, neuroendocrine, and behavioral changes that indicate a stress response." Following a painful procedure, animals could associate restraint with potential infliction of pain resulting in behavioral changes observed during handling. However, the opposite effect of castration on CS 4 wk after band application could possibly be attributed to decreased testosterone levels in castrates compared to intact bulls.

Overall EV did not differ ($P = 0.38$, Table 2.1) when castrates were pooled and compared to BULL and there were no main effects ($P > 0.65$) of KET or MEL. There were no effects of castration on EV observed throughout the observation period, although there was a tendency ($P = 0.10$) for BULL to have greater EV than pooled castrates on d 28. On the d of band application (d 0) there was a tendency for a main effect ($P = 0.10$) of KET to reduce EV.

The observed tendency for decreased EV on d 0 in animals receiving KET is most likely attributed to the effects of the combination of xylazine, butorphanol and ketamine. Baldridge et al. (2011) found that ketamine-stun reduced EV on the d of castration in dairy bull calves. The authors noted no other changes in EV following castration and dehorning (Baldridge et al.,

2011). This is consistent with the current study as there was a decreased EV observed from animals receiving KET.

There was no overall effect ($P = 0.81$, Table 2.2) of castration on SP concentration. There were no overall main effects of KET or MEL ($P > 0.19$) on SP concentration. When d was included in the model, KET reduced ($P = 0.05$) SP concentration on d 14.

Substance P is a neuropeptide that has been used to evaluate soft tissue injury and the efficacy of analgesics in human study (Coetzee et al., 2008). In the current study SP was not different between intact bulls and castrates. Repenning et al. (2013) also found no difference associated with band castration and suggested that band castration may not elicit a marked response in SP concentration as surgical castration does. In addition the 24 h interval of testing may be too long to capture the peak in SP concentration (Repenning et al., 2013). In the current study the low concentration of SP could be attributed to the methods of sample handling during and after collection (Mosher et al., 2014). The main effect of KET reducing SP concentration on d 14 is inexplicable.

There was an overall effect of castration ($P < 0.0001$, Table 2.2) to increase serum cortisol concentration. When d was included in the model, pooled castrates exhibited greater ($P < 0.001$) serum cortisol concentration than BULL on d 7, 21, and 28. Overall and on d 7, there was a main effect of KET ($P = 0.04$) to reduce serum cortisol concentration.

Cortisol is a glucocorticoid secreted in response to a stressful event (Anderson and Muir, 2005). Increased serum cortisol concentration isn't necessarily indicative of pain, since it can also be affected by stress (Baldridge et al., 2011). Earley and Crowe (2002) observed increased plasma cortisol concentration for at least 12 h following surgical castration compared to control animals. In the current study, castrates exhibited greater blood cortisol concentrations than intact

bulls for the duration of the study despite similar handling to all animals in the study. The duration of cortisol elevation could be attributed to the length of time it takes for necrosis of the scrotum compared to surgical castration.

Coetzee et al. (2010) and Baldridge et al. (2011) both observed lower serum cortisol concentrations in animals treated with ketamine-stun immediately after castration; however, the effect on serum cortisol was temporary and coincides with the length of duration of sedative effects. The time of onset is 5 to 10 min with 60 to 90 min duration (Coetzee, 2011). However, in the current study a main effect of KET on serum cortisol levels was observed overall and on d 7. This long duration of effect is likely attributed to the method of castration. Application of the band likely caused acute pain, followed by a prolonged inflammatory response as the tissue of the scrotum and testis became necrotic and sloughed off. Mitigation of acute pain associated with the tightening of the band, reduced the impact of central sensitization or "wind-up" which causes hypersensitivity to pain (Gottschalk and Smith, 2001). If this hypersensitivity was mitigated, even slightly, it could decrease the longer term serum cortisol concentrations.

Mean percent of animals lying down during the 15-min period immediately post-castration was greater ($P < 0.0001$, Table 2.3) for pooled castrates than bulls. Mean percent of animals lying was greater ($P < 0.0001$) for KET vs. no KET overall across time points. There was no ($P > 0.54$) effect of MEL at any time point or overall on post castration behavior. None of the sham bulls were observed lying down during the observation period.

Post-castration observations indicated KET increased the percent of cattle that lied down immediately after castration. A study using accelerometers to evaluate standing and lying behavior of cattle both before and after surgical castration found that calves spent significantly more time standing post-surgical castration (White, 2008). Although the method of castration

was different, data reported by the authors suggests that KET altered standing behavior. The results from the current study were also observed in a study using accelerometers in conjunction with ketamine-stun during surgical castration. Treatment in that study increased lying behavior immediately following castration (Pauly, 2012). Increased lying behavior could also be attributed to the potential sedative effect of the sub-anesthetic combination of xylazine, ketamine, and butorphanol, although it is intended to provide sedation without recumbency in cattle (Coetzee, 2011). Additionally, previous work indicated that more calves treated with ketamine and xylazine displayed unchanged attitude following castration compared to non-treated controls (Coetzee, 2010).

Dry matter intake did not differ ($P > 0.34$, Table 2.4) between bulls and castrates. There was no interaction ($P = 0.71$) between main effects on DMI. There were no effects of KET ($P = 0.55$) or MEL ($P = 0.38$) on DMI. Bulls exhibited greater ADG ($P < 0.001$, Table 2.4) and G:F ($P = 0.02$) than castrates. There was no interaction ($P \geq 0.60$) between main effects and no effect of KET or MEL on G:F ($P \geq 0.12$) or ADG ($P \geq 0.14$).

Average daily gain and G:F of castrates, regardless of treatment, was less than for bulls. However, DMI among castrates did not differ ($P = 0.34$) from the bulls. This clearly indicates castration negatively impacted feed efficiency and gain for the 28-d span after band application. These results could be based on the anabolic effects of testosterone in the intact bulls when compared to castrates since use of analgesia and sub-anesthetic did not mitigate negative effects in the current study. Results of a previous study that investigated oral MEL in association with surgical castration suggest that MEL impacted some aspects of morbidity, but no behavioral or feedlot performance parameters were different when compared to other castrates (Coetzee et al., 2011).

In the current study meloxicam dosage was administered at 3.0 mg/kg orally. This is an increased dosage intended to allow for prolonged effect of the anti-inflammatory effects. With a typical 0.5 mg/kg dosage the half-life is approximately 27 h (19.97 - 43.29 h range; Coetzee, 2011). Because meloxicam was administered in 7 d increments, the concentration in the blood would likely be immeasurable by the time of re-administration. Blood meloxicam concentrations were intended to be measured in order to determine the efficacy of higher dosage; however, laboratory availability limited the analysis of blood samples.

Implications

Based on behavior and feedlot performance response variables, castration resulted in more head motion, increased chute scores and increased plasma cortisol concentrations. Further, post-castration behavior and exit velocity were altered due to castration and use of KET. Data also suggest that neither KET nor MEL alter feed performance among bulls castrated by banding. Overall limited conclusions can be drawn about the effects of meloxicam and ketamine-stun on reducing negative impacts resulting from band castration in cattle of this age and body weight. Further investigation into the use of pain mitigation with castration on performance and behavior is necessary in order to address increasing demand by the public for humane production of beef. Increased knowledge of pain mitigation in livestock will be necessary to validate the usage of pain mitigation drugs in the future.

Table 2.1: Least square means (± SEM) for vertical head movement (DIST), chute score (CS), and exit velocity (EV) in response to castration and main effects of subcutaneous ketamine-stun and oral meloxicam after band castration or sham in weaned beef bulls

	Treatment[1]						Contrast, *P* =			
Item	BULL	CON	KET	MEL	MEL+KET	SEM	CAST[2]	INT[3]	KET	MEL
DIST[6], m	0.31[a]	0.60[b]	0.39[a]	0.28[a]	0.38[a]	0.062	0.06	0.001	0.12	0.01
CS[7]										
d 1	1.58	1.57	1.36	1.50	1.46	0.145	0.50	0.57	0.40	0.93
d 7	2.28	2.63	2.44	2.34	2.40	0.174	0.39	0.48	0.72	0.36
d 14	2.02	2.40	2.51	2.41	2.20	0.119	0.01	0.20	0.71	0.24
d 21	2.11	2.31	2.26	2.47	2.15	0.132	0.19	0.34	0.19	0.82
d 28	2.66	2.34	2.45	2.59	2.41	0.093	0.02	0.14	0.72	0.29
Overall	2.13	2.25	2.20	2.26	2.12	0.057	0.21	0.44	0.13	0.57
EV[8], m/s										
d 0	2.65	2.48	1.90	2.71	2.41	0.248	0.28	0.61	0.10	0.28
d 1	2.51	2.46	2.67	2.84	2.87	0.226	0.42	0.71	0.62	0.22
d 7	2.46	2.51	2.75	2.53	2.87	0.180	0.31	0.82	0.13	0.72
d 14	2.54	2.70	2.87	2.74	2.46	0.152	0.29	0.17	0.72	0.24
d 21	2.69	2.86	2.83	2.79	2.70	0.141	0.45	0.87	0.68	0.49
d 28	2.59	3.11	2.71	2.75	2.84	0.163	0.10	0.16	0.36	0.51
Overall	2.57	2.69	2.64	2.73	2.71	0.120	0.38	0.92	0.74	0.65

[1]BULL = sham procedure on d 0, CON = band on d 0, KET = band on d 0 with subcutaneous ket-stun, MEL = band on d 0 with oral meloxicam on d 0, 7, and 14, MEL+KET = band on d 0 with subcutaneous ket-stun and oral meloxicam on d 0, 7, and 14.
[2]CAST = Contrast between pooled CON, KET, MEL, MEL+KET means and BULL mean.
[3]INT = Interaction of main effects (KET and MEL).
[4]KET = Contrast between KET and non-KET treatments.
[5]MEL = Contrast between MEL and non-MEL treatments.
[6]Head movement at the time of castration was determined using video analyzing software in which the greatest difference in nose position longitudinally was measure during band castration or sham castration.
[7]Chute score was subjectively determined upon head restraint on a 15-cm line so that 0 = calm and 5 = aggressive (Gruber et al., 2010).
[8]Chute EV was collected as the velocity exhibited from 1.892 m to 3.784 m beyond the head catch using an electronic infrared barrier system.
[a,b]Means without common superscripts differ ($P \leq 0.05$).

Table 2.2: Least square means (± SEM) for substance P (SP) and plasma cortisol in response to castration and main effects of subcutaneous ket-stun and oral meloxicam after band castration or sham in weaned beef bulls

	Treatment[1]						Contrast, *P* =			
Item	BULL	CON	KET	MEL	MEL+KET	SEM	CAST[2]	INT[3]	KET	MEL
SP[6], pg/mL										
d 1	7.52	7.50	8.42	8.46	8.24	0.45	0.14	0.22	0.46	0.42
d 7	8.24	9.19	8.06	9.85	6.95	1.19	0.84	0.46	0.11	0.86
d 14	8.85	9.53	6.31	8.86	6.71	1.24	0.47	0.67	0.05	0.92
Overall	8.10	8.58	7.95	9.14	7.51	0.81	0.83	0.54	0.19	0.94
Cortisol[7], ng/mL										
d 1	25.4	31.3	27.8	28.2	28.1	2.1	0.13	0.44	0.40	0.51
d 7	21.2	32.7	26.6	32.4	28.7	2.1	0.001	0.59	0.04	0.69
d 14	30.0	38.9	35.2	39.4	35.1	3.3	0.11	0.93	0.29	0.95
d 21	27.3	42.6	38.7	40.3	38.5	2.5	<0.001	0.69	0.28	0.63
d 28	24.0	44.5	37.0	36.0	35.4	3.1	0.001	0.29	0.22	0.13
Overall	25.6	38.2	33.0	35.3	33.3	1.5	<0.0001	0.30	0.04	0.43

[1]BULL = sham procedure on d 0, CON = band on d 0, KET = band on d 0 with subcutaneous ket-stun, MEL = band on d 0 with oral meloxicam, MEL+KET = band on d 0 with subcutaneous ket-stun and oral meloxicam.
[2]CAST = Contrast between pooled CON, KET, MEL, MEL+KET means and BULL mean.
[3]INT = Interaction of main effects (KET and MEL).
[4]KET = Contrast between KET and non-KET treatments.
[5]MEL = Contrast between MEL and non-MEL treatments.
[6]SP concentrations were measured from blood via jugular venipuncture.
[7]Serum cortisol concentrations were measured from blood via jugular venipuncture.

Table 2.3: Least square means (± SEM) for percent of pen lying down at 3 min intervals for a 15-min period post castration and main effects of subcutaneous ketamine-stun and oral meloxicam after band castration or sham in weaned beef bulls

	Treatment [1]						Contrast, *P* =			
Time	BULL	CON	KET	MEL	MEL+KET	SEM	CAST[2]	INT[3]	KET[4]	MEL[5]
0 min	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-
3 min	0.0	0.0	30.0	0.0	28.3	13.6	0.35	0.95	0.05	0.95
6 min	0.0	0.0	50.0	10.0	43.3	10.7	0.05	0.45	0.002	0.88
9 min	0.0	4.2	60.0	10.0	48.3	12.4	0.04	0.49	0.003	0.82
12 min	0.0	5.2	76.2	11.1	62.7	9.6	0.02	0.30	<0.001	0.68
15 min	0.0	13.3	80.0	15.0	71.7	8.8	0.004	0.58	<0.001	0.58
Overall	0.8	4.4	50.1	8.3	40.2	5.4	<0.0001	0.17	<0.0001	0.54

[1]BULL = sham procedure on d 0, CON = band on d 0, KET = band on d 0 with subcutaneous ket-stun, MEL = band on d 0 with oral meloxicam on d 0, 7, and 14, MEL+KET = band on d 0 with subcutaneous ket-stun and oral meloxicam on d 0, 7, and 14.
[2]CAST = Contrast between pooled CON, KET, MEL, MEL+KET means and BULL mean.
[3]INT = Interaction of main effects (KET and MEL).
[4]KET = Contrast between KET and non-KET treatments.
[5]MEL = Contrast between MEL and non-MEL treatments.

Table 2.4: Least square means (± SEM) for ADG, DMI, G:F, initial BW and final BW in response to castration and main effects of subcutaneous ketamine-stun and oral meloxicam after band castration or sham in weaned beef bulls

	Treatment[1]						Contrast, *P* =			
Item	BULL	CON	KET	MEL	KET+MEL	SEM	CAST[2]	INT[3]	KET[4]	MEL[5]
ADG, kg	1.60	1.14	1.28	1.17	1.27	0.10	<0.001	0.80	0.14	0.93
DMI, kg	4.96	4.69	4.44	4.82	4.76	0.38	0.34	0.71	0.55	0.38
G:F	0.14	0.06	0.10	0.08	0.11	0.02	0.02	0.60	0.12	0.53
d -7 BW, kg	312.1	288.3	283.0	281.5	279.0	6.12	<0.001	0.81	0.51	0.36
d 28 BW, kg	338.4	294.6	291.3	291.1	296.3	7.72	<0.001	0.44	0.86	0.86

[1]BULL = sham procedure on d 0, CON = band on d 0, KET = band on d 0 with subcutaneous ket-stun, MEL = band on d 0 with oral meloxicam on d 0, 7, and 14, MEL+KET = band on d 0 with subcutaneous ket-stun and oral meloxicam on d 0, 7, and 14.
[2]CAST = Contrast between pooled CON, KET, MEL, MEL+KET means and BULL mean.
[3]INT = Interaction of main effects (KET and MEL).
[4]KET = Contrast between KET and non-KET treatments.
[5]MEL = Contrast between MEL and non-MEL treatments.

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CHAPTER III

Evaluation of a model demonstrating mitigation of nociceptive response to oxytetracycline injection site inflammation by flunixin meglumine in dairy cows

Two experiments were used to evaluate if pain (nociception) associated with oxytetracycline (**OT**) i.m. injection site inflammatory can be objectively measured using a pressure algometer. The second objective was to determine if flunixin meglumine (**FM**) can mitigate pain (nociception) associated with OT injection site inflammation, as measured objectively by a pressure algometer. In Exp. 1, non-lactating cull Jersey cows (n = 5) and in Exp. 2 non-lactating cull Jersey and Holstein cows (n = 10) were randomly assigned to 1 of 2 treatments: 1) flunixin meglumine 2.2 mg/kg (**FM**), or 2) equivalent volume 0.9% saline (**SALINE**). Both treatments were administered via i.v. at 24 h intervals. Researchers were blinded to all treatments. At 0 h animals were administered an i.m. injection of **OT** (LA-200, 200 mg/ml) at 5 mg/kg on one site in the neck and one site in the hind leg (Exp.1) or just the hind leg (Exp. 2) to induce an inflammatory response and stimulate acute pain. In both experiments mechanical nociception threshold (**MNT**) was measured at the OT site and the same location on the opposite neck/leg (**NON-OT**) using a pressure algometer. Pressure was applied to the site until the animal exhibited a conscious and visible reaction to the pressure. Pressure readings were taken in random order in triplicate and an average of the readings was used. In Exp. 2, blood samples were obtained every 24 h via jugular venipuncture to measure fibrinogen content. In Exp. 1, there were no differences ($P > 0.05$) in MNT between the OT site and the non-OT site in the neck, and there were no differences ($P > 0.05$) in MNT between treatments (FM vs. SALINE) at the OT site in the neck.

In both experiments there were differences ($P \leq 0.05$) in MNT observed between OT and non-OT sites in the leg starting on d 0 throughout the rest of the observation period. In Exp. 1 there were differences ($P \leq 0.05$) in MNT between treatments (FM vs. SALINE) at the OT site starting on d 3 through the rest of the observation period. In Exp. 2 there were also differences ($P \leq 0.05$) in MNT between treatments (FM vs. SALINE) at the OT site starting on d 3 through the rest of the observation period, with tendencies ($P \leq 0.10$) on d 5 and 8. Mean fibrinogen content in animals treated with FM was lower ($P \leq 0.05$) than CON animals at multiple time points. Data suggest OT induced an inflammatory reaction and FM was effective in reducing the pain sensitization associated with injection site inflammation, which was validated by the algometer readings.

Key Words: Algometer, Dairy cattle, Flunixin meglumine, Mechanical nociceptive threshold, Pain

Introduction

Interest in the ethical treatment of food animals and humane livestock practices has been increasingly important to consumers (Rollins, 2004). However, one challenge associated with pain mitigation is that currently there are no analgesic drugs approved for pain relief in livestock by the U.S. Food and Drug Administration (**FDA**; Smith, 2013). According to the FDA, "validated methods of pain assessment must be used in order for a drug to be indicated for pain relief in target species" (FDA-CVM, 2009). The ability to quantitatively assess pain in livestock is an important aspect in improving animal welfare (Stubsjoen et al., 2010). Because of the variable nature of pain associated with both chronic and acute pain, pain induction models are

thought to be a more robust approach for validating both pain measurement and mitigation efficacy (Tapper et al., 2013).

When pain studies are conducted on conscious animals they are designated "behavioral studies" since all of the responses (behavioral, physiological, performance) are used to measure the impact of nociception on the animal (Le Bars et al., 2001). Nociceptive models consist of an "input-output" system where a stimulus is applied and then the output, or reaction, by the animal is measured (Le Bars et al., 2001). Experimental studies of acute pain necessitate appropriate stimuli to provoke the intended "pain" stimuli whether caused through electrical, thermal, mechanical or chemical stimulation. In order for a behavioral pain model of nociception to be effective it should be characterized by specificity, sensitivity, validity, reliability and reproducibility (Le Bars et al., 2001).

Algometers have been used to quantitatively assess mechanical pain by application of pressure. The mechanical nociceptive threshold (**MNT**) is defined as the amount of pressure necessary to evoke a behavioral response indicative of pain (Haussler et al., 2007). Mechanical nociceptive thresholds can be used to measure pain as well as to assess the efficacy of analgesic interventions (Stubsjoen et al., 2010). In cattle pressure algometry has been most commonly used in lameness studies investigating lesions on feet and claws (Whay et al., 1998; Dyer et al., 2007; Schulz et al., 2011).

Flunixin meglumine (**FM**) is the only non-steroidal anti-inflammatory drug (**NSAID**) approved by the FDA for use in cattle (Davis et al., 2009). However, FM is only labeled for treatment of pyrexia associated with bovine respiratory disease, endotoxemia, and acute mastitis as well as associated inflammation (Davis et al., 2009). Validated data proving the efficacy of FM, or any NSAID, for treatment of pain is needed for approval by the FDA.

The objectives of this study were: 1) to evaluate if pain nociception associated with an oxytetracycline (**OT;** Liquamycin LA-200, Zoetis Inc., Kalamazoo, MI) i.m. injection site inflammatory response can be objectively measured and 2) if FM can mitigate nociception associated with OT injection site inflammation.

Materials and Methods

The protocol for this experiment was approved by the Colorado State University Institutional Animal Care and Use Committee

Exp. 1

Animals. Five culled non-lactating adult Jersey cows were acquired and delivered to the Colorado State Veterinary Teaching Hospital (**CSU VTH**). Animals were housed together in a dry lot and fed free choice grass hay and *ad libitum* water.

Experimental Design. Animals were restrained in a working facility at h -1, and a 3 x 3 cm area was clipped at 4 locations (Figure 3.1): left rear leg (site 1), right rear leg (site 2), left neck (site 3), and right neck (site 4). Animals were randomly assigned to 1 of 2 pain mitigation treatment groups and were administered either 2.2 mg/kg of FM (FM, n = 3) or equivalent volume of 0.9% saline (SALINE, n = 2) i.v. starting at h 0 (Table 3.1) and again every 24 h for a total of 5 administrations. Researchers were blinded to treatment. Starting at h 0, algometer pressure readings were taken in triplicate, in random order on each site. The pressure algometer (FPK, Wagner Instruments, Greenwich, CT) had a maximum pressure capacity of 13.61 kilograms of force (**kgf**) in increments on 1 kgf. Pressure was applied until the animal responded to the

pressure. Nociceptive responses included a kick, lifting a leg, or other movement to avoid the pressure. If there was no response at the maximum pressure, then 13.61 kgf was recorded.

After the initial algometer reading, animals were administered OT (200 mg/ml) at 5 mg/kg in the left rear leg (site 1, OT site) and right front neck (site 4, OT site) via a single i.m. injection to induce an inflammatory response. A sham procedure was performed on both of the non-OT sites (sites 2 and 3). Algometer readings were taken in triplicate in random order at each site at 1, 6, and 24 h after the initial OT injection. For the following 4 d, algometer pressure evaluations were performed at 1, 6, and 24 h after each IV injection of FM or saline. After each 24 h reading, the animals were re-administered the designated IV treatment for a total of 5 IV injections, 24 h apart (at 0, 24, 48, 72, and 96 h).

Experiment 2

Animals. Five culled non-lactating adult Jersey cows and 5 culled non-lactating adult Holstein cows were housed at the CSU VTH. Animals were housed together in a dry lot and fed free choice grass hay and *ad libitum* water.

Experimental Design. Animals were restrained in a working facility at 0 h and a 3 x 3 cm area was clipped at 2 locations (Figure 3.1): left rear leg (site 1), and right rear leg (site 2). The sites were located at the division between the caudal aspect of the semimembranosus and semitendinosus muscle bellies. Algometer (FPX, Wagner Instruments, Greenwich, CT) pressure readings were was taken as described in Exp. 1. Pressure was applied until the animal responded to the pressure. The pressure algometer had the capacity to measure up to 45.36 kgf in increments on 0.01 kgf, and maximum pressure was never reached. Animals were randomly assigned to 1 of 2 treatment groups. Animals were administered either 2.2 mg/kg of FM (n = 5) or equivalent volume of 0.9% saline (n = 5) IV starting at 0 h and re-administered every 24 h for

a total of 10 administrations. The observation and treatment period was extended from the 5 d in Exp. 1 to 10 d in Exp. 2 to observe the effects of both pain stimulus and analgesia for a longer period of time. Researchers were blinded to treatment. Starting at 0 h, blood samples were obtained via jugular venipuncture using a 16 g x 3.81 cm needle and 20 mL sterile syringe every 24 h for a total of 11 d. Samples were analyzed for fibrinogen content as an indicator of systemic inflammatory response.

After the initial algometer reading, animals were administered OT LA-200, 200 mg/ml) at 5 mg/kg administered randomly in either the left or right rear leg (by drawing, OT site) via single i.m. injection. A sham injection was performed in the opposite leg (non-OT site). The person assigned to collect algometer readings was blind to which site received OT injection. Algometer readings and blood collection followed by administration of pain mitigation treatment (FM or saline) were performed at 24 h intervals from h 0 for a total of 10 d. An additional algometer reading was taken daily 6 h after administration of pain mitigation treatment.

Fibrinogen Assay

Venous blood is collected via jugular venipuncture and anticoagulated with sequestrene (EDTA) and drawn into two microhaematocrit capillary tubes. These are sealed at one end and spun in a Hawksley microhaematocrit centrifuge (12,000 x g) for 5 min (Dintenfass and Kammer, 1976). One of each sample is then quantified using a refractometer to determine total protein. The other tubes are placed for 3 min in a water bath at 56°C, care being taken to ensure that the plasma columns are entirely under the water surface. The plasma becomes opaque due to precipitation of the fibrinogen which is then packed on top of the buffy coat by centrifugation. These samples are then spun in a Hawksley microhaematocrit centrifuge (12,000 x g) for 5 min

and then quantified using a refractometer. The second refractometer reading is subtracted from the total protein concentration and multiplied by 1,000 to determine fibrinogen concentration.

Statistical Analyses

Raw MNT means were obtained by averaging the triplicate pressure readings. The raw MNT mean for each time point and each animal was then used in the analyses. Animal was the experimental unit for analyses and the alpha level was set at 0.05. Differences were analyzed using PROC Mixed (SAS Institute, Cary, NC) in an analysis of variance model. Models initially included treatment, site, treatment x site, d, BCS, breed (Exp. 2 only), and breed x site (Exp. 2 only) as fixed effects and animal as a random effect. Readings taken on d 0 were used initially as a covariate; however, the covariate was not significant ($P = 0.41$) and removed from the model along with BCS. The model was analyzed as repeated measures, and although there was an effect of d ($P = 0.02$) there were no d x site ($P = 0.93$), d x treatment ($P = 0.39$), or d x site x treatment ($P = 0.66$) interactions. Because the main effects of the model did not change dependent on d, comparisons were made for each d rather than across all time periods.

Results

Exp. 1

There were no differences ($P > 0.05$; Figure 3.2) on any d for MNT observed between OT and non-OT sites in the neck of animals receiving saline. There were no differences ($P > 0.26$; Figure 3.3) in MNT at the OT site in the neck region for animals that received FM compared to animals that received saline at any time point. During algometer testing in the neck

region, the maximum amount of pressure the algometer was capable of measuring (13.61 kgf) was reached in 15.6% of data points.

There were no differences ($P = 0.23$) in MNT on d 0 in the leg. There were differences ($P \leq 0.05$; Figure 3.4) in MNT observed between the OT and non-OT sites in the leg starting on d 1 throughout the rest of the observation period in animals receiving saline. There were no differences ($P \geq 0.14$; Figure 3.5) in MNT at the OT site for animals that received FM compared to saline on d 0, 1, and 2. There were differences ($P \leq 0.05$) in MNT at the OT site for animals that received FM compared to saline on d 3, 4, and 5. During algometer testing on the leg region, the maximum amount of pressure the algometer was capable of measuring (13.61 kgf) was reached in 20.3% of data points.

Exp. 2

There were no differences ($P \geq 0.45$) in MNT on d 0. There were differences ($P \leq 0.05$; Figure 3.6) in MNT observed between OT and non-OT sites in animals receiving saline starting on d 1 throughout the rest of the observation period. Mechanical nociceptive threshold was greater ($P \leq 0.05$; Figure 3.7) at the OT site for animals that received FM compared to animals receiving saline on d 3, 4, 6, 7, and 9. There were tendencies ($P = 0.06$) on d 5 and 8 for greater MNT at the OT site in animals that received FM compared to animals receiving saline. The maximum amount of pressure the algometer was capable of reading (45.36 kgf) was never reached during pressure readings. On d 10 there was no difference ($P = 0.14$) between treatments at the OT site for animals that received FM compared to animals receiving saline.

Fibrinogen content was measured as an indicator of systemic inflammatory response. Mean serum fibrinogen concentration in animals receiving FM compared to control animals was lower ($P \leq 0.05$; Figure 3.8) on d 5 and 6 after an inflammatory pain response was initiated on d

0. There was no difference ($P = 0.45$) in mean fibrinogen content prior to administration of OT or treatment with FM.

Discussion

Oxytetracycline is a broad-spectrum antibiotic labelled for treatment of pneumonia, shipping fever, keratoconjunctivitis, foot-rot, bacterial enteritis, wooden tongue, leptospirosis, wound infections and acute metritis in beef cattle and non-lactating dairy cattle (FDA, 1997). Subcutaneous and i.m. injection is widely known to cause injection site inflammation and swelling as well as injection site lesions persisting past 28 d after injection (FDA, 1997). Use of OT as a chemical stimulus of inflammation and pain was chosen in the current model to cause an acute inflammatory response to test the efficacy of both the algometer and flunixin meglumine.

In order for a behavioral pain model of nociception to be effective, it should be characterized by specificity, sensitivity, validity, reliability and reproducibility (Le Bars et al., 2001). Specificity requires the stimulus to be nociceptive. If the stimulus does not cause nociception, the model is ineffective. In addition, it must be possible in the model to differentiate responses to the nociceptive stimuli from responses to extraneous stimuli. Sensitivity requires the ability to quantify the response and to correlate the variability to the intensity of the stimulus. The model needs to be sensitive enough to detect changes, such as pain mitigation interventions, that might reduce the nociceptive response. In order to be valid, the model has to allow for differentiation between nonspecific behavioral changes and behavioral changes caused by the stimuli. Reliability entails the consistency of scores when animals are retested with equivalent forms of the test. And lastly, reproducibility requires that the model, when repeated, should yield

the same results (Le Bars et al., 2001). With a well-designed model, researchers can measure the effect of the nociceptive stimuli and also measure any decrease in pain by use of mitigation strategies, validating the presence of pain as well as the efficacy of analgesia.

Results from Exp. 1 indicate that when using this model, the neck was not an effective area for testing mechanical threshold to pain since there were no differences observed between the OT and non-OT sites. As explained by Weary et al. (2006), it is crucial to have a response change associated with a condition assumed to cause pain in order to measure the efficacy of mitigation. Because the animal is more aware of the presence of the algometer and researcher applying pressure in the neck region, there may not be a reaction indicative of MNT and therefore this location is not an effective model for evaluating pain mitigation. Pain assessment in prey animals, such as cattle, is complex given the instinctual reaction to conceal pain (Underwood, 2002). The results are likely attributed to the inherent nature of the animals to resist head and neck manipulations. Stubsjoen et al. (2010) also observed similar fear-induced behavioral inhibition of response in ewes when using an algometer to test MNT.

There were no differences observed in MNT at the OT site in the neck between animals treated with FM and saline. Again, this is likely attributed to the nature of prey animals. Because the neck was observed to be an ineffective location to objectively indicate that something was painful in Exp. 1, it was not used in the Exp. 2.

In order to evaluate the hypothesis that nociceptive response to OT injection site inflammation can be objectively measured using an algometer, comparison between OT and non-OT site mean response was evaluated in both experiments. Prior to OT injection (0 h), both sites were measured for MNT and were not different ($P \geq 0.23$). Difference observed after OT injection between the 2 sites validates the use of OT as a chemical stimulus to elicit an acute pain

response. In addition to causing differences in response measurements, the stimulus must also be predictable and repeatable (Le Bars et al., 2001). By repeating the initial experiment using the same pain stimulus in Exp. 2, there is evidence of both repeatability as well as predictability of OT as a chemical stimulus of inflammatory response and local tissue pain. Further research is needed to determine the reproducibility of the model.

The second hypothesis of this study was that OT injection site inflammation could be mitigated by treatment with FM and objectively measured using a pressure algometer. Because a difference was detected between the OT and non-OT pressure thresholds at the leg site, it is also possible to look at whether treatment with an analgesic, such as FM, could decrease the sensitivity to mechanical stimulus. An increase in MNT indicated a reduction in pain associated with the pain stimulus site (Slingsby et al., 2001).

In both experiments there were differences detected in MNT to mechanical stimulation between saline and FM treatment when tested at the OT site in the leg. Based on results observed in the current study, FM effectively reduced sensitivity to mechanical pressure when compared to cattle not receiving FM. Lack of consistency at consecutive time points can likely be attributed to varying pain tolerance in the cows, as well as varying temperament of animals to the presence of the examiner with the algometer. Additionally, the relatively small number of animals in both experiments could contribute to decreased power to overcome the inherent variation between animal responses.

Variation accounted for in the model included a breed effect ($P = 0.05$) between Holstein and Jersey cows. There was a breed x site interaction ($P < 0.0001$; Figure 3.9), indicating that between the breeds there was a difference in the effect of the pain stimulus or a difference in sensitivity to pain. Jersey cows had a lower nociceptive threshold ($P = 0.01$) at the non-OT site

than Holstein cows. There were no differences in the nociceptive threshold ($P = 0.20$) between Jersey and Holstein cows at the OT site.

Animal was included as a random effect in the model to account for variability across cows. Stubsjoen et al. (2010) also found animal variability across MNT in a study using ewes to assess MNT in sheep. In the current study, difference in the physical size of the animals was accounted for in the dosage of both OT as well as treatment dosage (FM or saline). In addition, the effect of BCS was not significant ($P > 0.53$) when included in the model and was removed. Remaining variation is most likely due to temperament, pain sensitivity, and general nature of the cows towards the handlers.

At the site of tissue injury, there are numerous responses in both the tissue and its vasculature including plate aggregation, clot formation and cytokine production. This inflammation results in production and release of acute phase proteins, such as haptoglobin and fibrinogen from the liver (Faulkner et al., 1992). Animals treated with FM exhibited decreased fibrinogen content for a period of time in the middle of the experiment, suggesting that FM was effective at decreasing local inflammation and the cytokine signals that induce an acute phase response caused by the OT injection.

Mechanical nociceptive threshold determined using pressure algometry should continue to be evaluated because of its ability to objectively measure nociception. Used in conjunction with induced pain models, it offers a valid, reliable and repeatable model for validation of pain mitigation.

Table 3.1: Schedule of induction of pain using oxytetracycline (**OT**), algometer pressure measurements, and administration of treatment[4] (flunixin meglumine or saline) in cull dairy cows for evaluation of pain mitigation, in 2 experiments.

Exp. 1

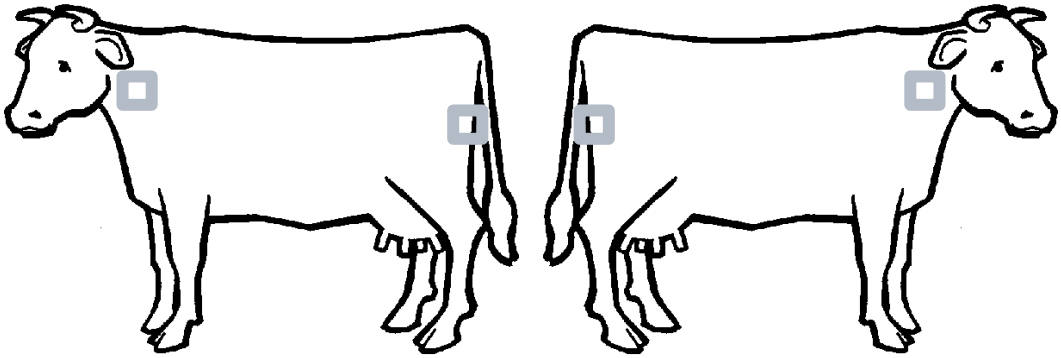
Day	Time (hours)	Procedure
0	0	Algometer reading & trmt administration OT injection[1]
	1	Algometer reading
	6	Algometer reading
1	24	Algometer reading & trmt administration
	25	Algometer reading
	30	Algometer reading
2	48	Algometer reading & trmt administration
	49	Algometer reading
	54	Algometer reading
3	72	Algometer reading & trmt administration
	73	Algometer reading
	78	Algometer reading
4	96	Algometer reading & trmt administration
	97	Algometer reading
	102	Algometer reading
5	120	Algometer reading

Exp. 2

Day	Time (hours)	Procedure
0	0	Algometer reading, blood sample[3] & treatment administration OT injection[2]
	6	Algometer reading
1	24	Algometer reading, blood sample & trmt administration
	30	Algometer reading
2	48	Algometer reading, blood sample & trmt administration
	54	Algometer reading
3	72	Algometer reading, blood sample & trmt administration
	78	Algometer reading
4	96	Algometer reading, blood sample & trmt administration
	102	Algometer reading
5	120	Algometer reading, blood sample & trmt administration
	126	Algometer reading
6	144	Algometer reading, blood sample & trmt administration
	150	Algometer reading
7	168	Algometer reading, blood sample & trmt administration
	174	Algometer reading
8	192	Algometer reading, blood sample & trmt administration
	198	Algometer reading
9	216	Algometer reading, blood sample & trmt administration
	222	Algometer reading
10	240	Algometer reading & blood sample

[1]Animals were administered OT (200 mg/ml) at 5 mg/kg i.m. in the left rear leg (site 1) and right front neck (site 4). A sham injection was performed in the opposite leg and neck.
[2]Animals were administered OT (200 mg/ml) at 5 mg/kg i.m. in either the left or right rear leg, randomly chosen. A sham injection was performed in the opposite leg.
[3]Blood samples were obtained via jugular venipuncture to measure fibrinogen content.
[4]Treatments: 1) 2.2 mg/kg flunixin meglumine, or 2) equivalent volume 0.9% saline.

Exp. 1:



Exp. 2:

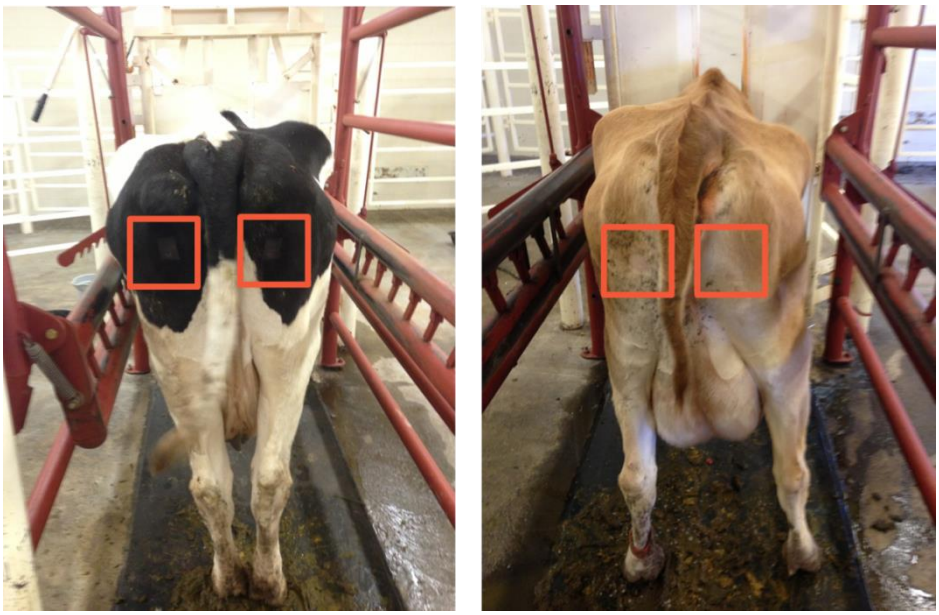


Figure 3.1: Location of mechanical nociceptive threshold readings for Exp. 1[1] and 2[2]. [1]Animals were administered oxytetracycline (200 mg/ml) at 10 mg/kg i.m. in the left rear leg (site 1) and right front neck (site 4). A sham injection was performed in the opposite leg and neck. [2]Animals were administered oxytetracycline (200 mg/ml) at 10 mg/kg i.m. in either the left or right rear leg, randomly chosen. A sham injection was performed in the opposite leg.

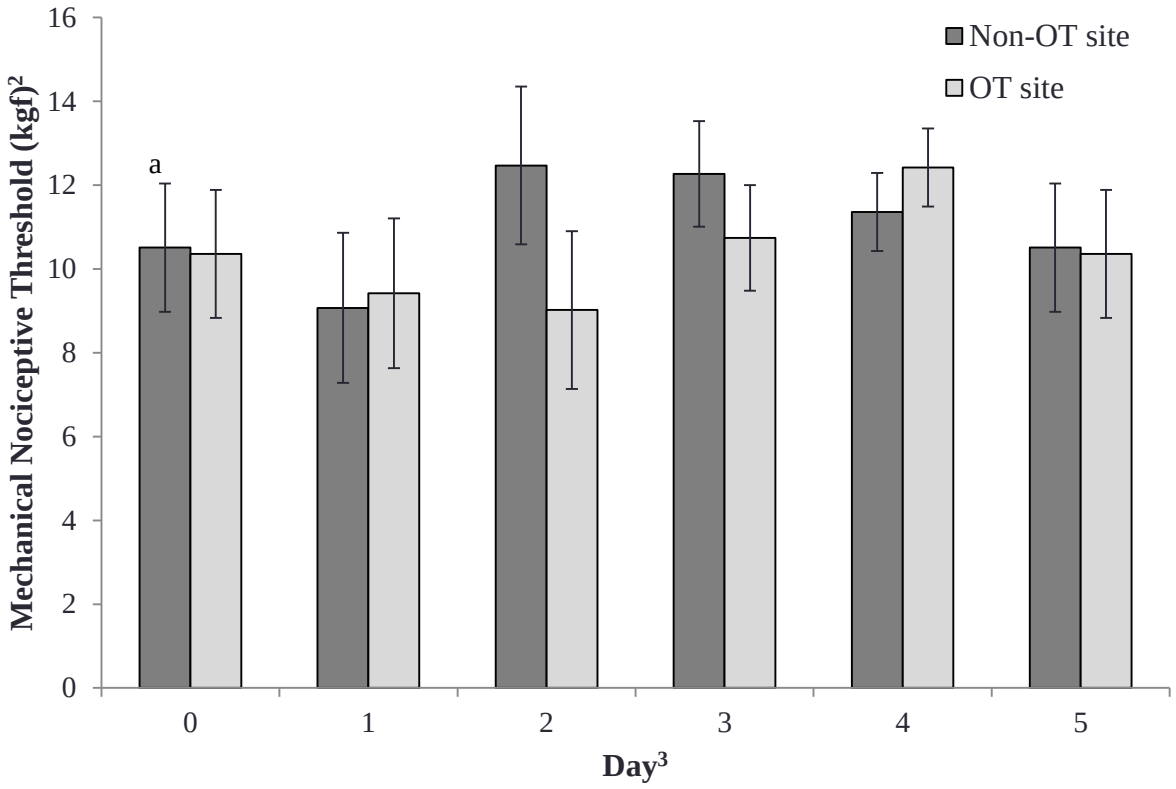


Figure 3.2: Mean response threshold (kgf) ± SEM to mechanical stimulation using pressure algometry after the i.m. injection of 5 mg/kg oxytetracycline (OT)[1] in the neck region of cull dairy cows receiving saline (n = 2, Exp. 1). [1]Animals were administered OT (200 mg/ml) at 5 mg/kg i.m. in the right neck. A sham injection was performed in the opposite left neck. [2]Pressure algometer capable of measuring up to 13.61 kgf. [3]Each d consisted of measurements taken at 0, 1, and 6 h, except d 0 which was only the measurement taken prior to OT administration. [a]No differences ($P > 0.05$) were observed.

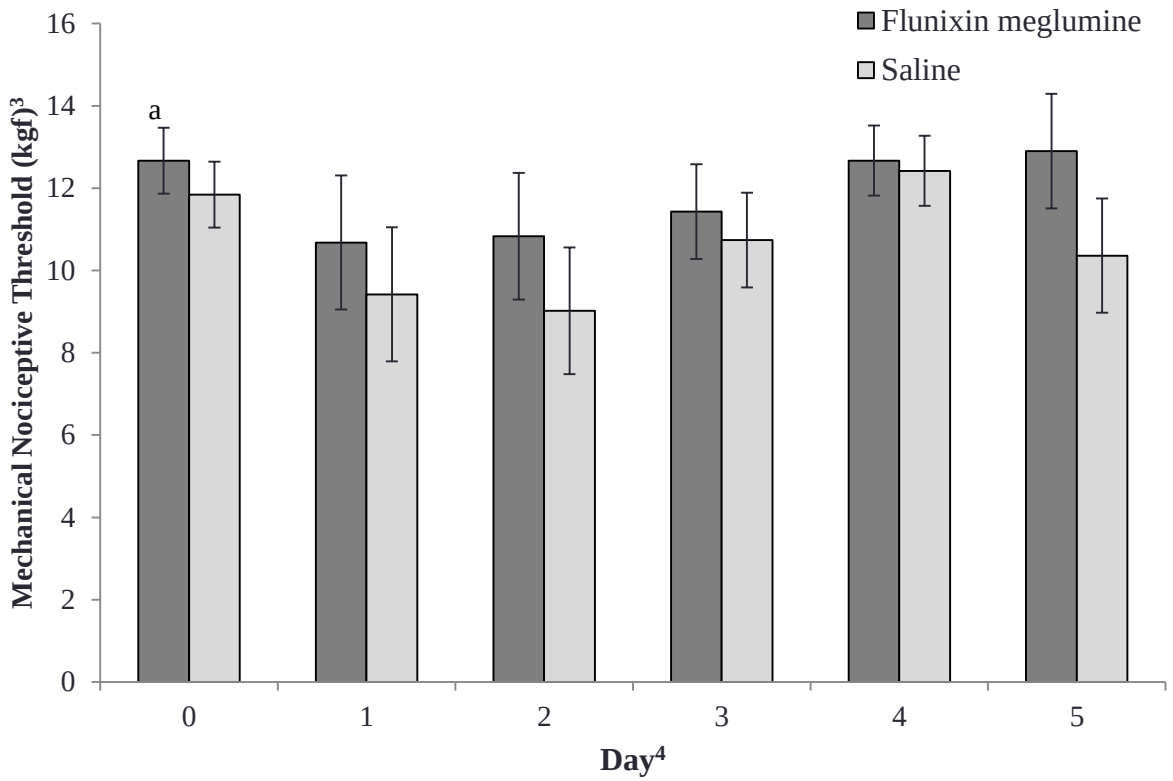


Figure 3.3: Mean response threshold (kgf) ± SEM to mechanical stimulation using pressure algometry after the i.m.injection of 5 mg/kg oxytetracycline (OT)[1] in 2 treatment[2] groups in the neck region of cull dairy cows (n = 5; Exp. 1). [1]Animals were administered OT (200 mg/ml) at 5 mg/kg i.m. in the right neck. [2]Treatments: 1) 2.2 mg/kg flunixin meglumine, or 2) equivalent volume 0.9% saline. [3]Pressure algometer capable of measuring up to 13.61 kgf. [4]Each d consisted of measurements taken at 0, 1, and 6 h, except d 0 which was only the measurement taken prior to OT administration. [a]No differences ($P > 0.05$) were observed.

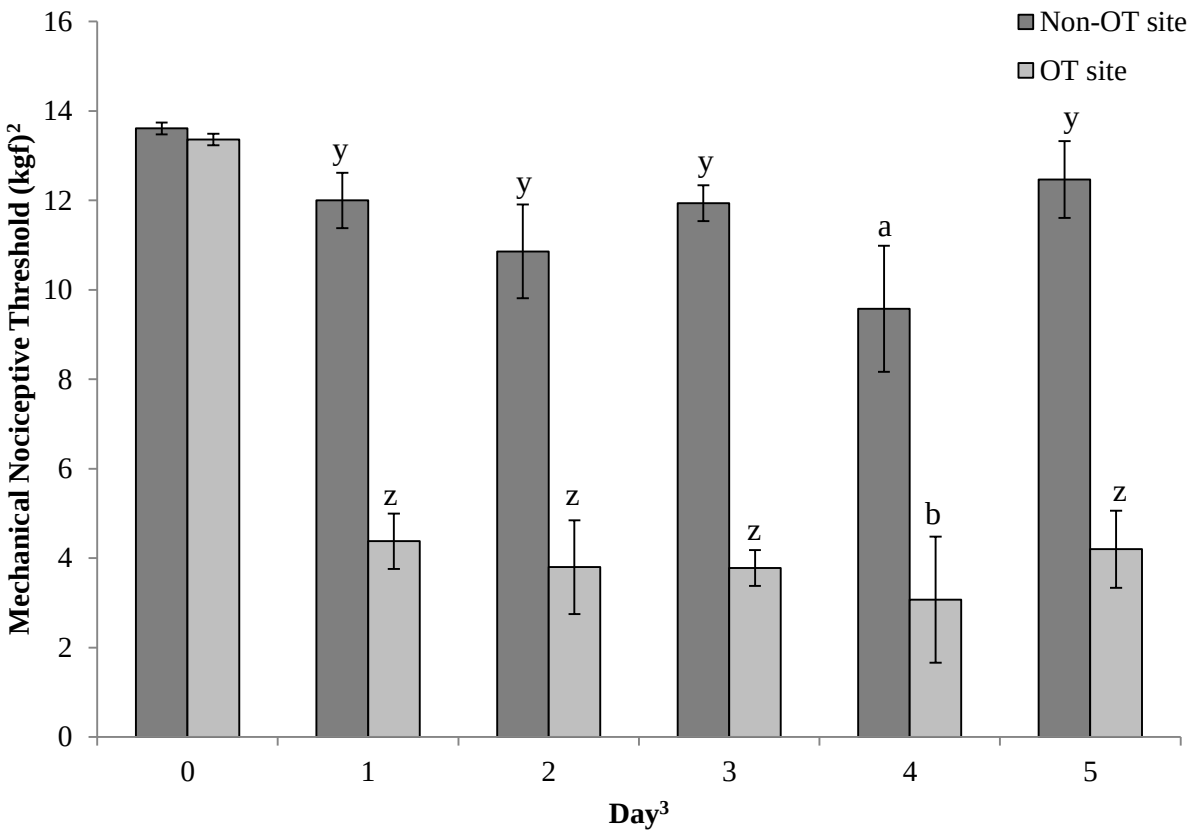


Figure 3.4: Mean response threshold (kgf) ± SEM to mechanical stimulation using pressure algometry after the i.m. injection of 5 mg/kg oxytetracycline (OT)[1], in the hind leg of cull dairy cows receiving saline (n = 3, Exp. 1). [1]Animals were administered OT (200 mg/ml) at 5 mg/kg i.m. in the left rear leg. A sham injection was performed in the opposite leg. [2]Pressure algometer capable of measuring up to 13.61 kgf. [3]Each d consisted of measurements taken at 0, 1, and 6 h, except d 0 which was only the measurement taken prior to OT administration. [a,b]Within d, means without common superscripts differ ($P \leq 0.05$). [y,z]Within d, means without common superscripts differ ($P \leq 0.01$).

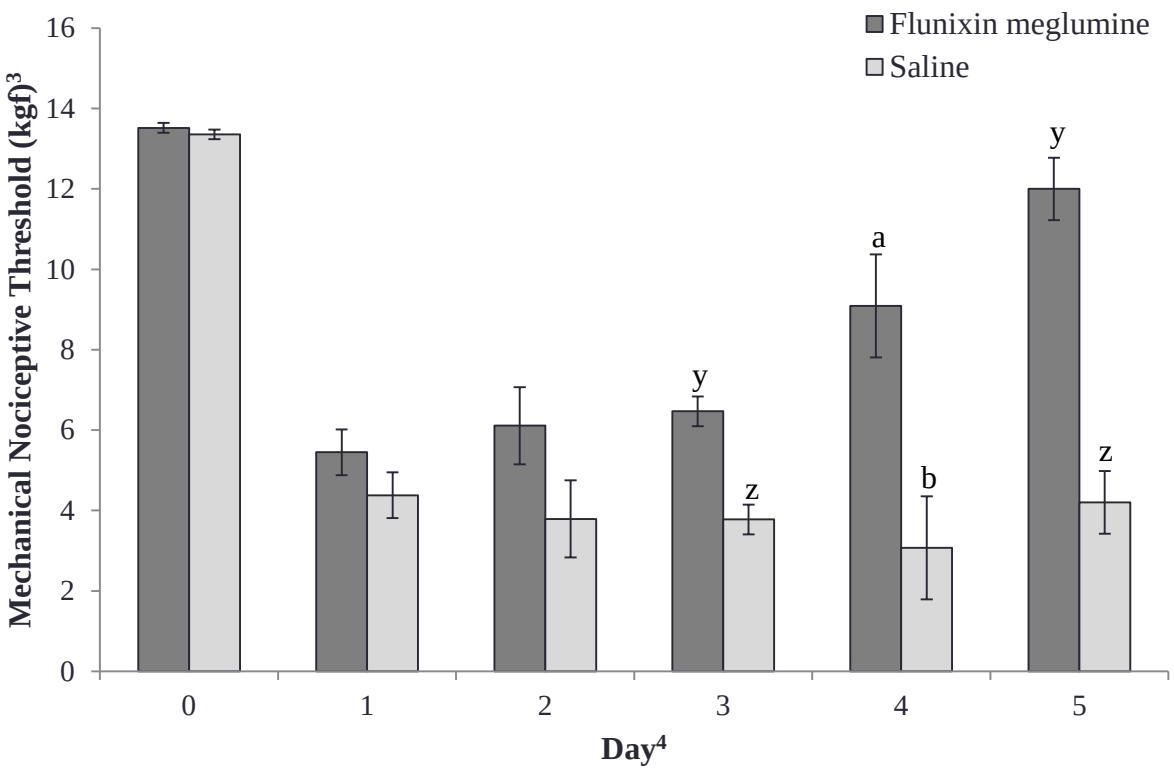


Figure 3.5: Mean response threshold (kgf) ± SEM to mechanical stimulation using pressure algometry after the i.m. injection of 5 mg/kg oxytetracycline (OT)[1] in 2 treatment[2] groups in the hind leg of cull dairy cows (n = 5; Exp. 1). [1]Animals were administered OT (200 mg/ml) at 5 mg/kg i.m. in the left rear leg. [2]Treatments: 1) 2.2 mg/kg flunixin meglumine, or 2) equivalent volume 0.9% saline. [3]Pressure algometer capable of measuring up to 13.61 kgf. [4]Each d consisted of measurements taken at 0, 1, and 6 h, except d 0 which was only the measurement taken prior to OT administration. [a,b]Within d, means without common superscripts differ ($P \leq 0.05$). [y,z]Within d, means without common superscripts differ ($P \leq 0.01$).

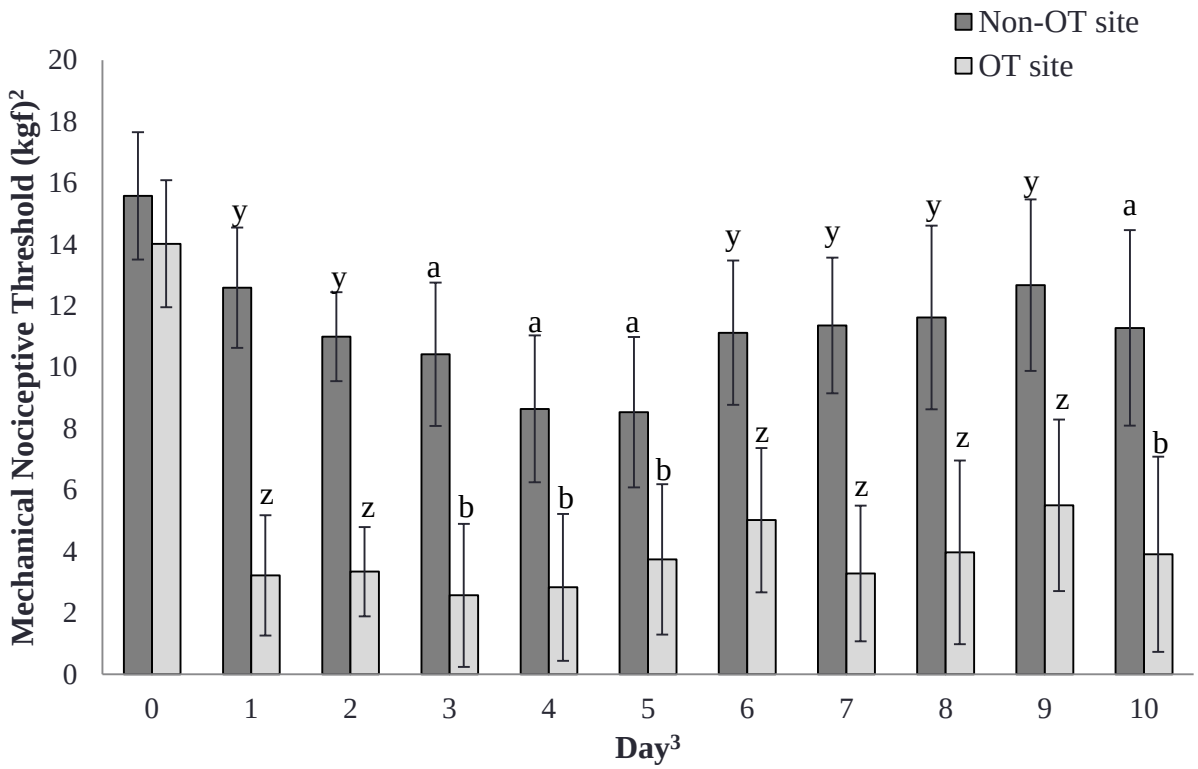


Figure 3.6: Mean response threshold (kgf) ± SEM to mechanical stimulation using pressure algometry after the i.m. injection of 5 mg/kg oxytetracycline (OT)[1] in the hind leg of cull dairy cows receiving saline (n = 5, Exp. 2). [1]Animals were administered OT (200 mg/ml) at 5 mg/kg i.m. in a randomly selected hind leg. A sham injection was performed in the opposite leg. [2]Pressure algometer capable of measuring up to 45.36 kgf. [3]Each d consisted of measurements taken at 0, 1, and 6 h, except d 0 which was only the measurement taken prior to OT administration. [a,b]Within d, means without common superscripts differ ($P \leq 0.05$). [y,z]Within d, means without common superscripts differ ($P \leq 0.01$).

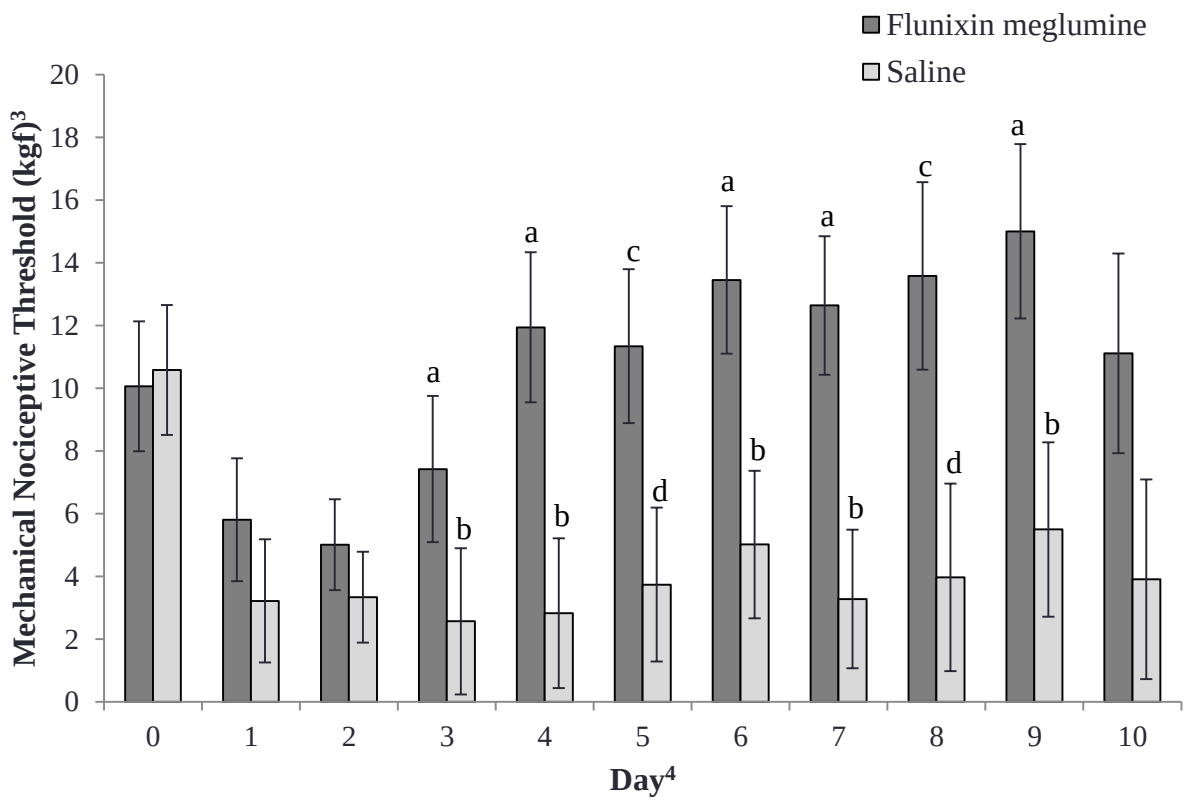


Figure 3.7: Mean response threshold (kgf) ± SEM to mechanical stimulation using pressure algometry after the i.m. injection of 10 mg/kg oxytetracycline (OT)[1] in 2 treatment[2] groups in the hind leg of cull dairy cows (n = 10; Exp. 2). [1]Animals were administered OT (200 mg/ml) at 5 mg/kg i.m. in the rear leg. [2]Treatments: 1) 2.2 mg/kg flunixin meglumine, or 2) equivalent volume 0.9% saline.[3]Pressure algometer capable of measuring up to 45.36 kgf. [4]Each d consisted of measurements taken at 0, 1, and 6 h, except d 0 which was only the measurement taken prior to OT administration. [a,b]Within d, means without common superscripts differ ($P \leq 0.05$). [d,c]Within d, means without common superscripts tend to differ ($P \leq 0.10$).

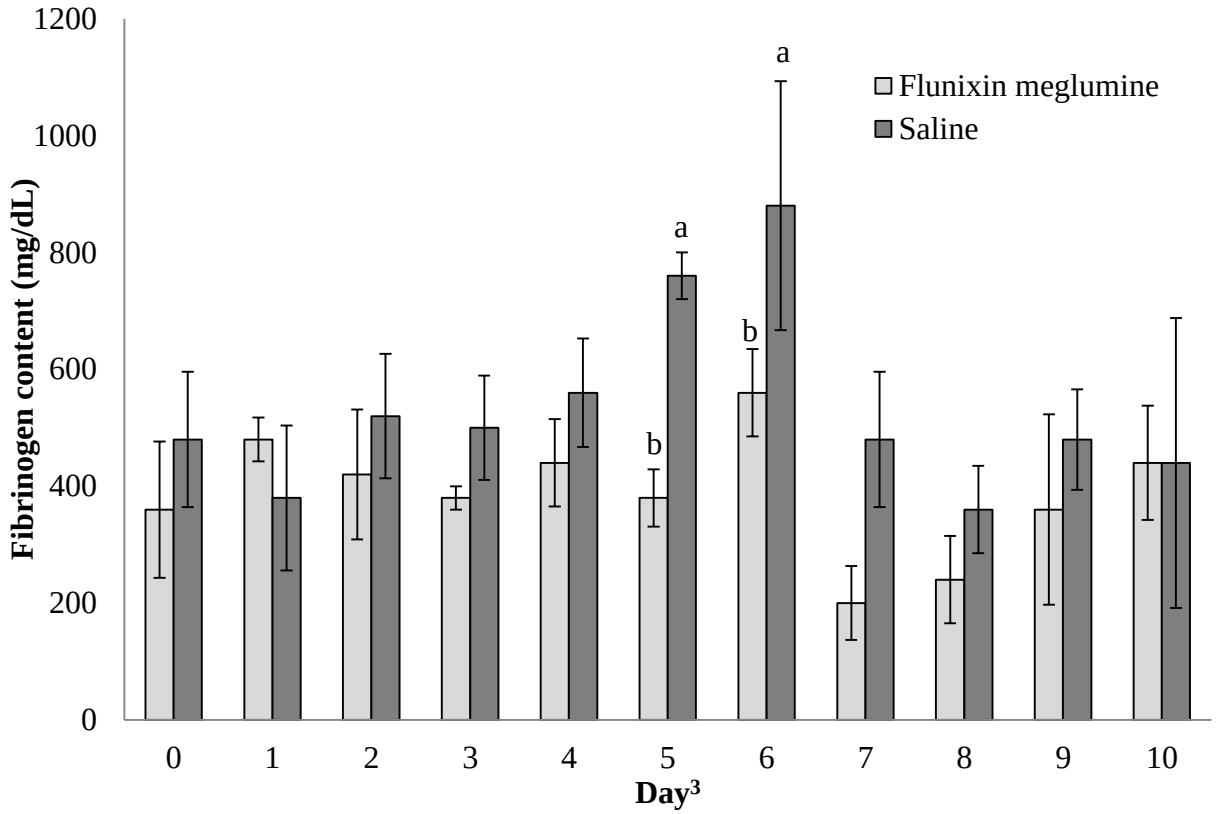


Figure 3.8: Mean fibrinogen content (mg/dL) ± SEM in cull dairy cows after chemical induction of an inflammatory response[1] on d 0 in animals treated with flunixin meglumine[2] compared to animals treated with saline. [1]Animals were administered oxytetracycline (200 mg/ml) at 5 mg/kg i.m. in the rear leg. [2]One of two treatments: 1) 2.2 mg/kg flunixin meglumine, 2) equivalent volume 0.9% saline. [3]Each d consisted of measurements taken at 0, 1, and 6 h, except d 0 which was only the measurement taken prior to OT administration. [a,b]Within d, means without common superscripts differ ($P \leq 0.05$).

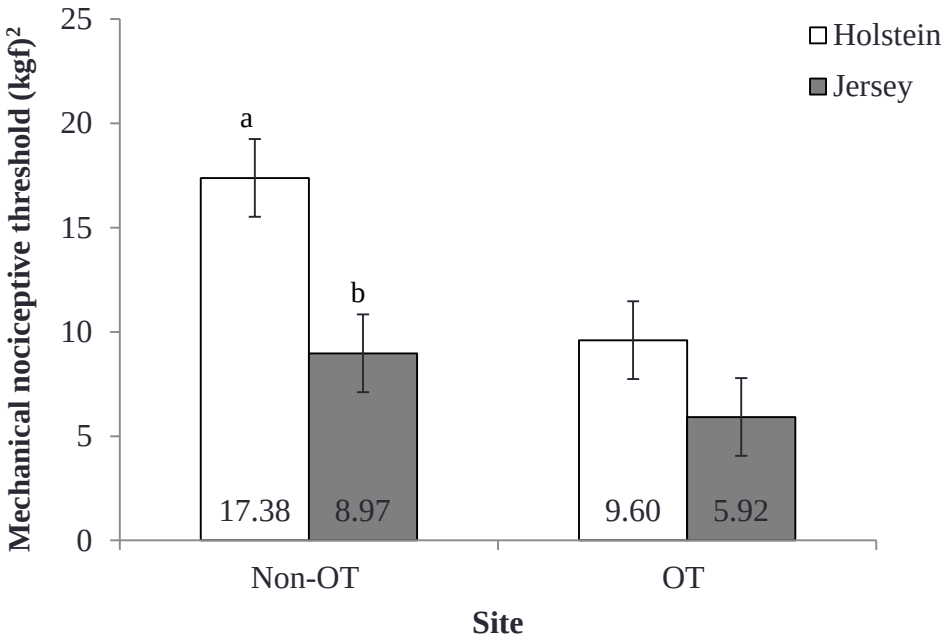


Figure 3.9: Breed comparison for mean response threshold (kgf) ± SEM to mechanical stimulation using pressure algometry after the i.m. injection of 5 mg/kg oxytetracycline (OT)[1] in the hind leg of cull dairy cows (n = 10, Exp. 2). [1]Animals were administered OT (200 mg/ml) at 5 mg/kg i.m. in one rear leg (OT) and nothing (non-OT) in the other. [2]Pressure algometer capable of measuring up to 45.36 kgf. [a,b] Within treatment, means without common superscripts differ ($P \leq 0.05$).

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APPENDIX

SAS Code for Chapter II

```
proc mixed data=HeadMove;
class Pen Treatment;
model mvmt=Treatment /ddfm=kr residual;
random Pen;
lsmeans Treatment/ pdiff adjust=tukey;
run;
proc mixed data=EV; where day gt -1; by day;
class trt day pen;
model ev=trt baseline/ddfm=kr;
random pen(trt);
lsmeans trt/pdiff;
contrast 'intact vs cast' trt 1 -.25 -.25 -.25 -.25;
contrast 'ket' trt 0 -1 1 -1 1;
contrast 'mel' trt 0 -1 -1 1 1;
contrast 'int' trt 0 1 -1 -1 1;
run;
proc mixed data=cortisol; where day gt 0;
class trt day pen;
model cortisol=trt|day baseline/ddfm=kr;
random pen(trt);
lsmeans trt|day/pdiff;
contrast 'intact vs cast' trt 1 -.25 -.25 -.25 -.25;
contrast 'ket' trt 0 -1 1 -1 1;
contrast 'mel' trt 0 -1 -1 1 1;
contrast 'int' trt 0 1 -1 -1 1;
run;
```

SAS Code for Chapter III

```
data rd1cows;
input ID Average Site Flu Time baseline baseline2 breed bcs;
if time lt 10 then per=0;
if time lt 31 and time gt 23 then per=1;
if time gt 44 and time lt 55 then per=2;
if time gt 55 and time lt 95 then per=3;
if time gt 95 and time lt 105 then per=4;
if time gt 105 and time lt 127 then per=5;
if flu eq 1 then flu2='saline';
if flu eq 2 then flu2='flunixin';
if site eq 1 then site2='con';
if site eq 2 then site2='oxytet';

proc sort data=rd1cows;
by flu id site time baseline;
proc means data=rd1cows nway noprint;
id flu2 site2;
class per id flu site ;
output out=rd1means mean=;
proc mixed data=rd1means; by per;
class id flu2 site2;
model average=flu2|site2/ddfm=kr outp=redfile;
random intercept/subject=id;
lsmeans flu2*site2/pdiff;
run;
proc gplot data=redfile;
plot resid*pred;
run;


data rd2cows;
input ID Average Site Flu Time baseline baseline2 breed bcs;
laverage=log10(average+1);
rtaverage=sqrt(average);
ftaverage=average**.25;
if time lt 31 and time gt 23 then per=1;
if time gt 44 and time lt 55 then per=2;
if time gt 55 and time lt 95 then per=3;
if time gt 95 and time lt 105 then per=4;
if time gt 105 and time lt 127 then per=5;
if time gt 127 and time lt 155 then per=6;
if time gt 155 and time lt 175 then per=7;
```

```
if time gt 175 and time lt 200 then per=8;
if time gt 200 and time lt 230 then per=9;
if time gt 230 then per=10;
if time lt 10 then per=0;
if flu eq 1 then flu2='saline';
if flu eq 2 then flu2='flunixin';
if site eq 1 then site2='con';
if site eq 2 then site2='oxytet';

proc sort data=rd2cows;
by flu id site time baseline;
proc means data=rd2cows nway noprint;
id flu2 site2;
class id flu site per;
output out=rd2means mean=;
proc sort data=rd2means;
by per flu2 id site2;
proc mixed data=rd2means; by per;
class id flu2 site2 breed;
model average=flu2|site2 breed breed*site2/ddfm=kr outp=redfile;
random intercept/subject=id;
*repeated per/subject=site*id type=ar(1);
lsmeans flu2*site2/pdiff;
lsmeans breed*site2/pdiff;
run;
proc gplot data=redfile;
plot resid*pred;
run;
```